JURNAL ILMIAH
MEDICAMENTO

VOLUME 11
NOMOR 2
EDISI SEPTEMBER 2025
ISSN: 2356 4814

UNMAS DENPASAR

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Table of Content Jinto Vol.11 No.2 September 2025

ORIGINAL ARTICLES

REVIEW ARTICLES

JURNAL ILMIAH MEDICAMENTO

Vol.11 No.2 (September 2025)
halaman 108-119
https://doi.org/10.36733/medicamento.v11i2.10708
e-ISSN: 2356-4814

Original Article

Hubungan Faktor Sosiodemografi dengan Perilaku Swamedikasi Obat Analgesik pada Masyarakat Kota Malang

The Relationship Between Sociodemographic Factors and the Self-Medication Behavior of Analgesic Drugs in the Community of Malang City

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Diajukan: 28-12-2024
Direview: 03-07-2025
Disetujui: 12-09-2025

Kata Kunci:
Swamedikasi, Faktor Sosiodemografi, Perilaku, Analgesik

Keywords:
Self-Medication, Sociodemographic Factors, Behavior, Analgesic

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Abstrak

Prevalensi swamedikasi di masyarakat Kota Malang mencapai 64,35% pada tahun 2022. Angka tersebut menunjukkan adanya potensi risiko yang cukup besar, terutama terkait perilaku yang kurang tepat dalam penggunaan obat. Analgesik merupakan jenis obat yang sering digunakan untuk mengatasi nyeri secara mandiri atau melalui swamedikasi. Penggunaan analgesik yang tidak tepat dapat menimbulkan efek merugikan, seperti gangguan pada lambung dan usus, reaksi hipersensitivitas, serta kerusakan pada ginjal dan hati. Penelitian ini bertujuan untuk mengetahui perilaku swamedikasi analgesik serta hubungan antara faktor sosiodemografi responden dengan perilaku tersebut. Penelitian ini merupakan studi analitik kuantitatif observasional dengan desain cross-sectional dan metode survei. Pengambilan sampel dilakukan secara non-probability sampling dengan melibatkan 100 responden yang memenuhi kriteria inklusi dan eksklusi. Analisis data dilakukan menggunakan uji statistik non-parametrik, yaitu uji Chi-Square untuk variabel jenis kelamin, pekerjaan, dan tempat tinggal, serta uji Spearman Rank untuk variabel usia, penghasilan, dan tingkat pendidikan. Hasil penelitian menunjukkan bahwa masyarakat Kota Malang memiliki perilaku swamedikasi analgesik yang tergolong "cukup" dengan persentase sebesar 72%. Uji statistik menunjukkan adanya hubungan yang signifikan secara statistik antara usia, penghasilan, tingkat pendidikan, dan wilayah tempat tinggal dengan praktik swamedikasi analgesik. Sebaliknya, variabel jenis kelamin dan pekerjaan tidak menunjukkan hubungan yang signifikan. Temuan ini memberikan dasar bagi penyusunan strategi edukasi dan kebijakan kesehatan yang lebih terarah untuk meningkatkan praktik swamedikasi yang aman dan rasional di masyarakat.

Abstract

The prevalence of self-medication among the population of Malang City reached 64.35% in 2022. This figure indicates a considerable potential risk, particularly related to inappropriate medication practices. Analgesics are among the most used drugs for pain relief through self-medication. Improper use of analgesics may lead to adverse effects, including gastrointestinal disturbances, hypersensitivity reactions, and damage to the kidneys and liver. This study aims to examine self-medication behavior involving analgesics and to analyze the relationship between respondents' sociodemographic factors and such behavior. The research employed quantitative analytic observational design with a cross-sectional approach and survey method. Sampling was conducted using a non-probability sampling technique, involving 100 respondents who met the inclusion and exclusion criteria. Data analysis was performed using non-parametric statistical tests, namely the Chi-Square test for gender, occupation, and residence variables, and the Spearman Rank test for age, income, and education level. The results showed that the self-medication behavior with analgesics among the people of Malang City was categorized as "moderate," with a prevalence of 72%. Statistical analysis revealed significant associations between age, income, education level, and residential area with analgesic self-medication practices. Conversely, gender and occupation did not show statistically significant relationships. These findings provide a foundation for developing targeted educational strategies and public health policies to promote safer and more rational self-medication practices.

Cara mensitasi artikel (*citation style*: AMA 11thEd.):

Yusuf MF, Sugihantoro H, Hakim A, Maulina N, Ummah SK. Hubungan Faktor Sosiodemografi dengan Perilaku Swamedikasi Obat Analgesik pada Masyarakat Kota Malang. *J. Ilm. Medicam.*, 2025:11(2), 108-119, DOI: 10.36733/medicamento.v11i2.10708

PENDAHULUAN

Masyarakat menggunakan swamedikasi sebagai alternatif untuk meredakan atau menyembuhkan keluhan ringan dan mengatasi gejala penyakit sebelum mencari pertolongan medis.[1] Kondisi yang paling sering ditangani melalui swamedikasi meliputi nyeri (22%), batuk dan sakit tenggorokan (19,5%), influenza, pilek, serta masuk angin (16%), dan demam (11,5%).[2] Penelitian lain menunjukkan bahwa demam (50,8%), pilek dan hidung tersumbat (41,5%), batuk (35,4%), nyeri/sakit kepala (33,8%), dan maag (27,7%) merupakan keluhan yang umum diatasi dengan swamedikasi.[3]

Pengobatan yang dilakukan secara mandiri atau swamedikasi adalah istilah yang digunakan untuk menggambarkan penggunaan obat-obatan yang dibeli di apotek tanpa resep dokter.[4] Masyarakat Indonesia yang melakukan swamedikasi memiliki persentase di atas 70%. Pada tahun 2024 mencapai 78,95%.[5] Sedangkan praktik swamedikasi masyarakat Jawa Timur pada tahun 2024 sebesar 79,93%.[5] Upaya dalam melakukan swamedikasi ketika mempunyai keluhan menjadi pilihan besar bagi penduduk Kota Malang, dengan persentase cukup tinggi mencapai 64,35% pada tahun 2022.[6] Tingginya prevalensi swamedikasi di masyarakat menawarkan risiko yang substansial, terutama jika tidak dilakukan dengan benar.[7] Ini disebabkan oleh ketersediaan luas obat-obatan bebas yang mudah diakses di apotek atau toko obat, untuk pengobatan mandiri tengah Masyarakat.[8]

Menurut teori Green Lawrence, elemen predisposisi seperti karakteristik sosiodemografis berperan dalam memengaruhi perubahan perilaku kesehatan individu terhadap tindakan tertentu.[9] Penelitian Prastiwi menunjukkan bahwa akurasi penggunaan obat dalam swamedikasi berkorelasi signifikan dengan faktor sosiodemografis seperti jenis kelamin, usia, tingkat pendidikan, pendapatan, dan jenis pekerjaan.[8] Penelitian lain juga menemukan bahwa usia, pendidikan, pekerjaan, dan tempat tinggal berpengaruh terhadap perilaku swamedikasi.[10] Namun, studi oleh Mardliyah menunjukkan tidak adanya hubungan antara perilaku swamedikasi dengan pekerjaan dan penghasilan.[11] Hal ini dapat disebabkan oleh perbedaan karakteristik sosiodemografi antar individu.[12]

Nyeri merupakan salah satu keluhan yang paling umum ditangani melalui swamedikasi. Jenis nyeri yang sering diobati meliputi sakit kepala, sakit gigi, nyeri otot, nyeri sendi, dismenorea, nyeri akibat luka, dan nyeri saat menelan. Kemudahan akses terhadap analgesik menjadikan obat ini pilihan utama bagi masyarakat yang mengalami nyeri ringan hingga sedang. Penelitian oleh Aria dkk., menunjukkan bahwa penggunaan analgesik secara swamedikasi mencapai 64,6%.[13] Masyarakat umumnya menggunakan analgesik golongan non-opioid, yang dianggap lebih aman karena tidak bersifat adiktif.[14]

Namun, penggunaan analgesik non-opioid secara berlebihan dapat menimbulkan efek samping seperti gangguan gastrointestinal, reaksi hipersensitivitas, serta kerusakan pada ginjal dan hati.[14] Meningkatnya ketersediaan obat bebas telah menimbulkan kekhawatiran di kalangan tenaga medis, terutama terkait penggunaan yang tidak tepat.[15] Penelitian di Kota Denpasar menunjukkan bahwa ketidaktepatan penggunaan analgesik secara swamedikasi mencapai 50,5%.[16]

Meskipun prevalensi swamedikasi telah banyak dilaporkan di berbagai daerah di Indonesia, sebagian besar penelitian masih berfokus pada aspek pengetahuan, sikap, dan kebiasaan penggunaan obat. Kajian yang secara spesifik meneliti hubungan antara faktor sosiodemografis dan perilaku swamedikasi analgesik, khususnya di Kota Malang, masih terbatas. Faktor-faktor seperti jenis kelamin, usia, pendapatan, tingkat pendidikan, jenis pekerjaan, dan domisili berpotensi memengaruhi keputusan masyarakat dalam mengonsumsi obat tanpa resep dokter. Keterbatasan data ini menimbulkan kesenjangan pemahaman mengenai determinan perilaku swamedikasi analgesik di tingkat masyarakat.

Oleh karena itu, penelitian ini penting dilakukan untuk memetakan secara rinci pola penggunaan analgesik tanpa resep serta mengidentifikasi hubungan antara faktor sosiodemografis dan perilaku swamedikasi di kalangan masyarakat Kota Malang. Temuan penelitian ini diharapkan dapat menjadi dasar dalam meningkatkan literasi penggunaan obat yang rasional serta mendukung penyusunan kebijakan kesehatan preventif guna menekan risiko penyalahgunaan obat di masyarakat.

METODE PENELITIAN

Penelitian ini dilaksanakan pada bulan September – Oktober 2024 dengan persetujuan etik dari Komisi Etik Penelitian Kesehatan (KEPK) Fakultas Kedokteran Dan Ilmu Kesehatan Uin Maulana Malik Ibrahim Malang dengan nomor kode etik No. 52/02/EC/KEPK-FKIK/09/2024.

Pada penelitian ini, menerapkan desain *cross-sectional* dalam kerangka studi observasional kuantitatif-analitik. Pengumpulan data dilakukan melalui survei menggunakan kuesioner sebagai instrumen utama. Seluruh penduduk Kota Malang menjadi populasi target. Teknik pengambilan sampel menggunakan metode *non-probability sampling* dengan pendekatan *purposive sampling*, di mana partisipan dipilih berdasarkan pertimbangan spesifik terkait tujuan riset. Kriteria inklusi meliputi: warga Malang berusia 17-65 tahun, memiliki literasi memadai (membaca & menulis), serta riwayat penggunaan analgesik dalam 30 hari terakhir. Kriteria eksklusi diberlakukan terhadap responden yang mengisi kuisioner secara tidak lengkap.

Penentuan jumlah sampel dalam penelitian ini menggunakan rumus Slovin dengan tingkat kesalahan sebesar 10%, yang dihitung sebagai berikut:

$$n = \frac{N}{1 + N\,(e)^2}$$

Keterangan:
n = Jumlah sampel
N = Jumlah Populasi
e 2 = Tingkat kesalahan 10%

Dengan demikian, hasil perhitungan sampel yang digunakan untuk penelitian ini adalah:

$$n = \frac{847.182}{1+847.182\,(0{,}1)^2} = 99{,}9$$

Sesuai dengan perhitungan besar sampel diperoleh yaitu 99,9 dan dibulatkan menjadi 100 orang responden.

Analisis Data

Pada penelitian ini, instrumen yang digunakan adalah kuesioner faktor sosiodemografi dan kuesioner perilaku dengan skala Likert yang terdiri dari sepuluh pertanyaan yang telah diujikan oleh 3 pakar farmasi dan selanjutnya dilakukan uji validitas pada sepuluh pertanyaan menunjukkan bahwa nilai r hitung lebih besar dari r tabel sebesar 0,361, yang mengindikasikan bahwa setiap item dalam kuesioner memiliki validitas yang baik.[17] Sementara itu, hasil uji reliabilitas menunjukkan nilai *Alfa Cronbach* sebesar 0,838, yang lebih besar dari batas minimum 0,60, sehingga instrumen ini dinyatakan reliabel.[18]

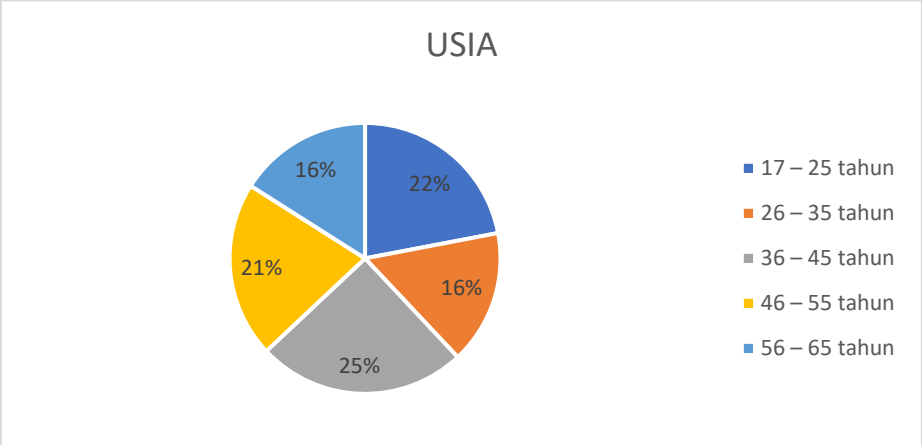
Analisi data secara univariat dan bivariat. Analisis univariat disajikan dalam bentuk ukuran statistik, tabel atau grafik. Analisis bivariat digunakan untuk uji hipotesis pada penelitian ini. Uji yang digunakan yaitu *chi-square* untuk jenis kelamin, pekerjaan dan tempat tinggal dengan perilaku menggunakan *p-value* <0,05 dan *p-hitung* untuk melihat korelasi antara variabel.[19] Sedangkan, *spearman rank* untuk usia, penghasilan dan pendidikan dengan perilaku menggunakan *p-value* <0,05 untuk melihat korelasi antara variabel dan *p-hitung* untuk melihat kekuatan dan arah korelasi antara variabel.[19]

Dalam interpretasi skala likert perlu dilakukan perhitungan persentase untuk mendapatkan persentase dari jawaban responden dengan rumus jumlah total jawaban responden/skor ideal x 100%.[17] Hasil persentase tersebut selanjutnya diinterpretasikan ke dalam tiga kategori. Responden dengan skor 76%–100% dikategorikan memiliki perilaku baik, yang menunjukkan bahwa responden telah melakukan swamedikasi analgesik sesuai prinsip penggunaan obat yang rasional. Responden dengan skor 56%–75% dimasukkan dalam kategori perilaku cukup, yang menunjukkan responden memiliki pemahaman atau praktik swamedikasi yang sebagian sudah tepat, namun masih terdapat kekurangan yang berpotensi menimbulkan risiko. Sementara itu, responden dengan skor <56% dikategorikan memiliki perilaku kurang, yang menandakan bahwa pola swamedikasi analgesik yang dilakukan belum sesuai prinsip penggunaan obat yang rasional dan berpotensi menimbulkan dampak negatif bagi kesehatan.

HASIL DAN PEMBAHASAN

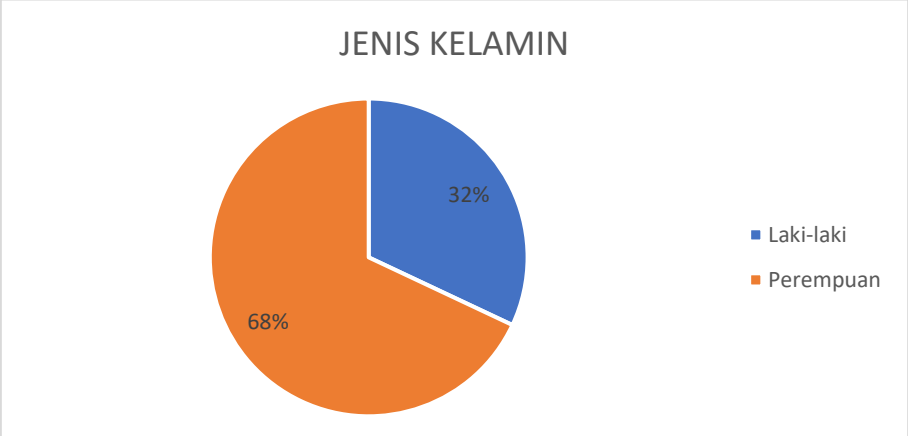
Karakteristik Responden

Berdasarkan **Gambar 1**, kelompok usia 36–45 tahun merupakan rentang usia yang paling banyak melakukan swamedikasi, yaitu sebanyak 25 responden (25%), yang umumnya termasuk dalam kategori dewasa akhir. Temuan ini menunjukkan bahwa kelompok usia dewasa akhir memiliki kecenderungan lebih tinggi dalam penggunaan analgesik secara mandiri dibandingkan kelompok usia lainnya. Data ini didukung oleh laporan Badan Pusat Statistik tahun 2023, yang menunjukkan bahwa pada tahun 2023, rentang usia 35–44 tahun merupakan kelompok usia terbanyak kedua di Kota Malang, dengan persentase sebesar 21,93%.[20] Hasil ini sejalan dengan penelitian sebelumnya yang menunjukkan bahwa kelompok usia 31–50 tahun merupakan kelompok yang paling banyak melakukan swamedikasi.[21]



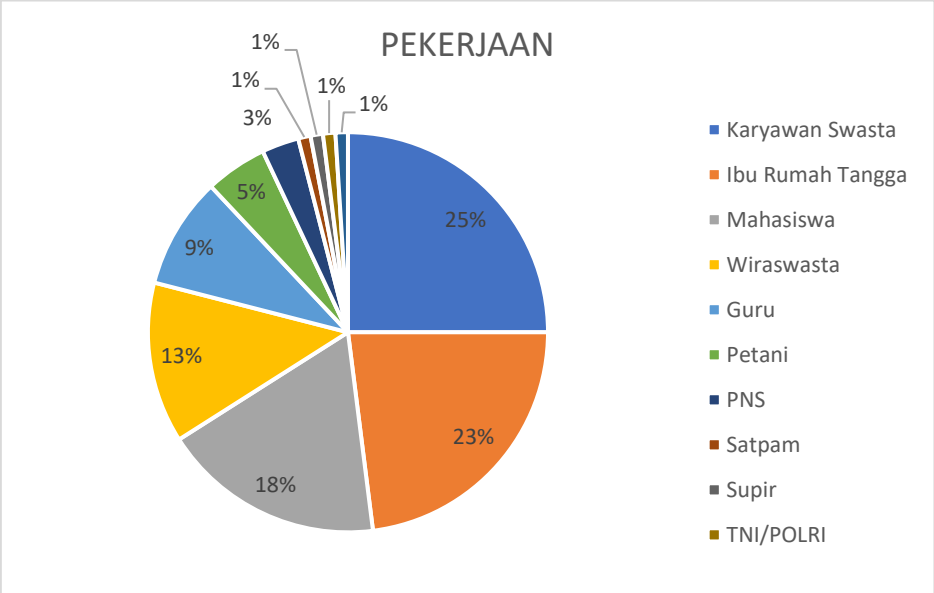
Gambar 1. Karakteristik Responden Berdasarkan Usia

Berdasarkan **Gambar 2**, diperoleh hasil bahwa perempuan lebih banyak melakukan swamedikasi menggunakan obat analgesik, yaitu sebesar 68%, dibandingkan dengan laki-laki yang sebesar 32% dalam penelitian ini. Hal ini didukung oleh data dari BPS (2023) yang menunjukkan jenis kelamin perempuan pada masyarakat Kota Malang sebesar 425.842 jiwa (50,27%) sedangkan pada laki-laki sebesar 421.340 jiwa (49,73%).[22] Temuan ini menunjukkan bahwa perempuan merupakan mayoritas peserta yang menggunakan obat analgesik untuk pengobatan mandiri. Hal ini disebabkan perempuan sering menggunakan obat analgesik untuk mengatasi nyeri haid. Penelitian lain juga menunjukkan bahwa pengobatan mandiri untuk nyeri menstruasi menjadi salah satu alasan utama perempuan lebih sering menggunakan analgesik.[23] Dalam studi terkait, data menunjukkan bahwa 95 responden, atau 73% dari total, adalah perempuan, sedangkan 35 responden, atau 27% dari total, adalah laki-laki. Proporsi ini semakin menguatkan bukti bahwa perempuan memiliki kecenderungan yang lebih besar untuk melakukan swamedikasi analgesik dibandingkan laki-laki, terutama untuk menangani kondisi nyeri tertentu.[24]



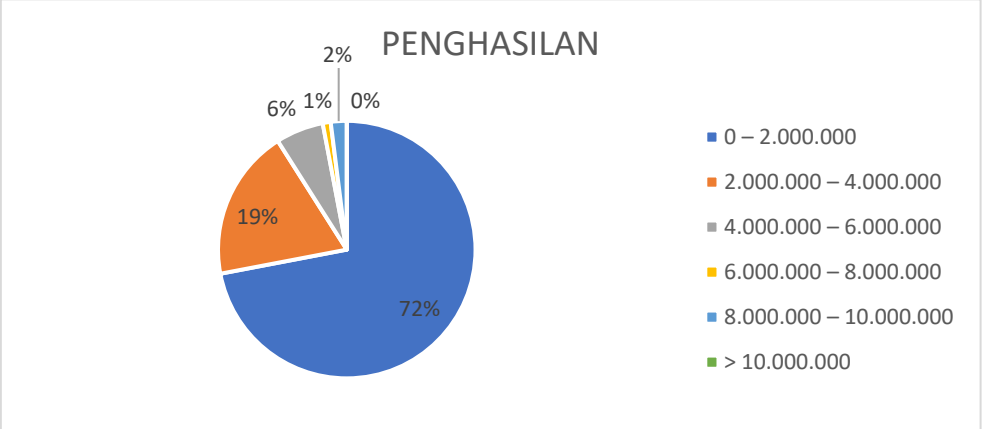
Gambar 2. Karakteristik Responden Berdasarkan Jenis Kelamin

Berdasarkan **Gambar 3**, jenis pekerjaan yang paling banyak dilakukan oleh responden adalah karyawan swasta, sebanyak 25 orang (25%). Temuan ini sejalan dengan data Badan Pusat Statistik pada tahun 2023, yang menunjukkan bahwa status pekerjaan utama masyarakat Kota Malang adalah sebagai karyawan, dengan jumlah mencapai 233.690 jiwa (54,51%).[25] Dapat disimpulkan bahwa jenis pekerjaan yang paling umum dijalani oleh masyarakat di Kota Malang adalah sebagai karyawan. Temuan ini sejalan dengan hasil penelitian lain yang menunjukkan bahwa pekerja di sektor swasta cenderung lebih sering melakukan swamedikasi, khususnya dengan menggunakan obat analgesik untuk mengatasi keluhan seperti sakit gigi. Hal ini mengindikasikan bahwa kelompok pekerja tersebut memiliki pola pengobatan mandiri yang lebih menonjol dibandingkan kelompok pekerjaan lainnya.[26]



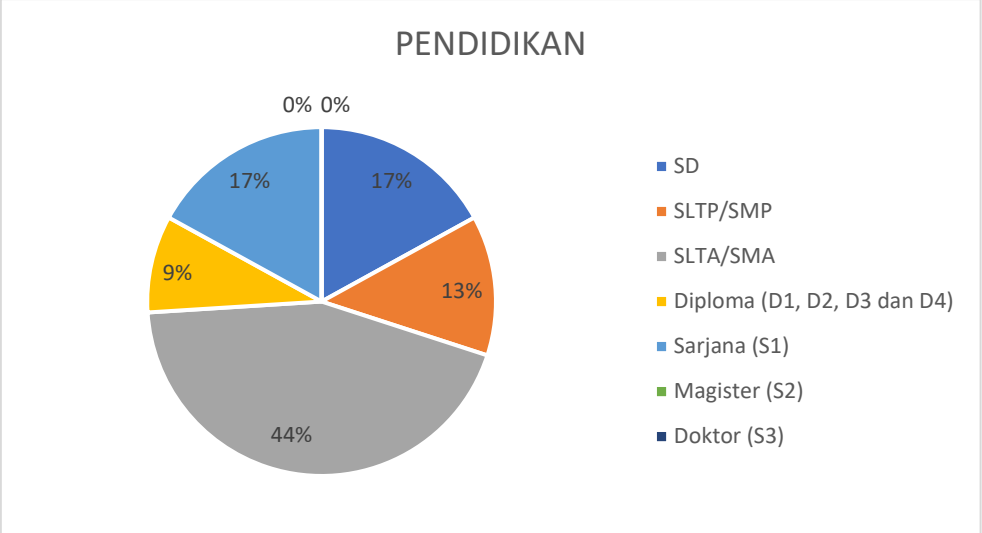
Gambar 3. Karakteristik Responden Berdasarkan Pekerjaan

Berdasarkan **Gambar 4**, mayoritas responden dalam penelitian ini memiliki penghasilan rendah, yaitu sebanyak 72 responden (72%). Temuan ini menunjukkan bahwa individu dengan penghasilan dalam kisaran Rp0–2.000.000 cenderung melakukan swamedikasi untuk mengatasi nyeri. Hal ini sejalan dengan hasil penelitian lain yang menunjukkan bahwa masyarakat berpenghasilan rendah lebih cenderung memilih swamedikasi sebagai upaya penghematan biaya pelayanan kesehatan.[27] Studi lain juga mengungkap bahwa individu dengan pendapatan kurang dari Rp2 juta per bulan memiliki kecenderungan lebih tinggi untuk menggunakan analgesik secara swamedikasi dibandingkan kelompok pendapatan lainnya. Fenomena ini dapat dikaitkan dengan keterbatasan akses terhadap layanan kesehatan, sehingga pengobatan mandiri menjadi alternatif yang lebih terjangkau dan praktis bagi kelompok berpenghasilan rendah.[28]



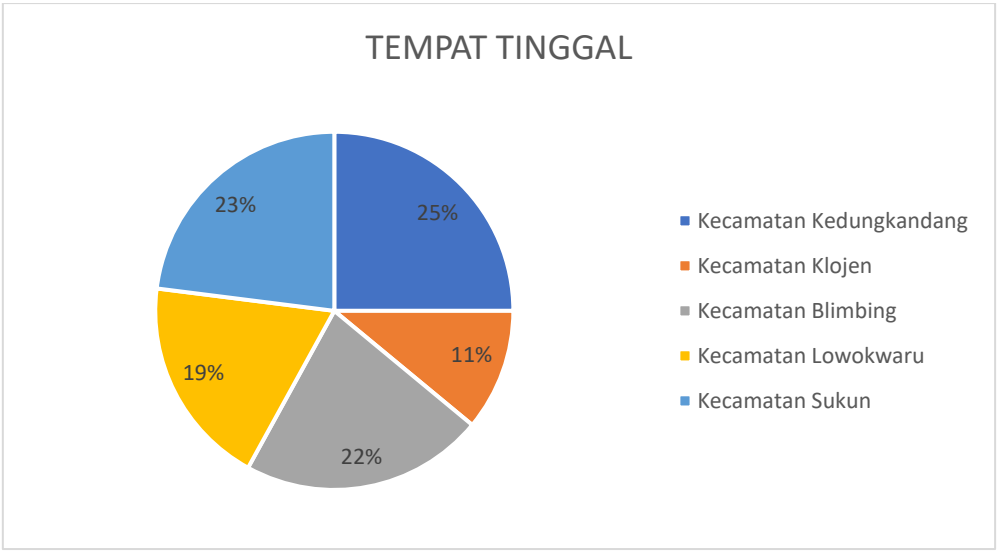
Gambar 4. Karakteristik Responden Berdasarkan Penghasilan

Berdasarkan **Gambar 5**, tingkat pendidikan terakhir responden yang paling banyak melakukan swamedikasi adalah SLTA/SMA, yaitu sebanyak 44 responden (44%). Temuan ini sejalan dengan data Badan Pusat Statistik Kota Malang, yang menunjukkan bahwa pada tahun 2023, pendidikan terakhir tertinggi yang dimiliki oleh masyarakat Kota Malang usia 10 tahun ke atas adalah SMA/sederajat, dengan persentase sebesar 35,22%.[29] Hasil ini sejalan dengan penelitian lain yang menemukan bahwa individu dengan pendidikan terakhir SMA/sederajat merupakan kelompok yang paling banyak melakukan swamedikasi.[30]



Gambar 5. Karakteristik Responden Berdasarkan Pendidikan

Sementara itu, berdasarkan **Gambar 6**, responden yang berdomisili di Kecamatan Kedungkandang tercatat sebagai kelompok terbanyak yang melakukan swamedikasi analgesik dibandingkan dengan kecamatan lainnya di Kota Malang. Data ini sesuai dengan informasi dari BPS (2023), yang mencatat bahwa Kecamatan Kedungkandang memiliki jumlah penduduk tertinggi, yaitu 209.375 jiwa, dibandingkan dengan Kecamatan Klojen (196.860 jiwa), Kecamatan Blimbing (182.851 jiwa), Kecamatan Lowokwaru (164.106 jiwa), dan Kecamatan Sukun (196.860 jiwa).[22] Tingginya jumlah penduduk di Kecamatan Kedungkandang dapat menjadi salah satu faktor yang memengaruhi tingginya angka swamedikasi di wilayah tersebut.



Gambar 6. Karakteristik Responden Berdasarkan Tempat Tinggal

Perilaku Swamedikasi Obat Analgesik

Berdasarkan kategori tingkat perilaku masyarakat kota Malang yang paling tinggi yaitu pada parameter tepat dosis sebesar 88,25% termasuk dalam kategori baik (**Tabel 1**). Hal ini dikarenakan responden sudah paham dalam menentukan dosis dan tidak diperbolehkan untuk menggandakan dosis secara mandiri

jika merasakan nyeri. Menurut studi yang berbeda, suatu zat dapat dikategorikan sebagai obat ketika digunakan untuk mengobati penyakit dengan waktu dan dosis yang tepat. Namun, penggunaan zat tersebut secara berlebihan dapat mengakibatkan keracunan dan menimbulkan efek samping yang berbahaya.[14] Temuan ini juga konsisten dengan studi lain yang menunjukkan bahwa 96% peserta mengonsumsi jumlah obat atau dosis yang tepat untuk mengobati rasa sakit mereka.[31]

Tabel 1. Hasil Persentase Swamedikasi Obat Analgesik berdasarkan Parameter Perilaku

Parameter Perilaku	Skor	Persentase Parameter	Total Persentase
Tepat Indikasi	523	65,37%	72%
Tepat Pemilihan Obat	491	61,37%	
Tepat Dosis	353	88,25%	
Tepat Efek Samping	623	77,87%	
Tepat Penilaian Kondisi Pasien	576	72%	
Tepat Tindak Lanjut	315	78,75%	

Parameter perilaku swamedikasi dengan persentase terendah di kalangan responden adalah aspek ketepatan pemilihan obat, yaitu sebesar 61,37%, yang termasuk dalam kategori cukup. Temuan ini menunjukkan bahwa responden memiliki pemahaman yang cukup dalam memilih obat yang sesuai untuk swamedikasi. Hasil ini sejalan dengan penelitian lain yang menunjukkan bahwa 72,22% responden mampu memilih obat analgesik dengan benar dalam praktik swamedikasi.[32]

Dengan persentase sebesar 72%, perilaku umum responden di Kota Malang dalam melakukan swamedikasi menggunakan obat analgesik dapat dikategorikan cukup baik. Temuan ini menunjukkan bahwa masyarakat telah memiliki keterampilan dasar dalam penggunaan analgesik untuk pengobatan mandiri, meskipun masih diperlukan pemahaman yang lebih mendalam mengenai aspek keamanan penggunaan obat. Hasil ini sejalan dengan penelitian lain yang menunjukkan bahwa perilaku swamedikasi masyarakat di Kota Magelang juga berada dalam kategori cukup.[33] Penelitian lain pun melaporkan bahwa perilaku responden dalam melakukan swamedikasi analgesik umumnya termasuk dalam kategori cukup.[34]

Hubungan Faktor Sosiodemografi Dengan Perilaku Swamedikasi

Berdasarkan **Tabel 2**, diperoleh koefisien korelasi sebesar 0,568 dengan nilai p sebesar 0,000 (< 0,05), yang menunjukkan adanya hubungan signifikan antara usia dan perilaku swamedikasi obat analgesik di Kota Malang. Temuan ini konsisten dengan hasil penelitian lain yang juga menunjukkan adanya hubungan signifikan antara usia dan perilaku swamedikasi. Penelitian lain turut mengungkap bahwa usia berperan penting dalam praktik pengobatan mandiri, khususnya dalam penggunaan obat analgesik untuk mengatasi nyeri.[2] Temuan ini mengindikasikan bahwa faktor usia memainkan peran penting dalam menentukan pola perilaku swamedikasi di masyarakat.[35]

Tabel 2. Analisis Hubungan Parameter Sosiodemografi dengan Perilaku Swamedikasi Obat Analgesik Menggunakan Uji *Chi-Square* dan *Spearman Rank*

Faktor Sosiodemografi	*p*-value	*p*-hitung	Keterangan
Usia	0.000	0.568	Adanya Hubungan
Penghasilan	0.006	0.272	Adanya Hubungan
Pendidikan	0.001	0.316	Adanya Hubungan
Tempat Tinggal	0.001	25.382	Adanya Hubungan
Jenis Kelamin	0.394	1.861	Tidak Ada Hubungan
Pekerjaan	0.201	13.413	Tidak Ada Hubungan

Nilai p sebesar 0,000 < 0,05 dan koefisien korelasi sebesar 0,568 menunjukkan adanya hubungan dengan kekuatan sedang antara usia dan perilaku swamedikasi obat analgesik di masyarakat Kota Malang. Korelasi ini bersifat positif, yang berarti bahwa semakin tinggi usia responden, semakin tinggi pula kecenderungan mereka untuk melakukan swamedikasi. Hal ini dapat dijelaskan oleh peningkatan pengetahuan

dan pengalaman yang umumnya dimiliki oleh kelompok usia dewasa, sehingga mereka lebih mampu melakukan pengobatan mandiri secara tepat dan bertanggung jawab.[2]

Koefisien korelasi pada penelitian ini sebesar 0,568 menunjukkan tingkat kekuatan korelasi yang sedang antara usia dengan perilaku swamedikasi obat analgesik pada masyarakat Kota Malang. Sedangkan korelasi menunjukkan arah yang positif atau bersifat searah. Oleh karena itu, dengan meningkatnya usia akan meningkatkan perilaku responden dalam melakukan swamedikasi. Hal tersebut dikarenakan dengan bertambahnya usia akan meningkatnya perkembangan pengetahuan, sesuai dengan pengetahuan yang telah didapatkannya, kelompok dewasa pada rentang tersebut memiliki pengalaman yang memadai sehingga dapat melakukan pengobatan secara swamedikasi dengan tepat[36].

Nilai p sebesar 0,006 < 0,05 untuk faktor sosiodemografi penghasilan, menunjukkan adanya hubungan signifikan dengan perilaku swamedikasi penggunaan obat analgesik di Kota Malang. Temuan ini konsisten dengan studi lain yang menunjukkan adanya korelasi yang kuat antara tingkat penghasilan dan praktik pengobatan mandiri.[37] Selain itu, penelitian sebelumnya juga menemukan bahwa penghasilan memiliki pengaruh signifikan terhadap perilaku swamedikasi, yang dapat memengaruhi seberapa sering individu menggunakan obat analgesik secara mandiri.[38]

Koefisien korelasi sebesar 0,272 menunjukkan adanya hubungan yang lemah namun signifikan antara tingkat penghasilan dan perilaku swamedikasi analgesik di masyarakat Kota Malang, dengan arah korelasi yang positif atau searah. Artinya, semakin tinggi penghasilan responden, semakin besar kecenderungan mereka untuk melakukan swamedikasi. Temuan ini tidak sepenuhnya sejalan dengan beberapa studi sebelumnya, seperti penelitian oleh Wulandari et al., yang menunjukkan bahwa swamedikasi lebih umum dilakukan oleh kelompok berpenghasilan rendah sebagai bentuk penghematan biaya kesehatan. Perbedaan ini dapat mencerminkan variasi dalam akses terhadap layanan kesehatan, preferensi individu, serta tingkat literasi obat di masing-masing wilayah. Meskipun pendapatan rendah sering dikaitkan dengan tingginya praktik swamedikasi, pemahaman yang tepat mengenai penggunaan obat tetap menjadi faktor krusial dalam mencegah efek samping yang merugikan, terlepas dari tingkat pendapatan.[27]

Analisis korelasi menunjukkan adanya hubungan signifikan antara tingkat pendidikan dan perilaku swamedikasi analgesik di Kota Malang, dengan nilai koefisien sebesar 0,316 dan nilai p = 0,001. Hal ini Menunjukkan hubungan signifikan antara tingkat pendidikan terhadap perilaku pengobatan mandiri. Temuan ini konsisten dengan studi oleh Chusun & Lestari (2020), yang juga melaporkan adanya hubungan bermakna antara tingkat pendidikan dan pola penggunaan analgesik secara mandiri.[39] Koefisien korelasi pada penelitian ini sebesar 0,316 menunjukkan tingkat kekuatan yang lemah antara hubungan antara pendidikan dengan perilaku swamedikasi obat analgesik pada masyarakat Kota Malang. Sedangkan korelasi memiliki arah yang positif atau bersifat searah. Pendidikan yang lebih tinggi memungkinkan individu untuk mengakses informasi kesehatan secara lebih efektif, sebagaimana dijelaskan oleh Pariyana et al. (2020), yang menyatakan bahwa pendidikan merupakan proses pembelajaran yang memperkaya kemampuan seseorang dalam menyerap dan memahami informasi, termasuk terkait penggunaan obat.[40]

Selanjutnya, terdapat hubungan signifikan antara tempat tinggal dan perilaku swamedikasi analgesik, dengan nilai p = 0,001 dan nilai Chi-Square sebesar 25,382, melebihi nilai kritis 15,507. Temuan ini dapat dikaitkan dengan distribusi fasilitas kesehatan, khususnya apotek, yang cukup merata di Kota Malang. Data tahun 2023 menunjukkan terdapat 282 apotek yang tersebar di wilayah tersebut, sehingga memudahkan masyarakat untuk memperoleh obat tanpa harus menempuh jarak yang jauh.[41] Kedekatan lokasi apotek dengan tempat tinggal memberikan kemudahan akses, efisiensi waktu, dan penghematan biaya, yang pada akhirnya mendorong praktik swamedikasi.[42] Penelitian oleh Subashini & Udayanga juga mendukung temuan ini, dengan menunjukkan bahwa lokasi tempat tinggal memiliki hubungan signifikan terhadap perilaku swamedikasi.[43]

Sebaliknya, tidak ditemukan hubungan signifikan antara jenis kelamin dan perilaku swamedikasi analgesik, dengan nilai p = 0,394 dan nilai Chi-Square sebesar 1,861, yang berada di bawah nilai kritis 5,991.

Hal ini menunjukkan bahwa jenis kelamin tidak memengaruhi kecenderungan individu dalam melakukan swamedikasi. Penelitian lain menunjukkan bahwa faktor seperti usia produktif, tingkat pendidikan, dan pengalaman lebih berperan dalam menentukan akurasi pengobatan mandiri, terlepas dari jenis kelamin. Temuan ini diperkuat oleh studi lain yang juga tidak menemukan korelasi signifikan antara gender dan perilaku swamedikasi.[44]

Demikian pula, tidak ditemukan hubungan signifikan antara jenis pekerjaan dan perilaku swamedikasi analgesik, dengan nilai p = 0,201 dan nilai Chi-Square sebesar 13,413, yang lebih rendah dari nilai kritis 18,307. Meskipun pekerjaan dapat memberikan pengalaman dan pengetahuan, terutama jika berkaitan dengan bidang kesehatan, hasil penelitian ini menunjukkan bahwa latar belakang pekerjaan bukanlah faktor penentu dalam praktik swamedikasi. Penelitian lain menyebutkan bahwa jenis pekerjaan dapat memberikan pengalaman dan pengetahuan kepada individu, baik secara langsung maupun tidak langsung.[45] Hasil penelitian ini juga sejalan dengan temuan studi lain yang menunjukkan bahwa sebagian besar responden bekerja di luar bidang kesehatan. Hal ini mengindikasikan bahwa pengetahuan atau pengalaman dalam bidang kesehatan bukan merupakan faktor penentu utama dalam praktik pengobatan mandiri. Dengan kata lain, meskipun responden tidak memiliki latar belakang pendidikan atau pekerjaan di bidang kesehatan, mereka tetap cenderung melakukan swamedikasi dengan tingkat akurasi yang serupa.[12] Temuan ini diperkuat oleh studi lain yang juga tidak menemukan hubungan signifikan antara jenis pekerjaan dan perilaku swamedikasi.[46]

Penelitian ini memiliki sejumlah kekuatan yang mendukung validitas hasil yang diperoleh. Pertama, partisipasi sebanyak 100 responden dianggap memadai untuk menggambarkan pola awal perilaku swamedikasi analgesik di wilayah Kota Malang. Kedua, penggunaan kuesioner yang telah melalui proses uji validitas dan reliabilitas turut meningkatkan akurasi dan kredibilitas data yang dikumpulkan. Selain itu, studi ini berhasil mengidentifikasi adanya hubungan signifikan antara beberapa faktor sosiodemografi seperti usia, tingkat pendidikan, penghasilan, dan lokasi tempat tinggal dengan perilaku swamedikasi. Temuan tersebut dapat dijadikan sebagai landasan awal dalam merancang program edukasi dan intervensi promotif yang disesuaikan dengan karakteristik populasi.

Namun demikian, penelitian ini memiliki keterbatasan, salah satunya adalah ruang lingkup geografis yang terbatas pada wilayah Kota Malang. Oleh karena itu, penerapan hasil penelitian ke wilayah lain dengan kondisi sosial ekonomi yang berbeda perlu dilakukan secara hati-hati dan mempertimbangkan konteks lokal. Selain itu, penggunaan kuesioner sebagai satu-satunya instrumen pengumpulan data belum mampu menggambarkan secara mendalam aspek kualitatif dari perilaku masyarakat dalam penggunaan obat analgesik secara mandiri.

SIMPULAN

Mayoritas responden (72%) di Kota Malang menunjukkan tingkat perilaku swamedikasi analgesik yang tergolong cukup. Analisis lebih lanjut mengungkap adanya hubungan yang signifikan secara statistik antara usia, penghasilan, tingkat pendidikan, dan wilayah tempat tinggal dengan praktik swamedikasi analgesik. Sebaliknya, variabel jenis kelamin dan jenis pekerjaan tidak menunjukkan hubungan yang signifikan. Temuan ini diharapkan dapat mendorong peningkatan kesadaran masyarakat dalam menerapkan swamedikasi analgesik secara tepat. Salah satu upaya yang dapat dilakukan adalah melalui penguatan regulasi dan kebijakan, di mana pemerintah daerah bersama Dinas Kesehatan dapat berperan dalam merumuskan kebijakan yang mewajibkan apotek memberikan edukasi kepada masyarakat saat membeli obat analgesik tanpa resep. Intervensi ini diharapkan menjadi langkah preventif dalam meminimalkan risiko penggunaan obat yang tidak rasional serta meningkatkan literasi masyarakat terkait penggunaan analgesik yang aman dan efektif.

KONFLIK KEPENTINGAN

Tidak ada konflik kepentingan antar penulis dari naskah.

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JURNAL ILMIAH MEDICAMENTO

Vol.11 No.2 (September 2025)
page 120-130
https://doi.org/10.36733/medicamento.v11i2.11156
e-ISSN: 2356-4814

Original Article

Antibiotic Consumption and Its Influence on *Escherichia coli* and *Klebsiella pneumoniae* Resistance: A Five-Year Ecological Study in a Regional Hospital

Penggunaan Antibiotik dan Resistensi *Escherichia coli and Klebsiella pneumoniae*: Studi Ekologikal Lima Tahun di Sebuah Rumah Sakit Umum Daerah

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Submitted : 28-02-2025
Reviewed : 07-07-2025
Accepted : 11-09-2025

Keywords: cross-resistance, DDD/100 bed-days, *Escherichia coli*, *Klebsiella pneumoniae*, meropenem.

Abstract

Antibiotic resistance is a growing global health threat, partly driven by high antibiotic consumption. The World Health Organization (WHO) has identified critical-priority bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*, due to their increasing resistance to multiple antibiotics. This study aimed to evaluate the correlation between antibiotic consumption and resistance rates in *Escherichia coli* and *Klebsiella pneumoniae*. This ecological study was conducted at a Regional Hospital in Indonesia based on retrospective inpatient data from January 2019 to December 2023. The population in this study is all data on systemic antibiotic consumption based on the J01 category of the Anatomical Therapeutic Chemical/Defined Daily Dose (ATC/DDD) classification system and antibiogram from inpatient databases. Pearson and Spearman's rank correlation analyses were performed to examine the associations between systemic antibiotic consumption levels and the percentage of *Escherichia coli* and *Klebsiella pneumoniae* resistance to other antibiotics. The most frequently used antibiotics were cefixime (305.664 DDD/100 bed-days), levofloxacin (139.552 DDD/100 bed-days), and ceftriaxone (109.805 DDD/100 bed-days). A strong and statistically significant correlation was observed between doxycycline consumption and *Escherichia coli* resistance to meropenem (r=0.894; p=0.041). Moreover, consumption levels of cefazolin, ceftazidime, cefepime, and ciprofloxacin were correlated with *Escherichia coli* resistance to ceftriaxone ($p<0.05$), while cefoperazone use demonstrated a very strong and statistically significant correlation with *Escherichia coli* resistance to ampicillin-sulbactam (r=0.952; p=0.012). Conversely, no significant correlation was found between antibiotic consumption and resistance in *Klebsiella pneumoniae*, suggesting that alternative factors such as intrinsic resistance mechanisms, mobile genetic elements, and environmental reservoirs may influence resistance development.

Kata Kunci: DDD/100 bed-days, *Escherichia coli*, *Klebsiella pneumoniae* meropenem, resistensi silang.

Abstrak

Resistensi bakteri merupakan ancaman kesehatan global yang semakin meningkat, yang sebagian disebabkan oleh tingginya tingkat penggunaan antibiotik. Organisasi Kesehatan Dunia (WHO) telah mengidentifikasi *critical-priority bacteria*, termasuk *Escherichia coli* dan *Klebsiella pneumoniae*, karena meningkatnya resistensi mereka terhadap berbagai jenis antibiotik. Penelitian ini bertujuan mengetahui korelasi antara tingkat penggunaan antibiotik dan tingkat resistensi pada *Escherichia coli* dan *Klebsiella pneumoniae*. Studi ekologikal ini dilakukan di sebuah Rumah Sakit Umum Daerah di Indonesia berdasarkan data retrospektif pasien rawat inap dari Januari 2019 hingga Desember 2023. Populasi dalam studi ini mencakup seluruh data penggunaan antibiotik sistemik berdasarkan kategori J01 dari sistem klasifikasi *Anatomical Therapeutic Chemical/Defined Daily Dose* (ATC/DDD), serta antibiogram dari basis data pasien rawat inap. Analisis korelasi Pearson dan Spearman dilakukan untuk menilai hubungan antara tingkat penggunaan antibiotik sistemik dan persentase resistensi *Escherichia coli* dan *Klebsiella pneumoniae* terhadap antibiotik lain. Antibiotik yang sering digunakan adalah sefiksim (305,664 DDD/100 hari rawat inap), levoflokasasin (139,552 DDD/100 hari rawat inap), dan seftriakson (109,805 DDD/100 hari rawat inap). Terdapat korelasi yang kuat dan signifikan secara statistik antara penggunaan doksisiklin dan resistensi *Escherichia coli* terhadap meropenem (r=0,894; p=0,041). Selain itu, penggunaan sefazolin, seftazidim, sefepim, dan siprofloksasin berkorelasi dengan resistensi *Escherichia coli* terhadap seftriakson ($p<0,05$), sementara penggunaan sefoperazon menunjukkan korelasi sangat kuat dan signifikan secara statistik dengan resistensi *Escherichia coli* terhadap ampisilin-sulbaktam

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(r=0,952; p=0,012). Sebaliknya, tidak ditemukan korelasi yang signifikan antara penggunaan antibiotik dan resistensi pada *Klebsiella pneumoniae*, yang mengindikasikan bahwa faktor lain seperti mekanisme resistensi intrinsik, elemen genetik mobil, dan reservoir lingkungan mungkin lebih berperan dalam perkembangan resistensi.

How to cite: (citation style AMA 11th Ed.)
Sanjaya DA, Meriyani H, Juanita RA, Siada NB, Mahaputra Y, Kamalia MGA. Antibiotic Consumption and Its Influence on *Escherichia coli* and *Klebsiella pneumoniae* Resistance: A Five-Year Ecological Study in a Regional Hospital. *J. Ilm. Medicam.*, 2025:11(2), 120-130, DOI: 10.36733/medicamento.v11i2.11156

INTRODUCTION

Global antibiotic consumption increased significantly by approximately 65% between 2000 and 2015.[1] In Indonesia, antibiotic consumption remains relatively high, with prevalence estimates ranging from 40% to 60%.[2] This upward trend has contributed to the accelerating problem of bacterial resistance, which now poses a major threat to achieving the Sustainable Development Goals (SDGs) by 2030. The emergence of antibiotic-resistant bacteria has led to increased rates of treatment failure, healthcare-associated infections (HAIs), and morbidity and mortality.[3,4] Furthermore, resistance amplifies healthcare costs, placing additional financial burdens on patients.[5–8]

The World Health Organization (WHO) has identified a group of critical-priority bacteria that pose an urgent threat to global public health due to their escalating resistance to multiple antibiotics. Infections caused by these bacteria are difficult to prevent because of their high transmissibility and the presence of global resistance mechanisms. Many of them have developed multidrug-resistant (MDR) strains, especially in specific populations or geographic regions. This multidrug resistance (MDR) severely limits available treatment options, complicating patient management and outcomes. Among the most concerning are *Escherichia coli* and *Klebsiella pneumoniae*, both of which are frequently implicated in life-threatening infections such as sepsis and pneumonia. Their widespread resistance patterns not only hinder therapeutic efficacy but also represent a formidable barrier to infection control efforts worldwide.[9–11]

A significant factor contributing to the development of resistance in various bacteria is the high level of antibiotic consumption. Several studies have shown a correlation between increased antibiotic consumption and and the development of resistance in *Escherichia coli* and *Klebsiella pneumoniae*, including the phenomenon of cross-resistance.[12,13] A study conducted at a hospital in Peru showed that using ceftazidime can enhance the resistance of *Enterobacter spp*. to piperacillin/tazobactam and ciprofloxacin.[12] Furthermore, research at a Malaysian hospital revealed that increased use of polymyxin antibiotics may lead to the development of carbapenem resistance in Enterobacteriaceae.[13] Klebsiella pneumoniae has developed resistance to four major classes of antibiotics, such as third-generation cephalosporins, aminoglycosides, fluoroquinolones, and carbapenems.[11,14] The development of carbapenem resistance in *Escherichia coli* and *Klebsiella pneumoniae* due to the influence of other antibiotics would significantly complicate and limit treatment options. This is particularly concerning because carbapenem antibiotics, such as meropenem, are considered last-line therapy for multidrug-resistant Gram-negative bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*.[15]

Given the regional variations in antibiotic consumption and resistance patterns, understanding these dynamics in local healthcare contexts is critical. The use of one antibiotic may not only induce resistance to itself but also promote resistance to other unrelated agents through cross-resistance.[9,15–17] Despite these global findings, there is a scarcity of studies in Indonesia that link specific antibiotic consumption to resistance trends in critical-priority bacteria, particularly *Escherichia coli* and *Klebsiella pneumoniae*. According to the 2023 report from Indonesia's national antimicrobial resistance surveillance network, Gram-negative bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae*, exhibit notably high resistance rates, especially against advanced-generation beta-lactam antibiotics. Resistance in *Klebsiella pneumoniae* remains a significant concern, with more than 65% of isolates exhibiting decreased sensitivity to third-generation cephalosporins, including ceftriaxone. Resistance to carbapenems, such as meropenem, has exceeded 20%, underscoring the increasing

presence of Carbapenem-Resistant Enterobacteriaceae (CRE) in clinical settings. Ampicillin resistance is widespread, with reported rates consistently surpassing 90% across clinical isolates. *Escherichia coli* also demonstrates an alarming resistance profile with more than 50% of isolates resistant to third generation cephalosporins and above 60% resistance rates to fluoroquinolones, including ciprofloxacin. The national surveillance system primarily collects and analyzes antimicrobial resistance data from hospitals across Indonesia to provide an overview of current conditions and resistance trends. However, it does not include correlation analysis with local or regional patterns of antibiotic consumption.[18] Understanding local resistance patterns is essential for guiding appropriate antibiotic selection. Therefore, this study aims to determine the extent of antibiotic consumption and investigate the correlation between specific antibiotics and the emergence of resistance, including potential cross-resistance pattern in critical-priority bacteria. It emphasizes the need for localized evidence to optimize antibiotic stewardship policies and improve patient outcomes.

RESEARCH METHOD

Study Design

This ecological study was conducted at a regional general hospital in Indonesia using retrospective inpatient data collected from January 2019 to December 2023. The hospital is a tertiary care facility with a capacity of 300 beds and reported bed occupancy rates (BOR) of 59%, 72%, 44%, 53%, and 59% annually from 2019 to 2023. Ethical approval for this study was granted by the hospital's ethics committee in May 2024 under approval number 070/18054/RSU.

Secondary data were used in this study, including records on antibiotic consumption, antibiograms, bed occupancy rates, and bed capacity. The independent variable was systemic antibiotic consumption, expressed in DDD per 100 bed-days. The dependent variable was the percentage of *Escherichia coli* and *Klebsiella pneumoniae* resistance. This study examines both the level of antibiotic consumption and the correlation between the use of specific antibiotics and the development of *Escherichia coli* and *Klebsiella pneumoniae* resistance to other antibiotics.

Population and Sample

The study population comprised inpatient antibiotic consumption data obtained from electronic pharmacy records and the corresponding percentages of *Escherichia coli* and *Klebsiella pneumoniae* resistance. All bacterial isolates collected between 2019 and 2023 were included, totaling 258 *E. coli* isolates and 129 *K. pneumoniae* isolates. Antibiotics analyzed were systemic agents classified under the J01 category according to the World Health Organization's Anatomical Therapeutic Chemical/Defined Daily Dose (ATC/DDD) classification system. Data were excluded if information on antibiotic consumption or resistance percentages was unavailable for any given timeframe within the study period.

The Level of Systemic Antibiotic Consumption

The level of antibiotic consumption was expressed as defined daily doses (DDD) per 100 bed-days. It was calculated using the following formula.[19]

$$\text{DDD/100 bed-days} = \frac{\text{Number of units administered in a given period (milligram)} \times 100}{\text{DDD (milligram)} \times \text{number of beds} \times \text{BOR} \times 365}$$

Percentage of *Escherichia coli* and *Klebsiella pneumoniae* Resistance

Antibiotic susceptibility data for *Escherichia coli* and *Klebsiella pneumoniae* were obtained from antibiogram records. The antibiogram were sourced from the Antimicrobial Resistance Control Program at the hospital where the study was conducted and included data on the percentage of *Escherichia coli* and *Klebsiella pneumoniae* susceptibility. *Escherichia coli* and *Klebsiella pneumoniae* isolates were collected from blood, sputum, and urine specimens. Susceptibility testing within the antibiograms followed the Clinical and

Laboratory Standards Institute (CLSI) guidelines, utilizing the disk diffusion method. The percentage of *Escherichia coli* and *Klebsiella pneumoniae* resistance was calculated by subtracting the susceptibility percentage from 100 percent.

Data Analysis

Data normality was assessed using the Shapiro–Wilk test. Variables conforming to the normality assumption were analyzed with Pearson's correlation, whereas those violating this assumption were analyzed with Spearman's rank correlation. Correlation analysis quantified the association between systemic antibiotic consumption and the percentage of *Escherichia coli* and *Klebsiella pneumoniae* isolates resistant to other antibiotics. Results are reported as correlation coefficients (r) with corresponding p-values.

The correlation coefficient (r) was used to assess the strength and direction of the relationship between variables, with values ranging from −1 (perfect negative correlation) to +1 (perfect positive correlation). Correlation strength was interpreted as follows: 0.90–1.00, very strong; 0.70–0.89, strong; 0.40–0.69, moderate; 0.10–0.39, weak; and 0.00–0.10, negligible. Statistical significance was set at $p < 0.05$. All analyses were performed using IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA).[20]

RESULT AND DISCUSSION

Antibiotic Consumption

Table 1 presents data on systemic antibiotic consumption at a Regional Hospital in Indonesia from 2019 to 2023. Over this five-year period, the most frequently used antibiotics were cefixime (305.664 DDD/100 bed-days), levofloxacin (139.552 DDD/100 bed-days), and ceftriaxone (109.805 DDD/100 bed-days). The findings of this study align with previous research conducted in inpatient departments of hospitals in China between 2013 and 2021, which identified third-generation cephalosporins as the most frequently used antibiotics.[21] Another study, covering hospital inpatient facilities in China from 2012 to 2022, indicated that third-generation cephalosporins, second-generation cephalosporins, and fluoroquinolones consistently ranked as the top three classes of antibiotics throughout the study period.[22] Similarly, a study at M. Natsir Hospital in Solok City in 2020 identified cefixime as the most widely used antibiotic, with a DDD/100 bed-days value of 67.791.[23] Third-generation cephalosporins are broad-spectrum antibiotics with a good pharmacokinetic profile, a low incidence of side effects, and relatively affordable prices. They show stronger activity against gram-negative bacteria.[24]

A recent cross-sectional study at Kiruddu National Referral Hospital in Uganda analyzed medicine delivery records for antibiotic consumption and utilization from 2021-2022, found third-generation cephalosporins and fluoroquinolones among the most consumed.[25] A study conducted in the inpatient ward of internal medicine at a hospital in Bandung in 2020 stated that the highest-consumed antibiotic was levofloxacin.[26] In addition to cefixime, levofloxacin also exhibited high total consumption between 2019 and 2023. Levofloxacin, a member of the fluoroquinolone class, provides broad-spectrum activity against both Gram-positive and Gram-negative bacteria. Moreover, it is a concentration-dependent antibiotic—higher concentrations enhance its bactericidal efficacy.[27]

Given their pharmacological advantages and broad-spectrum capabilities, both third-generation cephalosporins and fluoroquinolones are classified by the World Health Organization (WHO) under the Watch category in the AWaRe (Access, Watch, Reserve) framework. This classification indicates their elevated potential for resistance development and highlights the need for cautious and selective use. The inclusion of cefixime, ceftriaxone, and levofloxacin—commonly used antibiotics in this study—within the Watch group underscores the importance of reserving these agents for targeted therapy rather than empirical treatment. WHO recommends that Watch antibiotics be used only when Access group alternatives are ineffective due to clinical severity or microbial resistance.[28]

Table 1. The Level of Systemic Antibiotic Consumption at a Regional Hospital in Indonesia from 2019 to 2023

ATC Code	Antibiotic	Antibiotic Consumption (DDD/100 Bed-Days)					
		2019	2020	2021	2022	2023	Total 2019-2023
J01DD08	Cefixime	50.676	50.816	49.659	77.116	77.397	305.664
J01MA12	Levofloxacin	24.323	31.419	39.751	21.940	22.119	139.552
J01DD04	Ceftriaxone	15.968	16.242	20.024	29.694	27.877	109.805
J01FA10	Azithromycin	15.648	19.558	11.078	21.827	24.192	92.303
J01CA04	Amoxicillin	19.886	13.564	19.537	26.123	0.704	79.814
J01MA02	Ciprofloxacin	11.107	9.695	12.564	13.121	12.345	58.832
J01DB05	Cefadroxil	13.209	9.112	20.445	8.694	6.898	58.358
J01DD01	Cefotaxime	6.591	5.238	4.699	5.024	4.280	25.832
J01AA02	Doxycycline	5.241	3.425	5.063	3.610	2.914	20.252
J01MA14	Moxifloxacin	3.020	3.709	4.252	1.403	1.927	14.311
J01CR02	Amoxicillin and Clavulanic Acid	3.508	1.771	1.139	0.953	3.285	10.656
J01DB04	Cefazoline	1.525	0.938	2.053	2.511	1.833	8.860
J01DE01	Cefepime	1.156	2.253	0.637	0.442	0.765	5.253
J01DH02	Meropenem	0.369	0.800	1.916	1.285	0.796	5.166
J01DD12	Cefoperazone	0.611	0.385	1.775	0.637	1.011	4.418
J01DD02	Ceftazidime	0.500	0.090	0.446	1.251	0.719	3.006
J01CR01	Ampicillin and Sulbactam	1.766	0.670	0.041	0.129	0.212	2.818
J01CA01	Ampicillin	0.322	0.146	0.055	0.087	0.164	0.775
J01CE08	Benzylpenicillin	0.009	0.001	0.035	0.096	0.199	0.340
	Total	**175.433**	**169.831**	**195.170**	**215.944**	**189.637**	**946.016**

Resistance Profile of *Escherichia coli* and *Klebsiella pneumoniae*

Table 2 shows the percentage of resistance in *Escherichia coli* and *Klebsiella pneumoniae* from 2019 to 2022. Based on the 2021 resistance pattern shown in **Table 2**, *Escherichia coli* demonstrated multi-drug resistance (MDR), becoming resistant to three or more antibiotics, such as ampicillin-sulbactam, ceftriaxone, and ciprofloxacin. Meanwhile, *Klebsiella pneumoniae* exhibited resistance to ampicillin throughout the study period.

Table 2. Percentage of *Escherichia coli* and *Klebsiella pneumoniae* Resistance at a Regional Hospital in Indonesia from 2019 to 2023

Antibiotic	Percentage of Resistance (%)				
	2019	2020	2021	2022	2023
Escherichia coli					
Number of Isolate (n)	**19**	**17**	**54**	**65**	**103**
Ampicillin and Sulbactam	57.90	47.10	77.00*	50.00	57.30
Piperacillin and Tazobactam	15.80	0.00	13.00	6.30	10.80
Ceftriaxone	52.60	41.20	65.00*	74.60*	65.70*
Ceftazidime	47.40	18.70	43.00	31.20	52.90
Cefepime	0.00	11.90	43.00	20.30	29.40
Amikacin	0.00	0.00	0.00	0.00	3.90
Meropenem	5.30	0.00	2.00	0.00	0.00
Ciprofloxacin	84.20*	47.10	78.00*	68.70*	78.60*
Klebsiella pneumoniae					
Number of Isolate (n)	**10**	**7**	**28**	**34**	**50**
Ampicillin	100.00*	85.70*	100.00*	100.00*	100.00*
Ceftriaxone	50.00	28.60	43.00	50.00	36.00
Ceftazidime	50.00	14.30	39.00	41.20	35.40
Amikacin	0.00	0.00	0.00	0.00	0.00
Meropenem	0.00	10.00	0.00	2.90	0.00
Ciprofloxacin	80.00*	33.30	54.00	61.80*	54.00

*Highly resistance (more than 60%)

A study conducted in Thailand from 2017 to 2018 found that 41.3% of *Escherichia coli* isolates were classified as MDR.[29] Similarly, a 10-year retrospective study from a tertiary hospital in China, which analyzed antimicrobial resistance patterns in *Escherichia coli* isolates collected from various clinical samples and across different patient age groups, showed findings consistent with the present study. This study revealed a high prevalence of MDR *Escherichia coli*, with notable resistance to commonly used antibiotics, including ampicillin, ceftriaxone, and ciprofloxacin.[30] Comparable resistance patterns have also been reported in *Klebsiella pneumoniae*. A study conducted in Duhok City, located in the Kurdistan Region of Iraq between 2017 and 2019, documented a very high level of resistance of *Klebsiella pneumoniae* to ampicillin.[31] Likewise, a study conducted in Vietnam found that *Klebsiella pneumoniae* exhibited the highest resistance rates to ampicillin among all antibiotics tested.[32]

In gram-negative bacteria such as *Escherichia coli*, resistance to β-lactam antibiotics such as penicillins, cephalosporins, carbapenems, and monobactams occurs primarily through three mechanisms: the production of β-lactamases that hydrolyze antibiotics, increased expression of efflux pumps that expel antibiotics from the bacterial cel, and decreased expression or loss of porins, which limits antibiotic penetration into the cell[33]. Fluoroquinolone resistance in *Escherichia coli* involves several mechanisms, including chromosomal mutations that alter target enzymes such as the GyrA subunit of DNA gyrase and the ParC subunit of topoisomerase IV. Additionally, decreased intracellular drug concentrations may result from overexpression of efflux pumps and downregulation of porins. The emergence of plasmid-mediated quinolone resistance (PMQR) genes also contributes, involving qnr (protects target enzymes), *aac(6')-Ib-cr* (modifies quinolones), and *qepA* and *oqxAB* (enhance drug efflux).[34]

In *Klebsiella pneumoniae*, resistance to ampicillin is primarily mediated by the production of TEM-1 and SHV-1 β-lactamases, which are encoded chromosomally and efficiently hydrolyze penicillins.[35,36] Mutations in these genes can give rise to extended-spectrum β-lactamases (ESBLs), capable of hydrolyzing a broader range of β-lactams, including third-generation cephalosporins and monobactams. ESBLs are frequently plasmid-borne, facilitating horizontal gene transfer. Additionally, loss or modification of outer membrane porins (e.g., OmpK35, OmpK36) reduces antibiotic influx, further enhancing resistance when combined with β-lactamase activity. *K. pneumoniae* also employs efflux systems, such as AcrAB-TolC, to actively expel antibiotics like ampicillin. Moreover, biofilm formation contributes to resistance by creating a physical barrier that limits antibiotic penetration; ampicillin, in particular, exhibits poor diffusion through biofilms.[36]

Correlation between antibiotic consumption and the percentage of resistance to other antibiotics in *Escherichia coli* and *Klebsiella pneumoniae*

Table 3 shows a correlation coefficient between antibiotic consumption and the percentage of resistance to other antibiotics in *Escherichia coli*. A strong and statistically significant correlation was observed between the level of doxycycline consumption and *Escherichia coli* resistance to meropenem ($r=0.894$ and $p<0.05$). Doxycycline, a tetracycline-class antibiotic, induces the tet(A) gene in *Escherichia coli* isolates, which encodes efflux pumps that confer resistance to all tetracycline-class antibiotics.[37,38] Resistance genes such as tet(A) are found in multi-resistance plasmids such as the 225 kb IncHI2/IncP plasmid, which also carries the blaCTX, blaTEM-1, blaNDM-5, sul1, sul2, dfrA1, and aadA1 genes. The 90-120 kb IncI1 plasmid carries resistance genes such as tet(A) along with blaSHV-12, blaNDM-5, blaKPC-2, blaOXA-48, aadA1, cmlA1, and aadA2 genes. The 250 kb IncFIB/IncHI2 plasmid carries the tet(B) gene and the blaCTX-M-2, blaNDM-5, sul1, aadA29, strA, and strB genes. The IncFIC plasmid carries the tet(A) gene along with the blaCMY-2, blaNDM-5, blaKPC-2, blaOXA-48, cmlA, floR, strA, strB, sul1, sul3, and aadA7 genes. The 40 kb IncFIB/IncN plasmid carries the tet(A) gene and the blaNDM-5, sul1, dfrA16, and dfrA29 genes. These multi-resistance plasmids may subsequently be transferred horizontally by conjugation to other *Escherichia coli* bacteria. Thus, the use of doxycycline can accelerate the spread of resistance genes that cause cross-resistance to meropenem.[39–41]

Table 3. The Correlation Coefficient between Antibiotic Consumption and The Percentage of Resistance to Other Antibiotics in *Escherichia coli* at a Regional Hospital in Indonesia from 2019 to 2023

Antibiotic Resistance	Antibiotic Consumption					
	Doxycycline	Cefazolin	Ceftazidime	Cefoperazone	Cefepime	Ciprofloxacin
Ciprofloxacin Resistance	0.495	0.505	0.367	0.503	-0.735	0.593
Ceftazidime Resistance	0.253	0.343	0.233	0.504	-0.617	0.494
Meropenem Resistance	0.894*	-0.112	-0.224	0.112	0.112	-0.112
Amikacin Resistance	-0.707	0.001	0.354	0.354	0.001	0.001
Ampicillin and Sulbactam Resistance	0.624	0.310	-0.133	0.952*	-0.473	0.415
Cefepime Resistance	-0.281	0.492	0.141	0.868	-0.513	0.603
Piperacillin and Tazobactam Resistance	0.657	0.347	0.165	0.524	-0.595	0.466
Ceftriaxone Resistance	-0.090	0.980*	0.886*	0.456	0.957*	0.991*

*Significant (p<0,05)

Very strong correlation
Strong correlation
Moderate correlation
Weak-negligible correlation

In addition to doxycycline, other antibiotic classes such as cephalosporins also show significant associations with *Escherichia coli* resistance patterns. The consumption of antibiotics such as cefepime, ceftazidime, and cefazolin is correlated with *Escherichia coli* resistance to ceftriaxone ($p<0.05$), which is a third-generation cephalosporin antibiotic. In addition, the consumption of cefoperazone antibiotics is correlated with *Escherichia coli* resistance to ampicillin sulbactam ($r=0.952$ and $p=0.012$). Cephalosporin antibiotics induce resistance genes such as TEM, SHV, blaCTX-M, and OXA. These genes encode Extended-Spectrum Beta-Lactamase (ESBL) enzymes that confer resistance to the penicillin and cephalosporin groups (generations I, II, and III) and aztreonam[42]. In a study in Indonesia, the most common gene causing *Escherichia coli* resistance to third-generation cephalosporin antibiotics is the blaCTX-M gene, and globally, the most dominant gene causing resistance is blaCTX-M-15 and blaCTX-M-14, which contributes to a phenomenon known as "The CTX-M β-lactamase pandemic".[43,44]

This study also showed that the use of ciprofloxacin increased the percentage of *Escherichia coli* resistance to ceftriaxone ($r=0.991$ and $p<0.05$). Ciprofloxacin will induce the qnr gene, which protects DNA gyrase and topoisomerase IV from fluoroquinolone inhibition. This gene can be found on IncF, IncA/C2, and IncHI2 plasmids, which also carry resistance genes for ceftriaxone, namely blaCTX-M and blaCMY-2. The IncF, IncA/C2, and IncHI2 plasmids containing the qnr, aac(6')-Ib-cr, blaCTX-M, and blaCMY-2 genes can be transferred from one bacteria to another either horizontally or vertically.[45]

Table 4 shows that there is no significant correlation between antibiotic consumption and the percentage of resistance to other antibiotics in *Klebsiella pneumoniae*. However, antibiotics should not be administered without careful consideration. *Klebsiella pneumoniae* possesses intrinsic resistance mechanisms, such as reduced outer membrane permeability (e.g., porin loss), active efflux pumps, and chromosomally encoded β-lactamases. Moreover, resistance genes in *Klebsiella pneumoniae* are frequently located on mobile genetic elements, including plasmids, integrons, and transposons, which facilitate their rapid dissemination through horizontal gene transfer. Moreover, the persistence of resistance may be influenced by environmental contamination in hospital settings, including surfaces, medical equipment, and wastewater systems, as reservoirs for resistant strains of *Klebsiella pneumoniae*.[36]

In addition, there may be several inherent and ecological factors contributing to this lack of a statistically significant association of *Klebsiella pneumoniae* resistance with antibiotic consumption. *Klebsiella pneumoniae* has a more dynamic resistance profile and has more complex mechanisms that are less associated to only the level of antibiotics applied. A major contributing factor is the ability of the organism to form biofilms, which protect against environmental stresses and impede antibiotic penetration. Biofilm-associated cells exhibit altered metabolic states and reduced susceptibility, rendering them less responsive to antimicrobial pressure in a manner not directly reflected by antibiotic consumption. Furthermore, *Klebsiella pneumoniae* is characterized by innate resistance mechanisms and has the ability to acquire resistance genes

through horizontal gene transfer, primarily through plasmids encoding carbapenemases or extended-spectrum β-lactamases (ESBLs). These genetic traits may be disseminated regardless of the local use of antimicrobials, particularly in overcrowded healthcare-facilities and poor infection control environments.[46,47]

Table 4. The Correlation Coefficient between Antibiotic Consumption and The Percentage of Resistance to Other Antibiotics in *Klebsiella pneumoniae* at a Regional Hospital in Indonesia from 2019 to 2023

Antibiotic Resistance	Antibiotic Consumption					
	Doxycycline	Benzylpenicillin	Cefazolin	Ceftazidime	Cefoperazone	Ciprofloxacin
Ampicillin Resistance	0.354	0.707	0.707	0.707	0.707	0.707
Ceftriaxone Resistance	0.577	-0.056	0.689	0.655	0.131	0.603
Ceftazidime Resistance	0.575	0.137	0.618	0.552	0.287	0.616
Meropenem Resistance	0.667	-0.447	-0.224	-0.224	-0.671	-0.224
Ciprofloxacin Resistance	0.579	0.013	0.420	0.453	0.036	0.393

Strong correlation
Moderate correlation
Weak-negligible correlation

Despite these findings, the ecological design of this study imposes inherent limitations. This design only allows data analysis at the population level, preventing the identification of individual-level antibiotic exposure durations within the studied population. The duration of such exposure may significantly influence the percentage of antibiotic resistance in *Escherichia coli* and *Klebsiella pneumoniae*. Certain classes of antibiotics, such as beta-lactams, exhibit time-dependent pharmacodynamics, where their efficacy relies on the duration of time the drug concentration remains above the minimum inhibitory concentration (MIC).[48] Inappropriate use of time-dependent antibiotics—such as insufficient dosing or extended intervals between doses—can facilitate the emergence of resistance by exposing bacteria to suboptimal drug concentrations for prolonged periods, thereby allowing them to develop adaptive mechanisms against these agents.[49]

This analysis used Defined Daily Dose (DDD) calculation with Bed Occupancy Rate (BOR) as part of standard methodology to estimate antibiotic use.[50] It did not however, explicitly consider variations of BOR during the COVID-19 pandemic period (2020–2021) during which the general pattern of hospital admissions and occupancy rates was heavily influenced by public health measures and by changing clinical priorities. It is accepted that the changes in BOR during pandemic could have affected DDDs and estimated consumptions in both 2019 and 2020. However, these variations were not considered as a factor in this study. It is suggested that future research should consider more detailed hospital-level data to better control for such contextual variables and offer a more robust interpretation of consumption measures in times of disruption.

Although the study has certain methodological limitations, the results still offer meaningful insights that can support the development of antibiotic stewardship strategies. The findings of this study highlight the importance of improving antibacterial stewardship through monitoring antibiotic consumption and the integration of resistance data into prescribing practices. As such, clear institutional policies and focused education for the health care provider are critical to maintaining responsible use of antibiotics and prevent the development of antibiotic resistance. Therefore, developing clear institutional protocols and delivering focused educational initiatives for healthcare professionals represent fundamental measures in promoting responsible antibiotic consumption and slowing the advancement of antibiotic resistance.

CONCLUSION

This study identified significant correlations between antibiotic consumption and resistance in *Escherichia coli*, particularly for doxycycline, cephalosporins, and ciprofloxacin, whereas no significant

association was observed for *Klebsiella pneumoniae*. These findings underscore the multifactorial nature of antimicrobial resistance and highlight the need for comprehensive stewardship strategies that integrate optimized prescribing, robust infection prevention, environmental controls, and genomic surveillance. Targeted interventions should prioritize third-generation cephalosporins and fluoroquinolones, given their strong association with resistance. Furthermore, antibiotic use policies should be locally adapted and guided by resistance profiles to promote prudent prescribing and mitigate the emergence and dissemination of resistant strains.

ACKNOWLEDGEMENT

This study was supported by Universitas Mahasaraswati Denpasar (Grant number: K.171/B.01.01/LPPM-Unmas/IV/2024).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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JURNAL ILMIAH MEDICAMENTO

Vol.11 No.2 (September 2025)
page 131-138
https://doi.org/10.36733/medicamento.v11i2.11725
e-ISSN: 2356-4814

Original Article

Sunscreen Activity Assays of an Emulgel Formulation Containing Nanoencapsulated *Ipomoea batatas* L. (Antin-3 Variety) Leaf Extract

Uji Aktivitas Tabir Surya dari Formula Emulgel yang Mengandung Nanokapsul Ekstrak Daun Ubi Jalar Ungu (*Ipomoea batatas* (L.)) Varietas Antin-3

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Submitted : 23-05-2025
Reviewed : 16-08-2025
Accepted : 20-09-2025

Keywords:
Antin-3 Nanocapsule, Emulgel, Erythema, Pigmentation, SPF.

Abstract

Flavonoids in the purple sweet potato leaf extract (*Ipomoea batatas* (L.)) variety Antin-3 (Antin-3 extract) demonstrate effectiveness as natural sunscreen agents. Flavonoids are characterized by their large molecular weight and their susceptibility to degradation by light, high temperatures, and external environmental factors. Therefore, modification of the Antin-3 extracts through nanoencapsulation using chitosan and NaTPP is necessary to reduce particle size and protect the flavonoids, allowing them to remain longer in the skin layers and provide optimal sunscreen protection. This study aimed to formulate nanoencapsulated Antin-3 extract into emulgel sunscreen preparations and evaluate its sunscreen activity through SPF, erythema, and pigmentation values. The tested samples consisted of a base (control) and nanoencapsulated Antin-3 extract at concentrations of 0.3%, 0.6%, and 0.9%, labeled as Base, F1, F2, and F3, respectively. The physical characterization results showed pH values of 6.39 ± 0.04; 5.98 ± 0.28; 5.62 ± 0.25; and 5.3 ± 0.42 (sig. 0.027 < 0.05), and spreadability values of 5.91 ± 0.17; 5.89 ± 0.15; 6.08 ± 0.31; and 6.28 ± 0.16 cm^2 (sig. 0.266 > 0.05). The SPF values were 7.48 ± 0.01; 21.03 ± 0.03; 38.02 ± 0.10; and 38.29 ± 0.07 (sig. 0.00 < 0.05). The base formulation was categorized as having weak protection, the 0.3% Antin-3 emulgel provided moderate protection, while the 0.6% and 0.9% formulations were classified as offering high protection. All emulgel sunscreen formulations demonstrated the ability to protect the skin from redness and pigmentation caused by UV exposure. These findings highlight the potential of nanoencapsulated Antin-3 extract as a sustainable, plant-based alternative to synthetic sunscreen agents, contributing to safer and more eco-friendly photoprotection solutions.

Kata Kunci:
Emulgel, Erythema, Nanokapsul Antin-3, Pigmentasi, SPF.

Abstrak

Flavonoid dalam ekstrak daun ubi jalar ungu (*Ipomoea batatas* (L.)) varietas Antin-3 (ekstrak Antin-3) terbukti efektif sebagai agen tabir surya alami. Flavonoid memiliki karakteristik berat molekul yang besar dan rentan terhadap degradasi akibat cahaya, suhu tinggi, dan faktor lingkungan eksternal. Oleh karena itu, modifikasi ekstrak Antin-3 melalui nanoenkapsulasi menggunakan kitosan dan NaTPP diperlukan untuk memperkecil ukuran partikel dan melindungi flavonoid, sehingga dapat bertahan lebih lama di lapisan kulit dan memberikan perlindungan tabir surya yang optimal. Penelitian ini bertujuan untuk memformulasikan ekstrak Antin-3 yang dienkapsulasi dalam bentuk nano ke dalam sediaan emulgel tabir surya serta mengevaluasi aktivitas tabir surya melalui nilai SPF, eritema, dan pigmentasi. Sampel uji terdiri dari basis (kontrol) dan ekstrak Antin-3 nanoenkapsulasi dengan konsentrasi 0,3%, 0,6%, dan 0,9%, masing-masing diberi label Basis, F1, F2, dan F3. Hasil karakterisasi fisik menunjukkan nilai pH sebesar 6,39 ± 0,04; 5,98 ± 0,28; 5,62 ± 0,25; dan 5,3 ± 0,42 (sig. 0,027 < 0,05), serta nilai daya sebar sebesar 5,91 ± 0,17; 5,89 ± 0,15; 6,08 ± 0,31; dan 6,28 ± 0,16 cm^2 (sig. 0,266 > 0,05). Nilai SPF masing-masing adalah 7,48 ± 0,01; 21,03 ± 0,03; 38,02 ± 0,10; dan 38,29 ± 0,07 (sig. 0,00 < 0,05). Formulasi basis dikategorikan memiliki perlindungan lemah, emulgel Antin-3 0,3% memberikan perlindungan sedang, sedangkan formulasi 0,6% dan 0,9% diklasifikasikan memiliki perlindungan tinggi. Semua formulasi emulgel tabir surya menunjukkan kemampuan melindungi kulit dari kemerahan dan pigmentasi akibat paparan sinar UV. Temuan ini menegaskan potensi ekstrak Antin-3

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nanoenkapsulasi sebagai alternatif tabir surya berbasis tanaman yang aman, ramah lingkungan, dan berkelanjutan untuk menggantikan agen sintetis.

How to cite: (citation style AMA 11th Ed.)
Dipahayu D, Adenka DS, Srianto JI, Astutik ND. Sunscreen Activity Assays of an Emulgel Formulation Containing Nanocapsulated *Ipomoea batatas* L. (Antin-3 Variety) Leaf Extract. *J. Ilm. Medicam.*, 2025:11(2), 131-138, DOI: 10.36733/medicamento.v11i2.11725

INTRODUCTION

Ultraviolet (UV) radiation from sunlight comprises UVA (320–400 nm), UVB (290–320 nm), and UVC (200–290 nm) wavelengths. Prolonged UV exposure can cause erythema, hyperpigmentation, photoaging, and increase the risk of skin cancer.[1] Erythema, if untreated, may progress to pigmentation, while deeper UV penetration exacerbates carcinogenic risk. Sunscreens remain one of the most effective strategies for photoprotection.[2]

Growing concerns over the safety and environmental impact of synthetic UV filters have driven interest in natural alternatives with antioxidant and photoprotective properties. Purple sweet potato (Ipomoea batatas L.) leaves, particularly the Antin-3 variety, are rich in polyphenols and flavonoids. The extract contains 16.98 ± 0.77% flavonoids and 4.83 ± 0.07% polyphenols when extracted with 70% ethanol. At 900 ppm, Antin-3 extract demonstrates an SPF of 31.71 and exhibits strong antioxidant activity (IC_{50} = 47.99 ppm), approximately half that of vitamin C (20.18 ppm)[3] Despite these promising properties, incorporating Antin-3 extract into an emulgel sunscreen reduced its SPF to 6.5.[4] Emulgels—oil-in-water emulsions combined with a gel base—offer advantages such as improved spreadability, enhanced skin feel, and better solubility for hydrophilic actives.[4]

The reduction in SPF is attributed to the instability of polyphenols and flavonoids, which are prone to oxidation and degradation under environmental stressors such as heat and light. Encapsulation in biocompatible carriers, such as chitosan–sodium tripolyphosphate (NaTPP) nanoparticles, can enhance stability and skin penetration. [5] Chitosan, a biodegradable polysaccharide, forms nanoparticles via ionic gelation with NaTPP, creating a protective matrix for active compounds.[6] Nanoencapsulated Antin-3 prepared at a 1:5:1 ratio (extract : chitosan : NaTPP) demonstrated antioxidant activity (IC_{50} = 48.67 ppm) comparable to the crude extract.[7]

This study aimed to formulate sunscreen emulgels containing nanoencapsulated Antin-3 at concentrations of 0.3%, 0.6%, and 0.9%, and to evaluate their SPF values and protective effects against erythema and pigmentation.

RESEARCH METHOD

Tools. The tools used in this study were analytical balance (OHAUS-PA214), Spectrophotometer UV-Vis (Thermo Genesys 102), Centrifuge (PCL-03), Magnetic stirrer (Scilogex), mortar, stamper, beaker glass, spatula, watch glass.

Materials. Antin-3 purple sweet potato leaves were obtained from BALITKABI, Malang. Nanocapsules of Antin-3 extract were prepared at the Pharmaceutical Laboratory, Pharmacy Academy, Surabaya. Emulgel excipients included olive oil (cosmetic grade), cethyl alcohol (pharmaceutical grade), Tween 80 (pharmaceutical grade), Span 80 (pharmaceutical grade), 2-propanol (pharmaceutical grade), Carbopol 940 (pharmaceutical grade), propylene glycol (pharmaceutical grade), triethanolamine (TEA; pharmaceutical grade), methylparaben (nipagin; pharmaceutical grade), propylparaben (nipasol; pharmaceutical grade) sourced from Muda Berkah Jogja, and CO_2-free demineralized water (aqua demineralisata) obtained from Brataco, Surabaya.

Formulation and Preparation of Emulgel Sunscreen

Emulgel was prepared according to the formulation presented in **Table 1**. The gelling agent was prepared by dispersing Carbopol 940 into one-third of the total CO_2-free demineralized water and stirring slowly until homogeneous. Triethanolamine (TEA) was added dropwise to neutralize the dispersion, which was then allowed to hydrate for 30 minutes. The oil phase, consisting of olive oil, Span 80, and cethyl alcohol, was

melted in a water bath at 70 °C. The aqueous phase, comprising Tween 80, methylparaben, propylparaben, and the remaining water, was heated to the same temperature. The aqueous phase was gradually added to the oil phase under continuous stirring using a magnetic stirrer until an emulsion formed and cooled to 35 °C. The hydrated gelling agent was then incorporated into the emulsion, followed by the addition of propylene glycol, to obtain the emulgel base. Antin-3 extract nanocapsules, pre-dispersed in propylene glycol (1:1), were incorporated into the emulgel base at concentrations of 0.3%, 0.6%, and 0.9% to produce the final sunscreen emulgels.[8]

Table 1. Emulgel Sunscreen Formulation

Material	Function	Base	F1	F2	F3
Nanocapsule Antin-3 Extract	Active Ingredients	-	0.3	0.6	0.9
Olive oil	Oily base	10	10	10	10
Cethyl alcohol	Viscosity increasing agent	2.5	2.5	2.5	2.5
Span 80	Emulgator	8.4	8.4	8.4	8.4
Tween 80	Emulgator	5.6	5.6	5.6	5.6
Carbopol 940	Gelling agent	2	2	2	2
TEA	Alkalizing agent	2	2	2	2
Propylene glycol	Humectant	10	10	10	10
Methylparaben	Preservatives	0.8	0.8	0.8	0.8
Propylparaben	Preservatives	0.02	0.02	0.02	0.02
Aquadest CO_2 Free	Solvent	ad 100	ad 100	ad 100	ad 100

Emulgel sunscreen characterization

Organoleptic characteristic

The organoleptic evaluation assessed the sensory characteristics of the emulgel, including texture, color, and odor.[9]

pH value characteristic

The pH of the emulgel formulations was measured using a calibrated pH meter. Approximately 1 g of each sample was dispersed in 10 mL of CO_2-free demineralized water and mixed until homogeneous. The electrode was immersed in the dispersion, and the pH value was recorded once the reading stabilized.[8]

Spreadibilty characteristic

Spreadability was evaluated using the parallel plate method. Approximately 0.5 g of emulgel was placed on a glass plate and covered with another plate. Incremental weights of 50, 100, 150, and 200 g were applied sequentially, each for 60 seconds. The diameter of the spread was measured in multiple directions, and the mean value was recorded for analysis.[8]

In vitro SPF value, % erythema and % pigmentation measurement

Samples of Antin-3 leaf extract nanocapsule emulgel sunscreen (1,000 mg) were prepared at concentrations of 0.3%, 0.6%, and 0.9%. Each sample was dissolved in 2-propanol in a beaker, transferred to a 10 mL volumetric flask, and diluted to the mark with 2-propanol.[9] The solution was then filtered through filter paper into a clean beaker. An aliquot was placed in a cuvette and adjusted to the appropriate volume. UV-Vis spectrophotometric analysis was performed using 2-propanol as the blank.[10] Absorbance was measured across the wavelength range of 290–320 nm at 5 nm intervals to calculate the Sun Protection Factor (SPF). Transmission values within this range were recorded to determine the percentage of erythema protection. Additionally, transmittance was measured between 323–373 nm to calculate the percentage of pigmentation protection.[11]

SPF Value equation:

$$\text{SPF} = \text{CF} \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times \text{abs}(\lambda) \quad \text{......} \quad (1)$$

Description:

EE : Erythemal effect spectrum

I : Solar intensity spectrum

Abs : Absorbance of sunscreen product
CF : Correction factor (=10)

In vitro % erythema and % pigmentation

$$\%Te = \frac{Ee}{\Sigma Fe} = \frac{\Sigma(TxFe)}{\Sigma Fe} \quad \text{..... (2)}$$

$$\%Tp = \frac{Ep}{\Sigma Fp} = \frac{\Sigma(TxFp)}{\Sigma Fp} \quad \text{..... (3)}$$

Description:
% Te : percent erythema transmission value
% Tp : percent pigmentation transmission value
Ee : ∑(T x Fe)
Ep : ∑(T x Fp)

Statistical analysis

Data was analyzed using SPSS software. The Shapiro–Wilk test was applied to assess the normality of data distribution, while Levene's test was used to evaluate homogeneity of variances. When both assumptions were satisfied ($p \geq 0.05$), the data were further analyzed using one-way analysis of variance (ANOVA).

RESULT AND DISCUSSION

The organoleptic characteristics, pH values, and spreadability of the samples are presented in **Table 2**. Organoleptic evaluation showed that increasing extract concentration resulted in a darker brown color and a stronger extract aroma. The acceptable pH range for topical preparations is 4.5–5.5.[12] The base and emulgel formulations containing 0.3% and 0.6% nanocapsules exhibited neutral pH values, whereas the 0.9% formulation met the skin pH requirement. One-way ANOVA indicated significant differences in pH among all samples ($p = 0.027 < 0.05$).

The recommended spreadability range for emulgel is 2.58–5.00 cm.[13] None of the formulations met this criterion; however, a wider spread area is desirable to ensure uniform skin coverage.[14] Antin-3 extract nanocapsules exhibit acidic properties that influence the neutral pH of the emulgel base. Phenolic compounds—of which more than 8,000 have been identified—contain at least one aromatic ring and one or more hydroxyl groups. The delocalized electron system of the benzene ring stabilizes the phenoxide anion formed upon proton dissociation.[15] The acidic environment in Carbopol-based gels must be neutralized with triethanolamine (TEA) to optimize gel formation.[16] Increasing the concentration of Antin-3 nanocapsules reduced formulation viscosity, resulting in a thinner consistency. One-way ANOVA showed no significant differences in spreadability among samples ($p = 0.266 > 0.05$). For sunscreen formulations, ease of application and adequate coverage are essential to ensure effective facial protection.

Table 2. Evaluation results of basis and emulgel nanocapsule of Antin-3 extract sunscreen

Sample	Organoleptic	pH value	Spreadability
Base	Thick, white, odorless	6.39 ± 0.04	5.91 ± 0.17
F1	Thick, cloudy white, mild extract odor	5.98 ± 0.28	5.89 ± 0.15
F2	Thick, whitish-brown, mild extract odor	5.62 ± 0.25	6.08 ± 0.31
F3	Thick, whitish-brown, normal extract odor	5.3 ± 0.42	6.28 ± 0.16

The results for SPF, percentage erythema protection, and percentage pigmentation protection are presented in **Table 3**. Sunscreen effectiveness is primarily indicated by the Sun Protection Factor (SPF), which represents the ratio of the minimal erythema dose (MED) on protected skin to that on unprotected skin. MED is defined as the lowest UV radiation dose or exposure time required to produce erythema. This value reflects

the duration of protection provided by the active ingredients in the sunscreen, where an SPF of 1 is approximately equivalent to 15 minutes of protection.[17]

Table 3. Result of SPF Value

Sample	SPF Value				Sunscreen category
	Rep 1	Rep 2	Rep 3	Average	
Base	7.47	7.48	7.49	7.48 ± 0.01	Low (SPF 2-15)
F1	21.00	21.04	21.07	21.03 ± 0.03	Middle (SPF 16-29)
F2	37.89	38.03	38.13	38.02 ± 0.10	High (SPF 30-50)
F3	38.33	38.42	38.24	38.29 ± 0.07	High (SPF 30-50)

The statistical analysis of SPF values is presented in **Table 4**. The SPF data for the four samples were normally distributed and homogeneous. One-way ANOVA revealed a significant difference among the groups ($p < 0.05$). The emulgel base exhibited an SPF of 7.48, classified as low protection (SPF 2–15) and considered ineffective for adequate sun protection. The emulgel containing 0.3% nanocapsules demonstrated moderate protection (SPF 16–29), while formulations with 0.6% and 0.9% nanocapsules achieved high protection (SPF 30–50), comparable to formulations containing 9% extract. In tropical regions such as Indonesia, sunscreens with SPF ≥30 are recommended.[18] The high SPF values observed are attributed to the polyphenolic compounds in Antin-3 extract nanocapsules, which absorb UV radiation and neutralize reactive oxygen species.[18]

Table 4. Statistical result of SPF Value

Statistic Test	Sig Requirements	Sig result	Conclusions
Normality Test	> 0.05	0.12	Data normally distributed
Homogeneity Test	> 0.05	0.16	Data homogenously distributed
One Way Anova	< 0.05	0.00	Significant difference

The percentage erythema values are presented in **Table 5**. The data were normally distributed and homogeneous ($p > 0.05$). One-way ANOVA followed by Tukey's HSD test revealed significant differences between the base formulation and the emulgel containing 0.3% Antin-3 extract nanocapsules compared to formulations with 0.6% and 0.9% nanocapsules ($p < 0.05$). No significant difference was observed between the 0.6% and 0.9% formulations ($p > 0.05$). Polyphenols in Antin-3 extract reduce erythema by scavenging reactive oxygen species (ROS) and suppressing the inflammatory cascade responsible for redness and swelling.[20] UVB radiation is a major contributor to sunburn, causing erythema and irritation of the skin.[20]

Table 5. Result of % erythema value

Sample	% Erythema				Sunscreen category
	Rep 1	Rep 2	Rep 3	Average	
Base	9.91	9.93	9.97	9.93 ± 0.02	Suntan (6-12)
F1	0.41	0.41	0.40	0.41 ± 0.00	Sunblock (<1)
F2	0.01	0.01	0.01	0.01 ± 0.00	Sunblock (<1)
F3	0.01	0.01	0.01	0.01 ± 0.00	Sunblock (<1)

The percentage pigmentation values are presented in **Table 6**. The data were normally distributed and homogeneous ($p > 0.05$). One-way ANOVA followed by Tukey's HSD test revealed a statistically significant difference ($p < 0.05$) among the base formulation and the emulgel sunscreens containing Antin-3 extract nanocapsules at concentrations of 0.3%, 0.6%, and 0.9%, in terms of pigmentation protection. There is a known relationship between erythema and pigmentation, as erythema-induced inflammation can trigger biological processes that lead to hyperpigmentation. Antin-3 extract nanocapsules provide dual protection by reducing both erythema and pigmentation through their antioxidant activity.[21]

Table 6. Result of % pigmentation value

Sample	% Erythema				Sunscreen category
	Rep 1	Rep 2	Rep 3	Average	
Base	43.00	43.18	43.34	43.17 ± 0.14	Extra protection (42-86)
F1	7.72	7.80	7.78	7.77 ± 0.04	Sunblock (3-40)
F2	2.10	2.09	2.09	2.09 ± 0.00	Sunblock (3-40)
F3	1.13	1.14	1.14	1.14 ± 0.00	Sunblock (3-40)

The base formulation exhibited erythema values within the suntan category (6–12%) and pigmentation values within the extra protection category (42–86%). This indicates that while the base formulation offers some protection against pigmentation, it is insufficient to prevent erythema or skin irritation caused by UV exposure. Pigmentation is primarily triggered by UVB radiation (280–315 nm), which has a shorter wavelength than UVA (315–400 nm). The emulgel base contains olive oil, which provides partial protection against shortwave radiation but is not effective against longer wavelengths.[22] In contrast, emulgel formulations containing Antin-3 extract nanocapsules at concentrations of 0.3%, 0.6%, and 0.9%, as well as the formulation with 9% extract, demonstrated total sunblock protection, effectively preventing both erythema and pigmentation.[23] Purple sweet potato (*Ipomoea batatas*) leaves, the source of Antin-3 extract, are rich in flavonoids, which exhibit anti-inflammatory activity through multiple pathways, including inhibition of cyclooxygenase (COX) and lipoxygenase enzymes, suppression of leukocyte accumulation, inhibition of neutrophil degranulation, and reduction of histamine release. These mechanisms contribute to the extract's ability to mitigate UV-induced inflammation and pigmentation.[24] To further illustrate the comparative duration of skin protection, **Figure 1** presents the estimated protection times for each formulation.

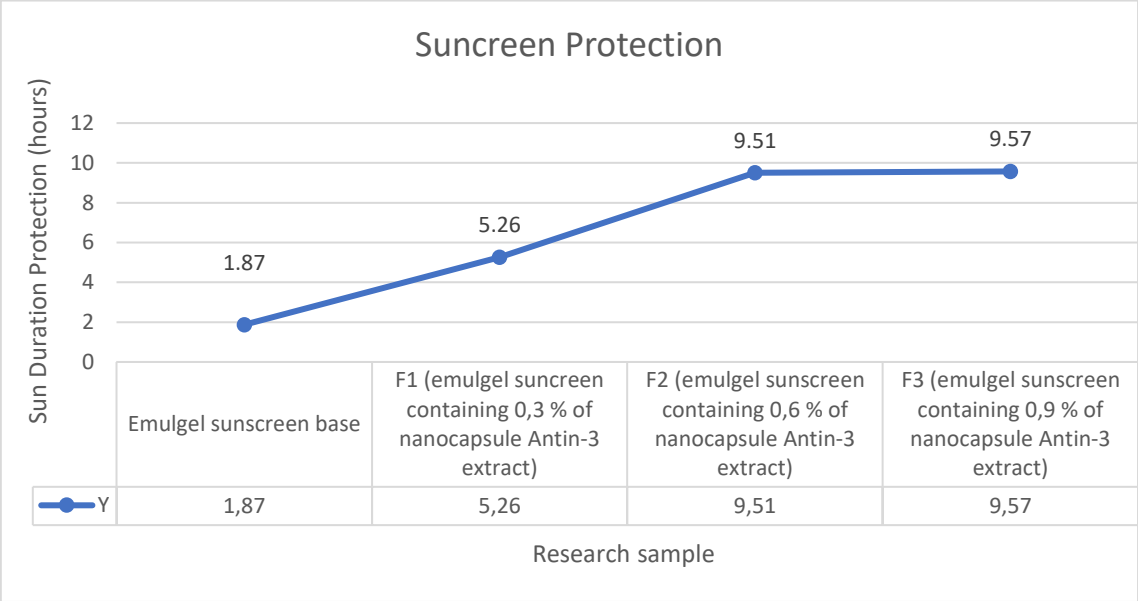

Figure 1. Sun duration protection of samples

As shown in Figure 1, increasing the concentration of nanocapsules enhanced skin protection against UV radiation. This effect is attributed to the higher flavonoid content in Antin-3 extract, which increases light absorption at specific wavelengths, thereby elevating absorbance values.[26] The 0.3% formulation provided only moderate protection with a shorter duration of UV defense, indicating that the conjugated double bonds of polyphenols and flavonoids at this concentration were insufficient for effective photoprotection.[27] In contrast, formulations containing 0.6% and 0.9% Antin-3 extract nanocapsules exhibited comparable durations of protection, although statistical analysis revealed a significant difference between them. Both concentrations effectively prevented erythema and completely inhibited skin pigmentation. These findings suggest that a 0.6% concentration of Antin-3 extract nanocapsules is adequate to protect against erythema and pigmentation through UV absorption and anti-inflammatory mechanisms.[20,21] Notably, the 0.3% difference between 0.6% and 0.9% concentrations resulted in only a 0.06-hour (3.6-minute) difference in protection time, implying that higher concentrations may offer minimal additional benefit. Further studies are warranted to determine whether this marginal increase in protection is clinically meaningful and to explore potential differences in antioxidant and anti-inflammatory activity between the two concentrations.

CONCLUSION

All emulgel sunscreen formulations containing Antin-3 extract nanoencapsulated (0.3%, 0.6%, and 0.9%) met the required physicochemical standards, including organoleptic properties, pH, and spreadability. Variations in nanocapsule concentration significantly influenced UV protection performance. Formulations with 0.6% and 0.9% Antin-3 extract nanocapsules demonstrated high photoprotective efficacy, providing estimated protection durations of 9.51 and 9.57 hours, respectively. In contrast, the 0.3% formulation offered only moderate protection, with a duration of 5.26 hours. These findings indicate that a 0.6% concentration is sufficient to achieve prolonged UV protection, while increasing the concentration to 0.9% yields minimal additional benefit. Further studies are recommended to evaluate whether the slight increase in protection time at higher concentrations is clinically significant and to explore potential differences in antioxidant and anti-inflammatory activity.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support provided by Akademi Farmasi Surabaya through the internal research grant (Agreement No. 031/AKFAR-SBY/PPPM/50.02/IV/2021). The authors also extend their sincere appreciation to Akademi Farmasi Surabaya for providing the facilities and resources necessary to conduct this study.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this manuscript.

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JURNAL ILMIAH MEDICAMENTO

Vol.11 No.2 (September 2025)
page 139-151
https://doi.org/10.36733/medicamento.v11i2.11418
e-ISSN: 2356-4814

Article Review

Bibliometric Analysis of Polyherbal Formulations as Aphrodisiacs: A Review of Current Research Trends

Analisis Bibliometrik Formulasi Poliherbal sebagai Afrodisiak: Tinjauan Tren Penelitian Terkini

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Submitted : 26-03-2025
Reviewed : 21-05-2025
Approved : 03-07-2025

Keywords: aphrodisiac, herbal combination, polyherbal formulation, sexual health, therapeutic potential.

Kata Kunci: afrodisiak, formulasi poliherbal, kesehatan seksual, kombinasi herbal, potensi terapeutik.

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Abstract

In recent years, the therapeutic potential of polyherbal formulations as aphrodisiacs, particularly as natural alternatives to synthetic treatments for sexual health disorders, has garnered significant attention. Polyherbal formulations are believed to enhance efficacy through synergistic effects, making them a preferred option in both traditional and modern therapeutic practices. This review aims to provide a comprehensive analysis of research trends relating to the use of polyherbal formulations for aphrodisiac purposes. A bibliometric analysis was conducted using the Scopus database, extracting relevant articles with the keywords 'Aphrodisiac AND Polyherbal OR Combination.' From an initial pool of 88 articles, 16 were selected for in-depth analysis. VOSviewer software was used to visualize collaboration networks, co-authorship patterns, and keyword trends, highlighting the increasing research interest in polyherbal formulations for enhancing sexual health. The narrative review supplements these findings by exploring study methodologies, sources of herbal ingredients, formulation compositions, and commonly used herbs. Results indicate that while polyherbal aphrodisiac formulations show promising therapeutic potential, research in this field remains fragmented, with limited clinical trials evaluating safety and efficacy. The most frequently studied herbs include *Tribulus terrestris*, *Mucuna pruriens*, and *Withania somnifera*, known for their effects on testosterone levels, vascular function, and libido enhancement. Aphrodisiac polyherbal formulations comprising 2 to 41 plant species operate through inter-plant synergistic mechanisms that enhance sexual function and support reproductive health comprehensively. These findings provide valuable insights for researchers and industries developing polyherbal-based nutraceutical and pharmaceutical products.

Abstrak

Dalam beberapa tahun terakhir, potensi terapeutik formulasi poliherbal sebagai afrodisiak, khususnya sebagai alternatif alami pengganti terapi sintetis untuk gangguan kesehatan seksual, telah menarik perhatian yang signifikan. Formulasi poliherbal diyakini meningkatkan efektivitas melalui efek sinergis, sehingga menjadi pilihan yang disukai baik dalam praktik pengobatan tradisional maupun modern. Tinjauan ini bertujuan memberikan analisis komprehensif mengenai tren penelitian terkait penggunaan formulasi poliherbal untuk tujuan afrodisiak. Analisis bibliometrik dilakukan menggunakan basis data Scopus dengan mengekstraksi artikel relevan menggunakan kata kunci "Aphrodisiac AND Polyherbal OR Combination". Dari 88 artikel awal, 16 artikel dipilih untuk analisis mendalam. Perangkat lunak VOSviewer digunakan untuk memvisualisasikan jaringan kolaborasi, pola kepenulisan bersama, dan tren kata kunci, yang menunjukkan meningkatnya minat penelitian terhadap formulasi poliherbal untuk meningkatkan kesehatan seksual. Tinjauan naratif melengkapi temuan ini dengan mengeksplorasi metodologi penelitian, sumber bahan herbal, komposisi formulasi, dan tanaman yang paling sering digunakan. Hasil menunjukkan bahwa meskipun formulasi afrodisiak poliherbal memiliki potensi terapeutik yang menjanjikan, penelitian di bidang ini masih terfragmentasi dengan uji klinis yang terbatas terkait keamanan dan efektivitas. Tanaman yang paling sering diteliti meliputi *Tribulus terrestris*, *Mucuna pruriens*, dan *Withania somnifera*, yang dikenal berpengaruh terhadap kadar testosteron, fungsi vaskular, dan peningkatan libido. Formulasi afrodisiak poliherbal yang terdiri dari 2 hingga 41 spesies tanaman

bekerja melalui mekanisme sinergis antar tanaman yang meningkatkan fungsi seksual dan mendukung kesehatan reproduksi secara komprehensif. Temuan ini memberikan wawasan penting bagi peneliti dan industri dalam pengembangan produk nutraseutikal dan farmaseutikal berbasis poliherbal.

How to cite: (citation style AMA 11th Ed.)
Kartika IGAA, Nugraha IS, Mahardika IBP, Suryani S. Bibliometric Analysis of Polyherbal Formulations as Aphrodisiacs: A Review of Current Research Trends. *J. Ilm. Medicam.*, 2025:11(2), 139-151, DOI: 10.36733/medicamento.v11i2.11418

INTRODUCTION

The global prevalence of sexual dysfunction has led to increasing interest in alternative and complementary therapies. Conventional pharmacological treatments, such as phosphodiesterase-5 inhibitors, are widely used but often come with side effects and contraindications,[1] leading many individuals to seek herbal-based solutions. Herbal medicines have been widely used in traditional healing systems, such as Ayurveda and Traditional Chinese Medicine (TCM), for managing sexual health disorders. With the growing demand for natural alternatives, scientific research has increasingly focused on validating the efficacy and safety of these herbal-based therapies.[2]

Among various herbal therapies, polyherbal formulations have gained significant attention due to their potential synergistic effects. These formulations combine multiple medicinal plants to enhance therapeutic efficacy while minimizing toxicity. Rather than using just one compound, polyherbal formulations offer a more targeted pharmacological approach making treatment more effective and safer.[3–6] *Eurycoma longifolia* and *Tribulus terrestris* are two important herbs that have been shown to increase sex hormone levels and enhance the function of vascular endothelial cells. This is one reason why these herbs are aphrodisiacs.[7,8] New meta-analyses show that polyherbal formulations are safe and effective for sexual health therapy in clinical settings.[9] A multi-herb formulation containing *Asparagus racemosus*, *Mucuna pruriens*, *Chlorophytum borivilianum*, *Asteracantha longifolia*, *Anacyclus pyrethrum*, and *Tribulus terrestris* significantly enhanced mating behavior, serum hormone levels, sperm count, and the testes-to-body weight ratio in a dose-dependent manner. The 600 mg/kg dose yielded effects similar to those observed with the standard treatment.[8]

Despite increasing interest in polyherbal aphrodisiacs, significant research gaps remain. Clinical trials evaluating their efficacy and safety are still limited, and the synergistic mechanisms between multiple herbal components are not well understood. Additionally, bibliometric studies on polyherbal aphrodisiac research are scarce, making it difficult to assess global research trends and collaborations. Addressing these gaps is crucial for advancing evidence-based applications of polyherbal formulations in sexual health therapy. To bridge this gap, this study aims to analyze bibliometric trends and provide a narrative review of polyherbal formulations as aphrodisiacs, focusing on their compositions, pharmacological mechanisms, and clinical potential.

METHODS

Data Source

The bibliometric analysis of the use of polyherbal formulations as aphrodisiacs is the main focus of this review article. To obtain relevant articles, we utilized the Scopus database as the primary source of bibliographic data, which provides a wide coverage of scientific research in various disciplines, including pharmacology and health sciences. Falagas et al. (2008) assert that Scopus offers extensive interdisciplinary coverage and stringent indexing, establishing it as a dependable source for high-quality, peer-reviewed literature.[10] The search strategy combined Boolean operators as follows: ("Aphrodisiac" AND "Polyherbal") OR ("Aphrodisiac" AND "Combination"). The Publish or Perish (PoP) version 8 application served as a supplementary tool for refining search queries and identifying relevant articles from the Scopus database, emphasizing search string optimization and duplicate screening to ensure data accuracy. To refine the results, we applied the following filters: (a) English language, (b) article-type documents (excluding conference proceedings and book chapters), and (c) studies available in full text. This study analyzed articles published from the earliest available record in 1974 to the most recent publications in 2024, without applying any restrictions on publication years. All

retrieved articles matching the specified keywords were included in the analysis. While Scopus provides extensive bibliographic data across disciplines, its limitation lies in the exclusion of certain regional or non-indexed journals. As a result, some relevant studies available in other databases, such as PubMed or Web of Science, may not be represented in our analysis.

Article Selection Procedure

An initial search identified 88 articles, which were screened based on predefined inclusion and exclusion criteria. Eligible studies included those published in any year that investigated aphrodisiac activity of polyherbal formulations through in vitro, in vivo, or clinical experiments, were written in English to ensure accessibility and consistency in data extraction, and provided clear information on pharmacological mechanisms or clinical outcomes related to aphrodisiac activity.

Articles were excluded if they did not meet these criteria. Specifically, review articles, meta-analyses, and opinion pieces were removed as they lack primary experimental data. Studies focusing exclusively on single herbal compounds rather than polyherbal formulations were also excluded to maintain the scope of analysis. Additionally, articles without detailed experimental methodologies, which hindered validity assessment, were excluded. Full-text availability was required; therefore, articles behind paywalls or inaccessible through institutional subscriptions were omitted. After applying these criteria, 16 articles were selected for final analysis.

Bibliometric Analysis

To analyze research trends in the use of polyherbal formulations as aphrodisiacs, we used VOSViewer software version 1.6.20 (Leiden University, The Netherlands). The software was used to create visual of networks of co-authors, keywords that appear together, and partnerships between institutions. The analysis applied a minimum threshold of two occurrences per keyword, grouping them into thematic clusters based on the strength of similarity. A network visualization was used to illustrate author collaborations, while a density visualization highlighted frequently occurring research topics. Cluster analysis further categorised keywords into distinct research themes, providing insights into dominant topics within the field.

Narrative Review

Along with the bibliometric analysis, we conducted a narrative review that examined at the study model, material sources, composition, and plant species used in polyherbal formulations. We extracted information from the 16 chosen articles about the types of experiments that were used (in vitro, in vivo, or clinical studies), where the herbal materials came from, and how they were categorized, what went into polyherbal formulations, and how often different plant species were used. The study identified patterns in the choice of herbal ingredients and how they were put together in previous research. This helped us understand the variety of polyherbal aphrodisiac preparations and how they might be used.

Data Analysis and Visualization

Bibliometric data obtained from Scopus were analyzed using VOSviewer to visualize co-authorship networks and keyword co-occurrence. Data analysis included year of publication, co-authorship based on country, author, and organization; co-occurrence based on keyword; analysis of citation based on document; and document number published by the publisher. We analyzed the narrative research data from selected articles employing descriptive statistics, which encompassed the identification of modes and percentage values. Graphs and tables were generated using Microsoft Excel and VOSviewer to systematically present bibliometric patterns and research trends.

Ethical Considerations

This study is based on publicly available bibliometric data and a narrative review of published literature. No human or animal subjects were involved, and therefore, ethical approval was not required. All sources used in this study have been properly cited to maintain academic integrity. Additionally, the authors declare no conflicts of interest in conducting or presenting this research.

RESULTS AND DISCUSSION

This study aimed to analyze bibliometric trends and provide a narrative review of polyherbal formulations as aphrodisiacs, focusing on their composition, pharmacological mechanisms, and clinical potential. Bibliometric analysis in natural product research has been applied to identify research trends, scientific collaborations, key topics across various fields, global scientific research output, as well as to analyze and predict hotspots for natural product research.[11–14]

In this study, bibliometric analysis identified key trends in publication growth, co-authorship networks, and keyword co-occurrence, reflecting research interests and collaboration patterns in this field. Meanwhile, the narrative review highlighted the most commonly used herbs, their synergistic effects, and potential therapeutic applications. These findings directly address the study objectives by mapping the research landscape and identifying gaps, such as the need for more clinical trials and standardization efforts, which are crucial for advancing polyherbal aphrodisiac formulations toward clinical use.

The use of polyherbal formulations, which involve a combination of various medicinal plants, is attractive in sexual health therapy because the synergistic effect is believed to be stronger than a single compound.[3–6] This effect prompts extensive research on polyherbal formulations as an alternative therapy for natural sexual dysfunction, with the expectation of minimal side effects.

Publications on polyherbal formulations of aphrodisiacs date back to 1974. The number of publications has shown an increasing trend since 2013, reflecting a growing interest in polyherbal research within the field of sexual health. The fluctuations observed in publication output may be influenced by research trends, funding availability, and policy changes in various countries. However, the limited number of publications since 1974 suggests a lack of widespread exploration of the potential of polyherbal aphrodisiacs.

Bibliometric analysis data show that eight countries have conducted research on polyherbal formulations. India has the highest number of publications in this field (10 articles). Other countries also contributed, albeit to a lesser extent. The United States contributed to two articles, while other countries such as Malaysia, Turkey, Indonesia, Italy, Morocco, and Taiwan only contributed one article each.

Traditional medicine, such as Ayurveda in India, has a rich history of applying various plants for sexual therapy, contributing to this dominance.[15,16] India has the highest number of publications on polyherbal formulations, a trend that aligns with the popularity of traditional Ayurveda medicine, particularly its *Vajikarana* branch of therapy, which aims to enhance vitality and sexual function.[17] The close collaboration between these countries indicates the existence of a strong research network and it serves as evidence that countries with rich local flora are trying to explore the aphrodisiac potential of their natural resources.

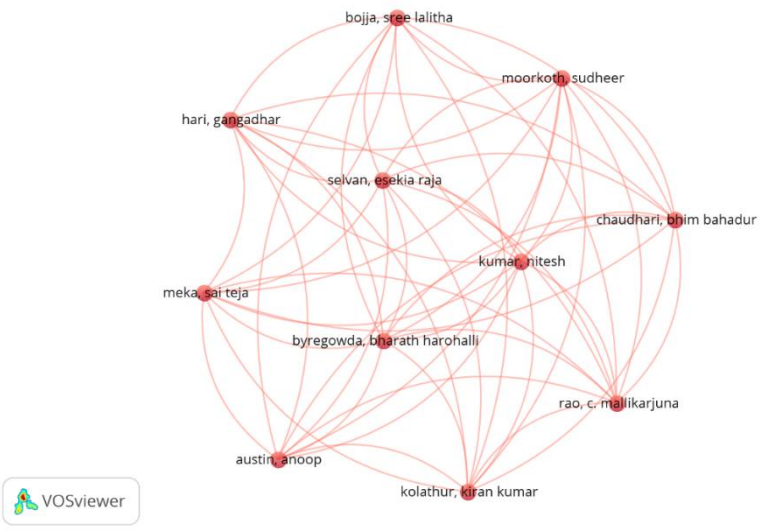

Figure 1. Co-Authorship Network Among Researchers. This figure visualizes the co-authorship network based on bibliometric analysis using VOSviewer software.

International collaboration plays an important role in advancing this research. Eighty-one authors contributed to the publication of 16 selected articles. No dominant author was identified in this research domain, but a network of collaboration among researchers is evident (**Figure 1**). Each author contributed only one article. This suggests that studies on polyherbal aphrodisiacs involve multiple research groups, though with relatively limited cross-group collaborations.

Most studies have been conducted by universities, pharmaceutical research institutions, and hospitals, with contributions from researchers in fields such as pharmacy, biomedical sciences, urology, nutrition, reproduction, pharmacology, biology, andrology, zoology, and natural product chemistry. Strengthening inter-institutional collaborations could enhance the quality and scope of future research. Based on the data, 47 organizations contributed to the publication of 16 selected articles. **Figure 2** presents the relationships between organizations.

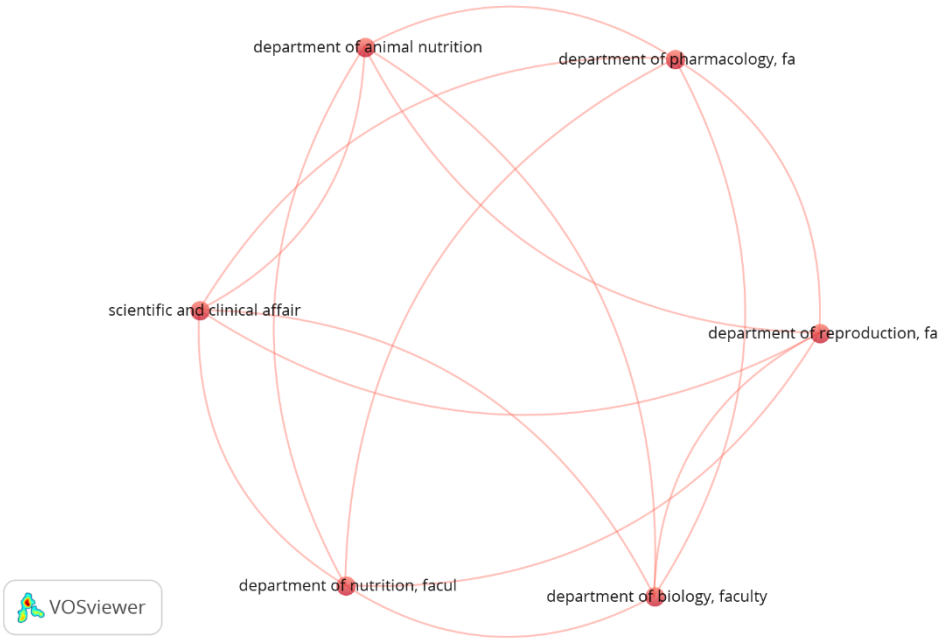

Figure 2. Institutional Collaboration in Polyherbal Aphrodisiac Research. This figure depicts the network of institutional collaborations in research on polyherbal aphrodisiacs.

Based on the co-authorship analysis (**Fig. 1** and **Fig. 2**), a network of collaborations exists among researchers. This indicates that research on polyherbal formulations as an aphrodisiac has the potential to become a multidisciplinary field that involves not only pharmacologists but also scientists in the fields of biomedicine, phytochemistry, urology, and andrology.

Table 1.* Keyword Clusters in Polyherbal Aphrodisiac Research

Cluster	Keywords	Focus correlation
1	Male infertility, *musalyadi churna*, nicotine, rats, sperm count, testicular degeneration	This cluster discusses the effects of nicotine and traditional medicine on male rats' fertility.
2	Aphrodisiacs, *Apium graveolens*, *Coriandrum sativum*, *Petroselinum crispum,* sildenafil	This cluster discusses the effects of natural ingredients as aphrodisiacs compared to standard drugs.
3	Ayurveda, ayurvedic *ghrita*, indian cow ghee, sexual performance, *vajikarana rasayana*	This cluster discusses polyherbal ingredients for sexual performance based on Indian culture.
4	Aphrodisiac activity, ejaculation latency, intromission latency, lordosis, mount latency	This cluster discusses the test parameters in measuring aphrodisiac activity.
5	Automated runway apparatus, copulatory arena, l-name induced female sexual dysfunction, polyherbal formulation	This cluster discusses various test models for polyherbal formulations as aphrodisiacs.
6	AndroCare, mice	This cluster discusses AndroCare and aphrodisiac test animals.

*This table categorizes the primary keywords used in studies on polyherbal aphrodisiacs into six clusters based on thematic relationships.

Based on the author keywords filter, we found a total of 68 keywords related to this aphrodisiac polyherbal publication. The most frequently occurring keywords include polyherbal formulation and aphrodisiac activity, show that a lot of research is being done on certain herbal ingredients and how they work in the body (**Figure 3**).

Figure 3 illustrates the keyword co-occurrence network, highlighting thematic clustering within research focus areas. This directly correlates with the six primary domains of study outlined in **Table 1**, based on similarity and frequency of occurrence, offering a visual confirmation of bibliometric patterns. The six main areas of study that make up polyherbal aphrodisiacs: (1) bioactive compounds and their pharmacological effects; (2) mechanisms of action related to hormone regulation; (3) experimental models used in aphrodisiac studies; (4) clinical applications of polyherbal formulations; (5) safety and toxicity issues; and (6) incorporating traditional herbal knowledge.

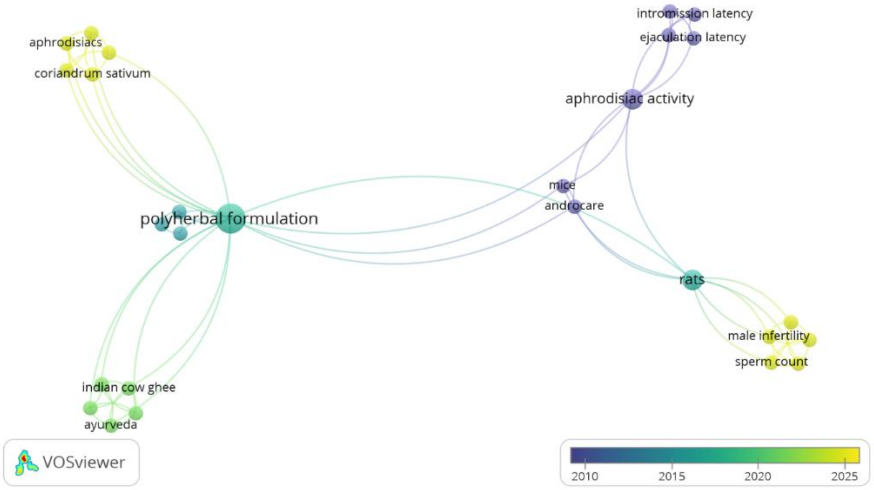

Figure 3. Keyword Co-Occurrence Analysis in Polyherbal Aphrodisiac Research. This figure presents a keyword co-occurrence network, clustering keywords into six thematic groups based on bibliometric analysis.

Keyword analysis reveals a focus on using animal models to test aphrodisiac effects, such as the Automated Runway Apparatus and Copulatory Arena, which serve to evaluate mating behavior. While these models can yield preliminary data, the application of animal results to humans is not always straightforward. Therefore, we need further research to test the safety and effectiveness of these plants in a broader human population, especially considering the differences in metabolism between humans and animals.

Analysis of citation based on document reveals that Arletti et al.'s 1999 article,[18] "Stimulating property of *Turnera diffusa* and *Pfaffia paniculata* extracts on the sexual behavior of male rats," received the most citations out of the 16 selected articles in this study, with 88 citations. Udani et al.'s 2014 article,[19] "Effects of a proprietary freeze-dried water extract of *Eurycoma longifolia* (Physta) and *Polygonum minus* on sexual performance and well-being in men: A randomized, double-blind, placebo-controlled study," garnered 36 citations, making it the second most cited article. Mahajan et al.'s (2012) article,[20] "Efficacy of aphrodisiac plants towards improvement in semen quality and motility in infertile males," received 21 citations, making it another article with a significant number of citations.

In total, 14 journals have accepted this polyherbal aphrodisiac research article for publication. The two journals with the most documents, namely two articles each, are Pharmacology online and Evidence-based Complementary and Alternative Medicine.

Based on the research model, studies on experimental animals still dominate the test models used in polyherbal research (13 studies), but there have also been tests on humans (2 studies). Polyherbal formulations that have been tested on humans include a combination of *Eurycoma longifolia* (Physta) and *Polygonum minus*; a combination of *Mucuna pruriens* Linn, *Chlorophytum borivillianum* (Sant and Fernand), and *Eulophia*

campestris Wall; and a TCM formula consisting of *Astragalus membranaceus*, *Lepidium meyenii* Walp. (maca), *Ophiocordyceps sinensis*, *Panax quiquefolium* (American ginseng), *Piper nigrum* (black pepper), *Rhodiola rosea*, and *Serpentes cnidium monnieri*.[19,21]

Variations in interspecies metabolism and human genetic diversity hinder the conversion of preclinical results into therapeutic applications. Supplementary methods, such as in vitro models, can improve predicted precision.[22] The focus of the aphrodisiac mechanism under study determines the diversity of the induction model in these experimental animals.[23] All studied polyherbal formulations use oral preparations as their administration method. However, researchers have developed topical formulations as aphrodisiacs, specifically in the form of nanohydrogels.[24]

The study analysis also considers the number of different types of herbal ingredients in a formula. This combination is expected to produce a positive synergistic effect both pharmacokinetically and pharmacodynamically. Polyherbal formulations used and studied as aphrodisiacs consist of a varying number of herbal items, from two to a maximum of 41 types of herbs (**Figure 4**). A polyherbal formulation with a mixture of 2–7 types of plants dominate the formula. There are two polyherbal formulations that contain only two plants: one made up of *Eurycoma longifolia* and *Polygonum minus,*[19] and another made up of *Turnera diffusa* and *Pfaffia paniculata*.[18] There are five polyherbal formulations comprising three plants. They comprise a formulation containing *Mucuna pruriens* (Linn), *Chlorophytum borivillianum* (Sant and Fernand), and *Eulophia campestris* (Wall);[20] a formulation with *Mucuna pruriens*, *Tribulus terrestris*, and *Withania somnifera;*[16] a formulation with *Tribulus terrestris*, *Eurycoma longifolia*, and *Pimpinela alpina;*[7] a formulation with *Chlorophytum borivilianum*, *Tribulus terrestris*, and *Withania somnifera;*[25] and a formulation with *Petroselinum crispum*, *Coriandrum sativum*, and *Apium graveolens*.[26] Two polyherbal formulations comprise six plants. They comprise a formulation containing *Mucuna pruriens*, *Withania somnifera*, *Pueraria tuberosa*, *Tribulus terrestris*, *Myristica fragrans*, and *Curculigo orchioides;*[27] and another formulation containing *Bacopa monnieri*, *Asparagus adscendens*, *Asteracantha longifolia*, *Asparagus racemosus*, *Mucuna pruriens*, and *Withania somnifera*.[28] There are three polyherbal formulations, each comprising seven plants. They comprise a formulation including *Tribulus terrestris*, *Curculigo orchioides*, *Allium tuberosum*, *Cucurbita pepo*, Elephant creeper, *Mucuna pruriens*, and *Terminalia catappa;*[8] as well as a formulation with *Chlorophytum borivilianum* Sant.F., *Pueraria tuberosa* DC, and Tinospora cordifolia (Willd) Miers. *Mucuna prurita* Hook, *Tribulus terrestris* Linn., *Bombax ceiba* DC, and *Phyllanthus emblica* Linn;[29] together with a formulation containing *Astragalus membranaceus*, *Lepidium meyenii* Walp., *Ophiocordyceps sinensis*, *Panax quinquefolius*, *Piper nigrum*, *Rhodiola rosea*, and *Cnidium monnieri*.[21]

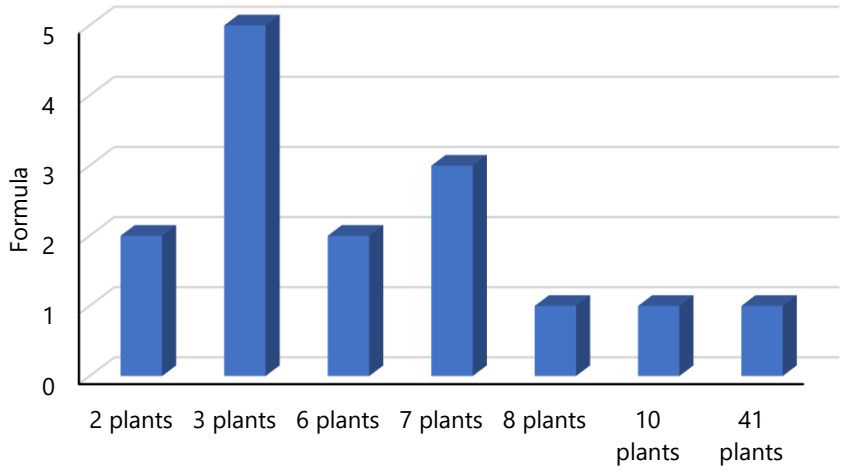

Figure 4. Type of Polyherbal Formulations Used. This figure categorizes polyherbal formulations studied for aphrodisiac effects based on the number of plant ingredients they contain. The formulations range from two-component combinations to complex mixtures of up to 41 herbs.

Various types of plants dominate the polyherbal formulations study (97%). However, there is also the use of animal and herbomineral materials. Cow Ghee[30] serves as the animal material in question, while Shilajit[31,32] serves as the hermomineral material.

While **Figure 4** illustrates the variation in the number of herbal components per formulation, **Table 2** provides complementary data on the most frequently included plant species, indicating clear trends in herbal selection across studies. Aphrodiac polyherbal formulations use seventy-three plant species. However, certain plant species are used more frequently. **Table 2** displays the list of plants utilized in multiple formulas. The *Tribulus terrestris* plant is the most frequently used and appears in nine formulas. This indicates that people consider this plant species to have the highest potential as an aphrodisiac. Meta-analysis research has proven the potential of this plant. *T. terrestris* has been shown to possess profertility and aphrodisiac activities.[33]

Table 2.* Most Frequently Used Plants in Polyherbal Aphrodisiac Formulations

No	Plant Name	Used in Formula (times)	References
1	*Tribulus terrestris*	9	7,8,16,25,27,29,31,32,34
2	*Mucuna pruriens*	7	8,16,20,27,28,31,34
3	*Withania somnifera*	7	16,25,27,28,31,32,34
4	*Myristica fragrans*	4	27,31,32,34
5	*Asparagus adscendenns*	3	28,31,34
6	*Asparagus racemosus*	3	28,31,32
7	*Chlorophytum borivilianum*	3	20,25,29
8	*Pueraria tuberosa*	3	27,29,31
9	*Curculigo orchioides*	3	8,27,32
10	*Astercantha longifolia*	2	28,31
11	*Eulophia campestris*	2	20,31
12	*Eurycoma longifolia*	2	7,19
13	*Syzygium aromaticum*	2	31,34
14	*Tinospora cordifolia*	2	29,31

*This table lists the plant species most frequently utilized in polyherbal formulations for aphrodisiac purposes, as identified from the 16 analyzed articles.

The most often utilized substances in ranks 2 to 5 are *Mucuna pruriens*, *Withania somnifera*, *Myristica fragrans*, and *Asparagus adscendens*, respectively. All of these herbs have demonstrated pharmacological effects as aphrodisiacs. *M. pruriens* has demonstrated aphrodisiac effects by enhancing male sexual function through mechanisms involving the Nrf2/HO-1 pathway, NF-κB inhibition, and androgenic activity, alongside potential benefits for diabetes-related sexual dysfunction.[35,36] Recent studies confirm that *W. somnifera* enhances male sexual function by improving sexual well-being, increasing serum testosterone levels, enhancing semen quality, and reducing male infertility.[37,38] *M. fragrans* (nutmeg) exhibits aphrodisiac properties by enhancing sexual behavior, increasing mounting frequency, and improving mating performance in male animal models without notable adverse effects.[39,40] The study by Bansode et al. (2015) demonstrated that *A. adscendens* root extract enhances anabolic, reproductive, and sexual behavioral activities in a dose-dependent manner, supporting its traditional use as an aphrodisiac.[41]

The main focus of polyherbal formulations' aphrodisiac research was found to be on how to increase testosterone levels, enhance vascular endothelial function, and find ways for medicinal plants to work together to make them more effective. This trend highlights the scientific effort to explore the potential of formulations that not only support sexual function but also impact overall reproductive health.

A review of the articles included in the study revealed that several polyherbal formulations had a synergistic impact. Synergistic effects in polyherbal formulations occur when the combined action of numerous herbs has a therapeutic impact greater than the sum of their individual effects, hence increasing efficacy

through complementary interactions. This is in contrast to additive effects, in which the herbs work separately to produce a total impact equal to the sum of their individual contributions, with no enhancement.[42]

A polyherbal formulation that demonstrates a synergistic effect consists of a hydroalcoholic extract of *Chlorophytum borivilianum*, an ethanolic extract of *Tribulus terrestris*, and a hydroalcoholic extract of *Withania somnifera*, all in a 1:1:1 ratio. The combination of these herbs exhibits a synergistic effect, allowing for potential dose reduction.[25] The combination of Apiaceae plant extracts (*Petroselinum crispum*, *Coriandrum sativum*, and *Apium graveolens*) also had a stronger aphrodisiac effect than their separate use, considerably increasing metrics such as mounting frequency, reduced latency times, and testosterone levels in male rats. This synergistic effect outperformed the results of individual extracts, demonstrating the combined formulation's higher efficacy in stimulating sexual activity and hormonal balance while minimizing side effects.[26] An additional interaction was identified between herbal therapy and a surgical technique. The synergistic interaction between an oral herbal formula and penile venous stripping (PVS) surgery in the treatment of erectile dysfunction results in substantially improved erectile function scores when the two therapies are combined. This combination offers patients complementary advantages, surpassing the efficacy of PVS or herbal treatment alone.[21]

Interactions between polyherbal formulations and conventional medications require scrutiny. Sildenafil, a Phosphodiesterase-5 (PDE-5) inhibitor commonly used as a conventional aphrodisiac, is recognized for its interactions with numerous herbal medications.[43] One of the herbal ingredients discussed in this study that interacts with sildenafil is pomegranate (*Punica granatum).* Recent studies suggest that pomegranate juice may be associated with low-flow priapism when ingested alongside sildenafil, as it potentially increases nitric oxide bioavailability and inhibits CYP3A4, the enzyme responsible for metabolising sildenafil, thereby raising its plasma levels[43,44] Clinical guidelines recommend against the simultaneous consumption of sildenafil and pomegranate juice to avert serious adverse effects such as priapism, and healthcare providers should educate patients about this interaction during treatment.

This study's findings suggest that developing polyherbal formulations as natural aphrodisiac products holds significant potential. In this regard, it is important to create formulations that are not only effective but also safe for long-term use. The pharmaceutical and nutraceutical industries can benefit from the results of this study by developing polyherbal-based products that meet clinical standards, especially for populations seeking natural alternatives to enhance sexual performance. When developing products, it is crucial to take into account the quality of raw materials and the sustainability of natural resources, particularly because some of the plants used in the formulation are rare and valuable.

The variability in plant composition caused by environmental conditions, harvest time, and processing processes can greatly affect the consistency of polyherbal formulations, which is essential for clinical applications. Standardizing raw materials by analyzing physical and chemical factors, including moisture content and active component concentrations, is crucial for ensuring quality and efficacy. Advanced methodologies such as high-performance liquid chromatography (HPLC) are frequently used for this objective.[45] Additional study is advised to establish more rigorous standardization techniques that account for heterogeneity among various plant species.

The increasing number of publications in recent years suggests growing scientific interest in polyherbal aphrodisiac formulations, aligning with the rising global demand for natural sexual health therapies. However, co-authorship analysis indicates that research remains fragmented with limited international collaboration, which may hinder the standardization and clinical translation of findings.

The narrative review provides further insight into how these bibliometric trends translate into research focus. The use of specific keywords, such as "aphrodisiac activity" and "polyherbal formulation" shows that a lot of work has gone into testing the effectiveness of combining several plants. The discovered synergistic effects of polyherbal formulations, which were discussed above, show how important it is to use multiple components in therapies to make them more effective and less harmful. These findings address the gaps

highlighted in the Introduction, emphasizing the need for further clinical validation and regulatory standardization of polyherbal formulations.

Further research is necessary to support the clinical translation of polyherbal formulations as aphrodisiac therapies. Well-designed randomized controlled trials (RCTs) are essential to validate their efficacy and safety in humans, determine optimal dosages, and assess long-term effects. Also, pharmacokinetic and pharmacodynamic studies are needed to learn about how the drug is broken down, how much of it is available in the body, and how it might interact with other drugs, such as phosphodiesterase-5 (PDE-5) inhibitors.

Standardization remains a major challenge in the clinical application of polyherbal formulations.[46,47] Variability in plant composition due to environmental factors, harvest timing, and processing techniques can affect consistency and therapeutic outcomes. Implementing Good Manufacturing Practices (GMP), quality control measures, and chemical fingerprinting techniques (e.g., HPLC) can help ensure product reliability and regulatory compliance. Greater collaboration between researchers, clinicians, and policymakers is necessary to harmonize regulations and facilitate product commercialization.

Furthermore, polyherbal formulations have the potential to be used as complementary therapies alongside synthetic drugs. Some studies show that some combinations of herbs may enhance the effects of PDE-5 inhibitors. In contrast, others show that there may be dangerous interactions between herbs and drugs that need to be carefully considered. Investigating these interactions through clinical trials and mechanistic studies is crucial to maximizing therapeutic benefits while minimizing risks. Addressing these aspects will contribute to the safe and effective integration of polyherbal formulations into modern clinical practice.

This study has several limitations. First, the reliance on bibliometric analysis means that while publication trends, co-authorship patterns, and keyword distributions were analyzed, the study does not assess the quality or methodological rigor of individual articles. Second, the small number of articles included in the narrative review (16 articles) may not fully represent the diversity of research on polyherbal aphrodisiacs, potentially introducing selection bias. Additionally, this study only utilized the Scopus database, which, while comprehensive, may not cover all relevant publications indexed in other databases such as PubMed or Web of Science. Moreover, only English-language articles were included, meaning that important studies published in other languages may have been excluded. These limitations should be considered when interpreting the findings, and future research should aim to incorporate multiple databases and broader language inclusion criteria to achieve a more comprehensive analysis.

CONCLUSION

This review offers an overview of recent research trends regarding polyherbal formulations with aphrodisiac potential, emphasizing the rise in publication output, common herbal combinations, and developing pharmacological themes. Polyherbal formulations are believed to exhibit greater synergistic effects than single-compound therapies, driving significant research interest in alternative treatments for sexual dysfunction. India is at the forefront of global research on polyherbal formulations, particularly in the area of aphrodisiacs. Bibliometric analysis reveals an increase in the number of publications, with research clusters focusing on bioactive compounds, pharmacological mechanisms, and clinical applications. Narrative analysis shows that *Tribulus terrestris*, *Withania somnifera*, and *Mucuna pruriens* are the herbs that have been studied the most, and they are all known for their aphrodisiac properties. Studies predominantly use animal models, emphasizing mechanisms such as testosterone regulation and vascular endothelial function to assess efficacy. Despite growing interest, challenges persist in standardization, clinical validation, and regulatory approval, with variability in herb compositions and a limited number of clinical trials hindering widespread adoption. Future research should focus on clinical validation, herb-drug interactions, and formulation standardization to facilitate the integration of polyherbal therapies into evidence-based sexual health treatments.

ACKNOWLEDGEMENT

The research did not receive funding. The research results have been presented in The 6th Jogjakarta Annual Meeting of Pharmacology and Therapy (Japemethe) 2024.

CONFLICT OF INTEREST

There is no conflict of interest.

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JURNAL ILMIAH MEDICAMENTO

Vol.11 No.2 (September 2025)
page 152-162
https://doi.org/10.36733/medicamento.v11i2.11434
e-ISSN: 2356-4814

Original Article

Anti-Inflammatory Activity Test of *Dendrophthoe glabrescens* (Blakely) Barlow Leaf Extract on Mice (*Mus musculus*) Induced with Carrageenan

Uji Aktivitas Antiinflamasi Ekstrak Daun Benalu Jeruk (*Dendrophthoe glabrescens* (Blakely) Barlow) terhadap Mencit (*Mus musculus*) yang Diinduksi Karagenan

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Submitted : 08-04-2025
Reviewed : 13-08-2025
Accepted : 20-09-2025

Keywords: antiinflammatory, carrageenan, citrus b*enalu* Leaf, GCMS

Kata Kunci: antiinflamasi, daun benalu jeruk, karagenan, GCMS

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Abstract

Inflammation is a natural defense mechanism of the body against injury or disease; however, excessive inflammation can lead to various chronic conditions. Prolonged use of synthetic anti-inflammatory drugs is associated with adverse effects, creating a need for safer natural alternatives. One such candidate is the citrus *benalu* leaf (*Dendrophthoe glabrescens* (Blakely) Barlow), a parasitic plant rich in flavonoids, saponins, and tannins. This study aimed to evaluate the anti-inflammatory activity of ethanol extract from citrus *benalu* leaves in carrageenan-induced mice. A laboratory experimental design using a Randomized Pre- and Post-Test Control Group approach was employed with 25 mice divided into five groups: negative control (CMC-Na 0.5%), positive control (sodium diclofenac), and three treatment groups receiving citrus *benalu* leaf extract at doses of 100, 200, and 400 mg/kgBW, respectively. The extract was administered orally 30 minutes after carrageenan induction, and paw edema was measured every 30 minutes for 180 minutes. One-way ANOVA revealed significant differences among groups, and LSD post hoc analysis indicated that all treatment groups differed significantly from the negative control ($p < 0.05$) but not from the positive control ($p > 0.05$). The 400 mg/kgBW dose demonstrated the greatest efficacy, reducing paw edema to 3.08%. These findings suggest that citrus *benalu* leaf extract has promising potential as a natural anti-inflammatory agent, offering an alternative to synthetic drugs with fewer side effects.

Abstrak

Peradangan (inflamasi) merupakan mekanisme pertahanan alami tubuh terhadap cedera atau penyakit; namun, peradangan berlebihan dapat memicu berbagai penyakit kronis. Penggunaan obat antiinflamasi sintetis dalam jangka panjang sering menimbulkan efek samping, sehingga diperlukan alternatif alami yang lebih aman. Salah satu kandidat adalah daun benalu jeruk (*Dendrophthoe glabrescens* (Blakely) Barlow), tanaman parasit yang kaya akan flavonoid, saponin, dan tanin. Penelitian ini bertujuan mengevaluasi aktivitas antiinflamasi ekstrak etanol daun benalu jeruk pada mencit yang diinduksi karagenan. Penelitian eksperimental laboratorium ini menggunakan rancangan *Randomized Pre- and Post-Test Control Group* dengan 25 ekor mencit yang dibagi menjadi lima kelompok: kontrol negatif (CMC-Na 0,5%), kontrol positif (natrium diklofenak), serta tiga kelompok perlakuan yang menerima ekstrak daun benalu jeruk dengan dosis 100, 200, dan 400 mg/kgBB. Ekstrak diberikan secara oral 30 menit setelah induksi, kemudian pembengkakan pada telapak kaki kanan mencit diukur setiap 30 menit selama 180 menit. Analisis one-way ANOVA menunjukkan perbedaan yang signifikan antar kelompok, dan uji post-hoc LSD menunjukkan bahwa semua kelompok perlakuan berbeda signifikan dengan kontrol negatif ($p < 0,05$), tetapi tidak berbeda dengan kontrol positif ($p > 0,05$). Dosis 400 mg/kgBB menunjukkan efektivitas tertinggi dengan penurunan edema hingga 3,08%. Temuan ini menegaskan potensi ekstrak daun benalu jeruk sebagai agen antiinflamasi alami yang berpeluang menjadi alternatif pengganti obat sintetis dengan risiko efek samping yang lebih rendah.

How to cite: (citation style AMA 11th Ed.)
Udayani NNW, Mahendra IPA, Widiasriani IAP, Mahardika IMA, Wardani IGAAK. Anti-Inflammatory Activity Test of *Dendrophthoe glabrescens* (Blakely) Barlow Leaf Extract on Mice (*Mus musculus*) Induced with Carrageenan. *J. Ilm. Medicam.*, 2025:11(2), 152-162, DOI: 10.36733/medicamento.v11i2.11434

INTRODUCTION

Inflammation is the body's initial defense mechanism against injury caused by internal or external factors, such as physical trauma, chemical agents, microbial infections, and cancer.[1] It is characterized by erythema, hyperthermia, pain, and edema, and involves immune cells such as neutrophils, mast cells, and macrophages, as well as key enzymes including cyclooxygenase (COX) and lipoxygenase (LOX).[1–5] Inflammation is closely linked to oxidative stress, which plays a critical role in initiating and exacerbating various degenerative

diseases.[6] According to the World Health Organization (WHO, 2024), degenerative diseases account for approximately 41 million deaths annually, underscoring their significant global health burden.[7]

Inflammation can be managed by alleviating pain or preventing tissue damage through the use of steroidal and non-steroidal drugs.[8] Both classes of anti-inflammatory agents function by inhibiting the release of prostaglandins at sites of tissue injury.[9] However, prolonged use of synthetic anti-inflammatory drugs is associated with significant adverse effects, including gastrointestinal disorders, peptic ulcers, and renal impairment. Steroidal drugs may also suppress immune function and lead to complications such as hypertension, moon face, and osteoporosis.[10] Consequently, there is a growing need for alternative treatments with minimal side effects, one of which involves the use of medicinal plants. In response to this need, the "back to nature" movement has gained traction, particularly in Indonesia, a country rich in biodiversity. The World Health Organization (WHO) has endorsed the use of traditional medicine derived from natural sources, citing its potential to reduce the side effects commonly associated with synthetic drugs.[11]

Bali Province, located in Indonesia, is home to several citrus production centers, including Jembrana, Tabanan, Badung, Gianyar, Klungkung, Bangli, Karangasem, Buleleng, and Denpasar City. Among these, Bangli Regency records the highest citrus yield, producing 93,162.3 tons annually from a harvest area of 38,140.21 hectares, with an average yield of 24.42 kw per hectare.[12] Local citrus farmers frequently encounter parasitic plants known as *benalu* (Balinese), which hinder citrus tree growth and reduce crop yields. These parasites are typically eradicated due to their detrimental effects. One such parasitic plant is the citrus *benalu* leaf, scientifically identified as *Dendrophthoe glabrescens* (Blakely) Barlow, which survives by attaching to citrus trees as its host.[13]

Despite its parasitic nature, the citrus *benalu* leaf exhibits potential as a medicinal plant. Its secondary metabolite profile varies depending on the host plant and includes alkaloids, steroids, triterpenoids, flavonoids, saponins, and tannins.[13] Among these, flavonoids, saponins, and tannins are recognized for their anti-inflammatory properties. A study by Neman et al.,[14] demonstrated that *benalu* leaves from kersen trees possess anti-inflammatory activity in formalin-induced rat edema, attributing this effect to the presence of flavonoids, saponins, and tannins. Additionally, research has shown that kersen *benalu* leaves exhibit strong antioxidant activity, with an IC_{50} value of 21.70 µg/mL.[15] Similarly, citrus *benalu* leaves have demonstrated potent antioxidant activity, with an IC_{50} value of 54.49 µg/mL.[13] Further support for the anti-inflammatory potential of citrus *benalu* leaves comes from Gas Chromatography–Mass Spectrometry (GC-MS) analysis, which identified additional bioactive compounds with anti-inflammatory properties.

This study employed mice (*Mus musculus*) induced with carrageenan to evaluate the anti-inflammatory efficacy of citrus *benalu* leaf extract. Carrageenan, a polysaccharide derived from seaweed species such as *Eucheuma*, *Chondrus*, and *Gigartina*, is commonly used to induce inflammation due to its irritant properties.[16] It offers several advantages as an inducer, including non-residual effects, minimal tissue damage, and heightened sensitivity to anti-inflammatory agents.[17] These characteristics make carrageenan a widely accepted model for evaluating anti-inflammatory activity.[18]

Based on the above considerations, this study aims to assess the effectiveness of citrus *benalu* leaf extract (*Dendrophthoe glabrescens* (Blakely) Barlow) in reducing carrageenan-induced edema in mice (*Mus musculus*), and to identify the bioactive compounds present in the extract through GC-MS analysis, thereby exploring its potential as a natural alternative to synthetic anti-inflammatory drugs.

RESEARCH METHODS

Tools. Analytical balance (Ohaus Pioneer PA 224C), Rotary evaporator (BUCHI R-300), oral sonde, glass jar, 100 ml beaker glass (PYREX), oven (MEMMERT), drinking bottle, plethysmometer (Orchid).

Materials. Citrus *benalu* leaf taken from Manikliyu village, Kintamani, Bali, carrageenan (PT. Brataco, Indonesia), distilled water, standard feed (Br-551), 96% ethanol (PT. Brataco, Indonesia), diclofenac sodium tablets (Kimia Farma), CMC Na (PT. Brataco, Indonesia).

Research Procedure

Preparation of Citrus *Benalu* Leaf Extract

The leaves of citrus *benalu* (*Dendrophthoe glabrescens* (Blakely) Barlow) were thoroughly washed, air-dried, and subsequently oven-dried at 50 °C for two days until completely desiccated. The dried leaves were then ground into a fine powder. A total of 100 g of powdered simplicia was subjected to maceration using 96% ethanol at a material-to-solvent ratio of 1:10 for three days. The resulting macerate was filtered through flannel cloth and concentrated using a vacuum rotary evaporator to obtain a thick extract. This maceration process was repeated twice to ensure optimal extraction.[13] The plant sample was taxonomically identified at the Biological Research Center, National Research and Innovation Agency (BRIN), Eka Karya Botanical Garden, Bedugul, under transaction number 1617-80983-1, dated February 27, 2023.

Preparation of Citrus *Benalu* Leaf Extract Test Solution

For the 100 mg/kg body weight (BW) dose, 1.8 mg of citrus *benalu* leaf extract was dissolved in 1% carboxymethyl cellulose (CMC) to a final volume of 10 mL. For the 200 mg/kg BW dose, 3.6 mg of extract was similarly dissolved in 1% CMC to a total volume of 10 mL. For the 400 mg/kg BW dose, 7.2 mg of extract was dissolved in 1% CMC, also adjusted to a final volume of 10 mL.

Preparation of 1% CMC-Na Suspension

A 1% carboxymethyl cellulose sodium (CMC-Na) suspension was prepared by dissolving 1 g of CMC in 10 mL of hot distilled water. The mixture was stirred until homogeneous, after which additional distilled water was added to reach a final volume of 100 mL.[19]

Preparation of Diclofenac Sodium Suspension

A 50 mg diclofenac sodium tablet was ground, and 45.410 mg of the resulting powder was taken. The powder was placed in a mortar, then mixed with a 0.5% CMC suspension until homogeneous, and the volume was adjusted to 10 mL.[16]

Preparation of 1% Carrageenan Induction

A total of 0.1 g of carrageenan was weighed and then dissolved in 0.9% sodium chloride in a 10 mL volumetric flask.[16]

GC-MS Testing

A total of 0.5 g of citrus *benalu* leaf powder was placed into a microtube containing 1.5 mL of methanol. The mixture was vortexed for 1 minute and centrifuged at 9,000 rpm for 3 minutes. The resulting supernatant was collected and used for Gas Chromatography–Mass Spectrometry (GC-MS) analysis. The GC-MS analysis was conducted over a 60-minute run time, with the injector temperature set at 260 °C, the detector temperature at 250 °C, and the column temperature at 325 °C. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. Bioactive compounds were identified based on chromatographic peaks and mass spectral (MS) data, which revealed the molecular weights of the detected compounds.[20]

Anti-inflammatory Testing

This study was conducted on 25 mice (Mus musculus) weighing between 20 and 30 g. The animals were randomly divided into five groups, each consisting of five mice. Ethical approval for the study was obtained from the Health Research Ethics Committee of Poltekkes Denpasar (Approval No.: DP.04.02/F.XXXII.25/0930/2024). Prior to testing, the mice were fasted for 18 hours. Each mouse was then induced with 0.2 mL of 1% carrageenan via subplantar injection in the paw.[16] Citrus *benalu* leaf extract was administered orally 30 minutes after carrageenan induction. Edema was measured over a six-hour period following the injection, with assessments taken at 0, 30, 60, 90, 120, 150, and 180 minutes using a plethysmometer.

The experimental groups were as follows:

1. **Group I** (Negative Control): Received 1% CMC-Na

2. **Group II** (Positive Control): Received sodium diclofenac
3. **Group III** (Treatment): Received citrus benalu leaf extract at 100 mg/kg BW
4. **Group IV** (Treatment): Received citrus benalu leaf extract at 200 mg/kg BW
5. **Group V** (Treatment): Received citrus benalu leaf extract at 400 mg/kg BW

Test Parameters

The parameter used in this study is the percentage of inhibition, which is calculated from the paw volume data of mice using the following formula:

$$\%\ edema = \frac{Vkt - Vko}{Vko} x\ 100\%$$

Description:
Vkt = Volume of mice paw at time x (ml)
Vko = Volume of mice paw at time 0 (ml)

Data Analysis

The spectra obtained from GC-MS analysis were processed using the instrument's integrated database library, generating chromatogram outputs. From these chromatograms, retention times were used to identify compounds present in the sample, while percentage area data were utilized to estimate the relative concentration of each compound.

Inflammation data were collected by measuring the thickness of edema in the hind paws of female mice (Mus musculus) induced with carrageenan. The data were categorized into control and treatment groups and analyzed statistically using a Completely Randomized Design (CRD). One-way Analysis of Variance (ANOVA) was employed to determine significant differences among groups. The ANOVA test was considered valid if the data met the assumption of normality ($p > 0.05$). If a significant difference was detected ($p \leq 0.05$), further analysis was conducted using a post-hoc test to identify specific group differences.

RESULTS AND DISCUSSION

Gas Chromatography–Mass Spectrometry (GC-MS) is a powerful analytical technique that combines gas chromatography for the separation of volatile compounds under high vacuum and low pressure with mass spectrometry for determining molecular weight, molecular formula, and generating charged molecular fragments.[21,22] GC-MS is widely used for the separation and analysis of complex mixtures, including plant secondary metabolites, due to its high sensitivity and ability to detect compounds at very low concentrations. The results are presented in the form of chromatograms and mass spectra, allowing for precise identification of bioactive constituents.[20,23]

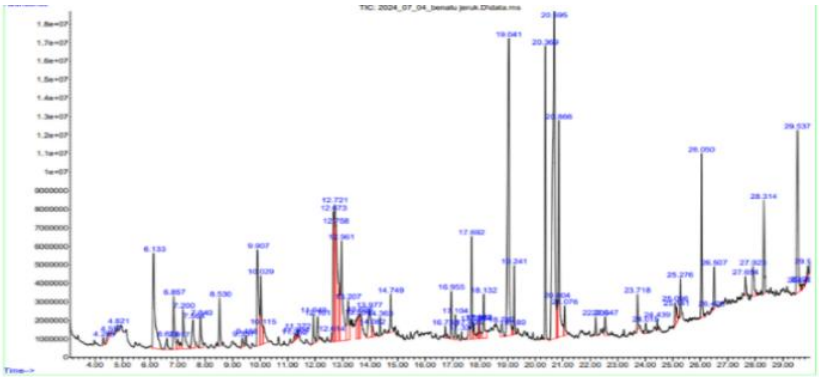

Figure 1. Chromatogram Graph of Citrus *Benalu* Leaf Extract Samples

In this study, GC-MS analysis of citrus *benalu* leaf extract revealed a total of 70 peaks in the chromatogram (**Figure 1**). Four of the most dominant peaks were selected based on their percentage area, as shown in **Table 1**. The most abundant compound was **9,12,15-Octadecatrienoic acid**, representing 15.14% of the total area with a retention time of 20.695 minutes. Also known as linolenic acid, this carboxylic acid

contains 18 carbon atoms and three cis double bonds. It is an essential fatty acid of plant origin, crucial to human health. Previous studies have associated 9,12,15-Octadecatrienoic acid with a range of pharmacological activities, including anti-inflammatory, antioxidative, anticancer, anti-obesity, antimetabolic, and cardiovascular-protective effects.[24] The second most prominent compound was **Octadecanoic acid**, accounting for 5.30% of the total area with a retention time of 20.866 minutes. Also referred to as stearic acid (not oleic acid, which is cis-9-Octadecenoic acid), Octadecanoic acid has the molecular formula $C_{18}H_{36}O_2$ and belongs to the saturated fatty acid group. Its straight-chain structure contributes to its solid form at room temperature.[25] Octadecanoic acid has been reported to exhibit antioxidant and anti-inflammatory properties,[26] and also plays roles in biological systems as an antibacterial and antifungal agent.[27] Both 9,12,15-Octadecatrienoic acid and Octadecanoic acid are bioactive compounds that may contribute to the anti-inflammatory potential of citrus *benalu* leaf extract. While these compounds are commonly found in flavonoid-rich plant matrices, they are classified as fatty acids rather than flavonoids.[28]

Table 1. Active Components Based on the Highest Outer Percentage in Citrus *Benalu* Leaf Extract Samples

Compound	Area (%)	Retention Time	Qual
9,12,15-Octadecatrienoic acid	15,14 %	20,695	97
Octadecanoic acid (CAS)	5,30 %	20,866	99
Phytol	4,69 %	20,369	91
1,2,3-Benzenetriol (CAS)	4,49 %	12,758	97

The third most abundant compound identified was **Phytol**, with a percentage area of 4.69% and a retention time of 20.363 minutes. Phytol is an acyclic diterpene alcohol derived from terpenoids.[29] It is naturally found in various foods, including fish, ruminant meat, green vegetables, and dairy products, and is a key component of chlorophyll, vitamin E, and vitamin K.[30] Phytol has demonstrated anti-inflammatory effects, including significant reduction of carrageenan-induced paw edema in animal models. It also inhibits edema induced by histamine, PGE_2, serotonin, and bradykinin, and suppresses leukocyte recruitment. Furthermore, phytol has been shown to reduce levels of myeloperoxidase (MPO), interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α), and malondialdehyde (MDA), while increasing glutathione (GSH) levels during acute inflammation.[30–32]

The fourth most abundant compound was **1,2,3-Benzenetriol**, also known as **pyrogallol**, a phenol derivative with strong anti-inflammatory and antimicrobial properties.[26,33] Pyrogallol has been identified as a major constituent in various plant parts[34] and is known for its ability to combat bacterial pathogens and reduce inflammation through its antioxidant activity.[33,35,36]

Based on the presence of these dominant compounds—9,12,15-Octadecatrienoic acid, Octadecanoic acid, Phytol, and 1,2,3-Benzenetriol—the citrus *benalu* leaf extract demonstrates promising potential as an anti-inflammatory and antioxidant agent. Anti-inflammatory activity is closely linked to antioxidant capacity, as oxidative stress plays a key role in the inflammatory process.[37,38] Antioxidants help neutralize reactive oxygen species (ROS), which, when present in excess, can damage cellular components such as lipids, proteins, and nucleic acids, leading to membrane instability and hemolysis.[33,39]

Inflammation testing was conducted by inducing mice with 0.2 mL of 1% carrageenan via subplantar injection. This method is known to stimulate an increase in cyclooxygenase-2 (COX-2) levels, a key enzyme involved in the inflammatory response.[40] The use of 0.2 mL of 1% carrageenan has been shown to reliably induce paw edema in mice, consistent with previous studies.[41]

The anti-inflammatory efficacy of the extract was evaluated using the paw edema method, with inflammation volume measured using a plethysmometer filled with mercury. This device operates on the principle of Archimedes' law,[42] allowing precise measurement of volume displacement caused by paw swelling. Although edema is typically monitored for six hours at 30-minute intervals,[16,43] in this study, the swelling had subsided by the 180-minute mark.

Carrageenan-induced inflammation occurs in two distinct phases. The first phase involves the release of histamine, serotonin, and bradykinin, while the second phase is characterized by the overproduction of prostaglandins, along with elevated levels of bradykinin, proteases, and lysosomal enzymes.[44] Edema generally persists for up to five hours post-injection and gradually resolves within 24 hours.[45] The percentage of edema observed in each group is presented in **Table 2** and **Figure 2**.

Table 2. Percentage of Paw Edema in Mice Following Carrageenan Induction

Average	Pretest (%)	After Induction (%)	30 Minutes (%)	60 Minutes (%)	90 Minutes (%)	120 Minutes (%)	150 Minutes (%)	180 Minutes (%)
Negative control	0.00	64.29	44.90	42.86	43.88	31.63	1.63	31.63
Positive control	0.00	119.18	131.51	73.97	34.25	24.66	10.96	2.74
Extract dose 100 mg/kgBW	0.00	148.21	119.64	107.14	98.21	71.43	51.79	26.79
Extract dose 200 mg/kgBW	0.00	125.86	77.59	67.24	68.97	50.00	37.93	10.34
Extract dose 400 mg/kgBW	0.00	110.77	69.23	81.54	58.46	40.00	26.15	3.08

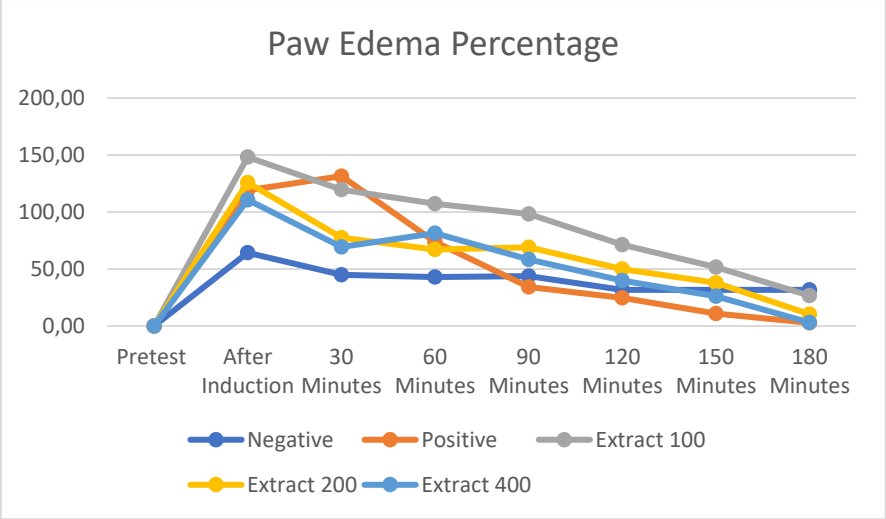

Figure 2. Percentage of Paw Edema in Mice Over Time Following Carrageenan Induction and Treatment with Citrus *Benalu* Leaf Extract

Following carrageenan induction, a significant increase in paw edema was observed across all experimental groups. The negative control group, which received 1% CMC-Na, exhibited consistently elevated edema levels throughout the 180-minute observation period. This outcome is attributed to the absence of anti-inflammatory activity in CMC-Na, leaving the inflammatory response entirely dependent on the animals' innate immune mechanisms.[46] Statistical analysis began with a normality test using the Shapiro–Wilk method, which yielded a significance value of $p > 0.05$ for the percentage reduction in edema from 30 to 180 minutes. This result confirms that the data were normally distributed. A homogeneity test using Levene's method also produced a significance value of $p > 0.05$, indicating that the data were homogeneously distributed across groups.[47] Given that both assumptions of normality and homogeneity were met, parametric analysis was conducted using one-way ANOVA.

The one-way ANOVA revealed statistically significant differences in the mean percentage reduction of edema among groups at all time points ($p < 0.05$, and in some cases $p < 0.001$), indicating that treatment with citrus *benalu* leaf extract significantly affected the inflammatory response compared to the negative control. Post hoc analysis using the Least Significant Difference (LSD) method confirmed that the negative control group had the highest edema percentage, significantly greater than all treatment groups.

The positive control group, which received diclofenac sodium, showed a marked reduction in edema compared to the negative control ($p < 0.05$), demonstrating the rapid and effective anti-inflammatory action of the standard drug.[48] Diclofenac sodium exerts its effect by non-selectively inhibiting cyclooxygenase (COX) enzymes and reducing the bioavailability of arachidonic acid.[49] Its selection for this study was based on its known pharmacokinetics, including high tissue penetration in inflamed regions such as the plantar surface of the paw.[50]

Treatment groups receiving citrus *benalu* leaf extract at varying doses exhibited dose-dependent anti-inflammatory effects. Notably, the group administered 400 mg/kg BW showed a significant reduction in edema, with results approaching those of the positive control group ($p > 0.05$), particularly between 60- and 180-minutes post-induction. These findings suggest that 400 mg/kg BW is the most effective dose, consistent with previous studies reporting optimal anti-inflammatory activity at this concentration.[51]

Flavonoids exert anti-inflammatory effects primarily by inhibiting key pro-inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), which are involved in the biosynthesis of prostaglandins and leukotrienes.[52,53] Additionally, flavonoids suppress neutrophil degranulation, thereby reducing the release of arachidonic acid and limiting the production of inflammatory mediators.[54,55] They also inhibit histamine release from mast cells and reduce leukocyte infiltration, contributing to the attenuation of the inflammatory response.[56]

Saponins demonstrate anti-inflammatory activity by inhibiting exudate formation and reducing vascular permeability, which helps to limit tissue swelling and leukocyte migration.[57,58] Certain saponin compounds have also been shown to modulate the NLRP3 inflammasome pathway, further suppressing the release of inflammatory cytokines such as IL-1β and IL-18.[59]

Tannins possess potent antioxidants and anti-inflammatory properties. Their antioxidant activity is linked to the inhibition of reactive oxygen species (ROS) production by neutrophils, monocytes, and macrophages, thereby reducing the formation of hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and hydroxyl radicals (-OH).[60] These actions help stabilize cellular membranes and prevent oxidative damage during inflammation.

The anti-inflammatory efficacy of the ethanol extract of citrus *benalu* leaves (*Dendrophthoe glabrescens* (Blakely) Barlow) is likely attributable to its bioactive constituents, including flavonoids, saponins, and tannins, which are known to modulate inflammatory pathways. However, a limitation of this study is the duration of observation, which was restricted to 180 minutes. While standard protocols often extend to 360 minutes,[61,62] the decision to limit the test duration was based on the observation that edema had nearly resolved by 180 minutes. Extending the test beyond this point could result in measurements that exceed baseline values, potentially confounding the interpretation of anti-inflammatory effects.

CONCLUSION

This study demonstrates that the ethanol extract of *Dendrophthoe glabrescens* (Blakely) Barlow, commonly known as citrus *benalu*, exhibits significant anti-inflammatory and antioxidant activities. GC-MS analysis identified key bioactive compounds—namely 9,12,15-octadecatrienoic acid, octadecanoic acid, phytol, and 1,2,3-benzenetriol—which are known to mitigate oxidative stress and modulate inflammatory pathways. In vivo evaluation using a carrageenan-induced mouse model confirmed the extract's anti-inflammatory potential, with the 400 mg/kg BW dose achieving a notable reduction in paw edema to 3.08%. These findings suggest that *D. glabrescens* leaf extract holds promise as a natural candidate for the development of alternative anti-inflammatory and antioxidant therapies. To advance its translational potential, further research is warranted, including comprehensive pharmacological profiling, toxicity assessments, and clinical trials to establish its safety, efficacy, and consistency in therapeutic applications.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Faculty of Pharmacy, Universitas Mahasaraswati Denpasar, for providing laboratory facilities and funding this research through the Internal Grant Program (Grant No. 1119.14/E.003/FF-UNMAS/XII/2024).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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JURNAL ILMIAH MEDICAMENTO

Vol.11 No.2 (September 2025)
page 163-171
https://doi.org/10.36733/medicamento.v11i2.11435
e-ISSN: 2356-4814

Original Article

Proliferation of Nano Chitosan and Platelet Rich Plasma from Pre-osteoblast Cell with Ki67 as a Surrogate Marker in Vitro

Proliferasi Nano Kitosan dan *Platelet Rich Plasma* dari Sel *Pre-Osteoblast* dengan Ki67 sebagai Pengganti *Marker In Vitro*

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Submitted : 08-04-2025
Reviewed : 08-07-2025
Accepted : 20-09-2025

Keywords: hydroxyapatite, nano-chitosan, pre-osteoblast, proliferation, PRP.

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Abstract

This study investigated the effect of nano-chitosan mix with platelet rich plasma (PRP) to proliferation rate of pre-osteoblast cell with incubation time used in vitro culture system. The culture media of pre-osteoblast cell MC3T3-E1 used alpha-MEM, 2mm L-glutamine, 1mm sodium pyruvate, 10% FBS and 10% pen strep in 25cm^2 flask bottle and incubated in an incubator with 5% CO_2 at a temperature of 37^{o}C until the cell was confluent 70-80% and planting in well-24 to give treatments. Treatment was divided into two groups, nano-chitosan+PRP and hydroxyapatite+PRP. The proliferation of pre-osteoblast cells saw with immunocytochemical staining and proliferation of cells were counted and investigated with confocal laser scanning microscope (CLSM). The normality of sample data was analyzed with Shapiro-Wilk. Comparison test used independent sample t-test and one-way ANOVA (F-test). All data were analyzed with SPSS software. The experiment results showed that nano-chitosan+PRP can accelerate proliferation than hydroxyapatite+PRP of 0% and 10% concentrations. The independent sample t-test showed there were a significant difference ($p=0.010<\propto$) from proliferation rate mean (0%) between treatment group nano-chitosan+PRP (1076.3±176.4au) and treatment group hydroxyapatite+PRP (659.5±272.7au) on five days incubation time, and proliferation mean (10%) between treatment group of nano-chitosan+PRP (710.3±109.7au) and hydroxyapatite+PRP (581.8±76.4au) on seven days incubation time. Based on proliferation mean (0%) and (10%), the treatment group of nano-chitosan+PRP with five- and seven-days incubation have higher mean than 0% and 10% on treatment group nano-chitosan and PRP and can accelerate bone healing with incubation time of five and seven days compared to treatment group of hydroxyapatite+PRP.

Kata Kunci: hydroksiapatit, *nano-chitosan*, proliferasi, PRP, sel preosteoblas.

Abstrak

Penelitian ini bertujuan untuk menyelidiki efek campuran nano-chitosan dengan plasma kaya platelet (PRP) terhadap laju proliferasi sel pre-osteoblast dalam sistem kultur in vitro dengan waktu inkubasi yang digunakan. Medium kultur sel pre-osteoblast MC3T3-E1 menggunakan alpha-MEM, 2 mm L-glutamin, 1 mm natrium piruvat, 10% FBS, dan 10% penisilin-streptomisin dalam botol flask 25 cm^2 dan diinkubasi dalam inkubator dengan 5% CO_2 pada suhu 37°C hingga sel mencapai kepadatan 70-80% dan ditanam dalam plate 24 sumur untuk pemberian perlakuan. Perlakuan dibagi menjadi dua kelompok, nano-chitosan+PRP dan hydroxyapatite+PRP. Proliferasi sel pre-osteoblast diamati dengan pewarnaan imunositokimia, dan jumlah sel yang berkembang biak dihitung dan dianalisis menggunakan mikroskop laser pemindaian konfokal (CLSM). Normalitas data sampel dianalisis dengan uji Shapiro-Wilk. Uji perbandingan menggunakan uji t sampel independen dan ANOVA satu arah (uji F). Semua data dianalisis menggunakan perangkat lunak SPSS. Hasil eksperimen menunjukkan bahwa nano-chitosan+PRP dapat mempercepat proliferasi dibandingkan dengan hidroksiapatit+PRP pada konsentrasi 0% dan 10%. Uji t sampel independen menunjukkan adanya perbedaan yang signifikan ($p=0.010<\alpha$) antara rata-rata laju proliferasi (0%) pada kelompok perlakuan nano-chitosan+PRP (1076.3±176.4au) dan kelompok perlakuan hidroksiapatit+PRP (659,5±272,7au) pada waktu inkubasi lima hari, serta rata-rata proliferasi (10%) antara kelompok perlakuan nano-chitosan+PRP (710,3±109,7au) dan hidroksiapatit+PRP (581,8±76,4au) pada waktu inkubasi tujuh hari. Berdasarkan rata-rata proliferasi (0%) dan (10%), kelompok perlakuan nano-chitosan+PRP dengan waktu inkubasi lima dan tujuh hari memiliki rata-rata yang lebih tinggi daripada 0% dan 10% pada kelompok perlakuan nano-chitosan dan PRP, dan dapat mempercepat penyembuhan tulang dengan waktu inkubasi lima dan tujuh hari dibandingkan dengan kelompok perlakuan hidroksiapatit+PRP.

How to cite: (citation style AMA 11th Ed.)
Wedagama DM. Proliferation of Nano Chitosan and Platelet Rich Plasma from Pre-osteoblast Cell with Ki67 as a Surrogate Marker in Vitro. *J. Ilm. Medicam.*, 2025:11(2), 163-171, DOI: 10.36733/medicamento.v11i2.11435

INTRODUCTION

Lately, many medical advancements are being made to aid bond defects caused by tumor re-sectioning of the bone, apex re-sectioning of tooth or bone caused by trauma. Room forming on surgical method required bone healing (bone remodeling).[1] To accelerate the process of bone healing, a bone graft material is needed which is biocompatible and made from natural chitosan. It is capable of acting as a physical barrier and serves in the osteo-conduction, osteo-induction, and osteogenesis.[2–4] Nano chitosan is a biopolymer which is biocompatible, biodegradable and anti-microorganism that can accelerate bone formation and wound healing through the properties of smaller particles, namely nano particles, where nano particles are smart materials that easily adapt to tissue.[5,6] The platelet-rich plasma (PRP) on nano chitosan as a scaffold is expected to function as the delivery vehicle for cells.[7] It also regulates the process of bone healing by acting as an extracellular matrix and maintain the space and shape of the bone defect remodeling. Thus, Hydroxyapatite (HA) is the crystal-like compound that acts as a scaffold.[8–10] Hypothetically, it is found out that nano chitosan plus a platelet-rich plasma (PRP) can accelerate proliferation compared with hydroxyapatite plus a platelet-rich plasma (PRP). For that reason, the process of bone healing by nano chitosan plus platelet-rich plasma (PRP) treatment is preferred.[11,12]

Modeling and Bone Healing

Bone Modeling is a term used to describe changes in bone structure during skeleton formation, growth and maturation.[13] Modeling leads to the process of changing the size and shape of bones which happens until the end of puberty, but the increase of density still occurs until four decades.[8] Medium regeneration of bone healing is a process that occurs continuously by replacing the old bone with new ones.[3,14] The place where remodeling occurred are termed basic multicellular remodeling units (BMus). It occurs between two to eight weeks as the formation of bone takes longer than the bone resorption. The remodeling process occurs since bone growth until the end of life.[10] The remodeling process includes two activities namely: bone resorption followed by bone formation process, the first process known as osteoclast activity while the latter known as osteoblast activity.[4,9,15] The remodeling process involves two main cell, osteoblasts and osteoclasts, and both are derived from marrow cells bone (bone marrow).[16] Osteoblasts are derived from pluripotent mesenchymal stem cells which is the fibroblast colony-forming units (CFU-F).[17,18] On the other hand, osteoclasts are derived from hematopoietic stem cells that is granulocyte-macrophage of colony-forming units (CFU-GM).[9,14,19]

MATERIALS AND METHODS

Ethical approval for the use of experimental animals was obtained from the Ethics Committee of Mahasaraswati University (Approval No. 03.0019/KEP-Unmas/VIII/2025). The primary material used in this study was nano-chitosan derived from Nephropidae shells collected from the Bali Sea, processed into nanoparticles using ball milling techniques. Platelet-rich plasma (PRP) was prepared from blood donated by the research team, with support from the Indonesian Red Cross, Malang Branch. PRP was isolated via centrifugation. The Ki67 proliferation marker was sourced from mouse origin, and pre-osteoblast MC3T3-E1 cells (ATCC murine cell line) were cultured in standard growth medium.

Pre-osteoblasts were seeded in multi-well plates and allocated into four experimental groups to assess proliferative and osteogenic responses under different treatments: (1) nano-chitosan + PRP, (2) micro-chitosan + PRP, (3) bone graft + PRP, and (4) ascorbic acid (positive control for osteogenic differentiation). Each group was prepared in multiple replicates to ensure statistical reliability. Cultures were incubated for two time points: 5 and 7 days. After each incubation period, assays were performed to quantify cellular proliferation and differentiation. Proliferation was evaluated using a standardized protocol, with measurements taken under two conditions (0% and 10%). Differentiation was assessed to determine the maturation of pre-osteoblasts into osteoblasts. All quantitative data for proliferation and differentiation were expressed in arbitrary units (AU).

Pre-osteoblast Cell Culture

MC3T3-E1 pre-osteoblast cells (ATCC, murine cell line) were cultured in α-MEM supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin in 25 cm^2 culture flasks. Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 until reaching 70–80% confluence. Upon confluence, cells were harvested and seeded into 24-well plates for experimental treatments. Platelet-rich plasma (PRP) was prepared from blood samples collected from the investigators, following standard centrifugation protocols, with assistance from the Indonesian Red Cross (PMI) Malang Branch. Pre-osteoblast cultures were treated with nano-chitosan and PRP and incubated for 5 or 7 days prior to analysis (**Figure 1**).

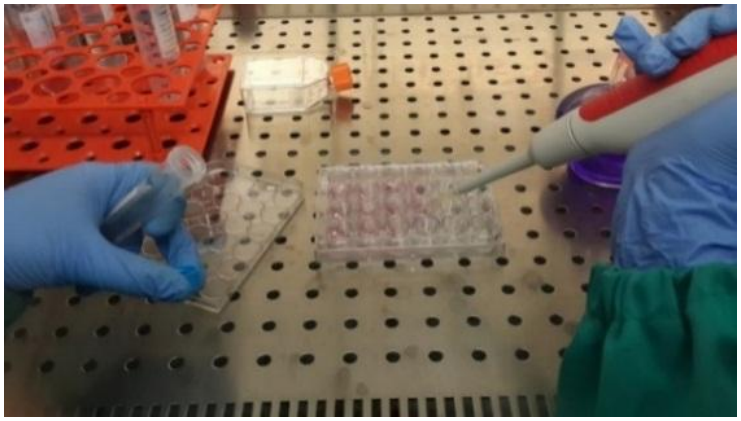

Figure 1. Platting Pre-Osteoblasts Cell Culture with Chitosan Nano Hydroxyapatite + + PRP and PRP

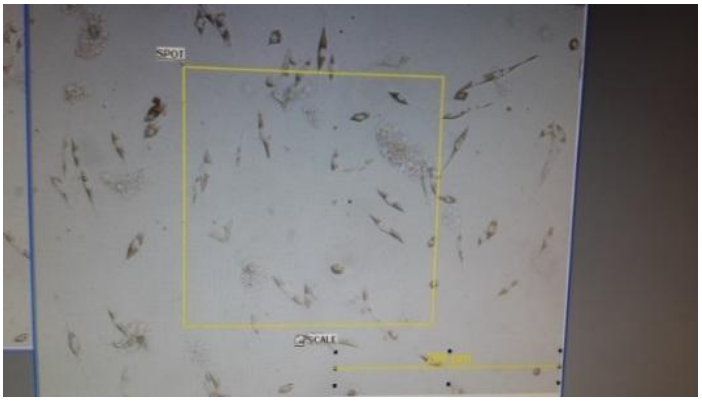

Figure 2. Growth Pre-Osteoblasts Cells after Incubation in an Incubator with an Electron Microscope (magnification 200µm)

Identification of Pre-osteoblast Cell Proliferation

Pre-osteoblasts proliferating cell staining were washed with PBS 3x10 min, incubation with 0.05 % Triton - X in PBS for 15 minutes. The color red ties Ki 67 primary antibody (mouse host) by administering anti-mouse secondary antibodies labeled rhodamine C. Proliferation of cells with test immunocytochemical was observed through a confocal laser scanning microscope (CLSM) (**Figure 2**).

Immunocytochemistry Featured

Immunocytochemistry was done to study the distribution of specific enzymes in the cell structure intact to normal or full band detect the cell component that is bio-macromolecules such as proteins, carbohydrates, among others. In this examination, it was done to see the proliferation of cell staining by pre-osteoblasts. Cell proliferation by immunocytochemistry was observed through a confocal laser scanning microscope (CLSM) (**Figure 3**).

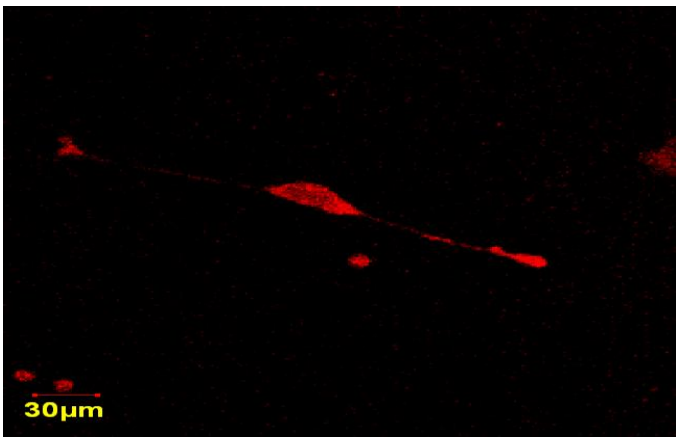

Figure 3. Overview of Pre-Osteoblasts Cell Proliferation in Immunocytochemistry*
*Note: the red Figure shows the absorption bonding primer Ki67 antibodies. It is with anti-mouse secondary antibodies labeled rhodamine C.

Data Analysis

Data analysis included six types of calculations. Normality of the sample data was assessed using the Shapiro–Wilk test. Group comparisons were performed using an independent samples t-test. All statistical analyses were conducted using SPSS software (version 22.0; IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Cellular proliferation of MC3T3-E1 pre-osteoblasts was quantified by Ki67 immunocytochemistry and expressed in arbitrary units (AU). Ki67 is a validated nuclear marker of cells in active phases of the cell cycle (G1, S, G2, M) and is absent in quiescent G0 cells, supporting its use for proliferation assessment in vitro.[20]

Table 1. Result of data normality test

Treatment Group	*p-value*	
	Proliferation (0%)	Proliferation (10%)
Nano Chitosan + PRP (5 days)	0.996	0.473
Nano Chitosan + PRP (7 days)	0.489*	0.867**
Hydroxyapatite + PRP (5 days)	0.122	0.620
Hydroxyapatite + PRP (7 days)	0.159	0.066

Interpretation: A p-value < 0.05 indicates that the data deviate significantly from a normal distribution, whereas a p-value > 0.05 suggests that the data are normally distributed.

The Shapiro–Wilk test indicated that the p-values for all observation groups were greater than the significance level (α = 0.05) (**Table 1**), confirming that the data met the assumptions of normality. Therefore, the dataset satisfied the prerequisites for parametric testing.

Table 2. Comparison results between incubation time of 0% proliferation

Treatment Group	Incubation Time		*p-value*
	5 days	7days	(t test)
Nano Chitosan + PRP	1076.3±176.4**	374.6±72.9	0.000 <∝
Hydroxyapatite + PRP	659.5±272.7*	368.7±95.8	0.033 <∝

Interpretation: A p-value < 0.05 indicates a statistically significant difference between groups, whereas a p-value > 0.05 indicates no statistically significant difference.

The independent samples t-test revealed a statistically significant difference ($p < 0.001$) in mean proliferation (0%) for the nano-chitosan + PRP treatment group between the two incubation periods: 5 days (1076.3 ± 176.4 AU) and 7 days (374.6 ± 72.9 AU). In contrast, the hydroxyapatite + PRP group showed no

statistically significant difference (p = 0.033 > α) between 5 days (659.5 ± 272.7 AU) and 7 days (368.7 ± 95.8 AU) (**Table 2**).

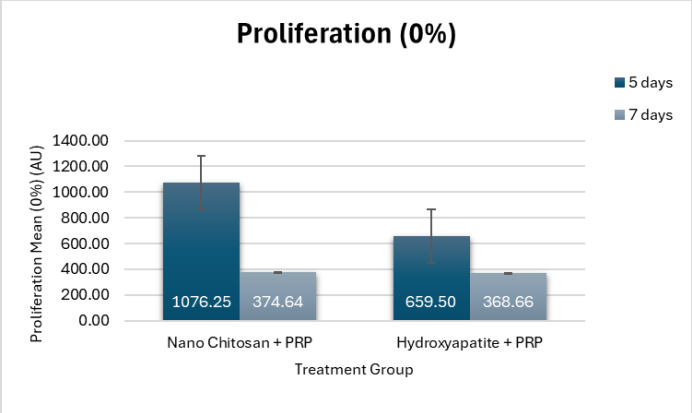

Figure 4. Histogram of Mean Proliferation (0 %) (incubation five days and seven days)*

Interpretation: For the treatment groups (nano-chitosan + PRP and hydroxyapatite + PRP), means sharing the same letter indicates no statistically significant difference, whereas means with different letters indicate a statistically significant difference.

Figure 4 presents a histogram of mean proliferation (0%) after 5 and 7 days of incubation in two treatment groups. The highest proliferation was observed in the nano-chitosan + PRP group at 5 days (1076.3 ± 176.4 AU), which was significantly greater than all other groups.

Table 3. Results of the comparison between the time incubation on proliferation (10 %)

Treatment Group	Incubation Time		*p-value*
	5 days	7days	
Nano Chitosan + PRP	241.9±96.7	710.3±109.7**	0.000 <∝
Hydroxyapatite + PRP	396.4±74.1	581.8±76.4*	0.002 <∝

Interpretation: For the t-test, a p-value < 0.05 indicates a statistically significant difference between groups, whereas a p-value > 0.05 indicates no statistically significant difference.

The independent samples t-test revealed a statistically significant difference (p < 0.001) in mean proliferation (10%) for the nano-chitosan + PRP treatment group between the two incubation periods: 5 days (241.9 ± 96.7 AU) and 7 days (710.3 ± 109.7 AU) (**Table 3**). Based on these values, proliferation after 7 days was substantially higher compared to 5 days, indicating that the combination of nano-chitosan and platelet-rich plasma (PRP) markedly enhances cellular proliferation over time. This suggests that nano-chitosan + PRP may accelerate bone healing within a 7-day incubation period.

In contrast, the hydroxyapatite + PRP group showed a smaller difference between incubation periods, with mean proliferation values of 396.4 ± 74.0 AU at 5 days and 581.8 ± 76.4 AU at 7 days. Although the p-value was 0.033 (which is below α = 0.05), the magnitude of change was less pronounced compared to the nano-chitosan + PRP group. This indicates that while hydroxyapatite + PRP also promotes proliferation over time, its effect is more moderate.

When comparing the two treatments, nano-chitosan + PRP demonstrated a significantly greater and faster proliferative response than hydroxyapatite + PRP after 7 days. These findings suggest that nano-chitosan + PRP is a more effective option for accelerating bone healing, whereas hydroxyapatite + PRP may be more suitable for applications requiring gradual, sustained regeneration, particularly when scaffold stability is a priority.

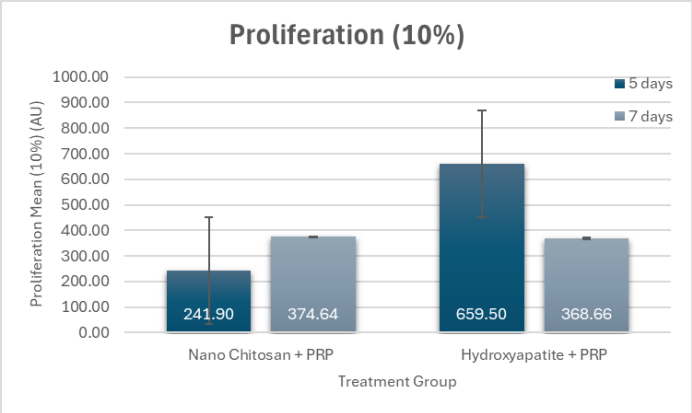

Figure 5. Histogram of Proliferation Mean (10 %) (incubation five days and seven days)

Interpretation: For the treatment groups (nano-chitosan + PRP and hydroxyapatite + PRP), means that share the same letter indicate no statistically significant difference, whereas means with different letters indicate a statistically significant difference.

Figure 5 illustrates a histogram showing the mean proliferation rate (10%) after 5-day and 7-day incubation periods for two treatment groups. The nano-chitosan + PRP group exhibited a markedly higher proliferation compared to the hydroxyapatite + PRP group, and this difference was statistically significant.

Table 4. The comparison results in proving the hypothesis

Treatment Group	Treatment group		*p-value*
	Nano Chitosan Mean ± SD	Hydroxyapatite Mean ± SD	
Proliferation (0%) (au)	1076.3±176.4**	659.5±272.7*	0.010 <∝
Proliferation (10%) (au)	710.4±109.7	581.8±76.4	0.040 <∝

Interpretation: For the t-test, a p-value < 0.05 indicates a statistically significant difference between groups, whereas a p-value > 0.05 indicates no statistically significant difference.

Table 4 presents the comparative results of mean proliferation rates (0% and 10%) for both treatment groups—nano-chitosan + PRP and hydroxyapatite + PRP—across 5-day and 7-day incubation periods. Based on the average proliferation values at both 0% and 10%, the nano-chitosan + PRP group consistently exhibited substantially higher proliferation compared to the hydroxyapatite + PRP group at both time points. This finding suggests that the combination of nano-chitosan and PRP significantly enhances cellular proliferation and may accelerate bone healing within 5 to 7 days of incubation, outperforming the hydroxyapatite + PRP treatment.

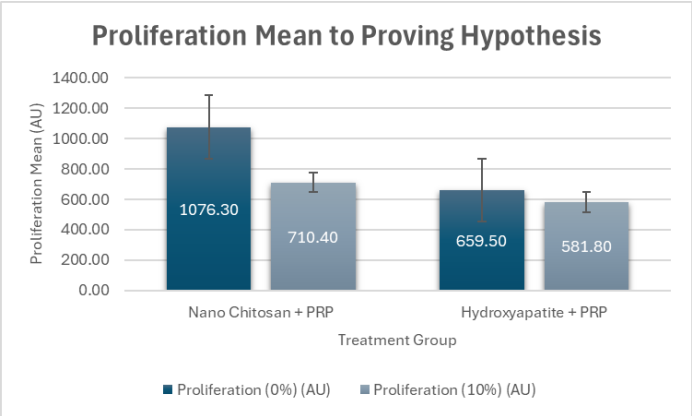

Figure 6. Histogram of Proliferation Mean to Proving Hypothesis*

* Description: The comparison involves two treatment groups: nano-chitosan + PRP and hydroxyapatite + PRP.

Figure 6 illustrates a histogram comparing the mean proliferation rate (0%) for the two treatment groups during the 5-day incubation period. The nano-chitosan + PRP group (1076.3 ± 176.4 AU) exhibited a higher proliferation rate than the hydroxyapatite + PRP group (659.5 ± 272.7 AU). This difference was

statistically significant ($p = 0.010 < \alpha$), indicating that nano-chitosan + PRP promotes greater cellular proliferation under these conditions.

Similarly, the mean proliferation (10%) in the nano-chitosan + PRP group after 7 days of incubation (710.3 ± 109.7 AU) was higher than that observed in the hydroxyapatite + PRP group (581.8 ± 76.4 AU) during the same period. This difference was statistically significant ($p = 0.040 < \alpha$), supporting the hypothesis that pre-osteoblast proliferation (Ki67) in vitro is greater with nano-chitosan + PRP compared to hydroxyapatite + PRP. These findings reinforce the role of PRP in accelerating bone regeneration during the early phases of healing, although previous studies have reported mixed results regarding its statistical significance across different models.[21]

Overall, there was a significant difference ($p < 0.05$) in mean proliferation (0% and 10%) between the two treatment groups across both incubation periods (5 and 7 days). The nano-chitosan + PRP group consistently demonstrated higher proliferation values than the hydroxyapatite + PRP group, indicating that nano-chitosan + PRP can accelerate bone healing more effectively. Notably, cell proliferation showed optimal results at 5% concentration during the 7-day incubation period. Previous research suggests that chitosan can minimize oxidative stress (as indicated by peroxide values), influence color stability, and reduce microbial load in samples.[22,23] Furthermore, nano-chitosan, with a deacetylation degree greater than 90%, has been shown to enhance pre-osteoblast proliferation and induce new bone formation more effectively than conventional chitosan, including in femoral defect models in goats.

The combination of chitosan and PRP has potential applications in complex or chronic wound healing and in soft and hard tissue regeneration, such as post-extraction sites requiring sustained release of growth factors. PRP provides concentrated bioactive mediators (e.g., PDGF, TGF-β, VEGF) that can stimulate cell proliferation and early osteogenic signaling, which is well-documented in oral and maxillofacial contexts.[24,25] In parallel, chitosan's cationic nature, biocompatibility, and modifiable nanoscale surface topography can improve protein adsorption, cell adhesion, and local factor presentation, thereby potentiating osteogenic responses. The synergy of nano-scale chitosan (greater surface area and adsorption capacity) with PRP likely underpins the stronger, time-dependent gains seen here.[20,26]

Within the limits of an in vitro model, the present findings suggest nano-chitosan + PRP may offer a more efficacious early-phase proliferative stimulus than hydroxyapatite + PRP—a desirable attribute for applications targeting rapid early cellular recruitment (e.g., alveolar ridge preservation or peri-implant defects). Nevertheless, translation to clinical outcomes depends on additional properties (matrix mineralization, vascularization, mechanical stability), domains where HAp's scaffolding and long-term dimensional integrity may remain advantageous.[27,28] Rigorous in vivo studies and controlled clinical trials are warranted to determine whether the proliferative advantage observed here translates into accelerated and durable bone formation in dental indications.[24,25,27]

CONCLUSIONS

The findings of this study indicate that the combination of nano-chitosan and platelet-rich plasma (PRP) resulted in higher mean proliferation values under both 0% and 10% conditions compared to hydroxyapatite + PRP, across both 5-day and 7-day incubation periods. These results suggest that nano-chitosan + PRP can accelerate pre-osteoblast proliferation more effectively than hydroxyapatite + PRP, thereby potentially enhancing the bone healing process within a shorter timeframe. The superior performance of nano-chitosan + PRP highlights its promise as a bioactive material for promoting early bone regeneration in dental applications.

RECOMMENDATIONS

Future research should further investigate the effect of nano-chitosan combined with platelet-rich plasma (PRP) on pre-osteoblast proliferation under varying concentrations (e.g., 0% and 10%) and extended

incubation periods in an in vitro culture system. Additional studies could also explore different PRP concentrations, nano-chitosan particle sizes, and their synergistic impact on osteogenic differentiation and mineralization to better understand their potential in accelerating bone regeneration for dental applications.

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JURNAL ILMIAH MEDICAMENTO

Vol.11 No.2 (September 2025)
page 172-184
https://doi.org/10.36733/medicamento.v11i2.11749
e-ISSN: 2356-4814

Original Article

The Evolving Trends In Vital Pulp Therapy Through the Application of Various Biomaterials: A Bibliometric Study

Perkembangan Tren Terapi Pulpa Vital melalui Aplikasi berbagai Biomaterial: Penelitian Bibliometrik

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Submitted : 01-06-2025
Revised : 01-09-2025
Accepted : 16-09-2025

Keywords: bibliometric, dentinogenesis, mineral trioxide aggregate, vital pulp therapy.

Kata Kunci: bibliometrik, dentinogenesis, mineral trioxide aggregate, terapi pulpa vital.

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Abstract

Vital pulp therapy (VPT) is a crucial dental intervention designed to maintain the vitality of the dental pulp, particularly in carious lesions or trauma. The evolution of biomaterials in VPT has significantly affected dental practices by providing less invasive alternatives that safeguard tooth vitality and functionality. Continued exploration of the properties and effects of these materials is crucial for enhancing treatment strategies and optimizing dental care outcomes. Bibliometric analysis allowed the assessment of trends in the biomaterials used for VPT. Data mining was performed using the Scopus database and Boolean expressions. Data extraction and analysis were conducted using VOSviewer version 1.6.20., VOSviewer thesaurus, and Microsoft Excel. A total of 856 documents were identified, and the United States had the largest number of documents and citations (110 documents, 6,938 citations). The top three sources were the Journal of Endodontics, International Journal of Endodontics, and Dental Materials Journal. Mineral trioxide aggregate (MTA) is still the dominant biomaterial used in VPT to this day. MTA can accelerate dentinogenesis in VPT. The prominence of "MTA" and "VPT" as keywords highlights research on prevalence, causes, prevention, and evaluation, emphasizing the need to investigate biomaterial-driven healing, regeneration, stem cell activity, and gene expression.

Abstrak

Terapi pulpa vital (*Vital Pulp Therapy*/VPT) merupakan prosedur terapeutik esensial dalam praktik kedokteran gigi yang bertujuan mempertahankan vitalitas pulpa gigi, pada kasus kerusakan akibat karies atau trauma. Inovasi penggunaan biomaterial memberi kontribusi besar terhadap pendekatan klinis yang lebih konservatif, dengan tetap menjaga integritas biologis dan fungsional gigi. Penelitian terhadap karakteristik fisikokimia dan bioaktivitas biomaterial menjadi aspek fundamental dalam upaya optimalisasi protokol terapi dan peningkatan luaran klinis. Evaluasi bibliometrik memberikan wawasan kuantitatif terhadap dinamika perkembangan penelitian biomaterial pada VPT. Data diperoleh melalui basis data Scopus dengan penelusuran berbasis Boolean. Proses ekstraksi dan analisis informasi menggunakan perangkat lunak VOSviewer versi 1.6.20 yang didukung oleh VOSviewer Thesaurus dan pengolahan lanjutan melalui Microsoft Excel. Sebanyak 856 publikasi berhasil diidentifikasi, dengan Amerika Serikat sebagai kontributor utama berdasarkan jumlah publikasi 110 dokumen dan total sitasi 6.938 kutipan. Tiga sumber literatur ilmiah yang paling banyak memuat publikasi terkait topik ini adalah Journal of Endodontics, International Journal of Endodontics, dan Dental Materials Journal. Di antara berbagai jenis biomaterial yang digunakan, Mineral Trioxide Aggregate (MTA) menjadi material yang paling banyak diaplikasikan dalam prosedur VPT hingga saat ini. MTA diketahui berperan dalam merangsang proses dentinogenesis, menjadikannya bahan yang sangat relevan dalam konteks terapi regeneratif pulpa. Frekuensi kemunculan kata kunci "MTA" dan "VPT" dalam publikasi ilmiah menunjukkan tingginya minat riset terhadap aspek prevalensi, etiologi, tindakan preventif, serta evaluasi klinis terapi ini. Fenomena ini menyoroti pentingnya eksplorasi lanjutan terhadap mekanisme penyembuhan jaringan, proses regenerasi, aktivasi sel punca, dan regulasi ekspresi gen yang dimediasi oleh biomaterial.

How to cite: (citation style AMA 11th Ed.)
Widyastuti W, Anggraini W, Fibryanto E, Roeslan MO, Gunardi I, Rizal MI, Khazin SM, Wedagama DM. The Evolving Trends In Vital Pulp Therapy Through the Application of Various Biomaterials: A Bibliometric Study. *J. Ilm. Medicam.*, 2025:11(2), 172-184, DOI: 10.36733/medicamento.v11i2.11749

INTRODUCTION

Maintaining the integrity of the dental pulp is essential due to its role in supplying nourishment, sensibility, and protection against infection.[1] Vital pulp therapy (VPT) methodologies are intended to maintain the vitality and functional integrity of the dental pulp following damage caused by trauma, dental caries, or restorative interventions.[2,3] VPT procedures have traditionally encompassed techniques such as indirect pulp capping, direct pulp capping, and partial and complete pulpotomy.[4] These techniques help to eliminate the inflammatory response irreversibly. VPT also facilitates the healing of the pulp tissue and safeguards it from external stimuli, including mechanical, bacterial, traumatic, chemical, iatrogenic, and thermal factors, thereby preventing extensive tissue damage.[1] VPT is initiated at sites of either near-pulpal or direct pulp exposure and involves the application of a protective dressing or base. This shields the pulp from further injury while facilitating its healing and repair. This procedure refers to placing a biomaterial over a nearly exposed coronal pulp after caries excavation.[3]

An ideal pulp-capping material should have the ability to stimulate reparative dentin formation, preserve pulpal vitality, release fluoride to inhibit secondary caries, exhibit bactericidal or bacteriostatic properties, adhere effectively to both dentin and restorative materials, withstand mechanical forces during restoration placement and throughout its functional lifespan, be sterile and radiopaque, and establish an effective bacterial seal.[2,5,6] Recently, the renewed interest in VPT can be attributed to advancements in diagnostic technologies, development of bioactive materials, improvements in magnification techniques, and an enhanced understanding of the pulp–dentin complex. The resurgence in interest has progressed notably since Hermann introduced calcium hydroxide ($Ca(OH)_2$) as a root-filling material in 1920. Notably, between 1928 and 1930, several studies investigating the use of $Ca(OH)_2$ concluded that it is a biocompatible material when applied to vital pulp tissue.[2] For several decades, $Ca(OH)_2$ has been the standard of excellence among pulp-capping materials.[7,8] $Ca(OH)_2$ has been widely utilized as a pulp-capping agent because of its antibacterial properties and capacity to promote the formation of a robust tissue barrier.[8,9]

In the early 1990s, MTA was introduced as a bioactive material. Its mechanism of action of MTA is similar to that of $Ca(OH)_2$. MTA was developed for clinical applications where maintaining a dry field is challenging, such as in retrograde endodontic filling and perforation repair. Extended usage includes apexification, dressing over pulpotomized, and pulp-capping material.[10] The benefits of MTA include its biocompatibility, effective sealing capability, bioactivity, and ability to induce the formation of mineralized tissue.[10] With the use of MTA, dentin bridge formation following pulp capping was observed as early as 1 week, with a consistent increase in both length and thickness over 3 months.[11] Nevertheless, the setting time, mechanical properties, potential for discoloration, ease of manipulation, and mud-like consistency of the cement have historically been significant technical challenges associated with traditional MTA.[10]

Calcium silicate cement (CSCs) have gained significant attention for their application in VPT. They include tricalcium silicates, dicalcium silicates, hydraulic calcium silicate cement, and "bioceramics." When MTA and other CSCs are used for VPT in permanent teeth with symptomatic or asymptomatic irreversible pulpitis, the success rate ranges from 85% to 100% at 1–2 years. Ca(OH)2, glass ionomer cement, and resin-based materials show less favorable clinical outcomes, with success rates ranging from 43% to 92%.[12] Compared with those of calcium hydroxide-based materials, the quality of the formed mineralized barriers using CSCs has improved.[12] During dental pulp procedures, silicate materials also exhibit advantageous physicochemical properties, such as high alkalinity, intratubular mineralization, inhibition of biofilm formation, and reduction in potent proinflammatory mediators. It also has improved setting times, including modified compositions that reduce tooth discoloration.[12]

In recent years, bibliometrics has emerged as a prominent trend in academic research. Bibliometric analysis involves a systematic examination of the scientific literature to identify patterns, trends, and effects within a specific field. The key steps in this process include collecting data from relevant databases, cleaning and refining the data, and applying various bibliometric methods to generate meaningful insights.[13] Bibliometrics reflects its applicability in handling huge scientific data and contributes significantly to impactful research.[13]

Despite increasing research on VPT, no bibliometric analysis has yet examined the most widely used materials from 2000 to 2024, the countries that utilize these materials most frequently, or the leading authors contributing to the study of pulp-capping materials in this field. Therefore, this review aimed to highlight the strengths and progress in the field by presenting the publication trends in dentistry related to VPT, identifying the most influential authors, sources, organizations, countries, and keywords, and describing the evolution of research on VPT needs over time.

METHODS

This bibliometric analysis report follows the guidelines of bibliometric reviews of the biomedical literature (BIBLIO). Bibliometric analysis is a systematic approach to examining scientific literature to identify patterns, trends, and effects within a specific field. The primary steps involve collecting data from relevant Scopus databases to perform a comprehensive literature search. Following data mining, cleaning, and refinement, data were analyzed using various bibliometric methods, a crucial step in deriving meaningful insights.[13]

Data sources and search strategy

This study conducted a bibliometric analysis of VPT-related scientific research. The aim was to assess the evolution of studies on VPT needs from 2000 to 2024 from Scopus. Scopus was selected because it includes peer-reviewed articles published by Elsevier, Springer, Wiley, Nature, and other reputable publishers.[14] Moreover, Scopus is regarded as the largest citation and abstract repository, encompassing a broader spectrum of topics than the Web of Science database. To the best of the authors' knowledge, this study has employed all relevant keywords used by various researchers, organizing them into a query search string with appropriate field codes and Boolean operators.

Bibliometric analysis broadly comprises three phases: Phase I, which involves identifying of sources and criteria; Phase II, which consists of selecting software and extracting data; and Phase III, which involves data analysis and interpretation. Before the bibliometric analysis, keyword selection was considered fundamental to the study's framework. To this end, the authors reviewed >1,000 articles, spanning both recent publications and earlier works with substantial citation counts, sourced from Scopus.[13]

A preliminary research topic search was conducted on December 3, 2024, via Boolean sentences in Scopus. To search for phrases in Scopus, double quotes (“), wildcards (*), and Boolean operators (OR, AND, NOT) were used. Double quotes were used to indicate “loose phrases,” ensuring that the words were kept together. Wildcards (*) were used to represent several characters, and Boolean operators were used to expand or narrow the search parameters when databases or search engines were used. The default search field in Scopus uses ALL with the description All Fields. The keywords used to guide the data search were ALL (“pulp inflammation”) OR ALL “pulp exposure”) OR ALL (“vital pulp therapy”) OR ALL (“pulp capping”) OR ALL (“direct pulp capping”) OR ALL (“indirect pulp capping”) AND ALL (“calcium hydroxide”) OR ALL (“mineral trioxide aggregate”) AND ALL (“dentin repair”) OR ALL (“dentin bridges”) OR ALL (“calcified barrier”) OR ALL (“dentinogenesis”)).

Data collection and analysis

Data were taken between 2000 and 2024. The data collection was initiated from the year 2000, as this period marks the beginning of a substantial increase in the number of publications indexed in Scopus

addressing the significance of vital pulp therapy. Do you want me to make it even more concise, like for a journal manuscript style?The query string initially yielded 1,282 articles. This analysis included all the literature about VPT, including articles, reviews, and case reports. Restricting to articles that used only the English language resulted in 1,254 articles. Other exclusion criteria were as follows: articles that did not contain the author name (blank) (n = 9); articles other than original articles, reviews, and cases (n = 110); articles with no specific titles (n = 122); and no specific keywords (n = 157). After the articles were filtered according to the exclusion criteria, up to 856 articles remained from Scopus.

All the acquired data were tabulated in Microsoft Excel 2019 (Microsoft Office, USA). Treatment needs to use Scopus. VOSviewer and Thesaurus were used to map and cluster the results based on the research questions. The bibliography analysis attributes in VOS viewer software 1.6.20 (Universiteit Leiden, Netherlands) include coauthorship, co-occurrence, and citation. After the data were processed using VOSviewer and several visualizations were generated, the information was further analyzed with VOSviewer thesaurus, and Microsoft Excel was employed to facilitate more interactive and user-friendly data visualization. The bibliometric analysis workflow is shown in **Figure 1**.

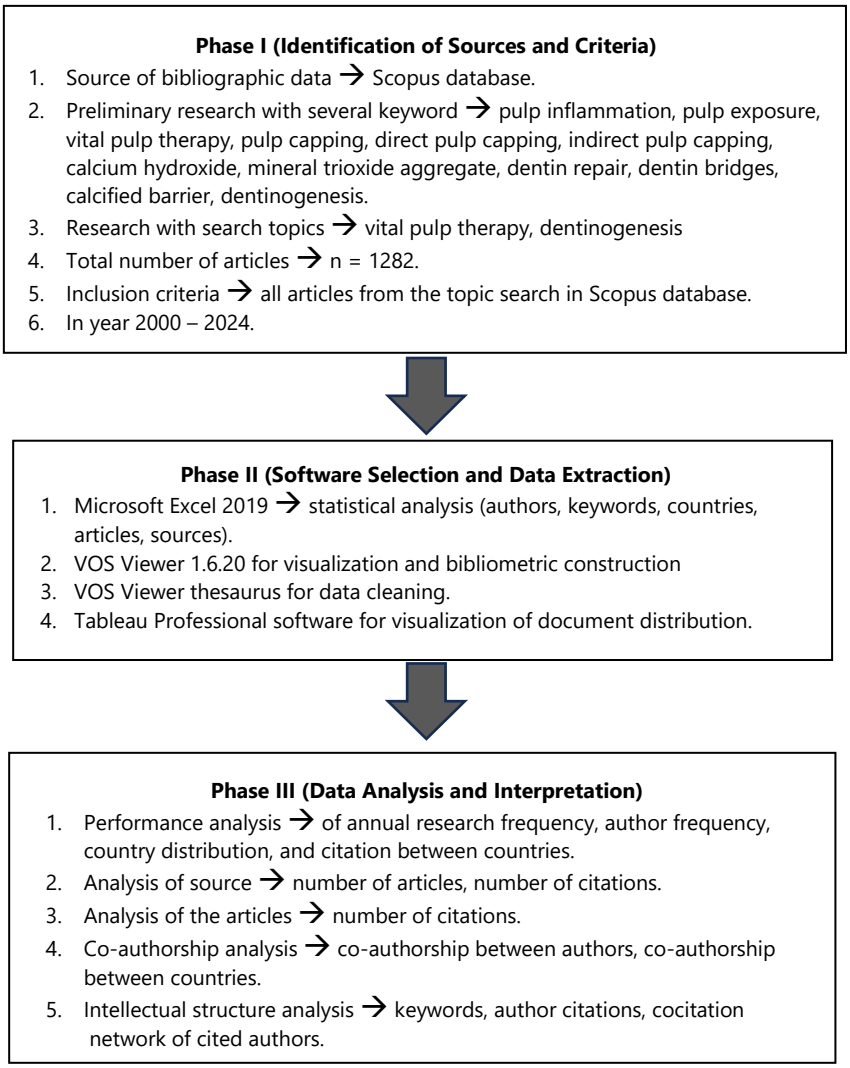


Figure 1. Flow diagram of the bibliometric analysis methodology process

DATA ANALYSIS RESULTS

1. Analysis of publication frequency by years

VPT has shown an increasingly interesting trend from year to year. This trend was evident from 2000 to 2004, when only 1–4 articles per year were identified. However, the number of articles increased significantly, starting from 10 articles in 2006 to 28 in 2010 and then 43 in 2014, and these figures were increasing through 2024. Notably, in 2021, 96 articles on VPT were published. As shown in **Figure 2**, a total of 3,617 citations across 29 articles were recorded in 2010.

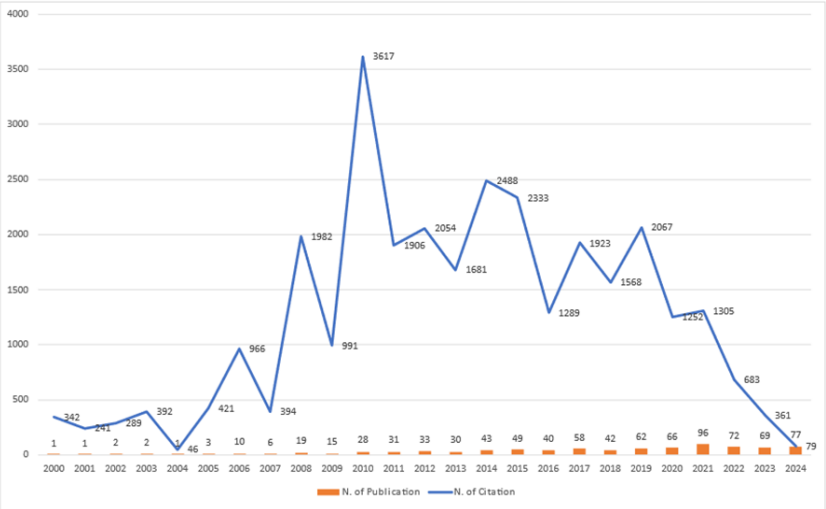


Figure 2. Trends in the number of publications and the number of citation articles from 2000-2024.

2. Analysis of countries to fields for VPT

To assess the number of articles published according to the author's country of origin, bibliometric coupling analysis was employed. Between 2000 and 2024, the 10 countries that published the most VPTs were the United States, with the highest number of articles at 192, followed by China with 147, Japan with 119, India with 116, Brazil with 100, United Kingdom with 100, Iran with 90, South Korea with 72, and Egypt and Turkey with 56 (**Figure 3**).

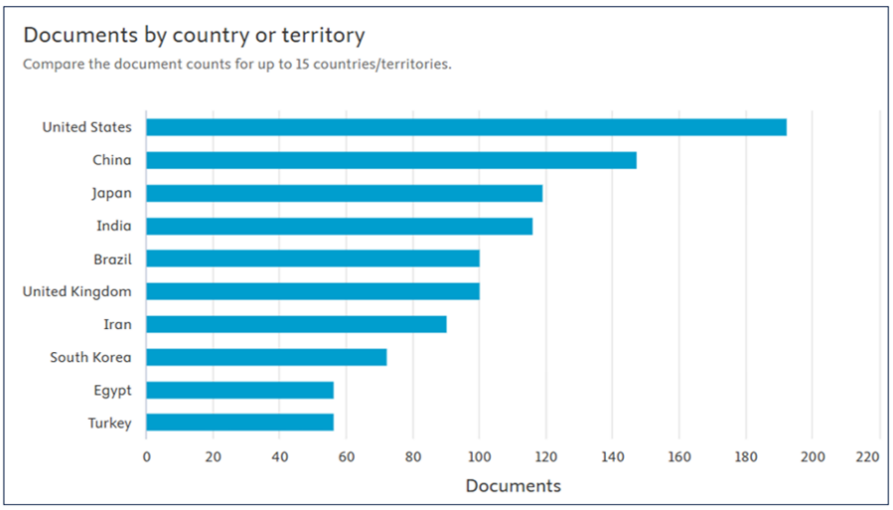


Figure 3. The trend of the distribution of articles by country or territory

Co-authorship analysis was conducted on country-level units, with a maximum of 25 countries per article and a minimum of five articles per country, excluding those with zero citations. We observed that 74 countries met these criteria; the largest group included 39 interconnected countries, along with 10 countries

exhibiting the highest levels of co-authorship (**Table 1**). The authors of these 36 countries were interconnected, except for the authors from three countries, namely, Serbia, Romania, and Mexico.

Table 1. Top 10 countries with the most significant number of documents and collaborating countries

Rank	Country	Region	Publication	Citation	Total link strength	Collaborating countries
1	United States	America	110	6,938	102	Brazil, Indonesia
2	China	China	105	2,474	40	Japan, Turkey, Belgium, Taiwan
3	Japan	East Asia	104	3,499	34	China, Turkey, Belgium, Taiwan
4	India	South Asia	79	1,170	17	Iran, South Korea, Egypt, Saudi Arabia, Australia, Canada, Malaysia, Jordan, Pakistan, Kuwait
5	Brazil	South America	66	2,133	33	United States, Indonesia
7	Iran	Middle East	63	3,546	22	India, South Korea, Egypt, Saudi Arabia, Australia, Canada, Malaysia, Jordan, Pakistan, Kuwait
8	South Korea	East Asia	60	1,573	13	India, Iran, Egypt, Saudi Arabia, Australia, Canada, Malaysia, Jordan, Pakistan, Kuwait
6	United Kingdom	Great Britain	50	4,430	80	Ireland, Switzerland, New Zealand, Denmark and Sweden
9	Egypt	Middle East	45	562	21	India, Iran, South Korea, Saudi Arabia, Australia, Canada, Malaysia, Jordan, Pakistan, Kuwait
10	Turkey	Middle East	39	763	7	Japan, China, Belgium, Taiwan

3. Analysis of the number of article citations by country

Citation analysis was performed on country-level units, with a maximum of 25 countries per article and a minimum of 10 articles per country (**Table 1**). The United States ranked first, with 6,938 citations from 110 publications.

4. Source analysis based on the number of documents

The analysis revealed that 239 Scopus-indexed sources contributed to the publication of 856 articles identified through data mining. The largest cluster of interconnected sources consisted of 13 journals. The Journal of Endodontics was the leading source, with 146 articles, followed by the International Endodontic Journal with 81 articles, Dental Materials with 27 articles, and Biomaterials with 21 articles (**Table 2**).

5. Analysis of the article

This analysis of articles and documents aimed to identify those that had the greatest influence on research trends related to VPT and biomaterials, or more specifically, research trends in VPT. VOSviewer was used for the analysis, with citation analysis as the method and documents as the unit of analysis. The number of citations for each document was set at 50. From 856 documents, 172 articles were found to have been cited 50 times, and 170 interconnected articles were identified.

The article titled, "Mineral Trioxide Aggregate: A Comprehensive Literature Review Part III: Clinical Application, Drawbacks, and Mechanism of Action," published in the *Journal of Endodontics* in 2010, has the highest citation count. Authored by Masoud Parirokh and Mahmoud Torabinejad (**Table 3**), this publication reflects the author's substantial contribution to the advancement of VPT.

Table 2. Top-ranking sources with a minimum of twenty documents

Rank	Sources	Country	ISSN	Publication	Citation	h-Index	Scimago	SJR (2023)
1	*Journal of Endodontics*	United States	00992399, 18783554	146	9,967	180	Q1	1.36
2	*International Endodontic Journal*	United Kingdom	01432885, 13652591	81	5,517	147	Q1	2.16
3	*Dental Material Journal*	Japan	02874547, 18811361	27	382	70	Q2	0.59
4	*Biomaterial*	United Kingdom	01429612, 18785905	21	1,452	435	Q2	3.02
5	*Clinical Oral Investigations*	Germany	14326981, 14363771	20	651	101	Q1	0.94
6	*Journal of Dental Research*	United States	00220345	20	971	211	Q1	1.91

Table 3. Top 10 most-cited articles

Rank	Title	Authors	Journal	Year	Citations
1	Mineral trioxide aggregate: a comprehensive literature review—Part III: Clinical applications, drawbacks, and mechanism of action	Masoud Parirokh and Mahmoud Torabinejad	*Journal of Endodontics*	2010	951
2	Mineral trioxide aggregate: a comprehensive literature review—part II: leakage and biocompatibility investigations	Mahmoud Torabinejad and Masoud Parirokh	*Journal of Endodontics*	2010	665
3	Calcium silicate bioactive cements: Biological perspectives and clinical applications	Carlo Prati and Maria Giovanna Gandolfi	*Dental Materials*	2015	390
4	Response of human dental pulp capped with biodentine and mineral trioxide aggregate	Alicja Nowicka, Mariusz Lipski, Mirosław Parafiniuk, Katarzyna Sporniak-Tutak, Damian Lichota, Anita Kosierkiewicz, Wojciech Kaczmarek, Jadwiga Buczkowska-Radlińska	*Journal of Endodontics*	2013	357
5	Mineral trioxide aggregate material use in endodontic treatment: a review of the literature	Howard W Roberts 1, Jeffrey M Toth, David W Berzins, David G Charlton	*Dental Materials*	2008	350
6	European Society of Endodontology position statement: Management of deep caries and the exposed pulp	H F Duncan, K M Galler, P L Tomson, S Simon, I El-Karim, R Kundzina, G Krastl, T Dammaschke, H Fransson, M Markvart, M Zehnder, L Bjørndal	*International Endodontic Journal*	2019	343
7	Designing new treatment strategies for vital pulp therapy	D Tziafas 1, A. J. Smith, H Lesot	*Journal of Dentistry*	2000	342
8	Biodentine (TM) induces TGF-β1 release from human pulp cells and early dental pulp mineralization	P Laurent, J Camps, I About	*International Endodontic Journal*	2012	339
9	Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview-Part I: vital pulp therapy	M Parirokh, M Torabinejad, P M H Dummer	*International Endodontic*	2018	325
10	The application of tissue engineering to regeneration of pulp and dentin in endodontics	Misako Nakashima, Akifumi Akamine	*Journal of Endodontics*	2005	323

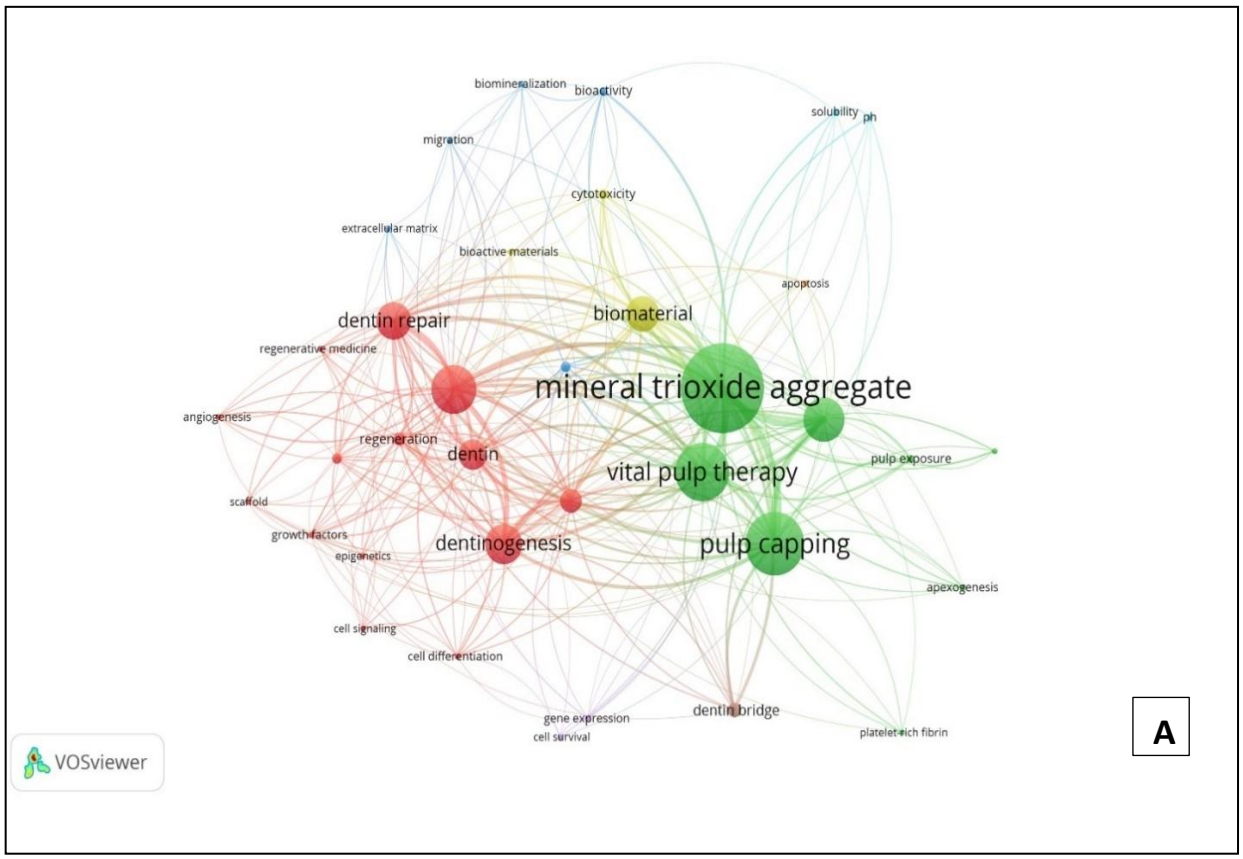
6. Analysis the intellectual structure of authors' keywords

Authors' keywords were analyzed to identify correlations between the keywords and the topics of the articles, thereby facilitating a deeper exploration of various aspects of research on the topic of VPT. The analysis was conducted using VOS Viewer, specifically employing a co-occurrence analysis of the authors' keywords with a minimum threshold of five occurrences per keyword. A total of 1,596 keywords were identified, of which 53 met the specified threshold.

The overlay visualization of the authors' keywords, organized into eight clusters with a total link strength of 2,025, highlights distinct research themes. Cluster 1 focuses on the biomolecular mechanisms underlying stem cell proliferation in the dental pulp. Cluster 2 emphasizes biomaterials, with MTA being the most frequently referenced, followed by $Ca(OH)_2$ and platelet-rich fibrin. Cluster 3 explores the cytotoxicity of the bioactive materials, whereas cluster 4 examines the interaction between the inflamed pulp tissue and the applied biomaterials. Cluster 5 addresses gene expression in dental pulp stem cells stimulated by biomaterial application, and cluster 6 investigates the physical properties of these biomaterials. Cluster 7 is involved in the apoptotic process, and cluster 8 focuses on the mechanisms of dentin bridge formation.

The overlay mapping reveals that several keywords were particularly prominent in the literature. "Mineral Trioxide Aggregate" is the most frequently used keyword, with 367 cooccurrences and a total link strength of 635. Other significant terms include vital pulp therapy (213 occurrences, total link strength of 381), dental pulp stem cells (172 occurrences, total link strength of 365), $Ca(OH)_2$ (154 occurrences, total link strength of 312), dentinogenesis (134 occurrences, total link strength of 270), biomaterial (117 occurrences, total link strength of 254), and dentin repair (125 occurrences and a total link strength of 268).

In addition to the prominent keywords, several less frequently encountered terms in the literature, including regenerative endodontics (2 cooccurrences, total link strength of 52) and gene expression (8 cooccurrences, total link strength of 17), were used. These terms indicate emerging research areas that are yet to gain widespread attention (**Figure 4**).



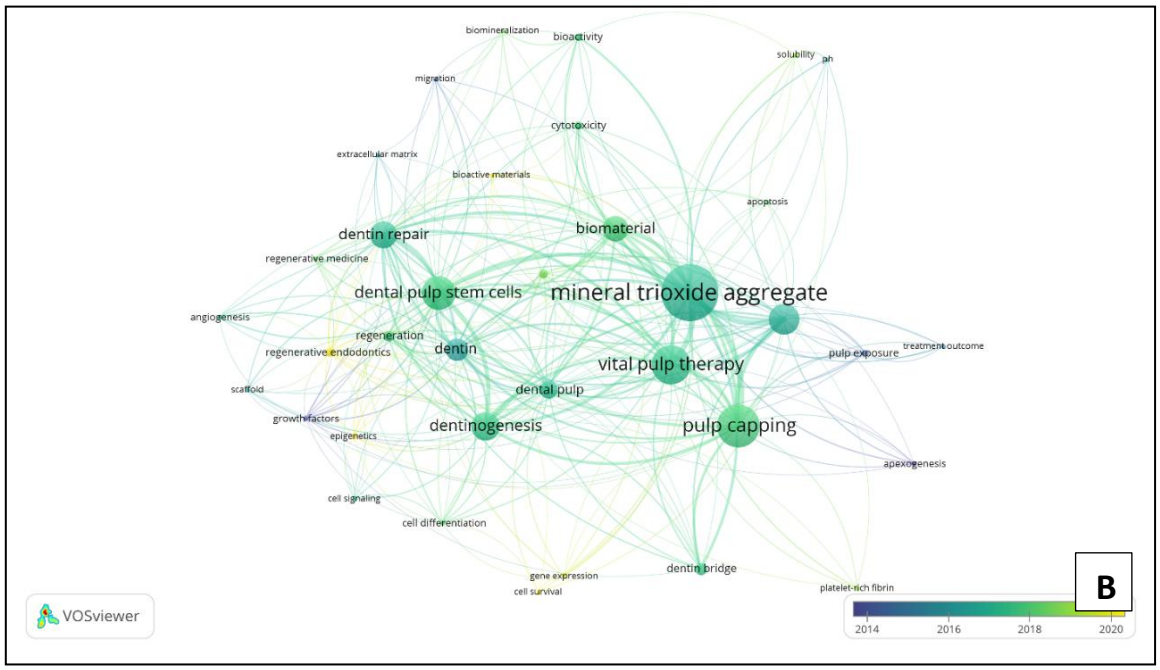


Figure 4. The overlay visualization **A.** Clustering map; **B.** Evolving research trends, indicating a shift in focus toward emerging areas such as regenerative endodontics, biomaterials, and gene expression. These trends reflect the current direction of scientific inquiry and highlight key priorities in contemporary medical research.

DISCUSSION

Scopus was chosen as the source of bibliographic data for this study because of its comprehensive coverage, superior data quality, and accuracy. It also offers advanced bibliometric analysis tools and is widely verified and academically acknowledged as a reliable resource for scholarly research.[15] Falagas et al.; evaluated the strengths and limitations of PubMed, Scopus, Web of Science, and Google Scholar. They noted that both PubMed and Google Scholar are freely available, with PubMed being suited for biomedical research, whereas Google Scholar has varied accuracy. In contrast, Scopus provides 20% more comprehensive citation analysis coverage than the Web of Science.[15,16] Sing et al.; compared the Web of Science, Scopus, and Dimensions. Nearly all journals indexed in the Web of Science are also included in Scopus and Dimensions. Scopus also contains 66.07% more unique journals than the Web of Science. Although both the Web of Science and Scopus primarily focus on the life sciences, physical sciences, and technology, Dimensions offers broader coverage in social sciences, arts, and humanities.[17] This bibliometric analysis revealed that journal age, open-access status, subject matter, quality, and impact factors critically influence the number of document citations.[15]

The bibliometric analysis, based on data retrieved from Scopus up to December 2024, identified 856 articles on VPT and related biomaterials. Publications on VPT showed a steady upward trend between 2000 and 2021. A marked increase was observed in 2010, when 28 articles collectively received 3,617 citations. The peak occurred in 2021, with 96 publications that together accrued 1,305 citations. After 2021, both the number of publications and their citation counts declined, likely reflecting shifts in treatment approaches and the introduction of diverse new medicament regimens.

The increasing number of published VPT articles may be due to the development of materials that support treatment. $Ca(OH)_2$, which was introduced by Hermann in 1930, is the main material used in VPT. Over time, MTA, another material that emerged, was able to replace the previous material.[2] However, this material is still unable to provide maximum results, so further research is needed on materials that can provide maximum results for VPT as early as possible. The limitations of biomaterials were previously the subject of various questions in this bibliometric analysis.

Pulp therapy intends to maintain the vitality of teeth damaged by caries or dental trauma, keep the tooth structure intact, and maintain optimal function. The pulp is crucial in preserving tooth vitality, as it contains various cellular components that respond to stimuli that affect the teeth.[18] Recent advancements in biotechnology and translational research have enabled the development of therapeutic strategies for vital pulp protection, with a focus on the modulation of reactionary and reparative dentinogenesis.[19] Regenerative treatment in VPT is intended to induce the differentiation of odontoblast-like cells, leading to the formation of tertiary dentin at the exposed site while preserving the integrity of the tissue structure. The success of this treatment is primarily determined by how the dental pulp cells respond to the materials in direct contact with the pulp. Several pulp-dressing agents have been used to promote the health of the radicular pulp.[20]

An analysis of global trends in VPT indicates that the United States holds the leading position in article production. The United States has published the highest number of articles, totaling 110, followed closely by China with 105 articles, Japan with 104, India with 79, and Brazil with 66. The United States has the highest number of articles published across countries and the highest citation count. However, an analysis of the most-cited countries revealed that a higher article count does not necessarily correlate with a greater citation impact. For instance, France ranks fifth among the top 10 countries with the highest number of citations, having garnered 3,117 citations from its 34 articles.

The Journal of Endodontics ranks as the most-cited journal, followed by the International Endodontic Journal, which has received 146 publication and 9,967 citations, respectively. The Journal of Endodontics stands out the most, the official publication of the American Association of Endodontists, publishes scientific articles, case reports, and comparative studies that assess materials and techniques related to pulp conservation and endodontic treatment. This journal has been in publication since 1975, with an h-index of 180 and a Q1 ranking.

The International Endodontic Journal is published monthly and aims to feature original, high-quality articles that contribute to the dissemination of scientific and clinical knowledge. Original scientific articles related to endodontic diseases and their management and the restoration of root-treated teeth have been published in the fields of biomedical science, applied materials science, bioengineering, epidemiology, and social science. The International Endodontic Journal was ranked Q1, with an h-index of 147.

The Dental Materials Journal ranked third, with 382 total citations from 27 articles. This journal is a publication of the Japanese Society for Dental Materials and Devices that aims to introduce the progress of basic and applied sciences in dental materials and biomaterials. The journal also encompasses clinical science related to dental materials and instrumental technologies. It covers materials such as synthetic polymers, ceramics, metals, and tissue-derived biomaterials. In addition, cutting-edge dental materials and biomaterials used in emerging fields such as tissue engineering, bioengineering, and artificial intelligence are favorably considered in this review. The Dental Materials Journal has been in existence since 1982, with the journal ranking Q2 and an h-index of 70.

Biomaterials ranks fourth, which has 1,452 citations, a Q1 ranking, and an h-index of 435. This international journal covers the science and clinical application of biomaterials. It covers a wide range of physical, biological, and chemical sciences that underpin the design of biomaterials and clinical disciplines in which they are used. Clinical applications include medical technology and regenerative medicine in all clinical disciplines and diagnostic systems that rely on innovative contrast and sensing agents.

The article titled, "Mineral Trioxide Aggregate: A Comprehensive Literature Review Part III: Clinical Applications, Drawbacks, and Mechanism of Action", holds the highest citation count, with 951 citations, securing the top position. It was authored by Masoud Parirokh and Mahmoud Torabinejad in 2010 from the Neuroscience Research Center at Kerman University of Medical Sciences, Iran. This publication highlights their significant contribution to VPT. The contents of this article are the results of MTA as a promising material for root-end filling, perforation repair, VPT, and apical barrier formation for teeth with necrotic pulps and open apices. Despite numerous case reports and case series concerning its applications, few studies have investigated the clinical applications of this material. MTA has several known drawbacks, such as a long setting

time, high cost, and potential for discoloration. Hydroxyapatite crystals form over MTA when they come in contact with the synthetic fluid. This can act as a nidus for the formation of calcified structures after using this material in endodontic treatments.[2,21,22]

The article titled, "Mineral trioxide aggregate: a comprehensive literature review--part II: leakage and biocompatibility investigations", was the second most-cited article. It was written by Mahmoud Torabinejad and Masoud Parirokh, with 651 citations. This study focused on the sealing ability and biocompatibility of MTA.[23] According to these two articles, MTA is the most commonly used material in VPT, offering advantages and limitations.[24,25] These limitations have driven ongoing research into identifying the optimal dental biomaterial for VPT and enhancing the regenerative processes of dental pulp stem cells to maintain pulp vitality for as long as possible.

The analysis of publications from 2000 to 2024 reveals a notable pattern of collaboration among authors. The authors in cluster 4 demonstrate strong connections with those in clusters 2, 3, and 1. Furthermore, the authors in clusters 2 and 1 have contributed to the most recent publications, reflecting the ongoing research activity in these groups. In addition to author collaboration, international collaboration patterns are also evident. The United States is leading in publication output and citation count and collaborates extensively with Brazil and Indonesia. Similarly, China has demonstrated strong research partnerships with Japan, Turkey, Belgium, and Taiwan.

The relationships between the keywords of existing articles are organized into clusters that represent the progression of VPT research. Cluster 1 focuses on the biomolecular mechanisms underlying stem cell proliferation in the dental pulp. Cluster 2 highlights commonly used biomaterials, including MTA, $Ca(OH)_2$, and platelet-rich fibrin. Notably, although $Ca(OH)_2$ has long been considered the gold standard in VPT, it has gradually been replaced by MTA. Cluster 3 addresses the cytotoxicity of bioactive materials, whereas cluster 4 explores the correlation between biomaterials and pulp inflammation. Cluster 5 investigates gene expression in dental pulp stem cells stimulated with biomaterials. Cluster 6 focuses on the physical properties of the biomaterials, cluster 7 examines the apoptosis process, and cluster 8 discusses dentin bridge formation, underscoring the effectiveness of these biomaterials in VPT. Across all the clusters, research areas such as regenerative endodontics, gene expression, and biomaterials remain underrepresented, with relatively few articles published in these fields.

This bibliometric analysis offers authors a quantitative and descriptive overview of country citations, journals, articles, authors, and authors' keywords related to VPT and the biomaterials used. Despite its utility, bibliometric analysis has several limitations. The key limitation is the necessity of open access to scientometric data, as obtaining data with sufficient accuracy is crucial. Accurate analysis requires crucial information, such as metadata, author data, affiliations, and citations. Another limitation involves the potential incompleteness of downloaded data, the presence of duplicates, and instances where articles are published in languages other than English, typically the language of the country of origin of the article. The complexity and diversity of the bibliographic data are the primary challenges in bibliometric analysis, which require authors to carefully consider various dimensions of data. In addition, the number of citations is directly related to the age of the publication, which indicates that older articles tend to accumulate more citations than those published more recently.

CONCLUSION

This bibliometric analysis reveals a clear paradigm shift in vital pulp therapy (VPT) research from calcium hydroxide toward mineral trioxide aggregate (MTA) and calcium silicate–based biomaterials between 2000 and 2024. MTA's dominance as both a clinical material and research keyword underscores its central role in regenerative approaches, particularly in dentinogenesis and pulp vitality preservation. The steady growth of publications confirms that VPT remains a dynamic and expanding research field, strongly shaped by advances in bioactive and bioceramic materials.

However, several methodological limitations must be acknowledged, including language and citation bias, restricted database coverage, and the inability of bibliometric mapping to assess qualitative outcomes such as clinical translation.

Future research should therefore broaden database inclusion, integrate non-English literature, and complement bibliometric methods with altmetric and qualitative analyses. Scientifically, the field would benefit from comparative studies of novel bioceramics, bioengineered scaffolds, and regenerative strategies involving stem cells and gene-editing technologies. These directions may facilitate the translation of laboratory discoveries into predictable, biologically based VPT protocols that improve long-term clinical outcomes.

CONFLICT OF INTEREST

There is no conflict of interest between the authors in this manuscript.

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JURNAL ILMIAH MEDICAMENTO

Vol.11 No.2 (September 2025)
page 185-192
https://doi.org/10.36733/medicamento.v11i2.11538
e-ISSN: 2356-4814

Original Article

In-use Stability of Hydrocortisone Injection Preparations at a Regional Referral Hospital in Central Java

In-Use Stability Sediaan Injeksi Hidrokortison di Rumah Sakit Rujukan Daerah Jawa Tengah

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Submitted : 01-05-2025
Revised : 19-08-2025
Accepted : 17-09-2025

Keywords: hospital, hydrocortisone, injection, in-use stability

Kata Kunci: hidrokortison, injeksi, *in-use stability,* rumah sakit

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Abstract

Hydrocortisone Sodium Succinate 50mg injection is widely used in perinatology wards. These wards cater to newborns (0-28 days), low birth weight (<2.5 kg), or premature babies (<37 weeks), requiring special handling. The use of this injection requires a very small dose, so one vial of hydrocortisone injection can be used for more than one patient. Therefore, the remainder of this hydrocortisone injection is often stored for 24 hours in the refrigerator which will later be reused on other patients. This study aims to determine the in-use stability of hydrocortisone injection stored for 24 hours at 4°C and 25°C. This is certainly to improve patient safety. The data collection technique uses an observational method on hydrocortisone injection samples with 3 replications. In-use stability is assessed from the results of organoleptic tests, pH tests, viscosity tests, determination of drug levels using UV-VIS spectrophotometry and sterility tests. Testing was conducted on days 0, 1, 2, 3, 7, 14 and 30. The results of the study showed that hydrocortisone injection preparations stored for 1 day at 4°C or 25°C in the perinatology ward were no longer physicochemically stable (concentration). Chemical degradation began on day 1, then microbial contamination occurred immediately after completion of compounding in the ward (day 0). This is because reconstitution was not carried out in a clean room in accordance with USP <797>. It can be concluded that hydrocortisone injection preparations prepared in this hospital are not recommended for administration to patients after 1 day of storage and must be compounded in a clean room.

Abstrak

Injeksi Hidrokortison Sodium Succinate 50mg banyak digunakan pada bangsal perinatologi. Bangsal ini merupakan bangsal dengan kondisi pasien bayi yang baru lahir (0-28 hari), berat badan lahir rendah (<2,5 kg) atau bayi premature (<37 minggu) sehingga membutuhkan penanganan khusus. Penggunaan injeksi ini membutuhkan dosis yang sangat kecil, sehingga satu vial injeksi hidrokortison dapat digunakan untuk lebih dari satu pasien. Oleh karena itu, sisa dari injeksi hidrokortison ini seringkali dilakukan penyimpanan selama 24 jam di refrigerator yang nantinya akan digunakan kembali pada pasien yang lain. Penelitian ini bertujuan untuk mengetahui *in-use stability* injeksi hidrokortison yang disimpan selama 24 jam pada suhu 4°C dan 25°C. Hal ini tentunya untuk meningkatkan keselamatan pasien. Teknik pengumpulan data menggunakan metode observasional terhadap sampel injeksi hidrokortison dengan replikasi 3 kali. I*n-use stability* dinilai dari hasil uji organoleptis (warna dan bau), uji pH, uji viskositas, penetapan kadar obat menggunakan spektrofotometri UV-VIS dan uji sterilitas. Pengujian dilakukan pada hari ke 0, 1, 2, 3, 7, 14 dan ke 30. Hasil penelitian menunjukkan bahwa sediaan injeksi hidrokortison yang disimpan selama 1 hari pada suhu 4°C atau 25°C di bangsal perinatologi sudah tidak stabil secara fisikokimia (viskositas, kadar). Degradasi kimia mulai terjadi pada hari ke-1, kemudian kontaminasi mikroba langsung terjadi setelah selesai peracikan di bangsal (hari ke-0). Hal ini karena rekonstitusi tidak dilakukan di ruang bersih sesuai dengan USP <797>. Dapat disimpulkan bahwa sediaan injeksi hidrokortison yang disiapkan di rumah sakit ini tidak direkomendasikan untuk diberikan kepada pasien setelah 1 hari penyimpanan dan harus diracik pada ruang bersih.

How to cite: (citation style AMA 11th Ed.)
Genatrika E, Sundhani E, Adriana EF. In-use Stability of Hydrocortisone Injection Preparations at a Regional Referral Hospital in Central Java. *J. Ilm. Medicam.*, 2025:11(2), 185-192, DOI: 10.36733/medicamento.v11i2.11538

INTRODUCTION

Hospital patients require diverse injection preparations, often necessitating compounding, so that a process of preparing or mixing injection preparations with each other is still required to meet patient needs.[1] The process of mixing injection preparations requires special attention, especially in several important aspects such as personnel, correct aseptic procedures and facilities so that the mixed preparations do not experience microbial contamination.[2,3] Microbially contaminated preparations can have a very fatal impact on patients, especially neonates.[4] Neonates are a population at high risk of infections (HCAIs). This is because their immune systems are immature, and they often come into contact with healthcare workers in the hospital environment. HCAIs that commonly occur include central-line-associated bloodstream infections, healthcare-associated pneumonia, soft tissue infections, and candidaemia. HCAIs can be minimized by improving hand hygiene.[5,6]

Dewi (2018) reported bacterial contamination in 2.3% of 43 tested IV admixtures. The frequency of contamination prepared in the ward was higher compared to preparations prepared in the pharmacy environment.[2] To avoid contamination, the mixing process for injection preparations should refer to existing guidelines such as the United States Pharmacopeia (USP) chapter 797.[7] Meanwhile, Indonesia also has basic guidelines that discuss and regulate the dispensing of sterile preparations which include all aspects starting from human resources, rooms and equipment, aseptic techniques, storage, distribution and documentation.[8,9] Furthermore, these aspects have been proven in research to have an influence on the quality of the preparation. This research has been stated in the basic guideline for the preparation of non-cytostatic sterile products.[9]

In a study conducted by Rambe (2023), the procedures for mixing sterile preparations did not comply with the guidelines for mixing injectable drugs. Incompatibility can occur if the process of mixing sterile preparations does not comply with aseptic techniques.[10] Then, if incompatibility occurs, it can affect the safety, medication, stability, dose changes and efficacy of the drug.[10] Then, research conducted by Genatrika (2022) stated that one of the hospitals in Purwokerto had not yet met the United States Pharmacopedia (USP) <797> guidelines and basic guidelines related to dispensing in preparing sterile preparations.[11] This research is also supported by the results of research conducted by Putri and Yuliani (2018), namely that the preparation of injection preparations in one of the hospitals in Semarang is still not in accordance with the Basic Dispensing Guidelines for Sterile Preparations and Guidelines for Mixing Injectable Drugs and Cytostatics.[12]

This Referral Hospital is a type B hospital located in Banyumas Regency, which has been accredited PARIPURNA in 2019 by the Hospital Accreditation Commission. This Referral Hospital provides friendly and good service, besides that this hospital has enough general practitioners and specialist doctors so that it is the choice of the Banyumas community. Many inpatients at this hospital receive sterile preparations that require reconstitution or mixing of drugs. One of the preparations is hydrocortisone injection. From data obtained from Banyumas Regional Hospital, the use in the last 3 months for hydrocortisone injection was 291 vials.

Hydrocortisone injections are administered in a perinatology ward that specializes in the care of newborns with conditions requiring intensive or specialized treatment. Therefore, hydrocortisone injections used in the perinatology ward are always in small doses so that the use of one vial of hydrocortisone injection can be used for more than one patient. This is what causes hydrocortisone injections to be stored if there is any leftover. The remaining reconstituted hydrocortisone injection is stored for 24 hours in the refrigerator which will then be reused on other patients if the storage is still under 24 hours. Every drug that has been opened from its primary packaging has a certain shelf life called the beyond use date (BUD). Although this BUD has been regulated by USP <797>, the BUD in this regulation is not specific to certain active substances and/or brands.[7]

Leanpolchareanchai et al. (2022) stated that hydrocortisone injection in NaCl 0,9% has stability in use for up to 48 hours when stored at room temperature or in the refrigerator. That study was conducted in Thailand, where the hydrocortisone injection preparation and solvent used were different from those used in this study.[13] Furthermore, a solution of dezocine 0.4 mg/ml mixed with 0.05 mg/ml tropisetron hydrochloride

in 0.9% NaCl has stability in use for 14 days when stored in a polyolefin bag or glass bottle.[14] Based on the descriptions above, this study aims to evaluate the in-use stability of hydrocortisone injection preparations which are often stored in the refrigerator with physical, chemical and microbiological quality parameters.

RESEARCH METHODS

Study Design

This research design is a non-experimental study to determine the in-use stability of hydrocortisone injection used in the Perinatology ward. This study has obtained ethical permission from the health ethics committee with number 286/KEPK-RSUDBMS/I/2024. In-Use Stability is determined based on the results of physical, chemical and microbiological stability tests. The physical stability tests carried out were organoleptic and viscosity tests, chemical stability tests were seen from the pH test and determination of drug levels. Furthermore, microbiological stability was seen from the sterility test of the preparation.

Sampling

The sample used was hydrocortisone injection prepared in the Perinatology Ward. Preparation was performed in the nurses' room using Laminar Air Flow (LAF). This room does not meet USP <797> standards. The reconstituted product will be sent to the microbiology laboratory for sterility testing.

Organoleptic Test

The reconstituted hydrocortisone injection preparation was stored in two different places, namely a room with a temperature of 25 ℃ and a temperature of 4 ℃. Then the change in the color of the hydrocortisone injection preparation will be seen for 30 days, replication 3 times. Furthermore, the changes will be seen on day 0, day 1, day 2, day 3, day 7, day 14 and day 30.[7]

Viscosity Test

The reconstituted hydrocortisone injection preparation will be stored in two different places, a room with a temperature of 25 ℃ and a room with a temperature of 4 ℃. Furthermore, the viscosity value was measured on days 0, 1, 2, 3, 7, 14 and 30 using a calibrated Brookfield DV2T Viscometer.[7(p20)] In this test, replication was carried out 3 times.

pH Test

The reconstituted hydrocortisone injection preparation will be stored in two different places, namely a room with a temperature of 25 ℃ and a temperature of 4 ℃. Furthermore, the pH value will be measured on day 0, day 1, day 2, day 3, day 7, day 14 and day 30 using a calibrated pH meter (OHAUS).[7(p20)] In this test, replication was carried out 3 times.

Hydrocortisone Level Determination

Determination of the levels of hydrocortisone injection samples using UV VIS Spectrophotometry. The sample with a concentration of 10 µg/mL were taken 10 µl, then put into a 10 ml measuring flask and Water for Injection (WFI) was added to the mark on the measuring flask. Next, 1 ml was taken and put into a 5 ml measuring flask then WFI was added to the mark on the measuring flask.[15] The sample that has been prepared is then read for absorbance at a wavelength of 248 nm and the concentration is calculated using the equation y = 0.0366x-0.024. This concentration determination will be carried out for 30 days, namely on day 0, day 1, day 2, day 3, day 7, day 14 and day 30.[7]

Sterility Test

Sterility testing was performed using Fluid Thioglycolate Media (FTM) and Soybean Casein Digest (SCD) media. Hydrocortisone injection samples prepared by staff at the perinatology ward of this referral hospital were directly inoculated into both FTM media and SCD media and incubated for 14 days, with three replicates, for FTM media at 30-35°C and SCD media at 20-25°C. If bacterial growth is observed within 14 days, the hydrocortisone injection can be said to be non-sterile.[16]

Data Analysis

The data obtained were analyzed descriptively and compared with the quality specifications for each quality parameter of Hydrocortisone injection.[16] Then, the chemical quality parameters (content) were analyzed using a two-way ANOVA test with the help of the IBM SPSS Statistics application.

RESULT AND DISCUSSIONS

Hydrocortisone Na Succinate injection samples experienced a color change on the 30th day stored at a temperature of 25°C, as seen in **Table 1**.

Table 1. Organoleptic Test Results

Day to	Organoleptic Observation	
	Temperature 4°C	Temperature 25°C
0	Clear, colorless	Clear, colorless
1	Clear, colorless	Clear, colorless
2	Clear, colorless	Clear, colorless
3	Clear, colorless	Clear, colorless
7	Clear, colorless	Clear, colorless
14	Clear, colorless	Clear, colorless
30	Cloudy, there are white particles	Cloudy, there are white particles

Hydrocortisone injections were known on day 0 to day 14 did not experience any color change and was not cloudy, both stored at 4°C and at 25°C. Then, on the 30th day the sample stored at 4°C was still clear, while the sample stored at 25°C had changed color, was cloudy and contained white particles. These results are in line with research conducted by Sagitha, et al (2023) which showed that the stability test of ampicillin sulbactam preparations after reconstitution was more stable when stored in the refrigerator.[17] Furthermore, for the viscosity test on the sample increased every day, the data can be seen in **Table 2**.

Table 2. Viscosity Test Results

Day to	Viscosity Value	
	Temperature 4°C	Temperature 25°C
0	62,08 cP	73,16 cP
1	60,88 cP	73,98 cP
2	63,56 cP	84,06 cP
3	65,88 cP	86,52 cP
7	78,56 cP	96,56 cP
14	86,64 cP	103,73 cP
30	96,56 cP	113,57 cP

The results of the viscosity test on day 0 to day 30 stored at a temperature of 4°C were in the range of 62-96 cP and stored at a temperature of 25°C were in the range of 73.16-113.567 cP. Berteau, et al (2015) stated that the viscosity that can be tolerated for subcutaneous administration is in the range of 15-20 cP.[18] Another study conducted by Ko Eunji, et al (2022) also stated that the viscosity of intravenous fluids, such as 0.9% physiological saline solution and Hartmann's solution usually ranges from 1.07-1.12 cP, and thick solutions, such as 6% hetastarch and 5% albumin have a viscosity of 2.77-1.86 cP respectively. Therefore, the viscosity value of this hydrocortisone injection is still too high when given intravenously or subcutaneously.[19] This is because the strength of the preparation affects the viscosity value of the solution, the higher the strength of the preparation, the higher the viscosity.[18–20] The viscosity values stored for 30 days at temperatures of 4°C and 25°C did not differ significantly ($p>0.05$), so it can be concluded that temperature does not affect the viscosity value. The solution for administering this preparation is to dilute it before giving it to the patient so that it is not too thick.

The chemical stability study in this study was seen from the pH parameters and drug levels. The pH value obtained was still within the range recommended by the Indonesian Pharmacopoeia, which is around 5-7,[16] as seen in **Table 3**.

Table 3. pH Test Results

Day to	pH value	
	Temperature 4°C	Temperature 25°C
0	7,34	7,31
1	7,41	7,33
2	7,32	7,26
3	7,29	7,26
7	7,25	7,15
14	7,25	6,96
30	7,32	6,87

The pH value produced after being stored for 30 days at a temperature of 4°C and at a temperature of 25°C is known to be in the range of 6 to 7, where according to the Indonesian Pharmacopoeia edition VI, the pH value required for hydrocortisone injection is between 5 and 7.[16] The pH value in this study still meets the pH range for hydrocortisone injection. The appropriate pH will help the safety of using injection preparations, such as if the pH is too low (below 3) it can cause pain when injected, while the pH is too high (more than 9) can cause tissue necrosis.[21] The pH value of injection preparations stored at 4°C and 25°C did not differ significantly (p> 0.05), so it can be concluded that temperature does not affect the pH value.

Then, this injection sample experienced a change in content and did not match the range specified by the Indonesian Pharmacopoeia (90-110%) after storage for 24 hours at both 4°C and 25°C.[16] The percentage of levels increased more than 110% after 24 hours of storage, this indicates that this injection is unstable after being stored for 24 hours at both temperatures. The results of this study are in line with research conducted by Leanpolchareancha et al (2022) which stated that hydrocortisone injection reconstituted using Water for Injection was stable for up to 24 hours at refrigerator temperatures (4±2°C), ICU temperatures (25±3°C), and room temperatures (30±2°C).[22] The data of hydrocortisone levels can be seen in **Table 4**.

Table 4. Results of Level Determination Test

Day to	Hydrocortisone Level (%)	
	Temperature 4°C	Temperature 25°C
0	100,728	98,816
1	155,373	132,695
2	138,888	114,663
3	146,357	150,364
7	145,719	150,455
14	148,360	151,548
30	134,244	119,854

Hydrocortisone injection stored for 24 hours was also determined for its active substance content. The results on day 0 showed that the content of hydrocortisone injection that had been reconstituted with WFI and would be stored at a temperature of 4°C was 100.728%, while that which would be stored at a temperature of 25°C was 98.816%. According to the Indonesian Pharmacopoeia edition VI, the content of the active substance hydrocortisone in injection is not less than 90.0% and not more than 110.0%.[16] The injection that will be stored at different temperatures can be said to be chemically stable on day 0. Then, after 24 hours to 30 days, it shows the level of active substances that have exceeded the levels required by the Indonesian Pharmacopoeia Edition VI (> 110%). This shows that the hydrocortisone injection that has been reconstituted with WFI is no longer stable after being stored for 24 hours. Then, the levels of hydrocortisone at each storage temperature were analyzed and a p value of 0.805 was obtained. It can be concluded that the levels of hydrocortisone stored at temperatures of 4°C and 25°C are not significantly different (p> 0.05).

The results of this study are consistent with those of Leanpolchareancha et al. (2022), which stated that hydrocortisone injection reconstituted using Water for Injection and 0.9% Sodium Chloride (1 mg/mL) was not affected by temperature.[22] The injection remained stable for 24-48 hours at refrigerator temperature (4±2°C), ICU temperature (25±3°C), and room temperature (30±2°C). However, this is inconsistent with other studies,

such as those conducted by Yusefa (2024), which stated that temperature can affect the levels of active substances with a difference in levels of 10.95%.[15,23]

Temperature changes are one of the external factors that cause instability in pharmaceutical preparations. Storing drugs in very hot conditions, high room humidity, and exposure to light can damage drug quality.[24] In this study, humidity is the factor suspected of influencing hydrocortisone stability. Humidity is closely related to water; the presence of water can cause hydrocortisone to degrade or undergo hydrolysis, particularly to 21-aldehyde.

Microbiological stability studies can be determined based on the presence or absence of bacterial growth. Samples inoculated into both test media showed bacterial growth, the results can be seen in table 5.

Table 5. Sterility Test

Day to	Bacterial Growth	
	Temperature 4°C	Temperature 25°C
0	+	+
1	+	+
2	+	+
3	+	+
7	+	+
14	+	+
30	+	+

Microbiological stability parameters also need to be tested to ensure that the injection preparations given to patients are not contaminated by microbes. To prove this, a sterility test was carried out on the hydrocortisone injection that had been reconstituted in the perinatology ward. The results showed that there had been microbial contamination of the injection. This is because the room used to prepare parenteral preparations in this referral hospital does not meet ISO standards and those required by USP <797).[7] If the microbially contaminated preparation is still given to the patient, it can endanger the patient, such as bloodstream infections, surgical site infections, ventilator-associated pneumonia, and urinary tract infections. Bloodstream infection and ventilator pneumonia can cause death.[25–27]

The limitation of this research lies in the lack of support from the government regarding this topic, so that researchers could only collect data from the perinatology ward because there were parties who did not agree with this at this referral hospital.

CONCLUSION

Hydrocortisone injection can only be stored for up to 24 hours at 4 °C and 25 °C based on physicochemical quality parameters, including organoleptic properties, viscosity, pH, and hydrocortisone content. However, this injection is not recommended for use in this hospital according to microbiological quality parameters (sterility), as contamination occurred on day 0. Hospitals must have a clean room that complies with USP <797> standards for the preparation of parenteral products to prevent microbial contamination. If microbial contamination does not occur, this hydrocortisone injection may be administered repeatedly within the 24-hour storage period. Implementing proper sterile compounding standards could significantly improve medication safety and resource efficiency in hospital settings.

ACKNOWLEDGEMENTS

The author would like to thank the hospital for its support in facilitating this research, and also extends gratitude to the Directorate of Research, Technology, and Community Service for the financial support provided through the Research Grant.

CONFLICT OF INTEREST

The author declared no conflict of interest.

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JURNAL ILMIAH MEDICAMENTO

Vol.11 No.2 (September 2025)
halaman 193-206
https://doi.org/10.36733/medicamento.v11i2.11465
e-ISSN: 2356-4814

Original Article

Skincare Antijerawat: Formulasi Masker Gel *Peel-Off* Etil P-Metoksisinamat dari *Kaempferia galanga* L dengan Polivinill Alkohol sebagai *Gelling Agent*

Acne Skincare: Formulation of Peel-Off Gel Mask with Ethyl p-Methoxycinnamate from *Kaempferia galanga* L. using Polyvinyl Alcohol as a Gelling Agent

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Diajukan : 20-04-2025
Direvisi : 31-08-2025
Disetujui : 16-09-2025

Kata Kunci: etil P-Metoksisinamat, formulasi, *Kaempferia galanga* L, masker gel *peel-off* antijerawat

Keywords: anti-acne peel-off gel mask, ethyl P-Methoxycinnamate, formulation, *Kaempferia galanga* L.

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Abstrak

Tanaman obat semakin banyak digunakan sebagai terapi antibakteri karena mudah diakses dan terjangkau. Rimpang *Kaempferia galanga* L. (kencur) mengandung etil p-metoksisinamat (EPMS), senyawa aktif dengan aktivitas antijerawat. EPMS pada konsentrasi 1,2% dalam sediaan krim terbukti memiliki aktivitas antibakteri terhadap *Propionibacterium acnes*, *Staphylococcus epidermidis*, dan *Staphylococcus aureus*. Namun, krim kurang sesuai untuk kulit berminyak karena berpotensi memperburuk jerawat. Gel *peel-off* menjadi alternatif yang lebih tepat karena tidak berminyak, mendukung eksfoliasi, merangsang regenerasi sel, memperkecil pori, serta membersihkan dan melembapkan kulit. Penelitian ini bertujuan memformulasi gel *peel-off* antijerawat yang mengandung EPMS dengan stabilitas fisik baik, menggunakan basis polivinil alkohol (PVA) 10% dan 12%. EPMS diisolasi dari rimpang kencur dan dicampurkan ke dalam basis gel. Evaluasi meliputi organoleptik, viskositas, homogenitas, pH, waktu mengering, daya sebar, serta uji stabilitas dipercepat pada suhu 27 ± 2 °C dan 40 ± 2 °C selama 21 hari. Hasil menunjukkan EPMS dapat diformulasikan dalam gel *peel-off* dengan PVA 10% dan 12%. Gel memiliki warna putih kekuningan, bau madu, homogen, pH 5,21–5,35, waktu mengering 17–30 menit, daya sebar 55–68 mm, dan viskositas 4.900–16.000 cPs. Semua formula menunjukkan stabilitas fisik sesuai standar gel *peel-off*. Penelitian ini memberikan dasar ilmiah untuk pengembangan produk kosmetik berbahan alami yang aman khususnya bagi kulit berminyak dan tidak memperburuk kondisi jerawat.

Abstract

Herbal medicines are increasingly used as antibacterial therapy due to their accessibility and affordability. *Kaempferia galanga* L. (kencur) rhizome contains ethyl p-methoxycinnamate (EPMS), an active compound with anti-acne properties. EPMS at 1.2% in cream formulations has shown antibacterial activity against *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. However, creams are unsuitable for oily skin as they may worsen acne. Peel-off gels offer a better alternative because it is non-greasy, supports exfoliation, stimulates cell regeneration, minimizes pores, and helps cleanse and moisturize the skin. This study aimed to formulate a physically stable anti-acne peel-off gel containing EPMS using polyvinyl alcohol (PVA) at 10% and 12%. EPMS was isolated from kencur rhizome and incorporated into gel bases. Evaluations included organoleptic properties, viscosity, homogeneity, pH, drying time, spreadability, and accelerated stability at 27 ± 2 °C and 40 ± 2 °C for 21 days. Results showed that EPMS can be successfully formulated into peel-off gels with PVA 10% and 12%. The gels exhibited yellowish-white color, honey-like odor, homogeneity, pH 5.21–5.35, drying time 17–30 minutes, spreadability 55–68 mm, and viscosity 4,900–16,000 cPs. All formulations maintained physical stability under test conditions. This study provides a scientific basis for the development of natural-based cosmetic products that are safe, particularly for oily skin, and do not worsen acne conditions.

Cara mensitasi artikel (*citation style*: AMA 11thEd.):

Kusuma IM, Indrawati T, Zulfah Z. *Skincare* Antijerawat: Formulasi Masker Gel *Peel-Off* Etil P-Metoksisinamat dari *Kaempferia galanga* L dengan Polivinill Alkohol sebagai *Gelling Agent*. *J. Ilm. Medicam.*, 2025:11(2), 193-206, DOI: 10.36733/medicamento.v11i2.11465

PENDAHULUAN

Pengobatan tradisional termasuk pengobatan berbasis tanaman lebih diminati karena lebih mudah diakses dan terjangkau daripada layanan kesehatan modern, terutama di daerah pedesaan dan daerah yang kurang terlayani. Hal ini menjadikannya pilihan praktis bagi banyak orang yang mungkin tidak memiliki akses mudah ke fasilitas medis modern.[1] Salah satu pengobatan tradisional yaitu dengan menfaatkan tanaman obat. *K. galanga* L secara tradisional telah dimanfaatkan sebagai ekspektoran, stimulan, diuretik, karminatif, antipiretik. Selain itu, *K. galanga* digunakan untuk mengobati diabetes, hipertensi, batuk, asma, patah tulang sendi, rematik, urtikaria, vertigo, dan cedera usus.[2]

Beberapa penelitian telah dilaporkan ekstrak *K. galanga* memiliki kandungan senyawa kimia dari pelarut non-polar dan polar yang bermanfaat sebagai antibakteri. Senyawa Etil P-Metoksisinamat (EPMS) dan etil-sinamat adalah senyawa utama yang dihasilkan pada ektrak dengan pelarut n-heksana, metanol dan diklorometan.[2] EPMS adalah senyawa turunan dari sinamat yang dapat berfungsi sebagai antibakteri. EPMS yang berasal dari ekstrak n-heksan rimpang kencur dengan kadar 1,2% dalam sediaan krim memiliki daya anti bakteri pada *P. acnes, S. aureus dan S. epidermidis* yang menyebabkan terjadinya jerawat.[3] Kristal EPMS dapat diformulasikan menjadi sediaan mikroemulgel antiinflamasi.[4] Sediaan kosmetik lainya yang dibuat dari zat aktif EPMS adalah tabir surya, yang dirancang untuk melindungi kulit terhadap radiasi UV yang dapat merusak kulit.[5]

Sediaan antibiotik dalam pengobatan jerawat topikal umumnya dalam bentuk hidrogel yang bermanfaat karena sifat reologinya yang baik, dalam pengaplikasianya dan penghantaran obat. Sediaan antijerawat dalam bentuk krim juga sering diresepkan. Krim merupakan sediaan semipadat yang banyak digunakan dalam industri farmasi, khususnya pada produk kosmetik dan obat topikal. Pemilihan bentuk krim pada sediaan kosmetik memiliki beberapa keunggulan antara lain kemampuan menyebar secara merata pada permukaan kulit, sehingga dapat mencegah peradangan serta memberikan efek protektif. Selain itu, krim juga dipilih karena sifatnya yang praktis, mudah dibilas, fleksibel dalam penggunaanya dan memberikan rasa yang nyaman.[6] Namun, sediaan krim tidak direkomendasikan untuk kulit berminyak yang rentan berjerawat karena dapat memperparah kondisi jerawat. Untuk mengatasi permasalahan diatas diperlukan alternatif sediaan antijerawat yang bersifat non-komedogenik, seperti gel. Sediaan gel *peel off* merupakan gel yang memiliki keunggulan dimana penggunaannya mudah untuk merawat kulit wajah, membantu mengurangi munculnya bekas jerawat dengan mendorong pengelupasan epidermis dan merangsang produksi sel kulit baru, mengecilkan pori-pori, membersihkan, mengobati kulit yang berjerawat serta melembabkan kulit.[7]

Formula gel *peel-off* terdiri dari bahan *gelling agent* dan zat aktif. Bahan pembentuk gel yang sering digunakan sebagai dasar *peel-off* meliputi Polivinil Alkohol (PVA), natrium alginat, dan karbomer. Agen ini dapat membantu membentuk lapisan film yang stabil sehingga mudah dikelupas.[8] PVA dapat dikombinasikan dengan polimer lain seperti Hidroksi Propil Metil Selulosa (HPMC) untuk meningkatkan stabilitas dan kenyamanan gel saat diaplikasikan. Misalnya, menggabungkan PVA dengan HPMC dapat meningkatkan stabilitas fisik dan mengurangi potensi iritasi pada masker.[9] PVA umumnya digunakan dalam masker *peel-off* karena sifatnya dapat membentuk lapisan tipis, biokompatibilitas, dan biodegradabilitas. Konsentrasi PVA dalam formulasi masker *peel-off* biasanya berkisar antara 8% hingga 14%, dengan variasi tergantung pada sifat masker yang diinginkan.[10] Hasil penelitian pada ekstrak buah alpukat yang diformulasi dalam sediaan gel *peel off* dengan konsentrasi formula basis PVA sebagai *film agent* 10%, 12%, dan 14% telah dilaporkan stabil secara farmaseutik.[11] Saat ini belum ada laporan mengenai formulasi maupun pengujian gel *peel-off* antijerawat yang mengandung EPMS dari *K. galanga*. Penambahan EPMS 1,2% sebagai zat aktif dengan basis PVA 10% dan 12%, serta dievaluasi melalui uji stabilitas dipercepat pada suhu 27°±2°C dan 40°±2°C. Gel *peel off* antijerawat yang dihasilkan diharapkan tidak hanya memberikan efek antibakteri terhadap jerawat, tetapi juga dapat mengendalikan laju pelepasan dan penetrasi senyawa ke dalam kulit. Penelitian sebelumnya melaporkan bahwa Oktil Metoksisinamat (OMC), yang memiliki kesamaan struktur dengan EPMS sebagai turunan p metoksisinamat, mampu mengendalikan laju pelepasan hingga 13–80%, sehingga berperan penting dalam pengendalian dosis sekaligus menekan risiko iritasi kulit yang dipengaruhi oleh perbedaan formula.[12]

Menjaga stabilitas sediaan gel *peel-off* sangat penting untuk mempertahankan efektivitasnya. Perubahan stabilitas fisik dapat memengaruhi kemampuan gel mengering dan terkelupas secara utuh, memicu pemisahan zat aktif dari basis, serta menyebabkan ketidaksesuaian pH, bentuk, bau, dan warna, yang pada akhirnya menurunkan kepuasan pengguna. Oleh karena itu, pengujian stabilitas gel *peel-off* perlu dilakukan.

Dari uraian tersebut, penelitian ini dilakukan dengan tujuan membuat sediaan gel *peel-off* antijerawat yang mengandung EPMS 1,2%, dengan PVA sebagai *gelling agent* pada konsentrasi 10% dan 12%, serta mengevaluasi stabilitas fisiknya. Penelitian ini penting dilakukan untuk menghasilkan sediaan topikal yang aman bagi kulit berminyak dan tidak memperburuk kondisi jerawat.

METODE PENELITIAN

Alat dan Bahan Penelitian.

Alat. Gelas Erlenmeyer (pyrex), rotary evaporator (Eye La), blender (Philips), batang pengaduk, timbangan digital (RadWag), spatula, pinset, kertas saring, alumunium foil, pipet tetes, toples kaca, cawan, lemari pendingin (Haier), bunsen, tabung reaksi (pyrex), *waterbath* (Memmert), gunting, gelas ukur (pyrex), beacker glass (pyrex), cawan penguap, kaca objek, kertas perkamen, oven (Memmert), pH meter, vial 10mL**,** *Apparatus melting point*, Plat KLT $G_{60}F_{254}$, pipa kapiler, chamber, viskometer (*Brookfield tipe LV*), Spektrofotometer *IR* (Shimadzu).

Bahan. Rimpang Kencur (*Kaempferia galanga* L) berasal dari Balai Pengujian Standar Instrumen Tanaman Rempah, Obat dan Aromatik (Bsip-Troa), PVA (Brataco), HPMC (Sigma Aldrich), propilen Glikol (Brataco), propil paraben (Gujarat Organics LTD, India), metil paraben (Gujarat Organics LTD, India), madu (PT. Madu Pramuka), etil asetat (Brataco) dan aquades (Brataco), etanol 96% (Brataco), n-heksan (Brataco).

Prosedur Penelitian

1. Pembuatan Ekstrak Rimpang Kencur dan Isolasi Kristal EPMS

Kencur yang diperoleh dari Bsip-Troa, Bogor Jawa Barat, selanjutnya dideterminasi di Badan Riset dan Inovasi Nasional (BRIN), Bogor. Rimpang kencur sebanyak 3 kg disortasi basah, dicuci pada air mengalir untuk menghilangkan kotoran, dan dipotong tipis dengan ketebalan 2-5 mm. Dikering anginkan selama 5 hari hingga kering. Simplisia kemudian diserbuk dan diayak dengan ayakan yang setara dengan mesh no. 40. Serbuk simplisia hasil pengolahan ditimbang dan disimpan dalam wadah yang tertutup rapat guna mencegah kontaminasi dan degradasi senyawa aktif. Ekstrak rimpang kencur dilakukan dengan metode maserasi menggunakan pelarut n-heksan untuk memperoleh fraksi non-polar. Perbandingan antara pelarut dan simplisia yang digunakan yaitu 1:4.[13] Proses remaserasi dilakukan sebanyak 4 kali hingga jernih. Maserat yang didapatkan selanjutnya disaring dengan kertas saring dan diuapkan pada suhu 48°C hingga menjadi ekstrak kental.[3]

Filtrat yang didapatkan selanjutnya dipindahkan ke dalam vial tertutup untuk penyimpanan dan didiamkan pada suhu ruang hingga kristal berwarna kekuningan terbentuk setelah pelarut menguap sepenuhnya. Kristal tersebut kemudian direkristalisasi sebanyak empat kali hingga diperoleh kristal murni, yang diketahui dari kejernihan kristal yang terbentuk.[13]

2. Identifikasi Kristal EPMS (Etil P-Metoksisinamat)

Pemeriksaan awal identitas kristal dilakukan secara organoleptis, pengukuran titik leleh, pengujian Kromatografi Lapis Tipis (KLT) dan analisis struktur dengan spektrofotometer IR. Pemeriksaan organoleptik dengan cara menguji kristal yang diperoleh melalui diidentifikasi secara fisik dengan mengamati warna, bentuk, dan baunya menggunakan panca indera untuk menilai sampel. Pengujian Kromatografi Lapis Tipis (KLT), bertujuan untuk mengetahui kandungan senyawa secara kualitatif. Ekstrak n-heksan dan kristal EPMS sampel yang diperoleh dilarutkan dalam pelarut heksan, kemudian ditotol pada lempeng KLT $G_{60}F_{254}$. Pengembangan dilakukan dengan fase gerak berupa campuran eluen heksan dan etil asetat (8:2), dan hasil kromatogram diamati di bawah lampu UV pada panjang gelombang 254 nm. Analisis struktur dilakukan menggunakan Spektrofotometer IR untuk membandingkan spektrum EPMS sampel dengan spektrum EPMS standar dari

literatur. Kemiripan spektrum menunjukkan bahwa kedua senyawa memiliki struktur yang identik. Pengujian lainya yaitu dengan penentuan titik leleh. Identifikasi titik leleh kristal dilakukan menggunakan *melting point apparatus,* dengan cara menempatkan sedikit sampel kristal ke dalam pipa kapiler, lalu dipanaskan secara bertahap untuk mencatat suhu saat awal kristal meleleh. Titik awal kristal EPMS mulai meleleh yaitu pada rentang 49-51°C.[13]

3. Formulasi Sediaan Gel *Peel-Off* EPMS

Pembuatan gel *peel-off* dengan metode pengembangan dan pencampuran pada konsentrasi PVA dibuat sebanyak 2 formula dan 2 blanko sebagai pembanding. Formula sediaan tertera pada **Tabel 1** yang telah dimodifikasi.[14] Gel *peel-off* EPMS dibuat dengan PVA didispersikan menggunakan air panas pada suhu 80^{o}C, setelah terdispersi sempurna dinginkan (massa A) kemudian, HPMC dilarutkan dengan air aduk hingga larut dan mengembang sempurna (massa B). Propil paraben dan metil paraben dilarutkan dalam propilen glikol (massa C), kemudian sebagian massa A ditambahkan ke dalam campuran massa B dan C, lalu diaduk secara merata hingga terbentuk campuran homogen. Madu dicampur dalam massa AB. Kristal EPMS dilarutkan dalam sedikit etanol, lalu dicampur dalam massa AB, homogenkan dengan menggunakan homogenizer. Sediaan yang dihasilkan dimasukkan dalam wadah kaca, sampai mencapai kesetimbangan gel.

Tabel 1. Formula Gel *peel-off* EPMS dari Rimpang Kencur[14]

Bahan	Konsentrasi (%)			
	Blanko 1 (BI)	Blanko 2 (BII)	Formula 1 (FI)	Formula 2 (FII)
EPMS	-	-	1,20	1,20
PVA	10,00	12,00	10,00	12,00
HPMC	1,00	1,00	1,00	1,00
Madu	5,00	5,00	5,00	5,00
Propilen Glikol	10,00	10,00	10,00	10,00
Metil Paraben	0,20	0,20	0,20	0,20
Propil Paraben	0,05	0,05	0,05	0,05
Etanol	qs	qs	qs	qs
Aquadest ad	100,00	100,00	100,00	100,00

4. Pengujian Mutu dan Stabilitas Fisik Gel *peel-off* EPMS

Evaluasi sediaan gel *peel-off* dilakukan dengan mengamati karakteristik organoleptik, pH, homogenitas, waktu mengering, daya sebar dan viskositas untuk menilai kualitas stabilitas fisik sediaan. Pada pengamatan organoleptik dilakukan terhadap bentuk sediaan, warna sediaan dan bau khas dari sediaan yang akan diuji. Pengujian homogenitas dilakukan dengan cara mengoleskan sediaan secara merata pada kaca objek, kemudian ditutup dengan kaca objek lainnya lalu amati homogenitas dan kehalusan merata dari sediaan tersebut. Nilai pH diukur dengan menggunakan pH meter. Pengukuran pH dilakukan dengan pH meter yang telah dikalibrasi dengan larutan buffer pH 7 (fosfat) dan pH 4 (kalium biftalat). Sebanyak 1 g gel dilarutkan dalam 10 mL akuades, kemudian elektroda dicelupkan hingga ujungnya terendam seluruhnya untuk membaca nilai pH. Pengujian waktu mengering dilakukan dengan mengoleskan 0,5 g gel *peel-off* pada kulit punggung tangan, kemudian waktu yang dibutuhkan hingga terbentuk lapisan film *peel-off* diukur menggunakan *stopwatch*. Waktu mengering yang baik berkisar antara 15-30 menit.[14] Uji kemampuan sebar dilakukan dengan mengoleskan gel 0,5 g ke pelat kaca berukuran 20x20 cm^2 pada salah satu slade. Gel diratakan menggunakan spatula hingga permukaanya rata dan bebas gelembung udara. Selanjutnya, slade lain diletakkan diatas gel telah diaplikasikan. Di atasnya diberikan beban tambahan seberat 125 g dan didiamkan selama 15 menit.[15] Diameter permukaan gel yang menyebar diukur menggunakan jangka sorong. Uji viskositas dilakukan untuk mengevaluasi tingkat kekentalan masing-masing formula, menggunakan *Viscometer Brookfield tipe LV* manual. Cara pengukurannya yaitu dengan meletakkan sejumlah gel di tabung silinder. Pasang spindel dengan nomer yang sesuai untuk sediaan gel tersebut dan masukkan dalam sampai batas yang telah ditentukan. Viscometer diputar pada kecepatan tertentu hingga jarum penunjuk menunjukkan nilai yang stabil, kemudian viskositas

gel dihitung. Uji stabilitas dipercepat dilakukan dengan menyimpan sediaan gel pada dua kondisi suhu, yaitu suhu kamar (27°±2°C), dan suhu tinggi (40°±2°C) selama 21 hari.[16] Selama penyimpanan, dilakukan pengamatan terhadap parameter organoleptik, homogenitas, pH, waktu mengering, daya sebar dan viskositas.

Analisis Data.

Daya sebar sediaan dihitung berdasarkan diameter permukaan gel yang menyebar, yang diukur menggunakan jangka sorong. Nilai daya sebar kemudian ditentukan menggunakan rumus (1) sebagai berikut:

$$F = \pi r^2 \text{ (mm)} \quad \ldots\ldots (1).$$

Dimana: F adalah kemampuan menyebar (mm^2), r = jari-jari (mm) dan π = 3,14.

Viskositas gel dihitung menggunakan persamaan (2) dan (3) berikut:

$$\text{Viskositas} = (\text{skala x factor perkalian}) \text{ cPs} \quad \ldots\ldots (2).$$

$$\text{Gaya} = (\text{skala x Kv}) \text{ dyne/cm}^2 \quad \ldots\ldots (3).$$

$$\text{Kv} = 637{,}7 \text{ dyne.cm}$$

Faktor perkalian viskositas ditentukan berdasarkan tabel yang sesuai dengan kecepatan (rpm) dan jenis *spindle* yang digunakan. Variasi kecepatan rotasi dilakukan untuk memungkinkan pengukuran viskositas pada berbagai tingkat rpm. Sifat alir sediaan ditentukan dengan memplot kurva antara kecepatan geser (rpm) dan gaya geser (dyne/cm^2), berdasarkan data yang diperoleh. Grafik diplot dengan gaya geser sebagai sumbu-x dan kecepatan geser sebagai sumbu-y. Analisis dilakukan terhadap pH, daya sebar, dan waktu mengering. Data dianalisis melalui uji normalitas, kemudian diproses menggunakan *ANOVA*, serta dilanjutkan dengan uji lanjut *Tukey HSD* untuk menentukan perbedaan signifikan antar formula.

HASIL DAN PEMBAHASAN

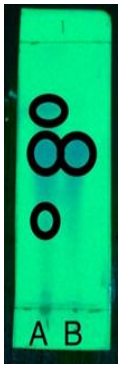
Ekstrak Rimpang Kencur dan Kristal EPMS

Tanaman kencur dideterminasi di Badan Riset dan Inovasi Nasional (BRIN), Bogor dan diketahui benar tanaman yang diteliti adalah kencur dengan nama latin *Kaempferiae galangal* L., sehingga dapat digunakan dalam penelitian ini. Serbuk kencur seberat 500 g diperoleh dari rimpang kencur segar seberat 3 kg. Proses ekstraksi dilakukan melalui metode maserasi dengan pelarut n-heksan sebagai media pelarut non polar. Dari hasil tersebut didapatkan ekstrak kental sebanyak 30,8 g dengan rendemen 6,16%. Ekstrak kental didiamkan dalam vial-vial pada suhu ruang hingga terbentuk kristal kekuningan, kemudian kristal yang masih berwarna kekuningan direkristalisasi dengan pelarut n-heksan hingga menghasilkan kristal murni sebanyak 8,30 g. Hasil kristal EPMS yang dihasilkan dalam penelitian ini jumlahnya lebih sedikit jika dibandingkan dengan jumlah pada penelitian sebelumnya yaitu sebesar 11,04 g.[3] Variasi hasil Kristal EPMS yang diperoleh dapat dipengaruhi oleh barbagai faktor internal dan eksternal. Faktor internal meliputi jenis dan usia tanaman yang digunakan dalam proses ekstraksi. Pada faktor eksternal terutama adalah lingkungan seperti air, cahaya, suhu, dan salinitas. Polusi lingkungan dan perubahan iklim saling terkait dan memengaruhi satu sama lain melalui interaksi yang kompleks.[17]

Identitas Kristal EPMS

Senyawa EPMS dari ekstrak n-heksan rimpang kencur yang dihasilkan secara organoleptik memiliki bau khas kencur, berwarna putih dan berbentuk kristal. Hasil tersebut sesuai dengan penelitian yang pernah dilaporkan, dimana menghasilkan EPMS dalam bentuk kristal, warna jernih dan bau khas sedikit kencur.[3]

Hasil uji identifikasi EPMS menggunakan Kromatografi Lapis Tipis (KLT) dengan fase diam silika $G_{60}F_{254}$ dan pengamatan di bawah lampu UV pada panjang gelombang 254 nm menunjukkan adanya kesamaan nilai retensi (Rf) sebesar 0,55 antara spot ekstrak kencur dan EPMS. Nilai Rf tersebut sesuai dengan laporan sebelumnya yang menggunakan perbandingan eluen n-heksan : etil asetat (4: 1).[3] Pengamatan terhadap spot EPMS menunjukkan terbentuknya satu noda tunggal, yang mengindikasikan kemurnian relatif senyawa. Selanjutnya, pada ekstrak rimpang kencur terdapat 3 spot, dimana ekstrak masih terdapat beberapa senyawa lain (**Gambar 1**).



Gambar 1. KLT (A) Ekstrak Rimpang Kencur; (B) EPMS

Hasil analisis struktur menggunakan spektrofotometer inframerah (*IR*), menunjukkan bahwa EPMS sampel memiliki pola spektrum yang konsisten dengan EPMS standar dari literatur, dengan identifkiasi gugus fungsi yang serupa. Dari hasil pengukuran spektrofotometer IR dengan membandingkan antara EPMS standar pada literatur dan EPMS sampel, kedua senyawa memiliki serapan gelombang daerah yang tidak jauh berbeda (**Tabel 2** dan **Gambar 3**).

Tabel 2. Intrepetasi data spektrum IR antara EPMS sampel dan EPMS standar

No	Karakteristik	Panjang Gelombang (cm^{-1}) EPMS	
		Sampel	Standar literatur [13]
1.	Gugus = C-H aromatik	2978.10	2981.00
2.	Gugus -C-H alifatik	2933.83	2937.00
3.	Gugus subsitusi cincin benzen 1,4 disbtitusi (para)	2036.90-1909.59	2036.83-1913.39
4.	Gugus C=O karbonil ester	1705.13	1701.00
5.	Gugus C=C alkena	1631.63	1628.00
6.	Gugus C=C aromatik	1602.80	1602.00

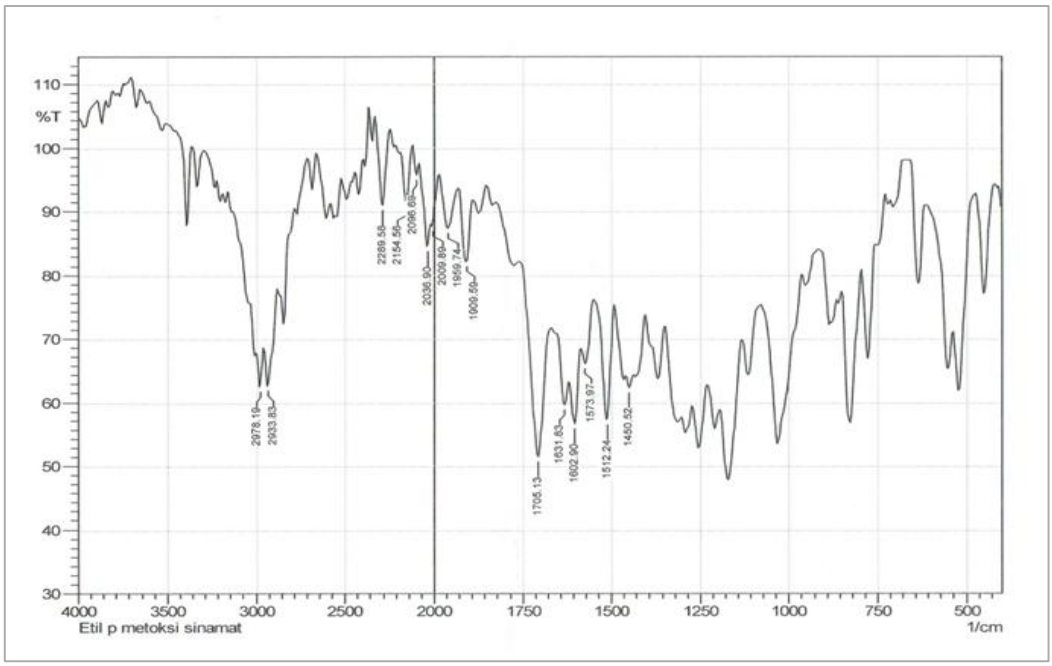
Senyawa Kristal EPMS memiliki gugus funsi khas berupa aromatik C-H, karbonil ester (C=O), dan cincin aromatik tersubsitusi 1,4 (para-disubsitusi). Berdasarkan literatur, ketiga gugus tersebut menunjukkan serapan IR berturut pada panjang gelombang 2981.00; 1701.00 dan 2036.83-1913.39 cm^{-1}. Hasil spectrum IR dari EPMS sampel menunjukkan pola serapan yang hampir identik, yaitu pada 2978.10; 1705.13 dan 2036.90-1909.59 cm^{-1}. Selain itu, terdapat kesesuaian tambahan pada gugus-gugus lainnya dengan EPMS standar dari literatur. Kesamaan pola spektrum ini menguatkan bahwa senyawa dalam sampel adalah senyawa etil p-metoksisinamat (EPMS).

Hasil pengujian dari titik leleh menggunakan alat *melting point apparatuss* diketahui bahwa krital EPMS sampel memiliki titik leleh pada 50°C. Nilai ini sesuai dengan data literatur, yaitu titik leleh EPMS standar berada pada rentang 49-50 °C.[13] Kesamaan tersebut mengidentifikasikan bahwa senyawa dalam sampel memiliki kemurnian dan identitas yang sesuai dengan etil p-metoksisinamat.

Sediaan Gel *Peel-Off* EPMS

Gel *peel-off* yang mengandung EPMS diformulasikan dengan konsentrasi PVA 10 % dan 12% yang terdiri dari blanko I (BI PVA 10%) dan II (BII PVA 12%) serta formula I (FI PVA 10% EPMS 1,2%) dan II (FII PVA 12% EPMS 1,2%). hasil pengamatan masker gel *peel-off* merupakan sediaan setengah padat berbentuk gel dengan warna blanko kuning bening dan formula warna krim dan berbau khas madu. Warna putih kekuningan dihasilkan dari senyawa EPMS dan bau khas madu dihasilkan dari bahan tambahan yaitu madu, ditampilkan pada **Gambar 2**.

Gambar 2. Gel *Peel-Off*



Gambar 3. Pengukuran Spektrum IR EPMS

Evaluasi dan Stabilitas Mutu Sediaan Gel *Peel-Off* Mengandung EPMS

Evaluasi sediaan dilakukan melalui beberapa tahapan yaitu pengamatan organoleptik, homogenitas, pH, viskositas, waktu mengering, sifat alir, dan kemampuan menyebar serta stabilitas fisik. Hasil pemeriksaan organoleptik diketahui sediaan berupa sediaan setengah padat berbentuk gel dengan warna blanko kuning bening (BI dan BII) dan formula (FI dan FII) berwarna putih kekuningan dengan berbau khas madu (**Tabel 3**). Warna putih kekuningan pada sediaan dihasilkan dari senyawa EPMS dan bau khas madu dihasilkan dari bahan tambahan madu.

Hasil pemeriksaan evaluasi homogenitas menunjukkan bahwa semua sediaan homogen (**Tabel 3**). Sediaan masker gel dikatakan homogen bila sediaan tersebar merata, permukaan halus merata dan tidak terdapat partikel kasar atau gumpalan yang teramati oleh mata. Cara pembuatan masker berpengaruh dalam tingkat homogenitasnya, termasuk optimasi kecepatan dan waktu pengadukan sediaan. Homogenitas dalam sediaan diperlukan agar zat aktif tersebar merata sehingga diperoleh dosis dan khasiat yang sama dalam setiap penggunaan.

Dari evaluasi pengukuran pH setiap sediaan berada direntang 5,21±0,03 sampai dengan 5,33±0,02 (**Tabel 3**). Pada formula II memiliki pH yang paling tinggi dan blanko I memiliki nilai pH yang paling rendah. Peningkatan kosentrasi PVA berpengaruh pada peningkatan pH sediaan. Peningkatan pH sediaan disebabkan oleh adanya gugus hidroksil (-OH) pada *polivinil alcohol* (PVA); semakin tinggi konsentrasi PVA, semakin banyak gugus -OH yang bereaksi, sehingga pH sediaan cenderung meningkat. Hal ini juga diperkuat oleh penelitian sebelumnya dimana dalam pengembangan formulasi gel masker *peel-off* minyak serai peningkatan konsentrasi PVA 8%, 10% dan 12% berpengaruh pada peningkatan pH sediaan menjadi 4,8; 5,2 dan 6,11.[18]

Besarnya pH yang dihasilkan dari seluruh formula penelitian ini masih berada dalam rentang pH fisiologis kulit (4,0-6,0), sehingga sediaan aman digunakan dan tidak menyebabkan iritasi pada kulit wajah.[19]

Tabel 3. Evaluasi dan Stabilitas Gel *peel off* EPMS

Evaluasi Gel *peel-off* EPMS					
Mutu Fisik		**Hasil**			
		BI	**BII**	**FI**	**FII**
Organoleptik		Sedian setengah padat, kuning bening, bau khas madu	Sedian setengah padat, kuning bening, bau khas madu	Sedian setengah padat, putih kekuningan, bau khas madu	Sedian setengah padat, putih kekuningan, bau khas madu
Homogenitas		Homogen	Homogen	Homogen	Homogen
pH		5,21±0,03	5,30±0,01	5,23±0,02	5,33±0,02
Daya Sebar (mm)		60±3,3	55±1,25	63±2,4	57±1,6
Waktu Mengering (menit)		18±0,82	20±1,25	15±1,25	18±0,82
Stabilitas Gel *peel-off* EPMS					
Suhu (°C)	Minggu	**pH sediaan**			
27	1	5,21±0,03	5,30±0,01	5,23±0,02	5,33±0,02
	2	5,19±0,01	5,20±0,01	5,21±0,02	5,31±0,04
	3	5,20±0,02	5,23±0,01	5,25±0,03	5,30±0,01
40	1	5,25±0,01	5,28±0,02	5,27±0,01	5,29±0,03
	2	5,24±0,03	5,32±0,03	5,29±0,01	5,32±0,02
	3	5,21±0,02	5,25±0,02	5,30±0,01	5,35±0,01
Suhu (°C)	Minggu	**Waktu Mengering (menit)**			
27	1	18±0,82	20±1,25	15±1,25	18±0,82
	2	16±1,25	18±1,25	14±1,70	17±1,25
	3	15±0,82	17±0,82	12±1,25	15±0,82
40	1	20±1,63	22±1,70	17±0,82	19±1,63
	2	22±1,70	27±1,63	19±1,63	25±0,82
	3	28±1,25	30±0,82	25±1,25	28±1,25
Suhu (°C)	Minggu	**Daya Sebar (mm)**			
27	1	60±3,30	55±1,25	63±2,40	57±1,60
	2	59±2,40	53±1,25	60±0,50	55±0,50
	3	55±1,60	50±1,60	57±1,25	53±1,60
40	1	63±1,25	56±1,25	60±1,60	55±1,70
	2	65±2,40	58±2,40	63±2,40	57±1,25
	3	68±1,60	60±0,50	65±1,25	59±0,50

Evaluasi dari waktu mengering sediaan gel *peel-off* masih memenuhi persyaratan yang ditetapkan yaitu dalam rentang 15±1,25 sampai dengan 20±1,25 menit (**Tabel 3**).[14] Berdasarkan hasil pengamatan, semakin tinggi konsentrasi PVA, menyebabkan waktu yang dibutuhkan sediaan untuk mengering menjadi lebih lama. Hal ini disebabkan oleh peningkatan konsentrasi PVA yang berbanding lurus dengan jumlah air yang dibutuhkan dalam formulasi, sehingga proses penguapan air saat pengeringan menjadi lambat. Konsentrasi air yang bertambah dalam sediaan menyebabkan waktu untuk penguapan air menjadi lebih banyak dan sediaan akan mengering dengan waktu yang lebih lama. Pengujian waktu kering gel bertujuan untuk mengetahui durasi yang dibutuhkan gel untuk mongering di atas permukaan kulit hingga membentuk lapisan film yang utuh. Lapiasan film yang terbentuk membuktikan kriteria masker gel *peel-off* dimana ketika diaplikasikan diatas kulit dan mengering akan membentuk lapisan film yang kompak, stabil, dan mudah dilepaskan dari permukaan kulit setelah kering yang menghilangkan sel kulit mati dan kotoran.[20] Proses durasi masker gel *peel-off* untuk mengering umumnya yaitu sekitar 20 menit.[21] Hasil data yang didapat, seluruh formula memenuhi kriteria waktu mengering yang sesuai dengan persyaratan untuk sediaan masker gel *peel-off*.

Hasil evaluasi daya sebar menunjukkan nilai yang berbeda dari setiap sediaan (**Tabel 3**). Kemampuan menyebar mengalami penurunan yang dipengaruhi oleh kandungan konsentrasi PVA yang lebih banyak sehingga daya sebar berkurang. Konsentrasi PVA yang meningkat akan menyebabkan viskositas (kekentalan)

sediaan meningkat atau membuat sediaan jadi lebih padat, sehingga mempengaruhi penurunan daya sebar. PVA bekerja sebagai *film forming agent* konsentrasi yang lebih tinggi meningkatkan muatan polimer dan viskositas, sehingga menyulitkan gel untuk menyebar. Hal ini sejalan dengan literatur yang menjelaskan bahwa viskositas dan *spreadability* berbanding terbalik, dimana semakin kental, maka semakin rendah kemampuan menyebar sediaan.[22] Berdasarkan hasil evaluasi kemampuan menyebar sediaan gel blanko dan formula memiliki diameter pada rentang 55-63 mm dan termasuk kategori daya sebar yang baik. Formulasi dengan *spreadability* rendah (<5 cm) terasa terlalu kental, berat, dan sulit diratakan. Sebaliknya, jika terlalu tinggi (>7 cm), gel bisa menjadi encer dan mudah mengalir, sehingga sulit membentuk film tipis *peel-off* yang baik.[23] Rentang yang ideal untuk daya sebar sediaan masker gel *peel-off* berada pada 50-70 mm, sesuai dengan kriteria mutu sediaan topikal.[24]

Dari pengujian viskositas gel *peel-off* dengan *viscometer Brookfield* tipe *LV* menggunakan spindel no 3 diperoleh antara 5.000-16.000 cPs. Viskositas merupakan ukuran hambatan suatu cairan terhadap aliran, semakin tinggi viskositas, semakin besar hambatan alirannya. Viscositas terendah terdapat dalam blanko I dengan konsentrasi PVA 10% dan viscositas tertinggi pada formula II dengan konsentrasi PVA 12%. Hal ini menunjukkan adanya rantai polimer pada EPMS dan PVA membentuk ikatan silang, sehingga semakin tinggi konsentrasi PVA semakin rapat dan semakin banyak ikatan silang yang terbentuk, sehingga konsentrasi PVA akan meningkatkan viscositas sediaan.

Pengujian stabilitas fisik gel *peel-off* EPMS mencakup evaluasi organoleptik, pH, waktu mengering, daya sebar, viskositas, sifat alir dan homogenitas. Berdasarkan pengamatan organoleptik selama penyimpanan pada suhu ruang (27°C) dan suhu tinggi (40°C) selama tiga minggu, seluruh sediaan menunjukkan bahwa semua formula stabilitas sediaan masker gel *peel-off* menunjukkan kestabilan pada bentuk, warna dan bau (**Tabel 3**). Pada BI dan BII yaitu bentuk sediaan setengah padat, warna kuning bening, bau khas madu, sedangkan pada FI da FII yaitu bentuk sediaan setengah padat, warna putih kekuningan, bau khas madu. Uji homogentitas menunjukkan bahwa semua formula (BI, BII, FI dan FII) tetap homogen tanpa perubahan selama penyimpanan pada suhu 27 °C dan 40 °C. Variasi konsentrasi PVA maupun adanya pemanasan tidak menunjukkan pengaruh terhadap homogenitas sediaan.

Hasil pengujian stabilitas pH sediaan gel pada suhu 27℃ selama tiga minggu berada di rentang nilai 5,19±0,01 sampai dengan 5,33±0,02; sedangkan pada suhu 40°C berada pada rentang 5,21±0,02 sampai dengan 5,35±0,01 (**Tabel 3**). Dari data nilai pH diketahui ada perubahan pH sediaan gel *peel off* di suhu 27℃ dan 40°C sesudah penyimpanan. Dari nilai pH menunjukkan adanya perbedaan signifikan ($p<0,05$) antara nilai pH di suhu yang berbeda setelah penyimpanan. Hal ini mengindikasikan bahwa terdapat perbedaan rata-rata pH akibat perbedaan suhu penyimpanan, yang menunjukkan bahwa penyimpanan gel *peel off* mempengaruhi nilai pH. Terjadi sedikit peningkatan pH selama penyimpanan pada FI dan II, serta penurunan pada BI dan II. Peningkatan pH akibat penambahan zat aktif juga terjadi pada penelitian sebelumnya, dimana penambahan ekstrak pandan wangi 1%, 2% dan 3% pada sediaan gel meningkatkan pH sediaan 5,92; 6,30; dan 6,43.[26] Namun pada penelitian ini nilainya masih berada dalam kisaran pH fisiologis kulit (4,0-6,0), sehingga tetap aman digunakan dan tidak menimbulkan iritasi pada kulit wajah.[19] Nilai pH yang terlalu basa dapat menyebabkan kulit menjadi kering, sementara pH yang terlalu asam (pH<3) beresiko menyebabkan iritasi dan kerusakan kulit.[27]

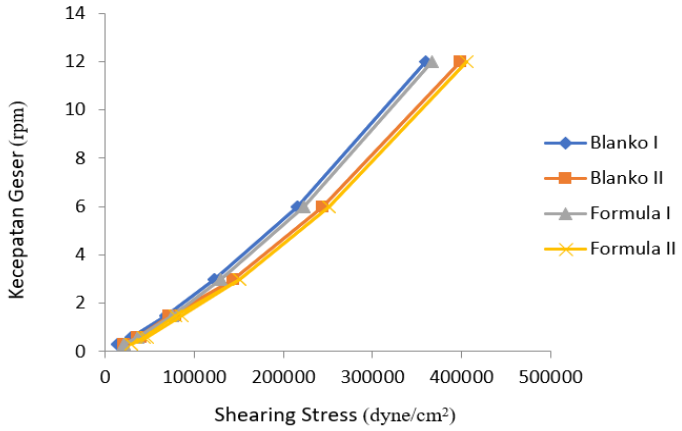
Sediaan pada suhu 27°C membutuhkan waktu 12±1,25 menit sampai dengan 20±1,25 menit untuk mengering dan sediaan pada suhu 40°C membutuhkan waktu 17±0,82 menit sampai dengan 30±0.82 menit untuk mengering, selama 3 minggu penyimpanan (**Tabel 3**). Hasil pengujian menunjukkan adanya perbedaan signifikan ($p<0,05$) antara waktu mengering sediaan sebelum dan setelah penyimpanan. Pada pengujian ini penyimpanan sediaan di suhu yang berbeda dapat meningkatkan waktu mengering sediaan gel *pell off* disemua formula. Waktu mengering sediaan setelah penyimpanan rata-rata sebesar 25±1,25 menit sampai dengan 30±0.82 menit. Peningkatan waktu mengering akibat penyimpanan juga terjadi pada evaluasi fisik sediaan masker gel *peel-off* kopi robusta sebelum dan sesudah penyimpanan, dimana waktu mengering gel

dengan ekstrak kopi robusta meningkat dari 16 menit menjadi 18 menit.[28] Waktu pengering pada semua formula masih masuk kedalam kisaran waktu mengering gel *peel off* yang ideal yaitu 15–30 menit.[29]

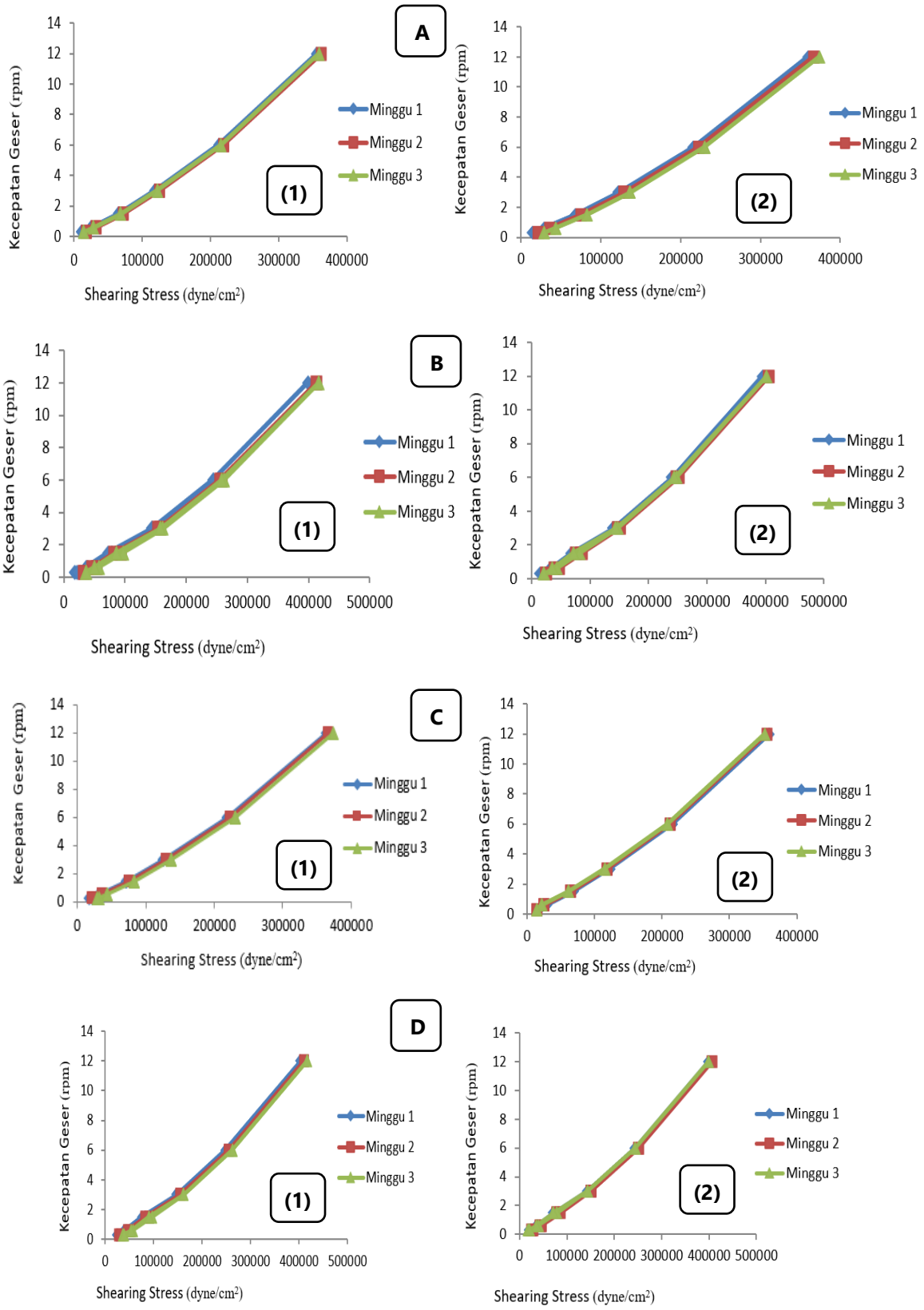
Daya sebar dari sediaan pada suhu 27°C lebih rendah dari pada sediaan pada suhu 40°C, selama penyimpanan 3 minggu. Kemampuan menyebar pada suhu kamar rata-rata pada BI, BII, FI, dan FII adalah 50±1,60 sampai dengan 63±2,40 mm. Sedangkan pada suhu 40°C, rata-rata pada formula BI, BII, FI, dan FII adalah 55±1,70 mm sampai dengan 68±1,60 mm (**Tabel 3**). Dari penyimpanan sediaan gel *peel off* di suhu yang berbeda menunjukkan adanya perbedaan signifikan ($p<0,05$) antara sedian gel *peel off* di awal dan setelah penyimpanan. Daya sebar sediaan gel *peel off* setelah penyimpanan mengalami peningkatan, hal ini dapat dipengaruhi oleh suhu tinggi, dimana ikatan antar molekul polimer pada sediaan cenderung melemah atau terputus, sehingga viskositas menurun dan sediaan menjadi lebih encer. Kondisi ini menyebabkan tahanan terhadap penyebaran menurun, sehingga sediaan lebih mudah menyebar di permukaan kulit.[30] Penelitian terdahulu menunjukkan bahwa penyimpanan gel ekstrak daun kelor selama uji stabilitas menyebabkan peningkatan nilai daya sebar. Semakin lama gel disimpan, daya sebar yang dihasilkan cenderung lebih besar. Kondisi ini terjadi karena sediaan menjadi lebih encer akibat basis tidak mampu mempertahankan air yang masuk ke dalam matriks gel.[31] Nilai daya sebar yang dihasilkan dari lama penyimpanan dengan suhu yang berbeda yaitu 59±0,50 mm sampai dengan 68±1,60 mm yang masih dalam nilai daya sebar yang ideal yaitu 5-7 cm.[32]

Berdasarkan hasil pengamatan terhadap rheogram (**Gambar 4**), BI, BII serta FI dan FII menunjukkan sifat alir pseudoplastis. Sifat ini umum ditunjukkan oleh bahan farmasi seperti gom alam dan sintetis seperti *disperse* cair *tragacnth*, natrium alginat, metil selulosa dan beberapa polimer dalam bentuk larutan. Aliran pseudoplastis ditandai oleh penurunan viskositas seiring peningkatan kecepatan geser (rpm), sehingga sediaan menjadi lebih mudah mengalir dari wadah. Ciri khas rheogram psedioplastis adalah lengkung alir yang dimulai dari titik (0,0) atau mendekatinya pada *rate of share* rendah dan tidak menunjukkan nilai *yield value*. Kurva ini terbentuk akibat gaya geser (*shearing*) yang bekerja pada molekul rantai panjang, seperti polimer, yang menyebabkan penurunan tahanan alir.[25]

Hasil pengamatan uji viskositas sediaan menunjukkan viskositas pada suhu kamar semua formula yaitu 5.000-22.000 cPs dan viskositas pada suhu 40°C yaitu 4.900-16.000 cPs dengan waktu penyimpanan 3 minggu. Sediaan pada suhu kamar memiliki nilai vikositas yang lebih besar dari pada yang disimpan pada suhu 40°C. Sediaan yang disimpan pada suhu 40°C lebih kecil nilai viskositasnya, disebabkan karena putusnya ikatan monomer dan polimer dari sediaan gel sehingga terjadinya penurunan viskositas pada sediaan. Suhu merupakan salah satu faktor yang dapat mempengaruhi kestabilan fisik sediaan. Namun, hasil pengamatan menunjukkan bahwa sediaan tetap mempertahankan konsistensi normalnya selama penyimpanan pada barbagai kondisi suhu.



Gambar 4. Kurva Evaluasi Sifat Alir Sediaan



Gambar 5. Kurva Stabilitas Sifat Alir Sediaan
(A) Blanko I — (1) pada suhu kamar (27°C) dan (2) pada suhu 40°C;
(B) Blanko II — (1) pada suhu kamar (27°C) dan (2) pada suhu 40°C;
(C) Formula I — (1) pada suhu kamar (27°C) dan (2) pada suhu 40°C;
(D) Formula II — (1) pada suhu kamar (27°C) dan (2) pada suhu 40°C.

Berdasarkan hasil pengamatan rheogram emulgel masker *peel-off* menunjukkan BI, BII dan FI, FII memiliki sifat alir pseudoplastis (**Gambar 5**). Sifat alir pseudoplastis ditandai dengan penurunan viskositas seiring peningkatan kecepatan geser (rpm), yang memungkinkan sediaan mengalir lebih mudah dari wadah. Lengkung rheorgam pseudoplastis terbentuk akibat gaya geser (*shearing*) terhadap molekul-molekul berantai panjang, seperti polimer linier. Kurva yang menunjukkan titik bertumpuk setelah gaya dilepaskan mengidentifikasikan bahwa sediaan juga memiliki sifat alir tiksotropik, yaitu kemampuan untuk pulih secara perlahan dari isothermal setelah kehilangan konsentrasi akibat gaya geser. Selama penyimpanan pada suhu 27℃, sifat alir sediaan mengalami peningkatan setiap minggunya. Sebaliknya, penyimpanan pada suhu tinggi menyebabkan penurunan sifat alir, yang diduga akibat terputusnya ikatan antar monomer pada molekul polimer dalam sediaan masker gel. Untuk mengetahui stabilitas sediaan gel *peel off* maka perlu dilengkapi pengujian daya lekat (uji adesi) yang menjadi keterbatasan pada penelitian ini, sehingga pengujian tersebut dapat mengevaluasi sediaan gel *peel off* berdasarkan daya lekat dan menilai stabilitas sediaan.

SIMPULAN

Masker gel *peel-off* yang diformulasikan dengan EPMS 1,2% dari *Kaempferia galanga* L. menggunakan basis PVA 10% dan 12% menunjukkan stabilitas fisik sesuai standar sediaan gel *peel-off*. Karakteristik yang diperoleh meliputi organoleptik yang stabil, homogenitas, pH 5,21 ± 0,02–5,35 ± 0,01, waktu mengering 17 ± 0,82–30 ± 0,82 menit, daya sebar 55 ± 1,70–68 ± 1,60 mm, dan viskositas 4.900–16.000 cPs. Penelitian ini memberikan dasar ilmiah untuk pengembangan sediaan topikal berbahan alami yang aman, khususnya bagi kulit berminyak, dan tidak memperburuk kondisi jerawat.

UCAPAN TERIMA KASIH

Ucapan terima kasih disampaikan kepada Fakultas Farmasi Institut Sains dan Teknologi Nasional (ISTN) dan Lembaga Penelitian dan Pengabdian Kepada Masyarakat ISTN (LPPM-ISTN) yang telah memberikan pendanaan hibah internal publikasi pada penelitian ini.

KONFLIK KEPENTINGAN

Penulisan naskah ini bebas dari konflik kepentingan.

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JURNAL ILMIAH MEDICAMENTO

Vol.11 No.2 (September 2025)
page 207-218
https://doi.org/10.36733/medicamento.v11i2.11811
e-ISSN: 2356-4814

Original Article

Analysis of Antibiotic Use with the ATC/DDD and DU 90% Methods at the Banjarbaru Selatan Health Center in 2023-2024

Analisis Penggunaan Antibiotik dengan Metode ATC/DDD dan DU90% di Puskesmas Banjarbaru Selatan Tahun 2023-2024

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Submitted : 21-06-2025
Revised : 16-08-2025
Accepted : 11-09-2025

Keywords: antibiotic quantity, control, prescription, resistance.

Kata Kunci: kuantitas antibiotik, pengendalian, resep, resistensi.

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Abstract

Antibiotic resistance is an escalating global health concern, with Indonesia increasingly reporting resistance across various bacterial species. This trend contributes to rising morbidity and mortality and influences patterns of antibiotic use, highlighting the need for systematic monitoring. The Anatomical Therapeutic Chemical/Defined Daily Dose (ATC/DDD) methodology is a widely accepted standard for evaluating antibiotic consumption and serves as a key indicator of antimicrobial stewardship effectiveness. This study aimed to: (i) identify the most consumed antibiotics based on DDD/1000 outpatient visits, (ii) compare consumption between 2023 and 2024, and (iii) determine the DU90% segment of antibiotic use at Banjarbaru Selatan Health Center. A quantitative, descriptive, cross-sectional design was conducted from January to May 2025, involving adult outpatients prescribed antibiotics during 2023–2024. Data analysis included DDD/1000 outpatient visit calculations and year-to-year comparisons using the Wilcoxon test. Amoxicillin consistently showed the highest consumption, with 272.94 DDD/1000 outpatient visits in 2023 and 274.26 in 2024. Statistical analysis revealed no significant difference in overall antibiotic use between the two years ($p = 0.063$). DU90% analysis identified Amoxicillin as the dominant antibiotic in 2023, while Amoxicillin and Cefadroxil led in 2024. Antibiotic consumption at Banjarbaru Selatan Health Center remained stable over the two years, with Amoxicillin as the predominant agent. These findings underscore the importance of routine monitoring using ATC/DDD and DU90% methodologies to inform targeted interventions and enhance antimicrobial resistance control strategies.

Abstrak

Resistensi antibiotik merupakan masalah kesehatan global yang semakin meningkat, dengan Indonesia terus melaporkan kasus resistensi pada berbagai spesies bakteri. Fenomena ini tidak hanya berkontribusi terhadap peningkatan angka morbiditas dan mortalitas, tetapi juga memengaruhi pola penggunaan antibiotik, sehingga diperlukan pemantauan yang sistematis. Metodologi *Anatomical Therapeutic Chemical/Defined Daily Dose* (ATC/DDD) diakui secara luas sebagai pendekatan standar dalam mengevaluasi konsumsi antibiotik dan menjadi indikator utama efektivitas program pengendalian resistensi antimikroba. Penelitian ini bertujuan untuk: (i) mengidentifikasi antibiotik dengan tingkat konsumsi tertinggi berdasarkan DDD/1000 kunjungan rawat jalan, (ii) membandingkan nilai konsumsi antara tahun 2023 dan 2024, serta (iii) menentukan segmen DU90% penggunaan antibiotik di Puskesmas Banjarbaru Selatan. Desain penelitian yang digunakan adalah kuantitatif, deskriptif, dan potong lintang, dilaksanakan pada Januari hingga Mei 2025, melibatkan pasien dewasa rawat jalan yang menerima resep antibiotik selama tahun 2023–2024. Analisis data mencakup perhitungan DDD/1000 kunjungan rawat jalan dan perbandingan antar tahun menggunakan uji Wilcoxon. Amoksisilin secara konsisten menunjukkan tingkat konsumsi tertinggi, yaitu 272,94 DDD/1000 kunjungan rawat jalan pada tahun 2023 dan 274,26 pada tahun 2024. Analisis statistik menunjukkan tidak terdapat perbedaan signifikan dalam konsumsi antibiotik secara keseluruhan antara kedua tahun ($p = 0,063$). Analisis DU90% mengidentifikasi Amoksisilin sebagai komponen dominan pada tahun 2023, sementara Amoksisilin dan Sefadroksil mendominasi pada tahun 2024. Konsumsi antibiotik di Puskesmas Banjarbaru Selatan tetap stabil selama dua tahun, dengan Amoksisilin sebagai agen utama. Temuan ini menegaskan pentingnya

pemantauan rutin menggunakan metodologi ATC/DDD dan DU90% untuk mendukung intervensi yang terarah dan memperkuat strategi pengendalian resistensi antimikroba.

How to cite: (citation style AMA 11th Ed.)
Sari OM, Intannia D, Lingga HN, Sandi DAD, Setiawan D, Andini RS, Dania SR. Analysis of Antibiotic Use with the ATC/DDD and DU 90% Methods at the Banjarbaru Selatan Health Center in 2023-2024. *J. Ilm. Medicam.*, 2025:11(2), 207-218, DOI: 10.36733/medicamento.v11i2.11811

INTRODUCTION

Antibiotic resistance is a growing global crisis, responsible for an estimated 1.27 million global deaths (GLASS report) and 133,800 deaths in Indonesia (Indonesian Health Survey).[1–4] Beyond its impact on mortality, antibiotic resistance modifies antibiotic utilization patterns and elevates healthcare costs.[5] Research conducted in Indonesia indicates substantial levels of antibiotic resistance among various bacteria, including coagulase-negative Staphylococcus resistant to Cefepime (78.19%), Meropenem (79.50%), and Ceftriaxone (97.75%), as well as *Escherichia coli* exhibiting high resistance to Ampicillin-sulbactam (90.27%).[6]

In this context, local epidemiological data further underscore the challenge. According to a report from the data.banjarbarukota.go.id website regarding the incidence of infectious disease cases in Banjarbaru City, it has been observed that Banjarbaru Selatan District experienced an increase in infectious disease cases, rising from 2,639 in 2023 to 3,773 in 2024. The infectious diseases reported include syphilis (among pregnant women), diarrhea, upper respiratory tract infections (URTI)/pneumonia, and tuberculosis.[7] As these illnesses are primarily due to bacterial infections, the elevated number of cases implies a potential increase in the prescription of antibiotics. It contributes to the development of antibiotic resistance.

Antibiotic resistance is the ability of bacteria to survive in the presence of antibiotics intended to inhibit or eliminate them.[3,8] Several factors contribute to antibiotic resistance, including the overuse of antibiotics.[9,10] The increased antibiotics use poses a critical concern, as higher prescribing rates contribute to the acceleration of antibiotic resistance.[1,11] Notably, a correlation has been observed between the use of cephalosporins and extended-spectrum beta-lactamase (ESBL) rates.[11] This condition highlights the critical importance of assessing antibiotic utilization as part of control antibiotic resistance.

In this regard, the evaluation of antibiotic consumption represents a critical quality indicator in antibiotic resistance control programs.[12,13] According to the Global Antimicrobial Resistance and Use Surveillance System (GLASS), antibiotic consumption in developed and developing nations varies between 12.3 and 31.2 Defined Daily Doses (DDD) per 1000 population per day. These figures highlight substantial variation in antibiotic usage between these countries.[1] The countries exhibiting the highest incidence of antibiotic resistance are those with elevated antibiotic consumption.[14] A systematic review study documented an increase in antibiotic consumption among inpatients in Indonesia from 2016 to 2021, compared to the period from 2000 to 2015.[15]

To address these variations and standardize measurement, the World Health Organization (WHO) endorses the use of the ATC/DDD method for the quantitative evaluation of antibiotic consumption.[8,12,16] The Anatomical Therapeutic Chemical (ATC) classification system categorizes antibiotics as pharmacologically active substances based on the organ or system they target, as well as their therapeutic, pharmacological, and chemical properties. DDD is defined as the average daily maintenance dose of an antibiotic prescribed for its main indication in adults.[8,12,17] A lower DDD value signifies more selective consumption of antibiotics. The Drug Utilization (DU) 90% method denotes the categorization of drugs within the 90% usage segment, often employed in conjunction with the DDD methodology for analyzing drug consumption. Investigating the specific drugs encompassed within the 90% segment is imperative for drug evaluation and regulation.[12]

Research conducted in various community health centers throughout Indonesia indicates that Amoxicillin exhibits the highest Defined Daily Dose (DDD) value, ranging from 39.39 to 2,077 DDD per 1000 outpatient visits. The 90% Drug Utilization (DU) segment comprises several antibiotics, including Amoxicillin, Ciprofloxacin, Cotrimoxazole, and Tetracycline.[18–21] This suggests that each region consistently uses the same type of antibiotic, as indicated by the highest consumption according to DDD metrics. However, the DDD

values of the antibiotics most frequently consumed differ, and the composition of the DU90% segment also demonstrates variability. Consequently, it is essential to research DDD values and DU90% of specific antibiotics in regions that have not yet conducted similar studies, with the intention of employing the findings as evaluative data for antibiotic consumption.

High antibiotic consumption increases the risk of resistance, making its evaluation essential. Using the DDD method allows one to compare antibiotic use.[22] There are no previous data on DDD-based antibiotic consumption at Banjarbaru Selatan Community Health Center, South Kalimantan. This study uses the 2025 ATC/DDD guidelines, which distinguishes it from past studies. Evaluating consumption helps to reduce resistance.[17] This study aims to calculate the highest amount of antibiotic consumption based on the DDD/1000 outpatient visits values, compare the DDD/1000 outpatient visits values between 2023 and 2024, and identify the segment of DU90% in antibiotic use at the Banjarbaru Selatan Health Center.

RESEARCH METHOD

This research employs a quantitative, descriptive, cross-sectional design, conducted from January to May 2025 at the Banjarbaru Selatan Community Health Center in Banjarbaru City, South Kalimantan. Data collection was done retrospectively using patient registration and prescription records from 2023 to 2024, provided by the Center's Pharmacy Department. Antibiotic use was assessed using ATC/DDD methodology. Ethical clearance was granted by Muhammadiyah University Banjarmasin, reference number 188/UMB/KE/IV/2025. The variables examined include: 1) antibiotic usage profile (class and name); 2) antibiotic consumption as DDD/1000 outpatient visits; and 3) segment of drug utilization (DU) of 90%.

Study Population

The study participants were adult outpatients who received antibiotics at the Banjarbaru Selatan Community Health Center in 2023 and 2024. This study employed a total sampling method. In this study, all members of the population who met the research criteria were sampled. The inclusion criteria covered patients under 18 years of age and those with complete outpatient registration and prescription records. Patients receiving non-systemic antibiotics or those using antibiotics without defined standard DDD values were excluded.

Research Procedures

Research methodology involves designing a data collection form. This form includes the patient's ID, age, gender, diagnosis, and medication details, including name, strength, class, form, dosage, and quantity, as well as the ATC code and DDD value. The Banjarbaru City Government Health Office approved the study, and an application for ethical clearance was submitted to Muhammadiyah University Banjarmasin prior to the research commencing. Data derived from registration records and prescriptions were filtered according to research criteria, leading to the finalization of the data form.

Evaluation of the quantity of antibiotic use

The evaluation of antibiotic use was evaluated using the DDD/1000 outpatient visits or *Kunjungan Pasien Rawat Jalan (KPRJ)* method. The ATC/DDD guidelines and the website https://atcddd.fhi.no/atc_ddd_index/ for the year 2025 served as references to establish the standard DDD value for antibiotics.

Data Analysis

The profile of antibiotic use, including nomenclature, class, dosage form, and disease diagnosis, was determined as a percentage and systematically presented in tabular form. The DDD/1000 outpatient visits value is calculated quantitatively, and the results are numerically characterized. The DU90% segment quantifies the percentage utilization of each antibiotic. The methodology for calculating the DDD/1000 outpatient visits value is delineated as follows:[23,24]

a. Calculating the total dose of antibiotics
   Total dose of a specific antibiotic = quantity used x strength of preparation
b. Calculating the total DDD of antibiotics
   Total DDD of a specific antibiotic = $\frac{specific\ antibiotic\ dose\ (grams)}{DDD\ values\ for\ specific\ antibiotics\ (WHO)}$
c. Calculate the DDD/1000 outpatient visits value
   DDD/1000 outpatient visits value for certain antibiotics = $\frac{total\ DDD\ of\ a\ specific\ antibiotic}{total\ outpatient\ visit}$ x 1000

A comparison of DDD/1000 outpatient visit values between 2023 and 2024 was conducted using the Wilcoxon test (significance level $p < 0.05$), following a normality test that indicated the data were not normally distributed. Statistical analyses were performed with SPSS version 25.

RESULT AND DISCUSSION

A total of 4,182 patients were included in this study, comprising 1,558 in 2023 and 2,624 in 2024. Of these, 3298 patients (1,344 in 2023 and 1,954 in 2024) were included in the final analysis after excluding 874 patients receiving non-systemic antibiotics and 10 patients receiving antibiotics without a DDD value. During 2023 and 2024, there were 35,334 outpatient visits to the South Banjarbaru Health Center.

The Antibiotic Use Profile

A total of 3,312 antibiotics were prescribed for 3,298 adult outpatients, comprising 1,348 in 2023 and 1,964 in 2024. During the period 2023-2024, the number of antibiotic prescriptions issued at the Banjarbaru Selatan Community Health Center increased from 1,348 to 1,964 (**Table 1**). This trend in antibiotic prescription data corresponds to the increase in infectious diseases within the Banjarbaru Selatan Subdistrict from 2023 to 2024.[7] The spectrum of antibiotic classes prescribed in 2023-2024 remained consistent, with penicillin being the predominant class. Similarly, the variety of antibiotics prescribed did not exhibit significant changes, with Amoxicillin being the antibiotic prescribed the most frequently between 2023 and 2024. This study identified acute pharyngitis as the predominant diagnosis that prompted antibiotic prescriptions during 2023-2024 (**Figs. 1 & 2**). These results align with previous studies, reinforcing that the Penicillin class and Amoxicillin are the antibiotics prescribed the most frequently in Community Health Centers.[25–27] In particular, Amoxicillin is included in the Indonesian National Formulary, thus ensuring provision of appropriate, effective, high-quality, safe, and affordable medications to patients.[28]

Table 1. Classes and Names of Antibiotics Prescribed at the South Banjarbaru Community Health Center

Classes	Antibiotics	Number of prescriptions		% Antibiotics	
		2023	2024	2023	2024
Penicillin	Amoxicillin	1,164	1,365	86.35	69.50
Cephalosporin	Cefadroxil	74	149	5.49	7.59
	Cefixime	3	0	0.22	0
Fluroquinolone	Ciprofloxacin	52	81	3.86	4.12
Amphenicol	Thiamphenicol	33	344	2.45	17.52
Nitroimidazole	Metronidazole	22	25	1.63	1.27
Total		1,348	1,964	100	100

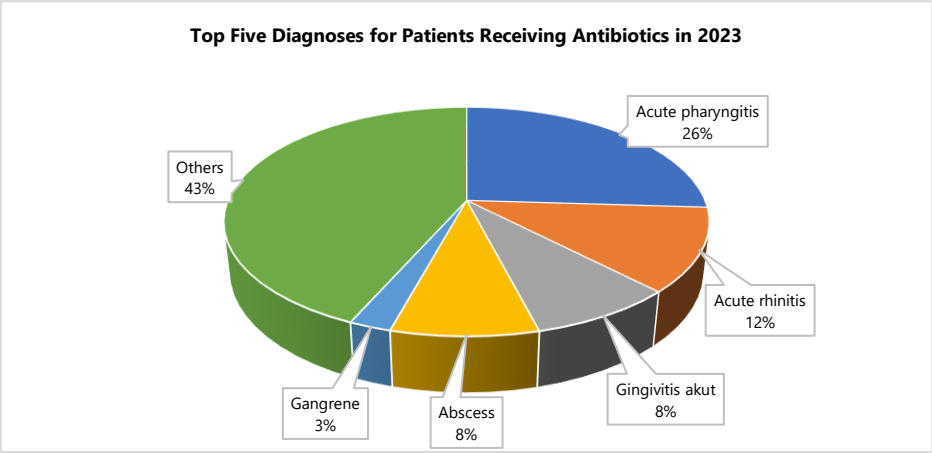

Figure 1. Top Five Diagnoses for Patients Receiving Antibiotics in 2023

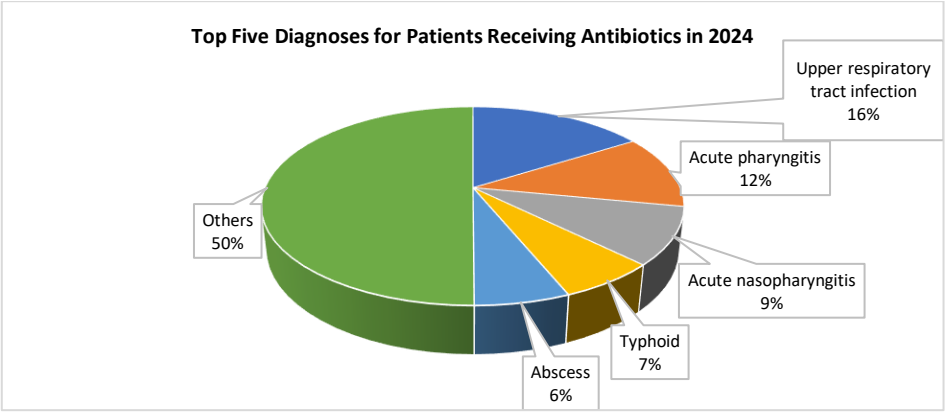

Figure 2. Top Five Diagnoses for Patients Receiving Antibiotics in 2024

The Quantity of Antibiotic Use

Based on the DDD method, total antibiotic consumption at the Banjarbaru Selatan Community Health Center increased from 315.57 DDD/1000 outpatient visits in 2023 to 377.38 DDD/1000 outpatient visits in 2024 (**Table 2**). Despite this increase, statistical analysis did not show significant differences in the DDD/1000 values between 2023 and 2024 ($p = 0.063$). This observation suggests that, despite an observed increase in antibiotic consumption at the Banjarbaru Selatan Health Center over the two years, the increase did not reach statistical significance. The rise in antibiotic consumption is correlated with various factors, including a high frequency of medication intake and a prolonged duration of medication use that exceeds the recommended guidelines for appropriate antibiotic use.[29] The total value of DDD/1000 outpatient visits is affected by the volume of outpatient visits.[23,24] In this study, the increase in the total DDD value per 1000 outpatient visits from 2023 to 2024 was concomitant with the rise in outpatient visits, increasing from 16,109 patients in 2023 to 19,225 in 2024. This trend has implications for the prescribing practices of antibiotics for patients.

Table 2. Antibiotic Consumption at the South Banjarbaru Community Health Center Based on the DDD Method

Antibiotics	ATC Code	Number of Doses (Gram)		Number of DDDs in 1 year		DDD value/1000 outpatient visits		p-value
		2023	2024	2023	2024	2023	2024	
Amoxicillin	J01CA04	6,595.25	7,909	4,396.83	5,272.67	272.94	274.26	0.063
Ciprofloxacin	J01MA02	260	405	260	405	16.14	21.07	
Cefadroxil	J01DB05	385	1,720	192.5	860	11.95	44.73	
Thiamphenicol	J01BA02	227.5	975	151.67	650	9.42	33.81	
Metronidazole	P01AB01	135	135	67.5	67.5	4.19	3.51	
Cefixime	J01DD08	6	0	15	0	0.93	0	
		Total				**315.57**	**377.38**	

Total outpatient visits in 2023 = 16109; Total outpatient visits in 2024 = 19225
The p-value represents the comparison of the DDD/1000 outpatient visits values between 2023 and 2024 using the Wilcoxon test.

The antibiotics used most frequently in the years 2023 and 2024 were Amoxicillin (**Table 2**). In 2023, Amoxicillin had a DDD value of 272.94 DDD per 1,000 outpatient visits, indicating that 273 out of 1,000 outpatient visits involved the administration of 1.5 grams of Amoxicillin.[23] In 2024, the DDD value increased slightly to 274.26 DDD per 1,000 outpatient visits, indicating that 274 out of 1,000 outpatient visits resulted in the administration of 1.5 grams of Amoxicillin. The DDD per 1,000 outpatient visits for Amoxicillin increased consistently throughout 2023 and 2024, suggesting a heightened clinical need for antibiotics that cover a variety of bacterial pathogens, especially those linked to respiratory infections. Consequently, health centers might consider broader-spectrum penicillin for empiric therapy.

Previous studies have supported these findings, with Amoxicillin reported as the most frequently used antibiotic in community health centers, according to DDD values.[18–21,30,31] However, current research has found that the DDD value for Amoxicillin was lower than that reported in multiple earlier studies.[19,20,30,31] This difference may be due to variants in patient diagnoses related to antibiotic usage, the geographical location where the study was conducted, and the period during which this research was conducted.

Amoxicillin is classified within the penicillin group of antibiotics and exhibits bactericidal activity against both Gram-positive and Gram-negative microorganisms. It is prescribed for conditions such as respiratory tract infections, otitis media, pneumonia, upper urinary tract infections, and skin infections.[28,32] The benefits of administering Amoxicillin include its broad-spectrum efficacy, suitability for oral administration, and favorable tolerability profile.[32,33] At the Banjarbaru Selatan Community Health Center, the predominant diagnosis among patients is pharyngitis. Empirical evidence suggests that individuals diagnosed with pharyngitis at this facility are more frequently prescribed Amoxicillin therapy.[34,35] Pharyngitis is identified as an infectious condition characterized by pharyngeal inflammation, often presenting with dysphagia and a sore throat. Streptococcus spp. is the primary bacterial pathogen responsible for pharyngitis.[36] Amoxicillin is designated as the first-line therapeutic agent for pharyngitis in the antibiotic guidelines of Indonesia, contributing to its widespread use.[34,36]

In this study, additional antibiotics recognized include Ciprofloxacin, Cefadroxil, and Thiamphenicol. The DDD/1000 outpatient visits for Ciprofloxacin and Cefadroxil observed in this research exceeded those recorded in the study by Perdaka et al.[21] Ciprofloxacin is classified as a fluoroquinolone antibiotic, which works by inhibiting the enzyme DNA gyrase. This enzyme is essential for bacterial replication and division.[37] Inhibition of DNA gyrase adversely affects DNA supercoiling and damages the double-stranded DNA structure, ultimately halting bacterial proliferation.[38] Ciprofloxacin is prescribed for various infections, including diabetic foot infections, respiratory tract infections, and urinary tract infections.[39] Research conducted at the Banjarbaru Selatan Community Health Center reveals that ciprofloxacin is the most commonly prescribed antibiotic, particularly among patients with urinary tract infections (UTIs).

Cefadroxil belongs to the cephalosporin class of antibiotics, which functions by inhibiting protein synthesis necessary for bacterial cell wall construction, thereby inducing damage and bacterial cell death, ultimately leading to the resolution of the infection. This antibiotic is effective only against bacterial infections and shows no efficacy against viral infections.[40] Cefadroxil is indicated for the treatment of uncomplicated urinary tract infections, respiratory tract infections, otitis media, and infections of the skin and soft tissues.[39] At the Banjarbaru Selatan Health Center in 2024, Cefadroxil was primarily administered to adults with skin infections, including abscesses, furuncles, and carbuncles, as well as acute pharyngitis and acute nasopharyngitis.

Thiamphenicol is classified as an Amphenicol antibiotic, functioning to inhibit bacterial protein synthesis by obstructing amino acid binding by tRNA.[41,42] This antibiotic is effective against Salmonella typhi bacteria, making it a common prescription for individuals suffering from typhoid fever.[42] At the Banjarbaru Selatan Community Health Center, the administration of thiamphenicol antibiotics was most prevalent among adult patients diagnosed with typhoid fever.

This investigation identified Cefixime as the antibiotic with the lowest consumption in 2023, while in 2024, Metronidazole was reported to have the lowest consumption. The results of this study differ from earlier research, which identified Erythromycin as the antibiotic with the least usage at the Jambi City Health Center.[18] Metronidazole is used in clinical practice and is the preferred drug for treating anaerobic infections due to its narrow antibacterial spectrum.[43,44] It is listed among the medications available at community health centers.[44] Metronidazole is used in the treatment of ulcerative gingivitis and acute oral infections.[39] According to the findings of a study conducted at the South Banjarbaru Community Health Center, Metronidazole is the most commonly prescribed antibiotic for patients diagnosed with periapical abscesses without sinus involvement, cellulitis, and oral abscesses. Cefixime, classified as a cephalosporin antibiotic analogous to Cefadroxil, shares a similar mechanism of action. The therapeutic indications for Cefixime include the treatment of uncomplicated urinary tract infections (caused by *Escherichia coli* and *Proteus mirabilis*), otitis media (caused by *Haemophilus influenzae* and *Streptococcus pneumoniae*), pharyngitis, and tonsillitis (caused by *Streptococcus pyogenes*). Cefixime is an antibiotic that should be available in advanced medical facilities such as hospitals. Its use is justified in particular high-risk resistance scenarios, necessitating careful monitoring of its application. This antibiotic can only be prescribed by specialist doctors or dentists and requires a pharmacist's review.[46]

This study found that DDD/1000 outpatient visits values differed when compared to earlier studies.[18,20,21,31] The variation in DDD/1000 outpatient visits values reflect differences in antibiotic use that may result from variations in disease prevalence, healthcare policies, or physicians' prescribing behaviors. This indicator is therefore essential for monitoring antibiotic consumption to control drug use and for organizing health services.[46] Furthermore, the varying DDD/1000 outpatient visit figures seen among health centers reflect the diverse rates of antibiotic use within these facilities.[47]

Antibiotic consumption data are crucial for assessing the effectiveness of antibiotic management programs implemented in healthcare settings.[48] The implementation of the ATC/ DDD system within these services offers reliable data for evaluating antibiotic consumption and functions as a global standard method.[8] The Defined Daily Dose (DDD) value represents the quantity of an antibiotic that is distributed. The decrease in DDD indicates that antibiotic prescriptions are being made more wisely, adhering to the principle of appropriate pharmacological use. On the other hand, high DDD can indicate improper or overuse of antibiotics. To improve the accuracy of DDD measurements, it is essential to include all antibiotic consumption within healthcare facilities.[49] This study demonstrates a decrease in the DDD value of metronidazole and cefixime use, which may be associated with changes in prescribing practices influenced by prescriber education and updated treatment guidelines.[50,51] By employing the ATC/DDD system, researchers can gain an understanding of changing trends in drug use and make well-informed choices about antibiotic prescriptions, thereby helping to combat the growing challenge of antimicrobial resistance.[17]

The Segment of Drug Utilization 90%

The composition of the DU90% segment exhibits variation over the two years. In 2023, Amoxicillin solely constitutes the composition of the DU90% segment. Conversely, in 2024, the segment comprises both Amoxicillin and Cefadroxil (**Fig. 3** & **Table 3**). This finding aligns with a study conducted at the Kebun Handil Community Health Center, which reported a change in the DU90% segment. In 2018, Amoxicillin was part of the DU90% segment, while in 2019, both Amoxicillin and ciprofloxacin were included in this category.[52]

Antibiotics within the DU90% segment represent those most frequently prescribed in practice, whereas those in the DU10% segment are prescribed less often.[53] This study demonstrated a change in prescribing patterns, with Amoxicillin maintaining its position and Cefadroxil emerging as a frequently used alternative. The findings of this study on antibiotic utilization can contribute to the development of essential drug lists for planning purposes. Furthermore, information regarding antibiotic usage in the DU 90% segment is utilized in the preparation of formularies. Pharmaceuticals included in the DU 90% segment are essential for inclusion in health service formularies such as those used by Community Health Center.[54]

The DU90% method emphasizes that frequently used antibiotics require careful management to prevent resistance.[55] Antibiotics within this segment carry a higher risk of resistance, emphasizing the need for cost-effective and efficient use.[56] High consumption and diversity of antibiotics further increase the risk of resistance, necessitating control measures such as limiting use and rotating antibiotic classes to maintain effectiveness.[46] The finding that Amoxicillin is included in the DU90% segment indicates that its frequent use may contribute to resistance if not adequately controlled. This highlights the need for rational prescribing, stronger pharmacist involvement in monitoring, and increased public awareness to prevent overuse.

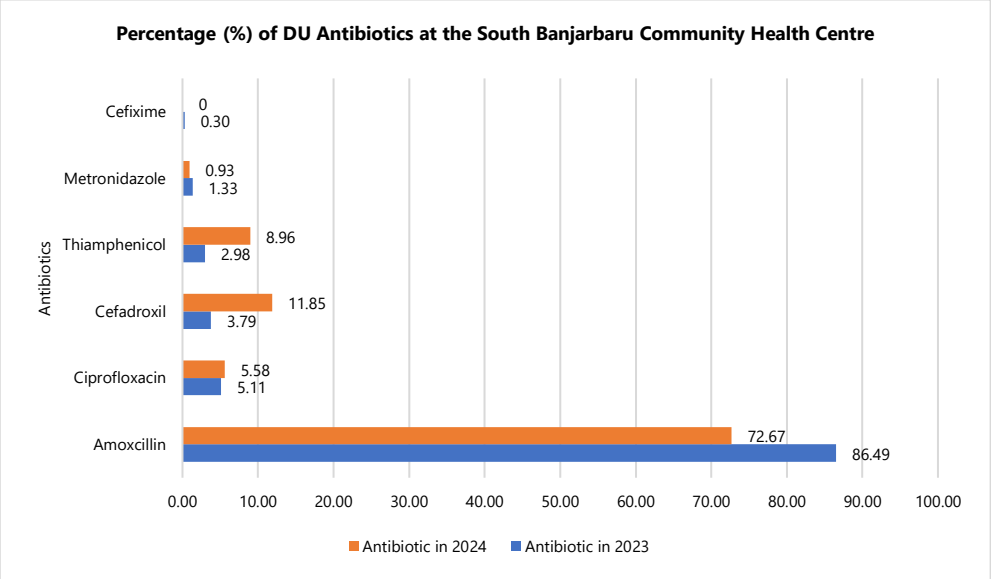

Figure 3. Percentage (%) of DU Antibiotics at the South Banjarbaru Community Health Center

Table 3. Compilation of the DU90% Antibiotic Segment at the South Banjarbaru Community Health Center Based on the DDD method

Year	Antibiotic	% DU	% DU Cumulative	DU Segment
2023	Amoxicillin	86.49	86.49	90% segment
	Ciprofloxacin	5.11	91.61	10% segment
	Cefadroxil	3.79	95.39	
	Thiamphenicol	2.98	98.38	
	Metronidazole	1.33	99.71	
	Cefixime	0.30	100	
2024	Amoxicillin	72.67	72.67	90% segment
	Cefadroxil	11.85	84.53	
	Thiamphenicol	8.96	93.49	10% segment
	Ciprofloxacin	5.58	99.07	
	Metronidazole	0.93	100	

Despite its contributions, this study has several limitations. The study utilized retrospective data from registration records and prescriptions, including only complete records to minimize bias. Furthermore, the Defined Daily Dose (DDD) method offers a standardized approach for comparing antibiotic use; however, it does not capture actual patient doses or the appropriateness of prescribing. Therefore, future research should integrate DDD analysis with patient-level and clinical data, complemented by qualitative assessments such as the Gyssens method, to provide a more comprehensive understanding of antibiotic utilization and its implications for resistance.

CONCLUSION

The relatively stable consumption of antibiotics, particularly the consistent predominance of Amoxicillin in the DU90% segment, reflects a prescribing pattern that, if not carefully managed, could accelerate antimicrobial resistance. This highlights the urgent need for rational prescribing, stronger pharmacist involvement, and public education on the responsible use of antibiotics. These findings offer actionable insights for strengthening antimicrobial stewardship and guiding policy interventions to preserve antibiotic efficacy.

ACKNOWLEDGEMENTS

The research was funded by the Institute for Research and Community Service (LPPM) of Lambung Mangkurat University in 2025, contract number 1687/UN8/LT/2025.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest regarding the publication of this manuscript.

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JURNAL ILMIAH MEDICAMENTO

Vol.11 No.2 (September 2025)
page 219-235
https://doi.org/10.36733/medicamento.v11i2.12233
e-ISSN: 2356-4814

Original Article

Spondias pinnata Leaf Extract Tablets: Impact of Primojel® and Maltodextrin Variations on Physical Properties, Stability, and Antioxidant Activity

Tablet Ekstrak Daun *Spondias pinnata*: Pengaruh Variasi Primojel® dan Maltodekstrin terhadap Karakteristik dan Stabilitas Fisik serta Aktivitas Antioksidan

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Submitted : 21-07-2025
Revised : 27-09-2025
Accepted : 30-09-2025

Keywords: antioxidant, *cemcem* leaf, maltodextrin, Primojel®, tablets physical quality.

Kata Kunci: antioksidan, *don cemcem*, maltodekstrin, mutu fisik tablet, Primojel®.

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Abstract

Cemcem leaves (*Spondias pinnata*) are rich in phenolic and flavonoid compounds with antioxidant potential. However, their traditional beverage form lacks stability, necessitating tablet formulation to improve stability and dosing convenience. This study evaluated the effect of varying concentrations of maltodextrin (binder) and Primojel® (disintegrant) on the physical quality and antioxidant activity of *cemcem* leaf extract tablets. Prior to formulation, extraction temperature optimization was performed using ultrasonic-assisted maceration at 30 °C and 45 °C, each for 3 minutes per cycle over three cycles. Three tablet formulations were prepared using optimized extract with different maltodextrin–Primojel® ratios: F1 (3%-8%), F2 (6.5%-5%), and F3 (10%-2%). Granule evaluation included moisture content, flow rate, angle of repose, and compressibility index. Tablets were assessed on days 1, 14, and 28 under room temperature storage for organoleptic properties, weight and size uniformity, hardness, friability, and disintegration time. Data were analyzed using Repeated Measures ANOVA and Friedman test at a 95% confidence level. Extraction at 30 °C yielded superior antioxidant activity, with lower IC_{50} values and higher total flavonoid content, and was therefore selected for formulation. All granules met physical quality standards; however, among tablet parameters, only disintegration time complied with pharmacopeial requirements. Formula F3, containing the highest maltodextrin concentration (10%) and lowest Primojel® concentration (2%), demonstrated the best physical stability from day 1 to day 28 ($p > 0.05$) and the strongest antioxidant activity, with the lowest IC_{50} value (23.88 µg/mL; AAI 1.68). These findings confirm a causal relationship between excipient concentration and tablet performance, supporting F3 as a promising prototype for antioxidant supplement development.

Abstrak

Daun *cemcem* (*Spondias pinnata*) kaya akan senyawa fenolik dan flavonoid yang berpotensi sebagai antioksidan. Namun, bentuk minuman tradisionalnya kurang stabil sehingga diperlukan formulasi tablet untuk meningkatkan stabilitas dan kemudahan pemberian dosis. Penelitian ini bertujuan mengevaluasi pengaruh variasi maltodekstrin (pengikat) dan Primojel® (disintegran) terhadap mutu fisik dan aktivitas antioksidan tablet ekstrak daun *cemcem*. Sebelum formulasi, dilakukan optimasi suhu ekstraksi menggunakan metode maserasi berbantu ultrasonik pada suhu 30°C dan 45°C, masing-masing selama 3 menit per siklus sebanyak tiga siklus. Tiga formula tablet disiapkan menggunakan ekstrak hasil optimasi dengan variasi maltodekstrin–Primojel®, yaitu F1 (3%-8%), F2 (6,5%-5%), dan F3 (10%-2%). Evaluasi granul meliputi kadar air, kecepatan alir, sudut istirahat, dan indeks kompresibilitas. Tablet diuji pada hari ke-1, 14, dan 28 yang disimpan pada suhu kamar terhadap parameter organoleptik, keseragaman bobot dan ukuran, kekerasan, friabilitas, serta waktu hancur. Data dianalisis menggunakan *Repeated Measures* ANOVA dan uji Friedman pada taraf kepercayaan 95%. Hasil optimasi menunjukkan ekstrak pada suhu 30 °C memiliki aktivitas antioksidan lebih baik dengan nilai IC_{50} lebih rendah dan kandungan total flavonoid lebih tinggi, sehingga digunakan untuk formulasi. Seluruh granul memenuhi kriteria mutu fisik, namun dari parameter tablet hanya waktu hancur yang sesuai standar. Formula F3 dengan konsentrasi

maltodekstrin tertinggi (10%) dan Primojel® terendah (2%) menunjukkan stabilitas fisik terbaik dari hari ke-1 hingga ke-28 ($p > 0,05$) serta aktivitas antioksidan tertinggi dengan nilai IC_{50} terendah (23,88 µg/ml; AAI 1,68). Temuan ini menegaskan hubungan kausal antara konsentrasi eksipien dan kinerja tablet, mendukung F3 sebagai prototipe yang menjanjikan untuk pengembangan suplemen antioksidan.

How to cite: (citation style AMA 11th Ed.)
Suena NMDS, Mayuni PDG, Dewi NNAP, Mendra NNY, Rahadi IWS, Wardani IGAAK. *Spondias pinnata* Leaf Extract Tablets: Impact of Primojel® and Maltodextrin Variations on Physical Properties, Stability, and Antioxidant Activity. *J. Ilm. Medicam.*, 2025:11(2), 219-235, DOI: 10.36733/medicamento.v11i2.12233

INTRODUCTION

Loloh don cemcem is a traditional Balinese herbal drink empirically consumed for general health maintenance;[1–5] however, standardized pharmacological evidence for many claimed benefits remains limited. The plant source, *cemcem* or forest hog plum (*Spondias pinnata* (L.f.) Kurz), contains secondary metabolites—including phenolics, flavonoids, terpenoids, and alkaloids—reported to exhibit antimicrobial and antioxidant activities.[6]

Previous studies found that *cemcem* in liquid "*loloh*" form shows relatively weak DPPH radical-scavenging activity, namely the loloh 1 sample of 471.9440 µg/mL and the loloh 2 sample of 322.2943 µg/mL,[7] whereas its ethanolic extract displays much stronger antioxidant capacity (IC_{50} value of 49.97 µg/mL).[8] Moreover, the beverage form is physically unstable, with a room-temperature shelf life of only about half a day,[9] limiting practical use and distribution. Converting *cemcem* into a tablet dosage form could improve stability, dose accuracy, portability, and patient acceptance.[10,11]

Successful tableting of botanical extracts depends on excipient selection and optimization to achieve acceptable weight uniformity, hardness, friability, and disintegration without compromising active release. Maltodextrin is a widely used binder with film-forming and compressible properties; typical use levels in wet granulation are about 3–10% w/w[12] and may also help protect labile phytochemicals during drying.[13,14] Primojel® (sodium starch glycolate) is a superdisintegrant that rapidly absorbs water and swells, promoting tablet breakup and potentially enhancing dissolution of herbal actives; its concentration can influence mechanical strength and performance. Literature further indicates that excipients are not always pharmacologically inert—variations in type or level can affect release, stability, and even biopharmaceutical behavior of actives.[15]

A previous study formulated cemcem extract tablets using maltodextrin as a binder and varied only the concentration of Primojel®; however, the resulting tablets exhibited poor antioxidant activity and suboptimal physical properties.[15] This suggests that optimizing both binder and superdisintegrant levels may be critical for achieving desirable mechanical integrity and functional performance.

To date, no published work has systematically evaluated the combined effect of binder and superdisintegrant levels on cemcem tablet quality and antioxidant activity. This represents a key gap in transforming a traditional herbal drink into a standardized, stable solid dosage form.

Therefore, the objective of this study is to formulate cemcem leaf extract into a tablet dosage form and to investigate how varying concentrations of maltodextrin (binder) and Primojel® (superdisintegrant) influence (1) precompression granule properties, (2) physical quality and short-term stability of the tablets, and (3) antioxidant activity (DPPH assay). The findings are expected to provide a scientific basis for developing a stable, user-friendly herbal tablet derived from *cemcem* leaves, contributing to the modernization and standardization of traditional Balinese medicine.

RESEARCH METHODS

A laboratory-based experimental design was employed to assess the effect of varying maltodextrin and Primojel® concentrations on the physical quality and antioxidant activity of cemcem leaf extract tablets. Independent variables were maltodextrin and Primojel® concentrations, while dependent variables included tablet physical quality, physical stability, and antioxidant activity. Three formulations were prepared with different maltodextrin–Primojel® ratios: F1 (3%, 8%), F2 (6.5%, 5%), and F3 (10%, 2%). Specific composition details are withheld due to intellectual property considerations.

Materials

Cemcem leaves (*Spondias pinnata* (L.f) Kurz) were collected from Penglipuran Village, Bangli Regency. The leaves were approximately 2–3 months old at harvest, mature but not yet yellowing, and hand-picked individually. Other materials used include ethanol 96% (Sabha Kimia, Indonesia), polyvinylpyrrolidone (PVP; Fadjar Kimia, Bogor), maltodextrin (Subur Kimia Jaya, Indonesia), lactose monohydrate (30320-Lactose-Edible-200M-25 KG FB, Grade A, Dairy Road & Hwy 49, USA), Primojel® (sodium starch glycolate USP, maize-based, Type A; Sigachi Industries LTD, India), magnesium stearate (Fadjar Kimia, Bogor), talc (PT. Karunia Sejahtera Abadi, Denpasar), Dragendorff and Liebermann-Burchard reagents, distilled water (aquadest), magnesium, hydrochloric acid, amyl alcohol, gelatin, ferric chloride ($FeCl_3$), ammonia 25%, chloroform, and ether.

Research Procedure

Plant Identification and Preparation of Simplicia

The botanical identification of *Spondias pinnata* (*cemcem* leaves) was conducted at the Characterization Laboratory of Eka Karya Botanical Garden – BRIN, Bali. *Cemcem* leaves were collected from Penglipuran Village, Bangli Regency. The leaves were sorted, washed thoroughly under running water, cut into small pieces, and air-dried at room temperature. The dried material was then oven-dried at 60°C for 6 hours. The dried simplicia was powdered using a blender and sieved through a mesh No. 20.[16]

Extraction of Cemcem Leaf

The powdered simplicia was extracted using ultrasonic-assisted maceration with 96% ethanol (1:8 ratio) in an ultrasonic bath (Elmasonic® S40H, Germany). Prior to tablet formulation, an initial optimization study was conducted to determine the most suitable extraction temperature. Among the tested conditions (30 °C and 45 °C), 30 °C yielded better extraction efficiency and was therefore selected for the current study. Ultrasonic waves at 20 kHz were applied for 3 minutes per cycle at 30 °C, repeated three times. The resulting extract was filtered using a Buchner funnel (PT. Mitra Sarana Instrumentasi, Indonesia) and concentrated under reduced pressure using a rotary evaporator (Buchi Rotavapor R-300 EL, Switzerland) at 50 °C to obtain a thick extract.[17]

Phytochemical Screening of Cemcem Leaf Extract

A test solution was prepared by dissolving 1 gram of the thick extract in 100 mL of hot water and filtering it to obtain filtrate A.[18]

1. Flavonoid Test
   Five milliliters of filtrate A were mixed with 0.1 g of magnesium powder, 1 mL of hydrochloric acid, and 2 mL of amyl alcohol. The mixture was shaken until layers separated. The presence of flavonoids was indicated by an orange color in the amyl alcohol layer.
2. Tannin Test
   Five milliliters of filtrate A were mixed with gelatin. The formation of a white precipitate indicated the presence of tannins.
3. Phenol Test
   Five milliliters of filtrate A were mixed with 1% ferric chloride solution. A dark blue coloration indicated the presence of phenolic compounds.
4. Alkaloid Test
   Two grams of thick extract were triturated with 5 mL of 25% ammonia and 20 mL of chloroform. The mixture was filtered, and the filtrate was spotted onto filter paper and treated with Dragendorff's reagent. An orange coloration indicated the presence of alkaloids.
5. Steroid or Triterpenoid Test
   One gram of thick extract was macerated in 20 mL of ether for 2 hours, then filtered. Five milliliters of the filtrate were evaporated in a porcelain dish and treated with Liebermann-Burchard reagent. A green-blue or purplish-red color indicated the presence of steroids or triterpenoids.

Tablet Formulation of Cemcem Leaf Extract

Three tablet formulations were prepared, each with a tablet weight of 650 mg, totaling 250 tablets per formula, with variations in the concentrations of maltodextrin and Primojel® [F1 (3%, 8%), F2 (6.5%, 5%), and F3 (10%, 2%)]. All equipment was prepared and materials weighed, following the wet granulation method adapted from Suena et al.[19] PVP and *cemcem* leaf extract (7%) were each dissolved in 15 mL of 96% ethanol, then combined and mixed until homogeneous. The lactose–maltodextrin mixture was gradually added to the PVP–extract solution until a kneadable wet mass formed. The wet granules were passed through a mesh No. 14 sieve (ASTM E-11, Indonesia) and spread on a parchment-lined tray. They were dried in an oven (Memmert UN110, Germany) at 50–60°C for 10–15 minutes, then re-sieved using mesh No. 20 (ASTM E-11, Indonesia). The granules were further dried to a moisture content of 2–4%. The dry granules were weighed, and Primojel®, magnesium stearate, and talc were added based on the percentage of dry granule weight. The mixture was homogenized in a plastic bag.

Granule Physical Quality Evaluation

1. Moisture Content
   Three grams of granules were placed on the pan of a moisture analyzer (MB90, Ohaus Corporation, USA). The instrument was tared and started. The test ended when the moisture percentage was displayed.[20]
2. Compressibility Test
   Ten grams of granules were placed in a 25 mL graduated cylinder, and the initial volume was recorded. The cylinder was placed in a tap density tester (LYCB220S, Linix) set to 300 taps. The final volume was recorded after the test.[15]
3. Flow Properties
   One hundred grams of granules were placed in a flow tester with the bottom outlet closed. The outlet was opened while simultaneously starting a stopwatch. The time taken for all granules to flow through was recorded. Flow rate was calculated. The diameter and height of the granule pile were measured to calculate the angle of repose.

Tablet Physical Quality Evaluation

Physical quality tests were performed on Days 1, 14, and 28 after tablet formulation. During the 28-day study period, tablets were stored in glass jars with silica gel desiccant to control moisture, kept at room temperature, and protected from direct sunlight.

1. Organoleptic Evaluation
   The tablets were observed for appearance, including aroma, color, taste, and shape.[21]
2. Weight Uniformity
   Twenty tablets were weighed individually and their average weight calculated. The deviation of each tablet from the average was assessed. For tablets >300 mg, no more than two tablets may deviate from the average weight by an amount greater than the limit specified in Column A (5%), and no single tablet may deviate from the average weight by more than the limit specified in Column B (10%).[22]
3. Size Uniformity
   Twenty tablets were randomly selected, and their diameter and thickness were measured using a caliper.[23]
4. Hardness Test
   Twenty tablets were tested using a hardness tester (Graigar YD-1, Mitra Medika Solo, China). Each tablet was placed horizontally, and the dial turned counterclockwise until the tablet broke. The breaking force (kg) was recorded.[15]
5. Friability Test
   Twenty dust-free tablets were weighed, placed in a friabilator (CS-0, UniLab, California), and rotated at 25 rpm for 4 minutes (100 rotations). Afterward, tablets were dusted and reweighed. The percentage weight loss was calculated.[21]

6. Disintegration Time

   One tablet was placed in each tube of a disintegration tester (BJ-3, Flight Pharmaceutical Machinery CO., LIMITED, China) filled with warm water at 37°C and covered with a disc. The timer was set for 15 minutes. All tablets should disintegrate completely within this time. If 1–2 tablets fail, the test is repeated with 12 additional tablets.[22,24]

Antioxidant activity assay

Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. A 40 µg/mL DPPH working solution was prepared by dissolving 4 mg of DPPH in 100 mL of 96% ethanol and homogenizing. The maximum absorption wavelength of the DPPH solution was determined by incubating it for 30 minutes and measuring absorbance across 400–800 nm. A 100 µg/mL vitamin C stock solution was prepared by dissolving 10 mg in 100 mL of ethanol, followed by serial dilutions to obtain concentrations of 2, 4, 6, 8, and 10 µg/mL. *Cemcem* leaf extract tablets (100 mg per formulation) were powdered, dissolved in 100 mL of ethanol, homogenized, and sonicated for 5 minutes. Serial dilutions of the sample solutions were prepared similarly to match the same concentration range. For each concentration, 2 mL of sample or vitamin C solution was mixed with 2 mL of DPPH solution, incubated for 30 minutes, and absorbance was measured using UV-Vis spectrophotometry at the determined maximum wavelength (typically 517–518 nm). A control was prepared by mixing 2 mL of DPPH with 2 mL of ethanol. Antioxidant activity was expressed as % inhibition, calculated using the formula (1).

$$\% \text{ Inhibition} = \frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance} \times 100\% \quad \text{.....(1)}$$

Notes:

The control absorbance refers to the DPPH solution mixed with solvent (96% ethanol), while the sample absorbance refers to the DPPH solution mixed with *cemcem* leaf extract tablets.

A regression curve was plotted using % inhibition versus concentration. The IC_{50} value (concentration required to inhibit 50% of DPPH radicals) was calculated by substituting y = 50 into the regression equation (y = bx + a), where y is % inhibition and x is the sample concentration. The Antioxidant Activity Index (AAI) was calculated using the formula: AAI = DPPH concentration (µg/mL) / IC_{50} (µg/mL).

Data Analysis

Data distribution was assessed using the Shapiro–Wilk test. All results are presented as mean ± standard deviation (SD) for consistency. For variables with normal distribution, between-group differences were analyzed using one-way ANOVA, with homogeneity of variances tested by Levene's test. For variables not normally distributed, differences were analyzed using the Kruskal–Wallis test, followed by Mann–Whitney U tests for post-hoc comparisons. Statistical significance was set at $p < 0.05$.

RESULT AND DISCUSSION

Plant Identification

Botanical identification of the *cemcem* plant was conducted at the Characterization Laboratory of Eka Karya Botanical Garden – BRIN, Bali. The results confirmed that the plant used in this study is *Spondias pinnata* (L.f) Kurz (ID: 71783), belonging to the genus *Spondias* L. and the family *Anacardiaceae* R.Br. The identification test was conducted to confirm the botanical authenticity of the plant material, ensuring it corresponds precisely to the intended species selected for the study. This step is essential to minimize the risk of misidentification, which could compromise the validity of subsequent experimental procedures.[15,25]

Extraction Yield of *Cemcem* Leaf Simplicia

Fresh *cemcem* leaves (9 kg) were dried to obtain 1.7063 kg of dry simplicia. The extraction process yielded 148 g of thick extract, corresponding to an extraction yield of 8.7%. Ultrasonic-assisted maceration was employed to enhance extract yield, reduce extraction time, and minimize solvent usage compared to conventional maceration.[15,26] However, this method may also lead to degradation of phenolic compounds due to hydrolysis, ionization, and oxidation reactions.[27,28]

Phytochemical Screening Results

Qualitative phytochemical screening of *cemcem* leaf extract was performed to identify the presence of secondary metabolites. The phytochemical screening of the *cemcem* leaf extract in this study revealed the presence of flavonoids, phenols, alkaloids, and steroids/triterpenoids, while tannins were not detected. These findings are partially consistent with previous reports, which identified flavonoids, alkaloids, tannins, saponins, glycosides, and steroids in ethanol extracts of *S. pinnata* leaves.[29,30] The absence of tannins in the present extract may be attributed to several factors. Previous studies have shown that extraction conditions, including solvent polarity and temperature, strongly influence tannin recovery.[31] Additionally, leaf maturity plays a critical role, as younger leaves typically contain higher tannin concentrations compared to mature leaves.[32] Furthermore, environmental factors such as climate and soil conditions can significantly affect tannin biosynthesis and composition.[33,34] Phytochemical screening is essential for identifying bioactive compounds in plant extracts, ensuring quality control, validating traditional medicinal uses, and supporting the discovery and development of novel therapeutic agents.[35–37]

Physical Quality of *Cemcem* Leaf Extract Granules

Granule quality was evaluated on Day 0 for three formulations varying maltodextrin–Primojel® ratios: F1 (3%–8%), F2 (6.5%–5%), and F3 (10%–2%), with results detailed in **Table 1**. Moisture content across all formulations showed no significant differences (F1 vs. F2: $p = 0.943$; F1 vs. F3: $p = 0.437$; F2 vs. F3: $p = 0.299$), indicating that variations in maltodextrin and Primojel® concentrations had minimal influence. All formulations met the pharmacopeial moisture standard (2–4%)[38] with values ranging from 2.78% to 3.27%, supporting optimal flowability for tablet compression. The relatively small SD values (≤0.5) indicate low variability within each formulation, suggesting consistent granule moisture distribution. Statistical analysis was performed using one-way ANOVA, as the data were normally distributed.

Previous literature has noted that maltodextrin, owing to its hygroscopic nature, tends to retain moderate moisture levels,[39,40] whereas Primojel® (sodium starch glycolate) demonstrates a higher water uptake capacity due to its pronounced swelling and hydration behavior.[41,42] In contrast, the relatively low and controlled moisture content observed in this study underscores the effectiveness of the formulation strategy, promoting granule stability and reducing the likelihood of moisture-induced processing complications. Excessive moisture can lead to particle agglomeration, adversely affecting flowability and uniformity during downstream processing.[43]

The average compressibility index values for formulations F1 and F3 were 20.33% and 16.63%, respectively, both falling within the "fair" category. Formulation F2 exhibited a slightly higher value of 21.21%, classified as "passable".[44] Statistically, F1 and F2 did not differ significantly ($p = 0.268$). However, F3, which showed the lowest compressibility index—indicating superior flowability—differed significantly from F2 ($p = 0.046$), and marginally from F1 ($p = 0.050$). The SD values (≤1.5) suggest moderate variability, with F2 showing slightly higher dispersion, consistent with its less favorable flowability. Because the data for F2 were not normally distributed, Kruskal–Wallis was applied, followed by Mann–Whitney for post-hoc comparisons.

The improved flow characteristics observed in F3 are attributed to its higher maltodextrin concentration, which likely enhanced granule cohesion during wet granulation. A lower compressibility index generally reflects better flowability, reducing the risk of processing issues such as poor die filling or weight variation during tablet compression.[45] Previous studies indicate that maltodextrin, due to its binding and particle-coating properties, can enhance granule flow and reduce compressibility index,[46,47] whereas formulations containing Primojel® (sodium starch glycolate) often exhibit slightly higher compressibility values because of its high water uptake and swelling capacity, which can increase interparticle friction.[42] Compared to these trends, the present study demonstrates that increasing maltodextrin concentration effectively improved flowability without compromising granule integrity, aligning with its reported role as a flow-enhancing binder.

Flow rate testing using 100 g of granules revealed that all three formulations exhibited free-flowing properties, with flow rates ranging from 13.22 to 14.22 g/s.[45] A good flow rate is typically greater than 10

g/second, while an acceptable angle of repose falls between 25° and 45°.[44,48] The SD values for flow rate (≤0.45) indicate high uniformity within each formulation, supporting consistent granule performance. Statistical analysis for flow rate and angle of repose was conducted using ANOVA, as both datasets were normally distributed. Previous studies have shown that increasing maltodextrin concentration generally improves flow rate and reduces the angle of repose, while combining maltodextrin with a higher concentration of Primojel® can further enhance flowability.[48] In the present study, F3 exhibited the highest flow rate, which was statistically significantly different from F1 ($p = 0.014$), whereas no significant difference was observed between F1 and F2 ($p = 0.489$) or between F3 and F2 ($p = 0.058$). This finding supports the reported positive effect of higher maltodextrin levels on granule flow.

Interestingly, F2 achieved the best angle of repose, although the differences were not statistically significant when compared with F1 ($p = 0.105$) or F3 ($p = 0.869$), and no significant difference was observed between F1 and F3 ($p = 0.202$). The SD values for angle of repose (≤0.6°) indicate minimal variability, with F3 showing slightly higher dispersion, suggesting consistent particle packing behavior within each formulation. These results suggest that a balanced ratio of maltodextrin and Primojel® may optimize particle packing and surface interaction.

Table 1. Physical Quality Test Results of *Cemcem* Leaf Extract Granules

Formula	Replication	Moisture content (%)	Compressibility index (%)	Flow rate (g/s)	Angle of Repose (°)
F1	1	3.11	19.05	13.14	32.97
	2	2.97	21.95	13.12	33.49
	3	2.56	20.00	13.39	33.36
Average±SD		**2.88±0.29^{a}**	**20.33±1.48^{ab}**	**13.22±0.15^{a}**	**33.27±0.27^{a}**
F2	1	3.09	20.45	13.85	30.96
	2	2.72	22.73	13.68	31.97
	3	2.54	20.45	13.00	31.61
Average±SD		**2.78±0.28^{a}**	**21.21±1.32^{a}**	**13.51±0.45^{ab}**	**31.51±0.51^{a}**
F3	1	3.67	16.67	14.43	32.97
	2	3.39	18.60	14.04	32.35
	3	2.74	14.63	14.20	30.31
Average±SD		**3.27±0.48^{a}**	**16.63±1.99^{b}**	**14.22±0.20^{b}**	**31.88±1.39^{a}**

Notes:
F1, F2, and F3 refer to granule formulations of cemcem leaf extract tablets with varying Maltodextrin–Primojel® concentrations: 3%–8%, 6.5%–5%, and 10%–2%, respectively.
Identical superscript letters indicate no statistically significant difference; differing letters denote a significant difference.

The angle of repose measurements for all three formulations fell within the "good" category, ranging from 31.51° to 33.27°.[44] Particle size, interparticle attraction, and frictional forces are key factors influencing the angle of repose.[49] There is an inverse relationship between flow rate and angle of repose—higher flow rates typically correspond to lower angles of repose, indicating better flowability into the hopper.[50]

Overall, these findings align with previous reports and highlight that the interplay between binder and disintegrant concentrations can influence different aspects of flowability. Granule flowability is influenced by factors such as particle size, surface characteristics, and moisture content.[49] Larger granule particles tend to have lower cohesive forces, which facilitates smoother flow.[50] Flowability is directly related to weight uniformity during tablet production; poor flow can lead to inconsistent filling of the die cavity, resulting in variable tablet weights.[51]

Tablet Physical Quality and Stability

Physical quality testing of cemcem leaf extract tablets was conducted on Days 1, 14, and 28 post-formulations. Throughout the 28-day study period, tablets were stored in glass jars containing silica gel desiccant, maintained at room temperature, and shielded from direct sunlight to control moisture exposure. The organoleptic assessment of the formulated herbal tablets revealed consistent characteristics across all formulations, including a distinct herbal aroma, greenish-brown coloration with a speckled appearance, a slightly sour and astringent taste, and a round tablet shape. Representative visual characteristics of the tablets are shown in **Figure 1**. The speckled appearance was likely associated with incomplete homogenization of the

extract during the formulation process rather than variations in binder composition. A similar observation was reported by Pradhan et al., where polyherbal tablets formulated using *Carica papaya*, *Emblica officinalis*, and *Foeniculum vulgare* and polyvinylpyrrolidone as the binder exhibited a speckled surface, attributed to non-uniform distribution of herbal powders during granulation.[52] In contrast, Ratrinia et al.[53] reported that binder type significantly influenced sensory attributes in *Sonneratia caseolaris* effervescent tablets. They compare the binders polyvinylpyrrolidone, gelatin, pulvis gummi arabic, and maltodextrin, with gelatin producing the most preferred aroma, color, and taste due to its ability to enhance homogeneity and maintain flavor stability under acidic conditions. Similar findings have been observed in other herbal tablet studies, where formulation factors such as binder choice and granulation technique affected sensory acceptability and physical uniformity. For example, Jatinkumar et al.[54] noted that polyherbal digestive tablets exhibited satisfactory taste and odor when wet granulation was optimized for uniformity. Likewise, Kushwah et al.[55] demonstrated that polyherbal chewable tablets achieved improved taste and overall acceptability through factorial optimization of excipients. Dhage et al.[56] also emphasized that nutraceutical herbal tablets formulated with multiple botanicals required careful process control to maintain consistent color and sensory properties. These comparisons suggest that while binder selection plays a critical role in effervescent systems, conventional herbal tablets rely more on process optimization to prevent visual inconsistencies and ensure desirable organoleptic characteristics.

Figure 1. Physical Appearance of *Cemcem* Leaf Extract Tablets

The results of the weight uniformity test are presented in **Table 2**. Formula F1 exhibited deviations beyond the specified limits on days 1, 14, and 28, with more than two tablets deviating by over 5%, thus failing Column A requirements, and at least one tablet exceeding 10%, thereby failing Column B.[22] Formula F2 met the requirements on days 1 and 14; however, on day 28, weight uniformity was not achieved due to more than two tablets deviating by over 5% (Column A). For F3, similar deviations to F1 were observed on days 1 and 14, but compliance was achieved on day 28, as no more than two tablets deviated by over 5% and none exceeded 10%. These findings indicate that none of the three formulations consistently met the uniformity criteria. Nevertheless, statistical analysis revealed no significant differences ($p > 0.05$) across observation days (1, 14, and 28) for all formulations (Table 4), suggesting overall weight stability. The actual tablet weights ranged from 409.2 mg to 597.8 mg, which is notably lower than the intended target of 650 mg per tablet. Weight uniformity is influenced by granule flow properties and bulk density, as represented by the compressibility index.[44] All three formulations demonstrated good flowability (free-flowing category), but compressibility indices were suboptimal: F1 and F3 were classified as fair, while F2 was passable. Variations in tablet weight can result in inconsistent active ingredient content, which may compromise dosage accuracy. Therefore, ensuring weight uniformity is essential, as it directly supports content uniformity—both being critical quality attributes that guarantee accurate dosing, consistent therapeutic performance, product safety, and regulatory compliance.[57,58]

In contrast, Ratrinia et al.[53] reported that all effervescent tablet formulations of *Sonneratia caseolaris* met the weight uniformity requirements, with deviations remaining within the 5% and 10% limits prescribed by the Indonesian Pharmacopoeia and USP standards. Their study emphasized that binder type did not affect weight uniformity, which was primarily influenced by tablet press consistency, die filling uniformity, and accurate granule weighing. Similar observations have been reported in other herbal tablet studies. Dhage et al.[56] found that nutraceutical herbal tablets prepared by wet granulation exhibited acceptable weight variation when granule flow and compression parameters were optimized. Kushwah et al.[55] demonstrated that polyherbal chewable tablets maintained weight uniformity across batches when factorial design was applied to optimize excipient ratios and compression force.

Table 2. Results of Weight Uniformity Test of *Cemcem* Leaf Extract Tablets

Formula	Day	Average Tablet Weight (g)	Average Weight Deviation		Conclusion
			A (5%)	B (10%)	
F1	1	0.515±0.027	5 tablets	1 tablet	Non-Compliant
	14	0.533±0.024	5 tablets	0 tablet	Non-Compliant
	28	0.527±0.037	5 tablets	3 tablets	Non-Compliant
F2	1	0.484±0.017	1 tablet	0 tablet	Compliant
	14	0.482±0.012	0 tablet	0 tablet	Compliant
	28	0.487±0.022	4 tablets	0 tablet	Non-Compliant
F3	1	0.525±0.027	5 tablets	1 tablet	Non-Compliant
	14	0.514±0.030	4 tablets	1 tablet	Non-Compliant
	28	0.526±0.014	1 tablet	0 tablet	Compliant

Notes:
F1, F2, and F3 refer to formulations of cemcem leaf extract tablets with varying Maltodextrin–Primojel® concentrations: 3%–8%, 6.5%–5%, and 10%–2%, respectively.

The dimensional uniformity test results for the three *cemcem* leaf extract tablet formulations (**Table 3**) indicate that none of the formulations complied, as the diameter-to-thickness ratio fell outside the specified range, i.e., D/T > 3 or D/T < 1⅓.[23] In comparison, Imtihani et al.[59] in their study, reported that the dimensional uniformity test results showed that the diameters of tablets F1 and F2 were identical at 1.004 cm. Consequently, both F1 and F2 did not meet the requirements because the tablet diameter was more than three times the tablet thickness. These findings from both studies highlight similar challenges in achieving dimensional compliance, which may be attributed to inadequate control of compression force and granule distribution during the tableting process. Based on statistical analysis (**Table 4**), F1 and F3 were stable with respect to dimensional uniformity from Day 1 through Days 14 and 28 ($p > 0.05$). In contrast, F2 was unstable from Day 1 to Day 28 ($p < 0.05$). Insufficient fill volume and compression force during tableting can lead to dimensional non-uniformity.[60] Non-uniform diameter and thickness (in addition to weight non-uniformity) may affect dosage accuracy and complicate packaging operations.[61]

Hardness testing (**Table 3**) revealed that none of the formulations met the requirement of ≥4 kgF.[62] However, F3 exhibited the highest hardness compared to F1 and F2. An increase in maltodextrin concentration as a binder and a decrease in Primojel® (disintegrant) were associated with improved hardness. Tablet hardness is influenced by several factors, including granulation method, compression force, granule strength, and the type and amount of binder used.[50] Low hardness values may result from insufficient compaction pressure, as lower pressure tends to produce friable tablets.[63] The combination of maltodextrin and polyvinylpyrrolidone (PVP) in cemcem extract tablets likely contributed to the relatively higher hardness observed in F3, which maintained the best hardness from Day 1 to Day 28. These binders can occupy inter-particulate voids in heterogeneous blends, producing more compact tablets.[64] Statistical analysis using repeated measures ANOVA (**Table 4**) indicated that all three formulations exhibited significant changes in hardness over time ($p < 0.05$).

Herlinawati[65] reported that varying maltodextrin concentrations (10%, 15%, and 20%) in tablet formulations resulted in hardness values within the acceptable range of 4–8 kgF,[66] with higher maltodextrin levels correlating with increased hardness. Manan et al.[67] also reported that an increase in maltodextrin concentration enhances tablet hardness. The present study reflects a similar trend, as F3—the formulation with the highest maltodextrin content—showed the greatest hardness, although all formulations failed to meet the pharmacopeial requirement of ≥4 kgF, likely due to the use of lower maltodextrin concentrations (3%, 6.5%, and 10%). Similarly, Syukri et al.[68] observed that tablet hardness decreased as Primojel® concentration increased. Their study evaluated five formulations containing Primojel® at 2%, 3.5%, 5%, 6.5%, and 8%, with the lowest concentration yielding an average hardness of 4.88 kgF. In the present study, hardness was also highest in F3, which contained the lowest Primojel® concentration, although all formulations exhibited hardness values below the acceptable range. This discrepancy may be attributed to the hydrophilic nature of cemcem leaf thick crude extract, which influences the physical characteristics of granules and tablets. While Herlinawati utilized dry extracts and Syukri did not employ natural extracts as active ingredients, the inclusion of hydrophilic cemcem extract in this study likely affected granule integrity and tablet hardness. Hydrophilic molecules can weaken interparticle bonding by increasing wettability and moisture uptake, leading to less compact granules during compression and consequently lower tablet hardness.[69]

Friability testing results (**Table 3**) indicated that all formulations complied with pharmacopeial limits for uncoated tablets (friability < 1%)[38,70] on Days 1 and 28, with values ranging from 0.20% to 0.93%. In contrast, none of the formulations met the requirement on Day 14, exhibiting friability between 2.88% and 10.12%. Consequently, overall compliance was not maintained throughout the study period. The highest friability was observed in F3, which contained the lowest Primojel® and highest maltodextrin concentrations, whereas F1 exhibited the lowest friability among F1–F3. This suggests that reducing maltodextrin (binder) while increasing Primojel® (disintegrant) enhances mechanical strength. These findings are consistent with previous reports. Imtihani et al.[59] noted that tablet friability increases at lower Primojel® concentrations, while Syukri et al.[68] demonstrated that increasing Primojel® from F1 to F4 reduced friability; however, at the highest concentration (F5, 8%), friability increased again, exceeding that of F1. Similarly, Kaewnoi et al.[71] reported that reducing maltodextrin concentration also decreases friability. Despite these trends, all formulations complied with pharmacopeial friability limits on Days 1 and 28. The pronounced increase on Day 14 may be attributed to storage-related changes and suboptimal granule compressibility, as inadequate compressibility typically results in friable tablets.[72]

Disintegration testing results (**Table 3**) showed that all formulations met the pharmacopeial criterion for uncoated tablets (<15 minutes)[38] and complied with safety and quality requirements for traditional medicines,[22] with mean disintegration times ranging from 3.52 to 10.88 minutes. Among the formulations, F1 disintegrated the fastest, whereas F3 was the slowest, consistent with its higher hardness and greater maltodextrin content. These observations align with Rijal et al.,[51] who reported that higher tablet hardness generally prolongs disintegration time because greater binder content strengthens granule bonding and increases hardness, thereby increasing resistance to water penetration. Similarly, the other previous studies[15,68] demonstrated that increasing Primojel® concentration reduced disintegration time, promoting faster breakdown. Primojel® acts by rapidly absorbing water and swelling upon contact, generating internal stress that facilitates tablet rupture and accelerates disintegration. Kaewnoi et al.[71] further noted that reducing maltodextrin concentration also shortened disintegration time, as lower binder levels weaken interparticulate bonding, enabling quicker dispersion. Overall, higher Primojel® (disintegrant) and lower maltodextrin (binder) concentrations were associated with shorter disintegration times.[51] Statistical analysis (Table 4) revealed that F1 and F2 exhibited significant variability in disintegration time from Day 1 through Days 14 and 28 ($p < 0.05$), whereas F3 remained stable across the study period ($p > 0.05$).

Table 3. Mean Data of Dimensional Uniformity, Hardness, Friability, and Disintegration Time on Days 1, 14, and 28

Formula	Day	Dimensional Uniformity (cm)		Hardness (kg)	Friability (%)	Disintegration time (minutes)
		Diameter	Thickness			
F1	1	1.115±0.016	0.340±0.019	1.67±0.20	0.20	6.15±0.39
	14	1.118±0.004	0.348±0.031	1.36±0.21	10.12	4.42±0.60
	28	1.119±0.003	0.356±0.012	1.41±0.18	0.47	3.52±0.53
F2	1	1.123±0.023	0.346±0.031	2.20±0.52	0.67	7.90±0.58
	14	1.123±0.023	0.348±0.031	1.66±0.14	7.13	6.80±1.27
	28	1.119±0.003	0.322±0.006	1.93±0.12	0.49	6.04±1.80
F3	1	1.118±0.003	0.350±0.024	1.75±0.45	0.93	10.02±1.01
	14	1.118±0.003	0.350±0.024	2.28±0.51	2.88	10.68±1.99
	28	1.115±0.005	0.354±0.008	2.52±0.20	0.61	10.88±3.45

Notes:
F1, F2, and F3 refer to formulations of cemcem leaf extract tablets with varying Maltodextrin–Primojel® concentrations: 3%–8%, 6.5%–5%, and 10%–2%, respectively.

Based on the evaluation results, the cemcem leaf extract tablet formulations exhibited suboptimal overall quality, as none of the three formulations consistently met all requirements throughout the 28-day observation period. All formulations complied with the disintegration time criterion; however, only F3 remained stable throughout the entire study. F1 failed to meet weight uniformity requirements at all time points, while F2 complied on Days 1 and 14 but not on Day 28. F3 initially did not meet weight uniformity requirements but achieved compliance by Day 28. Despite these variations, all formulations maintained relatively stable weight over the 28-day period. None of the formulations met dimensional uniformity requirements during the study, and all exhibited instability in hardness, failing to meet the specified limits. Friability requirements were met only on Days 1 and 28, but not on Day 14. Statistical analysis (**Table 4**) indicated that F3 demonstrated the best physical stability from Day 1 to Day 28.

According to Fiana et al.,[43] a higher concentration of maltodextrin increases total solids, thereby reducing granule moisture content. Consequently, the addition of 10% maltodextrin in F3 contributed to lower moisture content, improved granule flowability, and enhanced tablet properties. Furthermore, the presence of 2% Primojel® was sufficient as a disintegrant in the formulation.[73] Therefore, the combination of 10% maltodextrin and 2% Primojel® in F3 resulted in superior physical quality and stability compared with the other formulations.

Table 4. Results of Physical Stability Analysis of Cemcem Leaf Extract Tablets Using Repeated Measures ANOVA

Parameter	Formula	*p*-value	Interpretation
Weight uniformity	F1	0.165**	Stable
	F2	0.687*	Stable
	F3	0.235*	Stable
Dimensional uniformity	F1	0.071**	Stable
	F2	0.005**	Unstable
	F3	0.965**	Stable
Hardness	F1	0.000**	Unstable
	F2	0.000**	Unstable
	F3	0.000**	Unstable
Disintegration time	F1	0.002**	Unstable
	F2	0.004*	Unstable
	F3	0.607**	Stable

Notes:
p-value > 0,05: not significantly different
p-value < 0,05: significantly different
(*): tested using *repeated measures ANOVA*
(**): tested using *friedman*

Antioxidant Activity Test

The antioxidant activity of three *cemcem* leaf extract tablet formulations (F1, F2, and F3) and vitamin C were evaluated using the DPPH method. Five concentration levels (2, 4, 6, 8, and 10 µg/mL) were prepared, followed by the addition of DPPH solution and incubation for 30 minutes. The 30-minute incubation period was necessary because the reaction proceeds slowly, allowing sufficient time for the sample to interact with free radicals. The progress of the reaction was indicated by a color change in the sample—from purple to yellow—signifying the ability of the *cemcem* leaf extract tablets to act as antioxidants.[74]

For the DPPH assay, the absorbance of the control solution was measured, yielding a value of 0.746. Based on the absorbance values obtained for the five concentrations, the percentage inhibition (**Table 5**) for the DPPH solution combined with *cemcem* leaf extract tablets (F1, F2, and F3) at a concentration of 2 µg/mL, with a sample absorbance of 0.686 and a control absorbance of 0.746, is calculated as using Formula (1).

Based on the calculated percentage inhibition values for *cemcem* leaf extract tablets and vitamin C, a relationship between concentration and percentage inhibition was established, from which the following linear regression curve was obtained.

Table 5. Percentage Inhibition of Cemcem Leaf Extract Tablet Formulations and Vitamin C Against DPPH

Sample	Concentration (µg/mL)	Sample absorbance	Control absorbance	% Inhibition
F1	2	0,686	0,746	8,043
	4	0,681	0,746	8,713
	6	0,674	0,746	9,651
	8	0,671	0,746	10,054
	10	0,661	0,746	11,394
F2	2	0,671	0,746	10,054
	4	0,666	0,746	10,724
	6	0,655	0,746	12,198
	8	0,648	0,746	13,137
	10	0,641	0,746	14,075
F3	2	0,722	0,746	3,217
	4	0,680	0,746	8,847
	6	0,654	0,746	12,332
	8	0,625	0,746	16,219
	10	0,592	0,746	20,643

Sample	Concentration (µg/mL)	Sample absorbance	Control absorbance	% Inhibition
Vitamin C	2	0,566	0,592	4,392
	4	0,437	0,592	26,182
	6	0,337	0,592	43,074
	8	0,243	0,592	58,953
	10	0,148	0,592	75,000

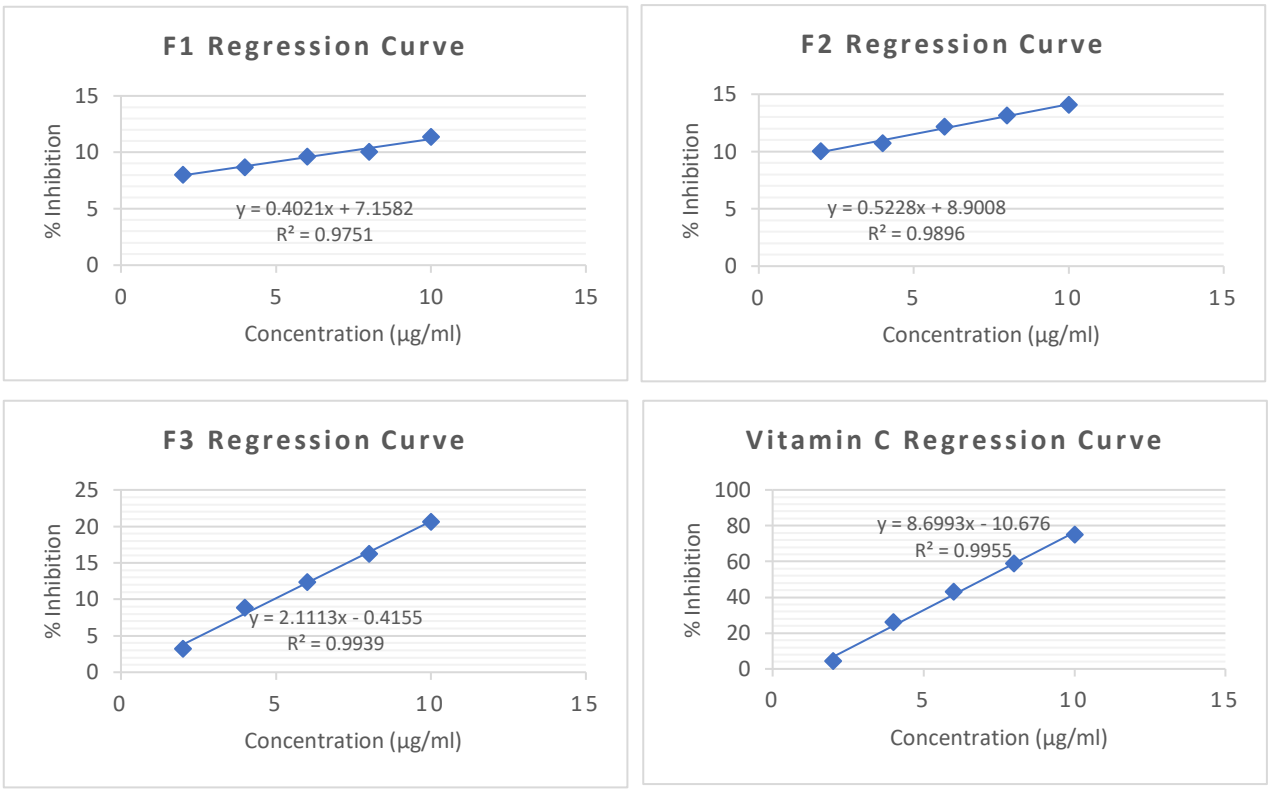

Figure 2. Linear Regression Curves of F1, F2, F3, and Vitamin C

Linear regression analysis was performed to establish the relationship between concentration and percentage inhibition for F1, F2, F3, and vitamin C. The regression equations and R^2 values were as follows: F1 ($y = 0.4021x + 7.1582$, $R^2 = 0.9751$), F2 ($y = 0.5228x + 8.9008$, $R^2 = 0.9896$), and F3 ($y = 2.1113x – 0.4155$, $R^2 = 0.9939$). IC_{50} values were determined by substituting $y = 50$ into the respective regression equations, where y represents % inhibition and x represents sample concentration (**Table 6**). The R^2 values obtained from the linear regression analysis—F1 (0.9751), F2 (0.9896), and F3 (0.9939)—indicate a strong linear relationship between concentration and percentage inhibition for all formulations. These high coefficients of determination reflect the reliability of the regression models in predicting antioxidant activity based on concentration. Notably, F3 exhibited the highest R^2 value, suggesting the most consistent and predictable antioxidant response.

Table 6. IC_{50} and AAI Values

Sample	IC_{50} (µg/mL)	IC_{50} Category	AAI	AAI Category
F1	106.545	Moderate	0.375	Mild
F2	78.614	Strong	0.509	Moderate
F3	23.879	Very strong	1.675	Strong
Vitamin C	6.975	Very strong	5.735	Very strong

Based on the above findings, the highest antioxidant activity was observed in Formula F3, which contained 2% Primojel® and 10% maltodextrin. In this study, increasing the concentration of maltodextrin correlated with stronger antioxidant activity in the *cemcem* leaf extract tablets. Maltodextrin protects key compounds, such as antioxidants, from extreme temperatures due to its ability to form a matrix and bind

strongly to encapsulate active substances.[75] Therefore, F3, which had the highest maltodextrin concentration (10%), exhibited the most potent antioxidant activity, as maltodextrin preserved the antioxidant components from thermal degradation during the wet granulation drying process in tablet formulation. Although heat exposure during processing can lead to oxidation and degradation of antioxidant compounds, maltodextrin helps retain these components, preventing complete loss of activity.[76] According to Aditya et al.,[77] maltodextrin concentrations between 6–12% show a positive parabolic trend in enhancing antioxidant activity, with 10% being the optimal concentration. Similarly, Purwati, Yuwanti, and Sari reported that effervescent tablets made from ant nest–roselle extracts using 10% maltodextrin as a binder exhibited higher antioxidant activity compared to those using dextrin, due to maltodextrin's protective effect on nutrient release.[78] Nurzarah et al. (2017) also found that tablets formulated with 5% maltodextrin had higher antioxidant activity than those with 5% dextrin.[64] Furthermore, maltodextrin concentrations up to 15% have demonstrated the ability to effectively protect encapsulated compounds.[79]

The antioxidant activity test on these three tablet formulations containing 7% cemcem leaf extract demonstrated significantly superior results compared to previous studies that used 2% and 5% extract concentrations along with 5% maltodextrin and 6% Primojel®.[15] The earlier research reported relatively low antioxidant capacities of 83.537 mg AEAC/100 g and 170.782 mg AEAC/100 g, respectively. These findings clearly indicate that increasing the cemcem leaf extract concentration to 7%, combined with optimized levels of maltodextrin and Primojel®, substantially enhances antioxidant activity in tablet formulations. Compared to formulations with lower extract concentrations, this approach achieved markedly higher antioxidant capacity, underscoring the critical role of extract loading and excipient synergy in improving functional properties. These results provide a strong foundation for developing antioxidant-rich herbal tablets with enhanced efficacy and suggest that formulation strategies focusing on extract concentration and excipient optimization can significantly influence therapeutic potential.

This study was limited to evaluating the short-term physical stability and antioxidant activity of cemcem leaf extract tablets over a 28-day period under controlled storage conditions. The formulations focused solely on varying concentrations of maltodextrin and Primojel®, without exploring other excipients or formulation techniques that may further enhance tablet performance. Additionally, the antioxidant activity was assessed using a single in vitro method (DPPH assay), which may not fully represent biological efficacy. Future studies should consider long-term stability testing under accelerated conditions, inclusion of alternative binders and disintegrants, and broader antioxidant assays—such as ABTS, FRAP, or cellular models—to validate and expand upon the observed bioactivity. Investigating the pharmacokinetics and in vivo antioxidant potential of the optimized formulation (F3) would also be valuable in supporting its development as a functional nutraceutical product.

CONCLUSION

This study demonstrated that varying concentrations of maltodextrin and Primojel® significantly influence the physical quality, short-term stability, and antioxidant activity of *cemcem* leaf extract (*Spondias pinnata* (L.f.) Kurz) tablets. Among the formulations, F3—containing the highest binder (10% maltodextrin) and the lowest disintegrant (2% Primojel®)—exhibited the most favorable tablet properties, including superior physical stability over 28 days and the strongest antioxidant activity (IC_{50} = 23.879 µg/mL). These results establish a clear causal relationship between excipient concentration and tablet performance, supporting F3 as a promising prototype for antioxidant supplement development.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the authorship or publication of this manuscript.

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JURNAL ILMIAH MEDICAMENTO

Vol.11 No.2 (September 2025)
halaman 236-244
https://doi.org/10.36733/medicamento.v11i2.9887
e-ISSN: 2356-4814

Original Article

Aktivitas Antidiabetes Kombinasi Ekstrak Daun Jambu Biji dan Daun Rambutan pada Tikus Putih Jantan Galur Wistar (*Rattus norvegicus*) yang Diinduksi *Streptozotocin*

Antidiabetic Activity of Guava and Rambutan Leaf Extract Combination in Streptozotocin-Induced Male White Wistar Rats (*Rattus norvegicus*)

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Diajukan : 16-03-2025
Direvisi : 24-08-2025
Disetujui : 28-09-2025

Kata Kunci: Antidiabetes, Daun Jambu Biji, Daun Rambutan, *Streptozotocin*

Keywords: Antidiabetic, Guava leaves, Rambutan leaves, *Streptozotocin*

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Abstrak

Diabetes melitus merupakan gangguan metabolisme yang ditandai oleh peningkatan kadar glukosa darah akibat defisiensi insulin atau resistensi terhadap insulin. Penggunaan obat antidiabetik oral dalam jangka panjang dapat menimbulkan efek samping dan biaya yang tinggi, sehingga diperlukan alternatif yang lebih aman. Pemanfaatan bahan alam sebagai obat herbal menjadi salah satu pilihan karena memiliki efek samping relatif lebih sedikit. Daun jambu biji (*Psidium guajava*) dan daun rambutan (*Nephelium lappaceum*) diketahui mengandung senyawa bioaktif yang berpotensi menurunkan kadar glukosa darah. Penelitian ini bertujuan menentukan dosis optimal kombinasi ekstrak etanol daun jambu biji (EEDJB) dan daun rambutan (EEDR) dalam pengendalian kadar glukosa darah pada tikus Wistar (*Rattus norvegicus*) yang diinduksi streptozotocin. Ekstraksi dilakukan dengan metode maserasi, dilanjutkan standarisasi dan uji Kromatografi Lapis Tipis (KLT) untuk identifikasi senyawa aktif. Sebanyak 32 ekor tikus dibagi menjadi delapan kelompok perlakuan: kontrol sehat, kontrol negatif (CMC-Na 0,5%), kontrol positif (glimepirid 0,036 mg/kgBB), ekstrak tunggal daun jambu biji (50 mg/kgBB), ekstrak tunggal daun rambutan (70 mg/kgBB), serta tiga kombinasi ekstrak (50%:50%, 75%:25%, dan 25%:75%). Induksi diabetes dilakukan dengan streptozotocin intraperitoneal. Kadar glukosa darah diukur setiap tujuh hari selama 21 hari menggunakan glukometer. Analisis statistik menggunakan One-Way ANOVA dan uji Post Hoc Games-Howell. Hasil menunjukkan ekstrak memenuhi persyaratan standarisasi dan mengandung flavonoid. Penurunan kadar glukosa tertinggi diperoleh pada kombinasi 25%:75% (EEDJB:EEDR) sebesar 72%, dengan perbedaan signifikan dibandingkan kontrol negatif. Kombinasi ekstrak daun jambu biji dan rambutan berpotensi dikembangkan sebagai kandidat antidiabetik berbahan alam.

Abstract

Diabetes mellitus is a metabolic disorder characterized by elevated blood glucose levels due to insulin deficiency or resistance. Long-term use of oral antidiabetic drugs can lead to adverse effects and high costs, making safer alternatives necessary. Herbal medicines derived from natural sources offer a promising option with relatively fewer side effects. Guava (*Psidium guajava*) and rambutan (*Nephelium lappaceum*) leaves are known to contain bioactive compounds with potential antihyperglycemic properties. This study aimed to determine the optimal dose combination of guava and rambutan leaf extracts for controlling blood glucose levels in streptozotocin-induced Wistar rats (*Rattus norvegicus*). The extracts were prepared using maceration, standardized, and analyzed by Thin Layer Chromatography (TLC) to identify active compounds. Thirty-two rats were divided into eight groups: healthy control, negative control (CMC-Na 0.5%), positive control (glimepiride 0.036 mg/kg BW), single extract of guava leaves (50 mg/kg BW), single extract of rambutan leaves (70 mg/kg BW), and three combinations of extracts (50%:50%, 75%:25%, and 25%:75%). Diabetes was induced intraperitoneally using streptozotocin. Blood glucose levels were measured every seven days for 21 days using a glucometer. Data were analyzed using One-Way ANOVA followed by Games-Howell post hoc test. The extracts met standardization requirements and contained flavonoids. The highest glucose reduction (72%) was observed in the 25%:75% combination (guava:rambutan), showing a significant difference compared to the negative control. These findings suggest that the combination of guava and rambutan leaf extracts has strong potential as a natural antidiabetic agent.

Cara mensitasi artikel (*citation style*: AMA 11thEd.):
Pradani LO, Widiastuti TC, Khuluq MH. Aktivitas Antidiabetes Kombinasi Ekstrak Daun Jambu Biji dan Daun Rambutan pada Tikus Putih Jantan Galur Wistar (*Rattus norvegicus*) yang Diinduksi Streptozotocin. *J. Ilm. Medicam.*, 2025:11(2), 236-244, DOI: 10.36733/medicamento.v11i2.9887

PENDAHULUAN

Diabetes melitus merupakan salah satu penyakit tidak menular dengan prevalensi yang terus meningkat secara global. Epidemiologi diabetes menunjukkan bahwa penyakit ini tidak hanya berdampak pada individu, tetapi juga menjadi masalah kesehatan masyarakat dengan pengaruh sosial-ekonomi yang signifikan.[1] Diabetes disebabkan oleh defisiensi insulin (tipe 1) atau resistensi insulin (tipe 2), yang menimbulkan hiperglikemia kronis, stres oksidatif, inflamasi, dan kerusakan organ target.[2] Obat diabetes yang tersedia saat ini, seperti metformin, sulfonilurea, dan insulin, memiliki keterbatasan berupa efek samping, biaya tinggi, serta kurang optimal dalam mencegah komplikasi. Salah satu solusi potensial adalah terapi berbasis bahan alam atau kombinasi pendekatan gaya hidup dan farmakoterapi.[3] Efektivitas terapi diuji melalui uji *in vitro*, *in vivo*, hingga uji klinis terkontrol acak (RCT), dengan analisis statistik seperti *t-test*, ANOVA, uji *post hoc*, interval kepercayaan 95%, dan ukuran efek untuk memastikan perbedaan signifikan secara ilmiah dan klinis.[4]

Hiperglikemia kronis merupakan ciri khas diabetes melitus (DM) dan sering disertai gangguan metabolisme lemak serta protein.[5] Kondisi ini dapat memicu stres oksidatif akibat pembentukan radikal bebas atau *Reactive Oxygen Species* (ROS), yang berpotensi merusak sel β pankreas dan menurunkan produksi insulin.[6] S*treptozotocin* (STZ) adalah senyawa diabetogenik yang bersifat radikal bebas dan umum digunakan untuk menginduksi diabetes pada hewan uji, baik untuk model diabetes tipe 1 maupun tipe 2.[7,8]

Menurut data *International Diabetes Federation* (IDF) tahun 2021, terdapat 537 juta orang berusia 20-79 tahun di seluruh dunia yang menderita diabetes. Indonesia menempati posisi lima besar negara dengan prevalensi diabetes tertinggi, yaitu lebih dari 19,5 juta jiwa pada rentang usia yang sama.[9] Berdasarkan data Dinas Kesehatan Kabupaten Kebumen, jumlah kasus diabetes melitus di wilayah tersebut mencapai 10.580 orang pada tahun 2020, menjadikannya salah satu masalah penyakit tidak menular yang perlu mendapat perhatian.

Pengobatan diabetes meliputi terapi farmakologi dan nonfarmakologi. Strategi nonfarmakologi mencakup perubahan pola makan, aktivitas fisik, dan olahraga teratur.[10] Sementara itu, terapi farmakologi melibatkan pemberian insulin dan obat antidiabetik oral.[11] Organisasi Kesehatan Dunia (WHO) merekomendasikan penggunaan pengobatan tradisional sebagai alternatif untuk menjaga kesehatan umum serta mencegah dan mengobati penyakit degeneratif, termasuk diabetes melitus.[12] Penggunaan obat herbal berbahan alami menjadi salah satu alternatif karena mudah diperoleh, terjangkau, dan memiliki efek samping yang relatif lebih sedikit.[13,14]

Salah satu tanaman yang berpotensi sebagai obat herbal adalah jambu biji (*Psidium guajava* L.), yang banyak ditemukan di Indonesia. Penelitian sebelumnya menunjukkan bahwa daun jambu biji mengandung flavonoid, terutama quercetin, yang diyakini dapat meningkatkan penyerapan glukosa oleh sel hati sehingga menurunkan kadar glukosa darah.[15] Berdasarkan penelitian Yanuarty, penapisan fitokimia mengidentifikasi kandungan flavonoid, alkaloid, tanin, saponin, dan polifenol pada simplisia daun jambu biji. Dilaporkan juga bahwa pemberian ekstrak etanol daun jambu biji pada tikus putih jantan yang diinduksi streptozotosin dosis 250 mg/kgBB mampu menurunkan kadar glukosa darah rata-rata sebesar 119,2 mg/dL.[16]

Selain jambu biji, rambutan (*Nephelium lappaceum* L.) juga dilaporkan memiliki efek antidiabetes. Penelitian Suliska menunjukkan bahwa ekstrak etanol daun rambutan memiliki aktivitas antihiperglikemik paling besar pada dosis 50 mg/kgBB. Penapisan fitokimia mengungkap kandungan flavonoid, tanin, dan saponin dalam daun rambutan.[17] Mekanisme kerja senyawa tersebut diduga terkait dengan aktivitas antioksidan yang mampu menghambat pembentukan radikal bebas atau meningkatkan efektivitas enzim pertahanan terhadap stres oksidatif.[18]

Penelitian Yanuarty menunjukkan bahwa ekstrak daun jambu biji memiliki potensi menurunkan kadar glukosa darah pada dosis 150 mg, 250 mg, dan 350 mg.[16] Sementara itu, penelitian Suliska membuktikan bahwa ekstrak daun rambutan dosis 50 mg/kgBB memberikan efek antihiperglikemia terbaik dibandingkan dosis 25 mg/kgBB dan 10 mg/kgBB,[17] yang menunjukkan bahwa peningkatan dosis memengaruhi efektivitas. Namun, mekanisme antihiperglikemia ekstrak daun rambutan belum sepenuhnya diketahui. Berdasarkan uraian tersebut, penelitian ini bertujuan menguji aktivitas antidiabetes kombinasi ekstrak etanol daun jambu biji (EEDJB) dan ekstrak etanol daun rambutan (EEDR) pada tikus putih jantan galur Wistar (*Rattus norvegicus*) yang diinduksi streptozotosin. Tujuan penelitian adalah mengevaluasi potensi sinergis kedua ekstrak, apakah kombinasi tersebut memperkuat efek antidiabetes atau justru menurunkannya akibat interaksi antar komponen.

METODE PENELITIAN

Alat. Alat yang digunakan mencakup berbagai perangkat laboratorium (PYREX) dan peralatan seperti *vortex mixer*, timbangan analitik (OHAUS), oven (MEMMERT), *rotary evaporator* (OHAUS), Kromatografi Lapis Tipis (MERCK), *water bath*, oral sonde, spuit berkapasitas 3 cc dan 5 cc, glukometer (Gluco-Dr), strip uji glukosa.

Bahan. Bahan yang digunakan adalah 32 ekor tikus wistar jantan berat badan 150-200 gram umur 2-3 bulan,, tanaman daun jambu biji dan daun rambutan (diambil dari daerah Ambal, Kebumen, Jawa Tengah), etanol 70% (Merck; Darmstadt, Jerman), air suling, metanol, quercetin (Sigma-Aldrich; St. Louis, AS), plat silika gel GF254, Na-CMC, kertas saring, NaOH, glimepiride, asam tanat, FeCl3, asam klorida, kloroform, etil asetat, asam asetat, butanol, makanan tikus standar (pelet), aluminium foil, kain kasa, dan kapas alkohol.

Prosedur Penelitian.

Pembuatan Simplisia dan Ekstraksi

Daun jambu biji dan daun rambutan segar yang telah dibersihkan dipotong kecil dan dikeringkan di bawah sinar matahari ditutup dengan kain hitam. Simplisia yang kering ditumbuk menjadi serbuk.[19] Prosedur maserasi digunakan untuk membuat ekstrak dari daun jambu biji dan rambutan. Proses maserasi dilakukan menggunakan etanol 70% serbuk daun dengan rasio 1:10 pada suhu kamar. Campuran diaduk selama 30 menit sebelum direndam selama 72 jam. Maserat yang dihasilkan disaring dan filtrat dipisahkan dari pelarut sampai diperoleh ekstrak kental, kemudian dikentalkan menggunakan rotary evaporator dengan suhu yang disesuaikan pada 50°C.[20]

Standarisasi Ekstrak[21]

Pengujian mutu ekstrak meliputi uji organoleptik, kadar air, kadar abu total, dan kadar abu tidak larut asam. Uji organoleptik dilakukan untuk menilai karakteristik fisik ekstrak, mencakup warna, tekstur, bau, dan rasa. Penentuan kadar air diawali dengan penimbangan wadah, kemudian menimbang ekstrak sebanyak 2 gram, yang selanjutnya dikeringkan selama 5 jam pada suhu 150°C dan dicatat beratnya. Prosedur ini diulang setiap satu jam hingga selisih berat antar pengukuran tidak melebihi 10%. Kadar abu total ditentukan dengan memasukkan 2 gram ekstrak ke dalam cawan silika yang telah dipanaskan dan ditimbang, kemudian sisa kertas saring dibakar dalam cawan yang sama. Filtrat diuapkan dan dibakar kembali hingga diperoleh berat konstan, dengan kadar abu total yang baik kurang dari 10%. Untuk kadar abu tidak larut asam, abu direbus bersama 25 ml asam sulfat encer selama 5 menit, kemudian disaring menggunakan kertas saring, dicuci dengan air panas, dikeringkan hingga berat konstan, dan ditimbang. Standar kadar abu tidak larut asam yang sesuai adalah 0,7%.

Skrining Fitokimia dan Konfirmasi Kandungan Senyawa

Skrining fitokimia dilakukan untuk mengidentifikasi kandungan senyawa aktif dalam ekstrak etanol daun jambu biji dan rambutan melalui uji flavonoid, tanin, dan saponin, serta konfirmasi dengan metode KLT. Uji flavonoid dilakukan dengan mencampurkan 4 ml larutan ekstrak dengan 1 ml metanol, kemudian dipanaskan sambil ditambahkan magnesium. Setelah itu, ditambahkan 5-6 tetes HCl encer; perubahan warna menjadi merah atau oranye menunjukkan adanya flavonoid.[22] Uji tanin dilakukan dengan melarutkan ekstrak

dalam air suling, menyaringnya, lalu menambahkan 1-3 tetes larutan $FeCl_3$ 1%; terbentuknya warna hitam atau biru kehijauan menandakan adanya tanin.[23] Uji saponin dilakukan dengan melarutkan 0,5 gram ekstrak dalam 10 ml air panas, didinginkan, kemudian dikocok kuat selama 10 detik; terbentuknya buih stabil selama 10 menit menunjukkan adanya saponin.[24] Konfirmasi kandungan senyawa dilakukan dengan metode uji tabung untuk flavonoid, tanin, dan saponin, serta KLT untuk mendeteksi senyawa beraktivitas antidiabetik, yaitu flavonoid. Pada uji KLT, pelat silika gel GF254 dipanaskan dalam oven 10 menit pada suhu 100°C untuk aktivasi, dengan fase gerak berupa campuran butanol:asam asetat:akuades (6:2:2). Quersetin digunakan sebagai pembanding untuk identifikasi flavonoid.[25]

Uji Aktivitas Antidiabetes

Uji aktivitas antidiabetes dilakukan sesuai standar etika penelitian hewan yang telah disetujui oleh Komite Etik Penelitian Universitas Ahmad Dahlan (Nomor: **022405062**). Hewan uji berupa tikus jantan *Rattus norvegicus* berusia 2-3 bulan dengan berat 150-200 g diadaptasi selama tujuh hari, kemudian dipuasakan 8-12 jam sebelum perlakuan.[26] Tikus dibagi menjadi delapan kelompok sebagai berikut:

1. Kelompok 1: Kelompok sehat tanpa perlakuan apapun,
2. Kelompok 2: Kontrol negatif (CMC-Na 0,5%/hari),
3. Kelompok 3: Kontrol positif (glimepirid 0,036 mg/kgBB)
4. Kelompok 4: diberi ekstrak etanol daun jambu biji (EEDJB) (50 mg/kgBB/hari),
5. Kelompok 5: diberi ekstrak etanol daun rambutan (EEDR) (70 mg/kgBB/hari),
6. Kelompok 6: diberi kombinasi ekstrak EEDJB-EEDR (50%:50%) per hari,
7. Kelompok 7: diberi kombinasi ekstrak EEDJB-EEDR (75%:25%) per hari, dan
8. Kelompok 8: diberi kombinasi ekstrak EEDJB-EEDR 25%:75%) per hari.

Induksi diabetes dilakukan pada hari ke-8 dengan pemberian *Streptozotocin* intraperitoneal dosis 45 mg/kgBB. Tikus dinyatakan diabetes bila kadar glukosa darah >200 mg/dL pada hari ketiga setelah induksi.[7] Perlakuan diberikan sekali sehari selama 21 hari. Pengambilan sampel darah dilakukan melalui vena ekor pada hari ke-0, 7, 14, dan 21 dalam kondisi puasa, kemudian diukur menggunakan glucometer. Hewan uji dipelihara dengan pemberian pakan dan minum setiap hari, kandang beralas serbuk kayu dengan penutup kawat. Pemusnahan dilakukan secara etis menggunakan metode *euthanasia* fisik (*cervical dislocation*) setelah hewan dianestesi, dan prosedur ini tidak dilakukan saat hewan dalam keadaan sadar.

Analisis Data.

Penurunan kadar glukosa darah dihitung menggunakan rumus pada Persamaan **(1)**. Persentase penurunan yang diperoleh dianalisis secara statistik menggunakan perangkat lunak SPSS versi 25. Analisis dilakukan dengan uji ANOVA satu arah (One-Way ANOVA) pada taraf kepercayaan 95% untuk menguji hipotesis komparatif, dilanjutkan dengan uji Post Hoc Games-Howell guna mengetahui perbedaan bermakna antar kelompok perlakuan. Perbedaan dianggap signifikan apabila nilai $p < 0,05$. Efektivitas perlakuan dalam menurunkan kadar glukosa darah ditentukan berdasarkan persentase penurunan kadar glukosa tikus putih (Rattus norvegicus) jantan galur Wistar yang telah dinyatakan diabetes, dengan membandingkan kadar awal dan kadar setelah perlakuan.[27]

$$\%Penurunan = \frac{(H0-H21)}{H0} X100\% \quad \textbf{(1)}$$

Keterangan:
H0 = Kadar gula darah awal
H21 = Kadar gula darah akhir

HASIL DAN PEMBAHASAN

Ekstraksi etanol daun jambu biji (*Psidium guajava* L.) dan daun rambutan (*Nephelium lappaceum* L.) menggunakan pelarut etanol 70% menghasilkan rendemen ekstrak sebesar 10% untuk masing-masing daun. Berdasarkan hasil skrining fitokimia, ekstrak etanol daun jambu biji mengandung tanin, saponin, dan flavonoid.

Temuan ini sejalan dengan penelitian Yanuarty yang melaporkan bahwa ekstrak etanol daun jambu biji mengandung flavonoid, alkaloid, tanin, saponin, dan polifenol.[16] Hasil skrining fitokimia yang ditampilkan pada **Tabel 1** juga menunjukkan bahwa ekstrak etanol daun rambutan mengandung flavonoid, tanin, dan saponin. Hal ini konsisten dengan penelitian Suliska yang menemukan komponen flavonoid, tanin, dan saponin dalam ekstrak etanol daun rambutan.[17]

Tabel 1. Skrining Fitokimia

Uji	Hasil		Keterangan
	EEDJB	EEDR	
Flavonoid	+	+	Merah
Tanin	+	+	Hitam
Saponin	+	+	Terbentuk buih

Keterangan: EEDJB = Ekstrak Etanol Daun Jambu Biji, EEDR = Ekstrak Etanol Daun Rambutan.

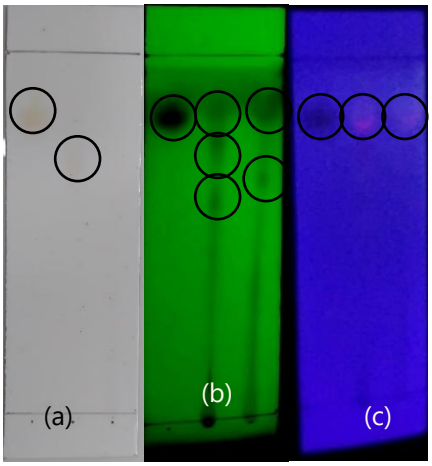
Hasil penetapan kadar air dan kadar abu total ekstrak etanol daun jambu biji (*Psidium guajava* L.) dan daun rambutan (*Nephelium lappaceum* L.) yang tercantum pada **Tabel 2** memenuhi standar <10% sesuai referensi.[21] Kedua ekstrak tidak memenuhi standar kadar abu tidak larut asam (**Tabel 2**), yang kemungkinan disebabkan oleh beberapa faktor, antara lain kesalahan teknik analisis, kontaminasi bahan baku, serta pengaruh suhu dan kondisi laboratorium terhadap hasil pengujian.[28]

Tabel 2. Standarisasi Ekstrak

No	Uji Standarisasi	Hasil	Standar[21]
1.	Organoleptis EEDJB		
	• Bau	Khas	Khas
	• Rasa	Pahit	Kelat
	• Warna	Hijau Pekat	Coklat Pekat
	• Bentuk	Kental	Kental
	Organoleptis EEDR		
	• Bau	Khas	Khas
	• Rasa	Pahit	Pahit
	• Warna	Hijau Pekat	Hijau Pekat
	• Bentuk	Kental	Kental
2.	Kadar Air		
	• EEDJB	6,7%	<10%
	• EEDR	6,8%	<10%
3.	Kadar Abu Total		
	• EEDJB	3,9%	<10%
	• EEDR	3,3%	<10%
4.	Kadar Abu Tidak Larut Asam		
	• EEDJB	4,7%	<0,7%
	• EEDR	4,6%	<0,7%

Keterangan: EEDJB = Ekstrak Etanol Daun Jambu Biji, EEDR = Ekstrak Etanol Daun Rambutan

Hasil uji Kromatografi Lapis Tipis (KLT) terhadap ekstrak etanol daun jambu biji dan daun rambutan menggunakan fase gerak campuran butanol:asam asetat:akuades (6:2:2) menunjukkan adanya senyawa flavonoid jenis kuersetin. Nilai Rf sebesar 0,88 yang diperoleh dari kedua ekstrak sesuai dengan nilai Rf senyawa pembanding kuersetin, mengindikasikan kesamaan komponen senyawa. Keberadaan flavonoid juga ditunjukkan oleh bercak berwarna merah pada sinar UV 366 nm. Secara umum, flavonoid ditandai dengan bercak berwarna biru, merah, atau hijau kekuningan di bawah sinar UV 366 nm, sedangkan pada sinar UV 254 nm bercak tampak gelap atau hitam dan berubah menjadi hijau kekuningan setelah disinari (**Gambar 1**).[25]



Gambar 1. (a) Pengamatan pada sinar tampak (b) Pengamatan pada λ 254 nm (c) Pengamatan pada λ 366 nm

Campuran ekstrak etanol daun jambu biji (*Psidium guajava* L.) dan daun rambutan (*Nephelium lappaceum* L.) diuji aktivitas antidiabetesnya pada tikus Wistar jantan. Pemilihan tikus jantan didasarkan pada stabilitas biologis yang lebih baik dibandingkan tikus betina, laju metabolisme obat yang lebih cepat, serta tidak terpengaruh oleh siklus estrus.[29] Hewan uji sebanyak 32 ekor dengan berat badan 150-200 g dibagi ke dalam delapan kelompok, yaitu kelompok sehat, kontrol negatif, kontrol positif, EEDJB 50 mg/kgBB, EEDR 70 mg/kgBB, kombinasi EEDJB:EEDR (50%:50%), (75%:25%), dan (25%:75%).

Induksi diabetes dilakukan menggunakan *streptozotocin*, senyawa diabetogenik yang bersifat toksik terhadap sel β pankreas melalui pembentukan radikal bebas dan spesies oksigen reaktif (ROS).[7] Mekanisme ini menyebabkan kerusakan sel β pankreas dan penurunan ekspresi GLUT2, sehingga mengganggu produksi insulin.[30] Dalam penelitian ini, *streptozotocin* diberikan secara intraperitoneal dengan dosis 45 mg/kgBB, yang ditetapkan berdasarkan berat badan tikus.[31] Dosis 40-45 mg/kgBB diketahui dapat menimbulkan gangguan insulin perifer dan menyerupai kondisi diabetes tipe 2.[32] Tikus dinyatakan menderita diabetes apabila kadar glukosa darah mencapai ≥200 mg/dL atau ≥135 mg/dL tiga hari setelah induksi *streptozotocin*.[7]

Tabel 3. Hasil Pengukuran Kadar Glukosa Darah Tikus Diinduksi STZ

Kelompok	Kadar Glukosa Darah (mg/dl)				Mean	%Penurunan KGD
	H-0	H-7	H-14	H-21		
Kontrol Sehat	94	105	106	89	98	5%
Kontrol Negatif	194	201	168	153	179	21%
Kontrol Positif	250	104	89	84	132	66%
EEDJB 50 mg/kgBB	200	183	159	132	167	34%
EEDR 70 mg/kgBB	238	177	163	146	181	39%
EEDJB;EEDR (50%;50%)	333	280	240	196	262	41%
EEDJB;EEDR (75%;25%)	317	278	213	131	235	59%
EEDJB;EEDR (25%;75%)	324	242	188	90	211	72%

Keterangan: EEDJB = Ekstrak Etanol Daun Jambu Biji, EEDR = Ekstrak Etanol Daun Rambutan

Tabel 3 menunjukkan bahwa kelompok kombinasi EEDJB:EEDR (25%:75%) memiliki persentase penurunan kadar glukosa darah tertinggi pada tikus, yaitu sebesar 72%. Penurunan ini lebih baik dibandingkan kelompok kontrol positif yang menunjukkan penurunan sebesar 66%, serta jauh lebih tinggi dibandingkan kelompok kontrol negatif yang hanya menurun 21%. Temuan ini mengindikasikan bahwa efektivitas penurunan kadar glukosa darah meningkat seiring dengan peningkatan dosis ekstrak.[33] Hal ini diduga karena pemberian dosis yang lebih tinggi meningkatkan konsentrasi senyawa aktif sehingga memperkuat sifat antidiabetik.[33]

Analisis statistik menggunakan uji Post Hoc Games-Howell menunjukkan bahwa kombinasi EEDJB:EEDR (50%:50%) dibandingkan dengan (75%:25%) memiliki nilai $p > 0,05$, yang berarti tidak terdapat perbedaan signifikan antara kedua kombinasi tersebut. Sebaliknya, kombinasi (75%:25%) dibandingkan dengan (25%:75%) menunjukkan nilai $p < 0,05$, menandakan adanya perbedaan signifikan. Demikian pula,

kombinasi (25%:75%) dibandingkan dengan (50%:50%) juga menunjukkan perbedaan signifikan ($p < 0,05$). Secara keseluruhan, kombinasi ekstrak daun jambu biji (*Psidium guajava* L.) dan daun rambutan (*Nephelium lappaceum* L.) memberikan penurunan kadar glukosa darah yang lebih tinggi dibandingkan ekstrak tunggal, sehingga terbukti lebih efektif. Efektivitas ini diduga terkait dengan peningkatan jumlah senyawa aktif seperti flavonoid, tanin, dan saponin dalam kombinasi, yang menciptakan interaksi sinergis dan memperkuat efek antidiabetes. Namun, kombinasi ekstrak tidak dapat dianggap sebagai terapi penyembuhan diabetes, melainkan berperan menurunkan kadar glukosa darah dan membantu mencegah komplikasi akibat hiperglikemia.

Daun jambu biji diketahui mengandung metabolit sekunder seperti flavonoid, alkaloid, tanin, saponin, dan polifenol,[16] sedangkan daun rambutan mengandung flavonoid, tanin, dan saponin.[17] Flavonoid merupakan senyawa utama yang berperan dalam aktivitas antidiabetes, dengan mekanisme meningkatkan sensitivitas insulin, melindungi sel β pankreas dari kerusakan akibat ROS, merangsang sekresi insulin, serta mendukung regenerasi sel β pankreas.[6,34,35] Selain itu, flavonoid, khususnya quercetin, dapat menghambat penyerapan glukosa melalui GLUT2 di mukosa usus sehingga menurunkan absorpsi glukosa.[36] Aktivitas antidiabetes kombinasi ekstrak etanol daun jambu biji dan daun rambutan terutama disebabkan oleh kandungan flavonoid yang memiliki sifat antioksidan, mampu mengikat radikal bebas, dan memaksimalkan produksi insulin. Penelitian sebelumnya juga mendukung efektivitas kombinasi ekstrak tanaman, seperti kombinasi daun salam dan jambu biji yang menurunkan kadar glukosa darah sebesar 50–60% dalam 15 hari.[14,23,37]

Berdasarkan hasil penelitian ini, kombinasi ekstrak etanol daun rambutan dan jambu biji menunjukkan efek lebih kuat dalam menurunkan kadar glukosa darah pada tikus diabetes dibandingkan ekstrak tunggal. Namun, perlu diperhatikan potensi toksisitas, terutama pada kombinasi EEDJB:EEDR (25%:75%) yang menyebabkan kematian tikus. Oleh karena itu, penelitian lanjutan diperlukan untuk menentukan dosis optimal dan melakukan uji toksisitas.

SIMPULAN

Kombinasi ekstrak etanol daun jambu biji (*Psidium guajava* L.) dan ekstrak etanol daun rambutan (*Nephelium lappaceum* L.) menunjukkan efek sinergis dalam menurunkan kadar glukosa darah pada tikus model diabetes, dengan hasil yang lebih signifikan dibandingkan pemberian masing-masing ekstrak secara tunggal. Dosis kombinasi optimal yang memberikan penurunan kadar glukosa tertinggi adalah EEDJB:EEDR (25%:75%) dengan konsentrasi ekstrak 50 mg/kgBB dan 70 mg/kgBB, yang menghasilkan persentase penurunan sebesar 72%. Temuan ini memberikan dasar ilmiah untuk pengembangan formulasi fitoterapi berbahan kombinasi ekstrak daun jambu biji dan rambutan sebagai kandidat antidiabetik alami yang berpotensi mendukung terapi komplementer dan mengurangi ketergantungan pada obat sintetik.

UCAPAN TERIMA KASIH

Terima kasih disampaikan kepada Universitas Muhammadiyah Gombong atas fasilitas dan dukungannya dalam menyelesaikan penelitian ini.

KONFLIK KEPENTINGAN

Penulis menyatakan bahwa tidak terdapat konflik kepentingan dalam penulisan naskah ini.

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JURNAL ILMIAH MEDICAMENTO

Vol.11 No.2 (September 2025)
halaman 245-254
https://doi.org/10.36733/medicamento.v11i2.11781
e-ISSN: 2356-4814

Original Article

Formulasi dan Penentuan Nilai SPF (*Sun Protection Factor)* Sediaan Gel Ekstrak Bunga Krisan (*Chrysanthemum indicum* L.)

Formulation and Evaluation of Sun Protection Factor (SPF) of *Chrysanthemum indicum* L. Flower Extract Gel

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Diajukan : 04-06-2025
Direvisi : 18-08-2025
Disetujui : 28-09-2025

Kata Kunci:
Chrysanthemum indicum L., gel, SPF

Keywords:
Chrysanthemum indicum L., gel, SPF

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Abstrak

Paparan sinar ultraviolet (UV) dapat menyebabkan kerusakan kulit, sehingga diperlukan upaya perlindungan yang efektif. Salah satu pendekatan yang umum digunakan adalah aplikasi tabir surya yang mampu menyerap atau memantulkan sinar UV sebelum mencapai lapisan kulit dalam. Ekstrak bunga krisan (*Chrysanthemum indicum* L.) diketahui mengandung senyawa aktif seperti flavonoid, tanin, dan alkaloid yang berpotensi sebagai bahan aktif tabir surya alami. Penelitian ini bertujuan untuk memformulasi sediaan gel tabir surya berbasis ekstrak bunga krisan serta mengevaluasi nilai Sun Protection Factor (SPF) dan karakteristik fisikokimianya. Formulasi dilakukan dengan tiga variasi konsentrasi ekstrak, yaitu F1 (5%), F2 (10%), dan F3 (20%), menggunakan basis gel yang terdiri atas Carbopol 940, trietanolamin, gliserin, metil paraben, dan aquadest. Evaluasi fisik meliputi pengujian pH, viskositas, dan daya sebar. Hasil menunjukkan bahwa seluruh formulasi memiliki karakteristik fisik yang sesuai standar sediaan gel, dengan pH berkisar antara 4,25-4,66; viskositas antara 22,70-38,46 Pa·s; dan daya sebar antara 5,04-5,21 cm. Penentuan nilai SPF secara in vitro menggunakan spektrofotometri UV-Vis (290-400 nm) menunjukkan peningkatan nilai SPF seiring dengan peningkatan konsentrasi ekstrak, yaitu kontrol negatif (1,395), F1 (1,795), F2 (2,506), dan F3 (5,041). Hasil ini mengindikasikan bahwa ekstrak bunga krisan berpotensi dikembangkan sebagai bahan aktif tabir surya alami yang aman dan ramah lingkungan.

Abstract

Exposure to ultraviolet (UV) radiation can lead to various forms of skin damage, necessitating effective protective measures. One commonly employed approach is the use of sunscreen formulations capable of absorbing or reflecting UV rays before they penetrate deeper layers of the skin. The extract of *Chrysanthemum indicum* L. flowers contains bioactive compounds such as flavonoids, tannins, and alkaloids, which exhibit potential as natural sunscreen agents. This study aimed to formulate a gel-based sunscreen containing Chrysanthemum indicum flower extract and to evaluate its Sun Protection Factor (SPF) and physicochemical characteristics. The formulations were prepared with three extract concentrations: F1 (5%), F2 (10%), and F3 (20%), using a gel base composed of Carbopol 940 (0.5%), triethanolamine (0.5%), glycerin (10%), methylparaben (0.2%), and purified water. Physical evaluations included pH, viscosity, and spreadability tests. The results showed that all formulations met the standard criteria for gel preparations, with pH values ranging from 4.25 to 4.66, viscosities from 22.70 to 38.46 Pa·s, and spreadability between 5.04 and 5.21 cm. SPF values were determined in vitro using UV-Vis spectrophotometry (290-400 nm), revealing an increase in SPF with higher extract concentrations: negative control (1.395), F1 (1.795), F2 (2.506), and F3 (5.041). These findings suggest that Chrysanthemum indicum flower extract holds promise as a safe and environmentally friendly active ingredient for natural sunscreen development.

Cara mensitasi artikel (*citation style*: AMA 11thEd.):

Wintariani NP, Suryaningsih NPA, Ardinata IPR, Wartana IGNAW. Formulasi dan Penentuan Nilai SPF (*Sun Protection Factor)* Sediaan Gel Ekstrak Bunga Krisan (*Chrysanthemum indicum* L.). *J. Ilm. Medicam.*, 2025:11(2), 245-254, DOI: 10.36733/medicamento.v11i2.11781

PENDAHULUAN

Indonesia merupakan negara beriklim tropis dengan intensitas paparan sinar matahari yang tinggi sepanjang tahun. Studi atmosferik di wilayah Jakarta menunjukkan bahwa radiasi UVB memiliki kontribusi signifikan terhadap variabilitas ozon dan kondisi meteorologis, menegaskan tingginya intensitas radiasi UV di kawasan tropis.[1] Data prakiraan resmi dari BMKG pada awal tahun 2021 juga melaporkan indeks ultraviolet (UVI) di beberapa wilayah Indonesia berada pada rentang 9–12, termasuk kategori bahaya sangat tinggi hingga

ekstrem.[2,3] Perlindungan terhadap efek merugikan radiasi ultraviolet (UV) sangat penting untuk mencegah kerusakan kulit akibat stres oksidatif dan fotodegradasi biomolekul. Salah satu upaya utama yang efektif adalah penggunaan tabir surya, yang bekerja dengan cara menyerap, memantulkan, atau menghamburkan sinar UV sehingga mengurangi intensitas radiasi yang mencapai lapisan kulit lebih dalam.[4] Mekanisme perlindungan tabir surya terhadap radiasi UV meliputi dua cara utama: memantulkan sinar UV agar tidak mengenai permukaan kulit dan menyerap sinar UV sebelum menembus jaringan kulit.[5]

Efektivitas tabir surya dinilai menggunakan indikator universal yang disebut *Sun Protection Factor* (SPF).[6] SPF didefinisikan sebagai perbandingan antara jumlah energi sinar UVB yang diperlukan untuk menimbulkan eritema minimal pada kulit yang dilindungi tabir surya dengan kulit yang tidak dilindungi.[7] Pengukuran SPF dapat dilakukan secara in vitro menggunakan spektrofotometri UV-Vis. Pemanfaatan bahan alam dalam formulasi tabir surya masih terbatas, sementara senyawa kimia sintetis mendominasi produksi.[8] Penelitian menunjukkan bahwa kosmetik berbahan herbal dianggap lebih aman dibandingkan zat aktif sintetis karena memiliki risiko efek samping yang lebih rendah. Keunggulan bahan alami terletak pada kemampuannya mengurangi iritasi dan lebih mudah diterima oleh kulit.[9]

Bunga krisan (*Chrysanthemum* sp.) merupakan tanaman hias yang mengandung senyawa fitokimia seperti flavonoid, terpenoid, dan tanin. Flavonoid, melalui sistem konjugasi aromatiknya, mampu menyerap radiasi UVA (320–400 nm) dan UVB (280–320 nm), sehingga menghambat penetrasi radiasi ke lapisan kulit lebih dalam.[10] Selain itu, flavonoid dapat mencegah pembentukan *Reactive Oxygen Species* (ROS) akibat paparan UV dan menetralkan radikal bebas melalui donasi elektron atau atom hidrogen, sehingga menekan peroksidasi lipid dan melindungi DNA dari kerusakan.[11] Flavonoid juga berperan dalam memodulasi jalur transduksi sinyal seluler.[12] Terpenoid berfungsi menghambat reaksi berantai peroksidasi lipid yang dipicu ROS pada membran sel.[13] Triterpenoid, seperti asam ursolat dan asam oleanolat, meningkatkan produksi kolagen serta menekan aktivitas matrix metalloproteinases (MMPs) yang diinduksi sinar UV.[14] Tanin, sebagai senyawa polifenol dengan struktur aromatik berkonjugasi, mampu menyerap radiasi UV dan menghambat penetrasi sinar ke jaringan kulit. Selain itu, tanin memiliki aktivitas antioksidan dengan menetralisir radikal bebas, sehingga mengurangi stres oksidatif akibat paparan UV serta melindungi DNA dan lipid membran.[15] Alkaloid pada bunga krisan juga diketahui memiliki aktivitas fotoprotektif. Studi menunjukkan alkaloid dari famili Amaryllidaceae, seperti tazettine, serta fraksi alkaloid dari bunga *Eucharis caucana* dan *Zephyranthes carinata*, mampu menurunkan produksi ROS dan IL-6 pada sel keratinosit yang terpapar UVB, sehingga memberikan efek perlindungan kulit dan aktivitas antiinflamasi.[16] Analisis UV-Vis terhadap flavonoid dan tanin menunjukkan panjang gelombang maksimum masing-masing 465 nm dan 765 nm.[17]

Secara umum, antioksidan adalah senyawa yang mampu menghambat atau menetralkan radikal bebas sehingga mencegah kerusakan sel dan jaringan. Radikal bebas merupakan molekul atau atom dengan elektron tidak berpasangan yang sangat reaktif dan dapat merusak DNA, protein, dan lipid.[18] Antioksidan fotoprotektif berfungsi melindungi kulit dari kerusakan akibat radiasi UV dengan menetralkan radikal bebas yang terbentuk saat kulit terpapar sinar matahari.[19] Paparan UV, terutama UVA dan UVB, dapat memicu stres oksidatif, kerusakan DNA, degradasi kolagen dan elastin, serta inflamasi kulit.[20] Antioksidan mampu menghentikan reaksi berantai oksidatif, termasuk peroksidasi lipid, melindungi DNA, dan mengurangi inflamasi, sehingga mencegah kerusakan kulit akibat radiasi UV.[21]

Dengan demikian, diperlukan sediaan kosmetik yang mampu melindungi kulit dari sinar UV sekaligus menangkal radikal bebas, salah satunya melalui formulasi dengan nilai SPF tinggi. Gel ekstrak bunga krisan dapat diformulasikan menggunakan carbopol sebagai agen pembentuk gel. Carbopol ideal untuk pembuatan gel karena pada konsentrasi rendah mampu menghasilkan gel yang kental dan stabil, sehingga efisien secara biaya dan mengurangi penggunaan polimer.[22] Carbopol juga membentuk gel yang jernih, sesuai untuk kosmetik, gel topikal, maupun sediaan oftalmik.[23] Selain itu, carbopol memiliki fleksibilitas formulasi sehingga dapat dipadukan dengan humektan, kopolimer, atau sistem penghantaran obat untuk menyesuaikan viskositas dan pelepasan zat aktif.[24]

Penelitian sebelumnya melaporkan aktivitas antioksidan bunga krisan dengan metode DPPH menunjukkan nilai IC_{50} sebesar 137,99 ppm pada suhu pengeringan 50°C.[25] Penelitian lain menyebutkan aktivitas antioksidan gel ekstrak bunga krisan termasuk kategori kuat dengan nilai IC_{50} sebesar 75,03 ppm.[26] Sediaan gel dengan bahan aktif ekstrak umumnya lebih stabil dibandingkan krim dan salep.[27] Berdasarkan uraian tersebut, penelitian ini dilakukan untuk membuat gel ekstrak bunga krisan dan menguji sifat fisika-kimia serta menentukan formulasi dengan nilai SPF tertinggi dari tiga konsentrasi, yaitu 5%, 10%, dan 20%.

METODE PENELITIAN

Alat dan Bahan Penelitian

Neraca digital (Acis AD-300i), *waterbath*, *blender* (Philips dan Miyako BL-152 GF), oven (Memmert), Viskometer Brookfield MLVT 115, pH meter, *mixer* (Maspion), *rotary evaporator* (Ika RV 10), spektrofotometer UV-Vis (Shimadzu UV mini-1240V), dan Elisa *reader* (Epoch 2). Bahan-bahan berupa etanol 96% teknis (Brataco), ekstrak bunga krisan (*Chrysanthemum indicum* L.), carbopol (0,5%), trietanolamin (0,5%), gliserin (10%), metil paraben (0,2%), dan aquadest.

Prosedur Penelitian

Pembuatan Simplisia Bunga Krisan

Bunga krisan diperoleh dari Desa Asah Panji, Buleleng. Dilakukan sortasi basah dengan pencucian menggunakan air mengalir untuk menghilangkan kotoran, kemudian dikeringkan di bawah sinar matahari dengan penutup kain hitam hingga kering. Setelah itu dilakukan sortasi kering. Simplisia kering dihaluskan menggunakan *blender*, kemudian diayak dengan ayakan mesh 40.

Pembuatan Ekstrak Bunga Krisan

Sebanyak 1 kg serbuk simplisia dimasukkan ke dalam wadah kaca bertutup dan dimaserasi menggunakan etanol 96% sebanyak 5 liter. Hasil maserasi disaring, kemudian diuapkan menggunakan *rotary evaporator* pada suhu 40 °C hingga diperoleh ekstrak kental.[28]

Pembuatan Gel Ekstrak Bunga Krisan

Formula gel ditampilkan pada **Tabel 1** (total 100 g). Carbopol dikembangkan dalam 100 mL aquadest, diaduk 10-20 menit hingga larut sempurna. Tambahkan triethanolamin sedikit demi sedikit sambil diaduk hingga terbentuk massa gel. Selanjutnya tambahkan gliserin dan ekstrak (5 g, 10 g, 20 g) yang telah dilarutkan, homogenisasi selama 5-10 menit. Metil paraben yang telah dilarutkan dalam air panas ditambahkan ke dalam massa gel, diaduk hingga homogen, kemudian dimasukkan ke wadah. Pengadukan dilakukan perlahan untuk menghindari pembentukan gelembung. Proses pembuatan gel dilakukan pada suhu kamar (20-25°C).

Tabel 1. Formulasi Gel Ekstrak Bunga Krisan (*Chrysanthemum indicum* L.)

No.	Nama Bahan	Formulasi dan Komposisi (%b/b)			
		K(-)	F1 (5%)	F2 (10%)	F3 (20%)
1.	Ekstrak bunga krisan	-	5	10	20
2.	Carbopol	2	2	2	2
3.	Trietanolamin	2	2	2	2
4.	Gliserin	10	10	10	10
5.	Metil Paraben	0,2	0,2	0,2	0,2
6.	Aquadest	ad 100 g	ad 100 g	ad 100 g	ad 100 g

Analisis Fisika-Kimia Gel Ekstrak Bunga Krisan

Analisis fisika-kimia terhadap gel ekstrak bunga krisan dilakukan melalui beberapa pengujian. Uji organoleptik dilakukan dengan mengamati warna, bau, dan tekstur sediaan menggunakan panca indera untuk menilai tampilan fisiknya. Pengukuran pH dilakukan menggunakan pH meter yang telah dikalibrasi, dengan cara mencelupkan elektroda ke dalam gel hingga nilai pH terbaca pada layar. Uji daya sebar dilakukan dengan menimbang 0,5 g gel, meletakkannya di atas kaca berukuran 20×20 cm^2, kemudian mengukur diameter sebaran. Setelah itu, beban tambahan sebesar 50 g, 100 g, 150 g, dan 200 g diberikan secara bertahap dengan

interval satu menit, dan diameter sebaran diukur kembali setiap kali penambahan beban. Viskositas gel diukur menggunakan viskometer Brookfield dengan kecepatan putar 60 rpm setelah menempatkan 100 g sampel dalam alat hingga spindel terendam. Uji homogenitas dilakukan dengan mengoleskan 1 g gel pada kaca transparan, kemudian diamati apakah sediaan menunjukkan susunan yang homogen tanpa adanya butiran kasar.

Pengujian SPF (In Vitro)

Sebanyak 0,1 g gel ekstrak bunga krisan ditimbang dan dilarutkan dalam 25 mL etanol 96%, kemudian dihomogenkan hingga diperoleh larutan uji yang seragam. Spektrofotometer UV-Vis terlebih dahulu dikalibrasi menggunakan etanol 96% sebagai blanko, kemudian dilakukan pengukuran absorbansi pada rentang panjang gelombang 290–320 nm (wilayah radiasi UV-B yang bersifat eritemogenik) sebanyak tiga kali replikasi. Nilai SPF (*Sun Protection Factor*) dihitung secara in vitro menggunakan metode spektrofotometri UV-Vis sesuai persamaan **(1)**. Perhitungan dilakukan dengan mengalikan nilai absorbansi pada setiap panjang gelombang dengan konstanta Erythemal Effectiveness (EE) × Intensity (I) sebagaimana tercantum pada **Tabel 2**. Hasil perkalian tersebut dijumlahkan dan dikalikan dengan faktor koreksi sebesar 10 untuk memperoleh nilai SPF akhir.[29]

$$SPF = CF\ x\ \sum_{290}^{320} EE(\lambda) x\ I\ (\lambda) x\ A\ (\lambda) \quad \textbf{(1)}$$

Keterangan:
EE(λ): Spektrum efek eritemal
I(λ): Intensitas spektrum sinar
A(λ): Absorbansi produk tabir surya
CF: Faktor koreksi (10)[30,31]

Tabel 2. Nilai EE x I pada Panjang gelombang 290 – 320 nm

Panjang Gelombang (λ nm)	EE x I
290	0,0150
295	0,0817
300	0,2874
305	0,3278
310	0,1864
315	0,0839
320	0,0180
Total	1

HASIL DAN PEMBAHASAN

Skrining fitokimia ekstrak bunga krisan

Hasil pengujian fitokimia terhadap ekstrak etanol 96% bunga krisan (*Chrysanthemum indicum* L.) menunjukkan adanya kandungan flavonoid, tanin, dan alkaloid (**Tabel 3**). Uji flavonoid memberikan hasil positif yang ditandai dengan perubahan warna larutan menjadi jingga. Flavonoid merupakan metabolit sekunder yang berperan dalam fotoproteksi melalui penyerapan sinar UV serta berfungsi sebagai penangkal *reactive oxygen species* (ROS) dengan mekanisme donor elektron/hidrogen; mekanisme struktur-fungsi ini telah didokumentasikan secara komprehensif.[32] Uji tanin juga menunjukkan hasil positif ditandai dengan perubahan warna menjadi biru kehitaman serta terbentuknya endapan jingga setelah penambahan reagen Dragendorff. Tanin memiliki kemampuan menyerap radiasi UV, khususnya pada rentang UV-B (280–320 nm), sehingga turut berkontribusi terhadap efek protektif kulit.[33] Selain itu, Selain itu, hasil positif pada uji alkaloid menunjukkan adanya senyawa alkaloid yang berperan dalam menangkap ROS dan menghambat reaksi oksidatif berantai, sehingga mampu mencegah peroksidasi lipid serta melindungi protein struktural kulit seperti kolagen dan elastin.[34]

Analisis fitokimia tersebut didukung oleh temuan penelitian sebelumnya, di mana analisis UPLC-Q-TOF/MS terhadap *Flos Chrysanthemi Indici* mengidentifikasi berbagai flavonoid dan asam fenolat, seperti luteolin dan linarin, sebagai konstituen dominan yang berkontribusi terhadap aktivitas antiinflamasi dan antioksidan ekstrak.[35] Penelitian lain oleh Zhou et al.[36] juga melaporkan bahwa profil UPLC-Q-TOF/MS ekstrak *C. indicum* menunjukkan keberadaan sejumlah flavonoid dan glikosida, termasuk buddleoside, sehingga memperkuat bukti kualitatif hasil skrining fitokimia dan menjelaskan potensi bioaktivitas ekstrak yang diamati dalam penelitian ini.

Tabel 3. Hasil Fitokimia Ekstrak Bunga Krisan (*Chrysanthemum indicum* L.)

No.	Senyawa Fitokimia	Pereaksi	Hasil pengujian	Keterangan
1.	Flavonoid	500 mg serbuk magnesium p + 1 ml HCl pekat	Larutan berwarna jingga	(+)
2.	Tanin	3 tetes larutan FeCl 1%	Biru kehitaman	(+)
3.	Alkaloid	Tabung I HCl 0,1 N + *Dragendrof* Tabung II HCl 0,1 N + *mayer*	Endapan jingga Endapan putih	(+) (+)
4.	Saponin	Aquadest 5 ml + HCl 1 N	Tidak berbusa	(-)

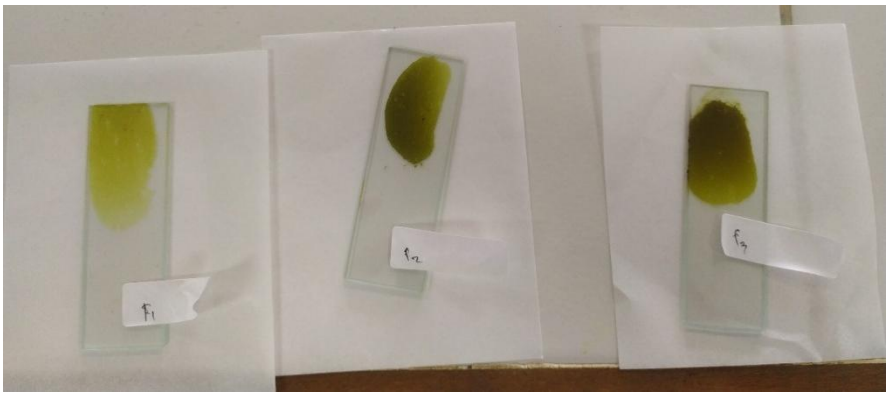
Keterangan:
(+): positif mengandung senyawa fitokimia
(-): negative mengandung senyawa fitokimia

Evaluasi fisikokimia gel bunga krisan

Uji organoleptik dilakukan untuk mengamati bentuk, warna, dan bau sediaan gel secara visual. Seluruh formula (F1 5%, F2 10%, F3 20%), dan kontrol negatif [K(-)]menunjukkan konsistensi setengah padat dengan warna kuning bening hingga kuning kecoklatan. Peningkatan konsentrasi ekstrak bunga krisan menyebabkan warna gel menjadi lebih pekat, sedangkan kontrol negatif tanpa penambahan ekstrak berwarna bening transparan.

Sediaan dengan penambahan ekstrak menunjukkan aroma khas aromatik yang berasal dari komponen volatil dalam ekstrak etanol 96% bunga krisan, sedangkan kontrol negatif tidak berbau. Perbedaan warna dan aroma antarformula disebabkan oleh peningkatan jumlah zat aktif yang terkandung dalam gel, sejalan dengan penelitian sebelumnya yang menyatakan bahwa peningkatan konsentrasi ekstrak berpengaruh terhadap intensitas warna dan aroma sediaan.[37] Hasil pengamatan visual terhadap perbedaan warna masing-masing konsentrasi dapat dilihat pada hasil pengujian homogenitas (**Gambar 1**).

Pengujian homogenitas menunjukkan bahwa sediaan gel antioksidan ekstrak etanol 96% bunga krisan memiliki distribusi komponen yang merata. Hal ini ditandai dengan tidak ditemukannya butiran kasar, gumpalan, maupun partikel yang tidak terlarut dalam basis gel. Homogenitas merupakan parameter penting karena memastikan bahan aktif dan bahan tambahan terdispersi secara seragam, sehingga setiap bagian sediaan memiliki sifat fisik, dosis, dan penampilan yang konsisten. Sediaan gel dinyatakan homogen apabila warna tampak merata dan tidak terdapat partikel dengan karakteristik berbeda.[38] Hasil pengamatan homogenitas dapat dilihat pada **Gambar 1**, yang menunjukkan sediaan gel dengan konsentrasi ekstrak 5%, 10%, dan 20%.



Gambar 1. Uji Homogenitas Gel Ekstrak Bunga Krisan (*Chrysanthemum indicum* L.)

Data hasil uji daya sebar sediaan gel ekstrak bunga krisan dapat dilihat pada **Tabel 4**. Nilai rata-rata daya sebar diperoleh sebesar 5,03 ± 0,00 cm untuk kontrol negatif, 5,04 ± 0,09 cm (F1), 5,21 ± 0,03 cm (F2), dan 5,21 ± 0,04 cm (F3). Nilai daya sebar yang relatif seragam menunjukkan konsistensi viskositas antarformula yang baik. Daya sebar yang optimal penting untuk memastikan kemampuan gel menyebar merata pada permukaan kulit, sehingga bahan aktif dapat berpenetrasi dengan baik.[39] Secara umum, peningkatan konsentrasi ekstrak sedikit meningkatkan daya sebar akibat perubahan densitas matriks gel.

Tabel 4. Hasil Uji Daya Sebar Gel Antioksidan Ekstrak Bunga Krisan (*Chrysanthemum indicum* L.)

Replikasi	Daya Sebar (cm)			
	K(-)	F1 (5%)	F2 (10%)	F3 (20%)
Replikasi 1	5,06	4,94	5,18	5,16
Replikasi 2	5,01	5,16	5,20	5,22
Replikasi 3	5,03	5,01	5,25	5,25
Rata-rata	5,03	5,04	5,21	5,21
SD	0,02	0,09	0,03	0,04

Uji pH bertujuan untuk memastikan keamanan sediaan terhadap kulit, karena pH yang terlalu asam dapat menyebabkan iritasi, sedangkan pH yang terlalu basa dapat menimbulkan kekeringan kulit.[5] Hasil uji pH ditampilkan pada **Tabel 5**, dengan nilai pH kontrol negatif sebesar 6,05 ± 0,04; F1 (4,66 ± 0,05); F2 (4,35 ± 0,08); dan F3 (4,25 ± 0,05). Seluruh formula memiliki pH yang masih berada dalam rentang ideal kulit, yaitu 4,5–6,5.[40]

Penurunan pH seiring peningkatan konsentrasi ekstrak disebabkan oleh sifat asam dari komponen fitokimia, terutama flavonoid dan tanin.[41] Temuan ini sejalan dengan penelitian Puspita dkk.,[42] yang menunjukkan bahwa peningkatan konsentrasi ekstrak etanol buah pepaya (1–5%) menurunkan pH sediaan krim secara signifikan ($p < 0,05$), serta penelitian Sari dkk.,[43] yang melaporkan penurunan pH emulgel daun mangga akibat penambahan ekstrak yang memiliki pH asam.

Tabel 5. Hasil Uji pH Gel Ekstrak Bunga Krisan (*Chrysanthemum indicum* L.)

Replikasi	pH			
	K(-)	F1 (5%)	F2 (10%)	F3 (20%)
Replikasi 1	6,06	4,72	4,45	4,31
Replikasi 2	6,00	4,64	4,32	4,23
Replikasi 3	6,09	4,61	4,27	4,20
Rata-rata	6,05	4,66	4,35	4,25
SD	0,04	0,05	0,08	0,05

Viskositas merupakan parameter penting yang menentukan stabilitas fisik dan kenyamanan pemakaian sediaan topikal. Pengukuran menggunakan spindle nomor 4 pada kecepatan 60 rpm menunjukkan bahwa seluruh formula memiliki viskositas dalam kisaran ideal untuk sediaan gel topikal, yaitu 2.000–50.000 cP.[44,45] Penurunan viskositas yang teramati seiring peningkatan konsentrasi ekstrak diduga terjadi akibat interaksi komponen aktif dengan basis gel yang melemahkan kekuatan jaringan polimer. Temuan ini konsisten dengan laporan Kusbandini pada gel semprot ekstrak daging buah alpukat[46] dan Limpongsa *et al*. pada gel *Curcuma comosa*,[47] yang menunjukkan bahwa variasi kadar ekstrak dapat memengaruhi viskositas sediaan. Mekanisme penurunan viskositas juga berkaitan dengan penurunan pH akibat sifat asam ekstrak, yang menurunkan derajat ionisasi gugus karboksilat pada Carbopol. Akibatnya, pembengkakan rantai polimer dan gaya tolak antarmolekul berkurang sehingga viskositas menurun. Fenomena ini sejalan dengan hasil penelitian Jaworski *et al*. dan Shiehzadeh et al., yang menjelaskan bahwa viskositas Carbopol sangat bergantung pada tingkat ionisasi polimer pada pH tertentu.[48,49] Secara reologis, hubungan terbalik antara viskositas dan daya sebar yang ditemukan dalam penelitian ini mendukung prinsip bahwa penurunan viskositas akan meningkatkan kemampuan sebar dan kenyamanan aplikasi sediaan.

Tabel 6. Hasil Uji Viskositas Gel Ekstrak Bunga Krisan (*Chrysanthemum indicum* L.)

Replikasi	Viskositas (cP)			
	K(-)	F1 (5%)	F2 (10%)	F3 (20%)
Replikasi 1	36,00	38,29	32,09	22,50
Replikasi 2	36,09	38,50	32,00	22,70
Replikasi 3	36,20	38,59	31,90	22,90
Rata-rata	36,10	38,46	32,00	22,70
SD	0,08	0,13	0,08	0,16

Pengujian Nilai SPF *(Sun Protection Factor)*

Hasil pengujian (**Tabel 7**) menunjukkan nilai SPF masing-masing formula: F1 (1,795), F2 (2,506), dan F3 (10,398). Berdasarkan klasifikasi tingkat proteksi, F1 dan F2 termasuk kategori proteksi rendah (SPF 2–9), sedangkan F3 menunjukkan proteksi sedang (SPF 10–29). Peningkatan nilai SPF seiring bertambahnya konsentrasi ekstrak menunjukkan korelasi positif antara kandungan senyawa aktif dan kemampuan penyerapan sinar UV.

Hasil ini sejalan dengan penelitian pada krim ekstrak bunga telang yang menunjukkan peningkatan nilai SPF dari 3,58 (1%) menjadi 12,83 (5%),[8] serta penelitian pada gel semprot bunga rosella yang menghasilkan nilai SPF 2,953–5,026 pada konsentrasi 10–20%.[50] Perbedaan nilai SPF antar penelitian dapat disebabkan oleh variasi jenis senyawa fitokimia, terutama flavonoid, tanin, dan alkaloid,[4] yang berperan dalam penyerapan radiasi UV. Dengan demikian, peningkatan konsentrasi ekstrak bunga krisan dalam sediaan gel berbanding lurus dengan nilai SPF yang dihasilkan, menunjukkan potensi ekstrak bunga krisan (*Chrysanthemum indicum* L.) sebagai bahan aktif tabir surya alami dengan efektivitas proteksi sedang.

Tabel 7. Hasil Uji Nilai SPF Gel Ekstrak Bunga Krisan (*Chrysanthemum indicum* L.)

Sampel	Replikasi	Kadar SPF	Rerata Kadar SPF	Kategori Proteksi
K (-)	1	1,329	1,395	Tidak Ada
	2	1,329		
	3	1,401		
F1 (5%)	1	2,798	1,795	Proteksi rendah
	2	1,257		
	3	1,329		
F2 (10%)	1	2,405	2,506	Proteksi rendah
	2	2,403		
	3	2,709		
F3 (20%)	1	10,353	10,398	Proteksi sedang
	2	10,425		
	3	10,416		

SIMPULAN

Ekstrak bunga krisan (*Chrysanthemum indicum* L.) terbukti mengandung senyawa bioaktif utama berupa flavonoid, tanin, dan alkaloid yang berpotensi sebagai bahan aktif tabir surya alami. Formulasi gel yang dikembangkan menunjukkan karakteristik fisikokimia yang stabil dan sesuai dengan standar mutu sediaan topikal. Aktivitas fotoprotektif yang diperoleh menunjukkan bahwa peningkatan konsentrasi ekstrak berbanding lurus dengan nilai SPF, hingga mencapai tingkat proteksi sedang pada konsentrasi tertinggi yang diuji. Temuan ini mengindikasikan potensi pemanfaatan ekstrak bunga krisan sebagai agen tabir surya berbasis bahan alam yang aman dan ramah lingkungan. Penelitian lanjutan disarankan untuk mengevaluasi aktivitas tabir surya secara in vivo, melakukan analisis kuantitatif senyawa aktif, serta mengoptimalkan konsentrasi ekstrak guna memperoleh efektivitas proteksi yang lebih tinggi.

UCAPAN TERIMA KASIH

Terima kasih kepada Universitas Bali Internasional yang telah memfasilitasi terlaksananya penelitian ini.

KONFLIK KEPENTINGAN

Penulis menyatakan tidak ada konflik kepentingan pada penelitian ini.

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JURNAL ILMIAH MEDICAMENTO

Vol.11 No.2 (September 2025)
page 255-266
https://doi.org/10.36733/medicamento.v11i2.11352
e-ISSN: 2356-4814

Original Article

Formulation and Compound Identification of *Citrus hystrix* DC Essential Oil by GC-MS for Mosquito Repellent Activity Against *Aedes aegypti*

Formulasi dan Identifikasi Senyawa Minyak Atsiri Citrus hystrix DC. dengan GC-MS untuk Aktivitas Repelan terhadap Nyamuk Aedes aegypti

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Submitted : 14-03-2025
Revised : 19-09-2025
Accepted : 30-09-2025

Keywords: *Aedes aegypti*, essential oil, GC-MS, kaffir lime fruit, repellent.

Kata kunci: *Aedes aegypti*, buah jeruk purut, GC-MS, minyak atsiri, repelan.

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Abstract

Plant-based mosquito control offers a promising alternative to reduce the health and environmental risks associated with chemical insecticides. *Citrus hystrix* DC. (kaffir lime), a member of the Citrus genus, produces essential oils rich in bioactive compounds such as limonene, citronellal, and terpinolene, which are known for antimicrobial and insecticidal properties. This study focused on extracting essential oil from kaffir lime fruit, identifying its chemical constituents using gas chromatography–mass spectrometry (GC-MS), and evaluating the repellent efficacy of its spray formulation against *Aedes aegypti*. Essential oil was obtained through steam distillation and analyzed by GC-MS to determine its chemical profile. Repellent activity was assessed experimentally using 100 adult mosquitoes divided into five treatment groups, with spray formulations prepared at concentrations of 5%, 10%, and 15%. Data was analyzed using one-way ANOVA followed by post-hoc tests. GC-MS analysis revealed 90 compounds, with five major constituents: D-limonene (14.57%), limonene (13.92%), citronellal (6.66%), terpinolene (6.47%), and α-terpineol (5.74%). Repellent testing demonstrated that the 15% concentration provided the highest protection against mosquito landings. These findings confirm the potential of kaffir lime essential oil as an effective natural mosquito repellent and provide a scientific basis for developing eco-friendly, plant-derived vector control products. This research contributes to reducing dependence on synthetic insecticides and supports sustainable strategies for vector management that prioritize human health and environmental safety.

Abstrak

Pengendalian nyamuk berbasis tanaman merupakan alternatif yang menjanjikan untuk mengurangi risiko toksisitas insektisida kimia. *Citrus hystrix* DC. (jeruk purut) adalah anggota genus Citrus yang menghasilkan minyak atsiri dengan potensi bioaktivitas, termasuk limonena, sitronelal, dan terpinolena yang diketahui memiliki sifat antimikroba dan insektisida. Penelitian ini bertujuan untuk mengekstraksi minyak atsiri dari buah jeruk purut, mengidentifikasi komponen kimia menggunakan gas chromatography–mass spectrometry (GC-MS), serta mengevaluasi efektivitas sediaan *spray* minyak atsiri sebagai repelan terhadap *Aedes aegypti*. Minyak atsiri diekstraksi melalui metode destilasi uap-air, kemudian dianalisis dengan GC-MS untuk menentukan profil senyawa. Uji repelan dilakukan secara eksperimental menggunakan 100 ekor nyamuk *Aedes aegypti* yang dibagi ke dalam lima kelompok perlakuan, dengan tiga variasi konsentrasi minyak atsiri (5%, 10%, dan 15%). Analisis data dilakukan menggunakan uji ANOVA satu arah dan uji lanjut post-hoc. Hasil GC-MS menunjukkan 90 senyawa teridentifikasi, dengan lima komponen dominan yaitu D-limonene (14,57%), limonene (13,92%), sitronelal (6,66%), terpinolena (6,47%), dan α-terpineol (5,74%). Uji efektivitas menunjukkan bahwa konsentrasi 15% memberikan daya proteksi tertinggi terhadap pendaratan nyamuk. Temuan ini menegaskan potensi minyak atsiri jeruk purut sebagai repelan alami yang efektif dan memberikan dasar ilmiah untuk pengembangan produk pengendalian nyamuk yang ramah lingkungan dan berkelanjutan. Hasil penelitian ini berkontribusi pada upaya pengurangan ketergantungan terhadap insektisida sintetis dan mendukung strategi pengendalian vektor yang lebih aman bagi kesehatan dan lingkungan.

How to cite: (citation style AMA 11th Ed.)
Juliadi D, Cahyaningsih E, Suena NMDS. Formulation and Compound Identification of *Citrus hystrix* DC Essential Oil by GC-MS for Mosquito Repellent Activity Against *Aedes aegypti*. *J. Ilm. Medicam.*, 2025:11(2), 255-266, DOI: 10.36733/medicamento.v11i2.11352

INTRODUCTION

Aedes aegypti is the primary vector of dengue virus, which causes Dengue Hemorrhagic Fever (DHF), a major public health concern in tropical countries including Indonesia.[1,2] Indonesia's tropical climate supports high mosquito density, contributing to recurrent dengue epidemics with significant morbidity and mortality.[3,4] Over the past five decades, the incidence of dengue hemorrhagic fever (DHF) in Indonesia surged from 0.05 cases per 100,000 person-years in 1968 to 77.96 cases in 2016, following a cyclical pattern with peaks every 6–8 years. Recently, Bali recorded the highest incidence with a case fatality rate below 1%, whereas non-endemic regions such as Papua experienced outbreaks marked by elevated fatality rates.[5] Mosquito control is considered the most reliable strategy to reduce human exposure to vectors.[6] Chemical repellents containing N,N-diethyl-meta-toluamide (DEET) are widely used but associated with skin irritation, hypersensitivity, and environmental concerns, while prolonged insecticide use can lead to resistance and ecological impact.[7,8] These limitations underscore the need for plant-based repellents that are safer and biodegradable.[9,10]

Indonesia is a major producer of essential oils, which are volatile compounds widely applied in perfumery, cosmetics, food, and pharmaceuticals.[11] Essential oils, also known as ethereal or volatile oils, are characterized by their distinctive aroma, volatility at room temperature, and solubility in organic solvents.[12] Among essential oil sources, *Citrus hystrix* DC. (kaffir lime) is notable for its high oil content and traditional culinary and medicinal uses. Its essential oil contains monoterpenes and sesquiterpenes such as limonene, citronellal, linalool, α-pinene, and terpinolene, which exhibit antimicrobial, antioxidant, and insecticidal properties.[13,14] Previous studies have identified limonene and citronellal as major constituents, but composition varies with plant part and extraction method.[15,16]

Steam distillation is a conventional and efficient technique for extracting essential oils from citrus matrices.[17,18] For chemical profiling, gas chromatography-mass spectrometry (GC-MS) is considered the gold standard, enabling separation and identification of complex mixtures with high accuracy, including minor components that influence aroma and bioactivity.[19,20] Standardized chemical characterization is critical to link composition with biological efficacy and ensure reproducible formulations.

Spray formulations offer practical advantages for repellent delivery, including ease of application, reduced microbial contamination, and prolonged contact time compared with other dosage forms.[21,22] Although kaffir lime oil has been explored for insecticidal and aromatherapy applications,[23,24] data on its effectiveness as a standardized spray repellent against *A. aegypti* remain limited. Furthermore, few studies integrate GC-MS-based profiling with repellent efficacy testing across multiple concentrations.

This study addresses these gaps by extracting essential oil from *C. hystrix* fruit via steam distillation, characterizing its chemical constituents using GC-MS, and evaluating the repellent activity of spray formulations at 5%, 10%, and 15% concentrations against *A. aegypti* under controlled laboratory conditions. The novelty lies in combining comprehensive chemical profiling with formulation and efficacy assessment, providing a scientific basis for developing eco-friendly, plant-derived mosquito repellents.

RESEARCH METHOD

Tools and Materials.

The equipment used included a steam distillation apparatus, analytical balance (Ohaus Pa 214), digital scale (ACIS BC-500), homogenizer (IKA T25 Digital Ultra Turrax®, Germany), Ostwald viscometer (Silberbrand, Germany), and universal pH indicator (Macherey-Nagel, Germany). Fresh *Citrus hystrix* (kaffir lime) fruits were sourced from Battal, Panji District, Situbondo, East Java (Identification: No. ID ELSA 60450). Additional materials

included propylene glycol (PT. Karunia Sejahtera Abadi SABA KIMIA, Indonesia), 96% ethanol (PT. Brataco, Indonesia), and spray containers, obtained from the Faculty of Pharmacy, Universitas Mahasaraswati Denpasar.

Study Design

This study employed a mixed-method approach combining descriptive and experimental designs. GC-MS analysis was conducted to characterize the chemical composition of kaffir lime essential oil.[25,26] Subsequently, an experimental evaluation assessed the physical properties and repellent efficacy of spray formulations against *Aedes aegypti* mosquitoes.[22,27]

Plant Determination

Botanical identification of kaffir lime (*Citrus hystrix* DC.) was performed at the Characterization Laboratory of the Eka Karya Bali Botanical Garden (BRIN). Morphological characteristics were compared with descriptions in standard botanical references to confirm species identity.

Distillation of Kaffir Lime Essential Oil

Kaffir lime (*Citrus hystrix* DC.) essential oil was obtained using the steam–water distillation method, a commonly applied and efficient technique for isolating volatile compounds from plant materials.[28] A total of 24 kg of fresh kaffir limes were sorted and washed to remove impurities, then cut into small pieces and reweighed. The prepared plant material was placed in the distillation chamber, while the lower compartment of the apparatus was filled with water and boiling chips prior to heating. Steam–water distillation was carried out for approximately 6–7 hours, and the extraction was terminated once no additional oil droplets were observed. The collected distillate was treated with 20 g of anhydrous sodium sulfate (Na_2SO_4) to remove residual moisture, and the resulting essential oil was stored in airtight, light-protected containers to prevent oxidative and photolytic degradation.[12,29] The chemical composition of the essential oil was subsequently analyzed using GC–MS to obtain an accurate profile of its volatile constituents.[30]

GC–MS Analysis of Essential Oil

Chemical profiling of the essential oil was performed using Gas Chromatography–Mass Spectrometry (GC–MS) in accordance with established analytical procedures for Citrus essential oils. The analysis was conducted with helium as the carrier gas at a flow rate of 1 mL/min and an injection temperature of 250 °C. The oven temperature program was set as follows: an initial temperature of 50 °C with a 2-minute hold, followed by a ramp of 2 °C/min to 80 °C, 5 °C/min to 150 °C, 10 °C/min to 200 °C, and 20 °C/min to 300 °C, with a final hold of 5 minutes. The MS interface was operated at a source temperature of 230 °C and a quadrupole temperature of 150 °C. For sample preparation, 25 µL of the essential oil was diluted in 1 mL of n-hexane, filtered through a 0.22 µm syringe filter, and transferred into GC vials, from which a 1 µL aliquot was injected for analysis. Compound identification was achieved by comparing retention times and mass spectra with reference spectra from the instrument's library database, using similarity indices, molecular weights, and peak intensities as confirmation criteria.[16,31] GC–MS is widely recognized as a robust and validated technique for the characterization and chemometric evaluation of Citrus essential oils.

Spray Formulation and Preparation

Four formulations with varying essential oil concentrations (including 0%, 5%, 10%, and 15%) were prepared (**Table 1**). Ethanol (96%) was used as a volatile solvent and vehicle in the spray formulation to facilitate solubilization of essential oil components and rapid evaporation upon skin application. Ethanol concentrations of 60–80% v/v are commonly employed in topical antiseptic and repellent sprays and are considered safe for use on intact skin. Previous studies have shown that ethanol at these concentrations is well tolerated, causing at most mild and reversible dryness without significant disruption of the skin barrier.[32–34] The inclusion of a humectant (propylene glycol) in the formulation helps maintain skin hydration and enhances user comfort.[35]

Table 1 Spray repellent Formula

Material	Quantity (ml)				Function
	F1	F2	F3	F4	
Kaffir lime fruit essential oil	-	1	2	3	Active Ingredients
Propylene Glycol	4	4	4	4	Co-solvent/ humectant
Ethanol 96%	ad 20	ad 20	ad 20	ad 20	Solvent

Remarks: Formulation codes represent increasing concentrations of kaffir lime fruit essential oil: F1 (0%), F2 (5%), F3 (10%), and F4 (15%).

The essential oil was first transferred into a beaker, followed by the addition of propylene glycol as a solvent. The mixture was homogenized until a uniform solution was obtained. Ethanol was then added gradually while maintaining mixing with the homogenizer. The resulting spray solution was transferred into a calibrated container and adjusted to a final volume of 20 mL using 70% ethanol.[22,36]

Physical Evaluation of Spray

Organoleptic evaluation was performed by observing the color, odor, and texture of the preparation. Homogeneity was assessed using glass slides to ensure uniform distribution of the active ingredients and solvents; a homogeneous preparation is indicated by the absence of precipitation and visible contamination.[37] The pH of the formulation was measured using universal pH paper to ensure its suitability for topical application, as the ideal skin pH ranges from 4.5 to 6.[38] Viscosity was determined using an Ostwald viscometer to characterize the flow properties of the preparation, as viscosity is closely related to its resistance to deformation and flow.[39]

Anti-Mosquito Repellency Assay

The anti-mosquito spray assay was conducted to evaluate the repellent activity of Citrus hystrix DC. essential-oil spray against Aedes aegypti. All procedures involving animals were carried out in accordance with the guidelines of the Universitas Udayana Animal Ethics Committee and were approved under ethical clearance No: B/280/UN14.2.9/PT.01.04/2022.

Repellency testing was performed in a 30 × 30 cm laboratory mosquito cage following standard entomological bioassay protocols.[40,41] Five rabbits (2.5–3.0 kg; 4–5 months old) obtained from Dusun Dukuh Pulu Tengah, Mambang Village, East Salamadeg, were used due to their sufficiently large dorsal surface area, which enables consistent and standardized exposure during repellency evaluation. All animals underwent a one-week acclimatization period under controlled conditions with ad libitum access to food and water.

On the day of testing, the dorsal hair of each rabbit was shaved evenly to ensure a uniform exposure area. The shaved skin was then cleansed using sterile gauze moistened with physiological saline. The mosquito spray formulation was applied evenly to the exposed dorsal area, after which each rabbit was placed into an individual mosquito cage containing adult Aedes aegypti. A control group received no treatment or a formulation containing 0% essential oil.

Observations were performed hourly from 0 to 6 hours following formulation application. During each interval, mosquito landing attempts, biting behavior, and avoidance responses were recorded. These parameters were used to quantify the repellency rate and overall protection efficacy of each test formulation. The study included five experimental groups: Group I served as the negative control (untreated animals), while Groups II, III, IV, and V received spray formulations containing 0%, 5%, 10%, and 15% essential oil, respectively. Repellent efficacy was assessed by comparing the proportion of mosquitoes repelled from animals treated with the test formulations to that of the control groups, which either received no treatment or a formulation lacking the active essential oil.[42,43] Mosquito repellency was calculated using the following formula:[44]

$$\%\text{Protection} = \frac{(C-T)}{C} X\ 100 \quad \text{..... (1)}$$

where C represents the number of mosquito landings or bites on the control area and T denotes the number of landings or bites on the treated skin area.

Statistical Analysis

Data was analyzed using SPSS version 21. Normality was assessed using the Shapiro–Wilk test, with $p > 0.05$ indicating a normal distribution. Differences among groups were evaluated using one-way ANOVA followed by appropriate post-hoc tests.[45] GC–MS results were presented descriptively based on chromatogram peak areas.

RESULTS AND DISCUSSION

This study aimed to characterize the chemical composition of kaffir lime (*Citrus hystrix* DC) essential oil using gas chromatography-mass spectrometry (GC-MS). Fruits were collected from Situbondo, East Java, and identified at the Characterization Laboratory of the Eka Karya Bali Botanical Garden (BRIN). Essential oil was extracted by steam distillation and subsequently analyzed using GC–MS under predetermined operational parameters. The steam-distilled essential oil yield obtained in this study was 0.363%. This value is lower than many published yields for Citrus hystrix reported in the literature, where yields typically range from approximately 0.9% to 1.8% depending on plant part and extraction conditions. For example, hydrodistillation of kaffir lime leaves has been reported to produce yields of ~0.98–1.38% (fresh vs. dried material), while steam-distillation of fruit peel/leaf samples in other studies reached yields up to ~1.8%.[15,29] Several factors likely account for this discrepancy, including the plant part used (fruit flesh vs. peel or leaves), pre-treatment (fresh vs. dried), harvest location and plant maturity, steam-distillation parameters (sample mass, distillation time, condenser efficiency), and possible post-harvest losses during storage. Meta-analyses and comparative studies highlight that peel and leaf material generally yield higher essential-oil percentages than whole fruit pulp, and optimized pre-treatments (e.g., drying, fermentation, steam-explosion) can substantially increase recoverable oil. Thus, the lower yield observed here does not negate the sample's chemical relevance but indicates differences in raw material and/or processing that should be noted when comparing across studies.[24,46]

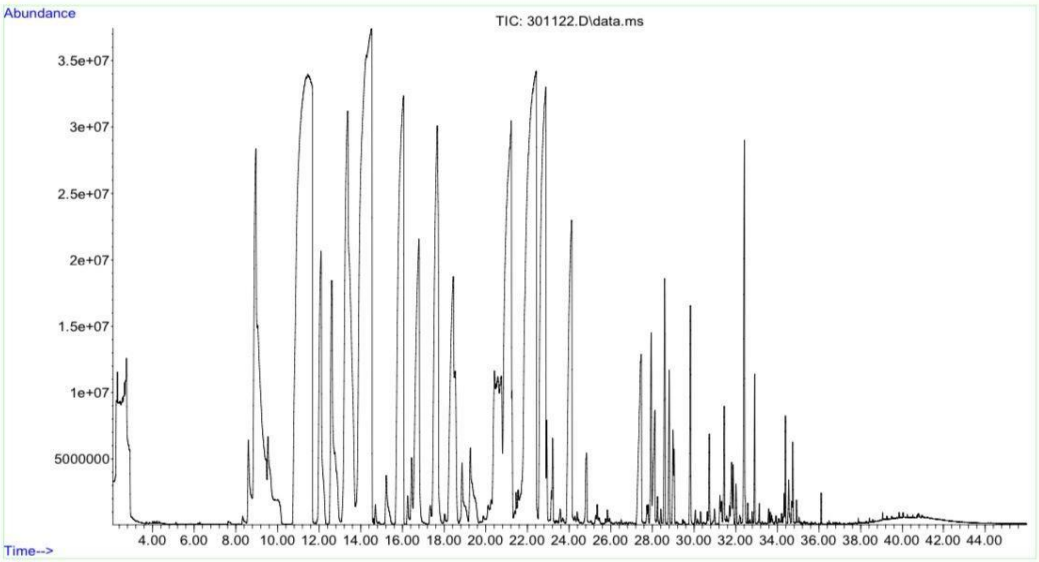

Figure 1. GC-MS chromatogram of kaffir lime (*Citrus hystrix* DC) essential oil showing the distribution of identified volatile constituents.

The resulting chromatogram (**Figure 1**) revealed 90 distinct peaks corresponding to 90 identified compounds, all exhibiting quality values above 80.[47,48] A summary of the 20 most abundant compounds among the 90 identified constituents of kaffir lime essential oil is presented in **Table 2**. Relative abundances were determined based on peak area percentages. The five most abundant constituents were D-limonene (14.57%, retention time 14.268 min), limonene (13.92%, retention time 11.376 min), citronellal (6.66%, retention time 21.222 min), terpinolene (6.47%, retention time 13.372 min), and α-terpineol (5.74%, retention time 22.876 min). Compared with previous studies analyzing peel or twig-derived oils, this investigation identified a greater

number of compounds, while key constituents such as limonene, sabinene, and citronellal remained consistent with earlier findings. Notably, the essential oil also contained linalool (46%), a compound recognized for its mosquito-repellent properties and its role as a contact toxin in insects. Overall, the GC–MS chromatogram (**Figure 1**) provides a comprehensive chemical profile of kaffir lime fruit essential oil, underscoring its potential as a natural and effective mosquito repellent.

The compound profiling results obtained in this study show notable differences from previous reports analyzing kaffir lime peel essential oil. The number of detected compounds was substantially higher than that reported by Iryani and Deka,[49] who identified only 27 constituents, although several major compounds—such as limonene, sabinene, and citronellal—were consistently observed in both studies. Simanjuntak et al.,[50] who examined essential oil derived from kaffir lime twigs, reported 12 compounds with citronellal as the major component. It is well known that essential-oil composition may vary according to plant part, cultivation location, and distillation conditions.[51,52] In this study, the kaffir lime fruits were sourced from Situbondo, a different geographical region from those investigated in previous studies, which may account for the identification of 90 distinct compounds. Furthermore, temperature control during the steam distillation process can influence the characteristics of the essential oil. Excessive temperature may cause oil droplets from the fruit to rise, resulting in a slightly yellowish coloration. This represents one of the limitations of the present study. Nevertheless, the number of identified secondary metabolites remained greater than that reported in several earlier investigations.

Table 2. Top 20 Chemical Constituents Identified by GC–MS from a Total of 90 Compounds in Kaffir Lime (*Citrus hystrix* DC) Fruit Essential Oil.

No	Compound name	Qual	%Area	Total
1	*D-limonene*	96	8.29%	14.57%
	D-limonene	96	6.28%	
2	*Limonene*	87	11.21%	
	Limonene	87	1.90%	13.92%
	Limonene	87	0.81%	
3	*Citronellal*	93	6.66%	6.66%
4	*Terpinolene*	98	6.47	6.47%
5	*Gamma terpinene*	97	6.16%	6.16%
6	*Alpha terpineol*	91	5.74%	
7	*Alpha pinene*	94	2.61%	5.16%
	Alpha pinene	96	2.55%	
8	*Isoterpinolene*	98	4.14%	4.14%
9	*Linalool*	96	2.51%	3.22%
	Linalool	97	0.69%	
10	*Ethyl propan carbonate*	94	2.85%	2.85%
11	*Citronellol*	97	2.66%	2.66%
12	*Alpha phallandrene*	90	1.55%	2.11%
	Alpha phallandrene	93	0.55%	
13	*Beta myrcene*	94	2.06%	2.06%
14	*Cyclofenchene*	87	2.00%	2.00%
15	*3-carane*	91	1.79%	1.79%
16	*Menthoglycol*	91	1.20%	1.20%
17	*Camphene*	97	0.87%	1.09%
	Camphene	97	0.87%	
18	*Neo-isopulegol*	99	1.01%	1.01%
19	*isoregol*	98	0.88%	0.88%
20	*Copaene*	99	0.62%	0.62%

Prior to assessing mosquito-repellent efficacy, the spray formulations underwent a four-week physical stability screening, conducted as a short-term preliminary evaluation to examine the integrity of the formulations during storage. The physical quality assessments included organoleptic evaluation, homogeneity testing, pH measurement, and viscosity determination. The organoleptic assessment aimed to characterize the

formulations in terms of physical appearance, color, and odor, as well as to evaluate their sensory acceptability for topical use.[53] All formulations appeared as clear liquids with the characteristic aroma of kaffir lime, and no changes in appearance, color, or odor were observed throughout the four-week storage period. These findings indicate organoleptic stability under the short-term screening conditions.

The pH measurement was included because pH is a critical chemical parameter for topical preparations that affects both formulation stability and skin tolerability. Maintaining an appropriate pH is crucial to prevent skin irritation, with the recommended range for topical products being 4.5–6.0.[38] The measured pH of the mosquito-repellent spray was 6.0, which falls within the acceptable range and suggests that the manufacturing and storage conditions maintained formulation integrity at room temperature. Homogeneity testing assessed the presence of undispersed particles or agglomerates; a formulation is considered homogeneous if no solid particulates or clumps are detectable.[53] No particulate matter or agglomeration was observed in the spray formulations, indicating satisfactory homogeneity and uniform dispersion of ingredients. Viscosity testing was carried out to determine the formulation's rheological properties, which influence sprayability and spreadability. All formulations exhibited viscosities below 150 cP (**Table 3**), a level considered suitable for easy dispensing via pump or trigger spray bottles and for achieving good spray distribution.[54] Taken together, the physical quality evaluations demonstrate that the spray formulations exhibited satisfactory short-term physical stability and retained appropriate characteristics for topical administration during the four-week storage period.

Table 3. Viscosity Measurements of Kaffir Lime Essential Oil Spray Formulations

Formula	Replication	Viscosity (cPs) on Week-			
		1	2	3	4
F1	1	1.849	1.444	1.775	1.459
	2	1.565	1.595	1.793	1.644
	3	1.793	1.778	1.729	1.694
	mean ± SD	1.736±0.150	1.606±0.167	1.766±0.033	1.599±0.124
F2	1	1.698	1.581	1.606	1.548
	2	1.596	1.669	1.718	1.557
	3	1.609	1.487	1.686	1.672
	mean ± SD	1.634±0.056	1.579±0.091	1.670±0.058	1.592±0.069
F3	1	1.683	1.873	1.805	1.668
	2	1.795	1.669	1.610	1.664
	3	1.787	1.646	1.825	1.714
	mean ± SD	1.755±0.062	1.729±0.125	1.747±0.119	1.682±0.028
F4	1	1.548	1.624	1.629	1.6293
	2	1.737	1.649	1.718	1.6300
	3	1.693	1.625	1.693	1.6884
	mean ± SD	1.659±0.099	1.633±0.014	1.680±0.046	1.649±0.034

Remarks: Formulation codes represent increasing concentrations of kaffir lime fruit essential oil: F1 (0%), F2 (5%), F3 (10%), and F4 (15%).

The mosquito-repellent activity of kaffir lime (*Citrus hystrix* DC) essential oil spray formulations was evaluated over a 6-hour observation period. The total number of mosquito landings and the calculated hourly protection percentages are presented in **Table 4** and **Table 5**, respectively. The base formulation (F1; 0% essential oil) demonstrated a low mean protection rate of 12.46%. Increasing concentrations of essential oil produced a clear concentration-dependent improvement in repellent efficacy: formulations containing 5% (F2), 10% (F3), and 15% (F4) essential oil achieved mean protection rates of 35.89%, 57.76%, and 93.76%, respectively. The F4 formulation provided the highest level of protection, maintaining 100% protection during the first three hours and 93.33% at hour four before a gradual decline occurred.

Table 4. Total Number of Mosquito Landings for Each Treatment Group During 6-Hour Observation

Treatment groups	Total Number of Mosquito Landings at Hour–					
	1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour	6th Hour
C	8	8	16	16	18	27
F1	7	6	14	15	16	25
F2	3	3	11	12	13	13
F3	2	2	5	8	10	10
F4	0	0	0	1	3	3

Remarks: F1–F4 correspond to spray formulations containing 0%, 5%, 10%, and 15% kaffir lime essential oil, respectively. C represents the negative control.

Statistical evaluation confirmed these observations. Shapiro–Wilk tests indicated normal distributions for all groups ($p > 0.05$), and Levene's test showed homogeneity of variance ($p = 0.150$). One-way ANOVA detected a significant effect of formulation on protection ($p < 0.001$). Post-hoc pairwise comparisons revealed significant differences between all treatment pairs ($p < 0.05$), indicating that each incremental increase in essential-oil concentration produced a statistically meaningful improvement in protection.

Table 5. Protection Percentage of Kaffir Lime Essential Oil Spray Formulations Over 6 Hours

Treatment	Protection percentage (%)						Average (%)
	1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour	6th Hour	
F1*	12.50	25.00	12.50	6.25	11.11	7.41	12.46
F2*	57.14	50.00	21.43	20.00	18.75	48.00	35.89
F3*	71.43	66.67	64.29	46.67	37.50	60.00	57.76
F4*	100.00	100.00	100.00	93.33	81.25	88.00	93.76

Remarks: F1–F4 correspond to spray formulations containing 0%, 5%, 10%, and 15% kaffir lime essential oil, respectively. The asterisks on the formulations indicate significant differences.

The observed concentration-dependent efficacy is consistent with reports for other botanical repellents (e.g., citronella), where higher active concentrations yield greater protection.[44,55] The activity of kaffir lime oil likely reflects the combined effects of major volatile constituents identified in this study—citronellal, limonene, linalool, and α-pinene—which are known to disrupt mosquito olfactory and sensory pathways. In particular, linalool has documented repellent and contact-toxic properties that can provoke avoidance or knockdown in insects.[56,57]

Female *Aedes aegypti* (2–5 days old) were used in accordance with standard testing guidelines to ensure biological relevance.[58,59] Testing conditions (controlled lighting, standardized spray volume) were applied to minimize procedural variability and support comparability across formulations.

Temporal decline in protection is attributable to volatilization of active components—a recognized limitation of essential-oil–based repellents. While regulatory guidance ideally targets ≥90% protection for six hours, many plant-derived repellents fail to sustain that duration because of rapid evaporation.[55,60] In this study, F4's maintenance of >90% protection for ≈4 hours nevertheless represents superior short-term performance relative to several reported botanical formulations. To advance product development, further work should focus on extending residual activity (e.g., microencapsulation, use of fixatives or extender matrices), testing across larger sample sizes and additional mosquito species, and conducting comprehensive stability and safety assessments (including skin-irritation and microbial tests) to support commercialization and regulatory claims.

CONCLUSION

Based on the findings of this study, the essential oil of kaffir lime was identified to contain 90 distinct compounds, with the five most abundant constituents being D-limonene (14.57%), limonene (13.92%), citronellal (6.66%), terpinolene (6.47%), and α-terpineol (5.74%). The formulated kaffir lime essential oil spray demonstrated effective mosquito repellent activity. Among all formulations, F4—containing 15% kaffir lime essential oil—exhibited the highest repellent efficacy, providing 93.76% protection. These results indicate that

kaffir lime essential oil possesses significant potential as a natural, plant-based mosquito repellent and may serve as a promising alternative to conventional synthetic repellents.

ACKNOWLEDGMENTS

The researchers would like to express their sincere gratitude to the Characterization Laboratory of the Botanical Garden, National Research and Innovation Agency "Eka Karya" Bali (BRIN), for their assistance in the botanical determination of the kaffir lime plant. Appreciation is also extended to the Faculty of Pharmacy, Universitas Mahasaraswati Denpasar, for providing financial support for this research.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this publication.

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