JURNAL ILMIAH MEDICAMENTO

Vol.11 No.1 (March 2025)
page 58-66
https://doi.org/10.36733/medicamento.v11i1.9914
e-ISSN: 2356-4814

Original Article

In-Vitro Cholesterol Reduction Using Three Dosages of Chitosan from Mangrove Crab Shells (*Scylla serrata*) in Effervescent Granules Suspension

Uji In-*Vitro* Penurunan Kadar Kolesterol dengan Perbandingan Tiga Dosis Kitosan Cangkang Kepiting Bakau *(Scylla serrata)* pada Suspensi Granul Effervescent

Erna Sri Novyanti[1], Fateha Rizka Andina[1], Laura Andrea Subekti[1], Hilya Nur Imtihani[2*]

[1]Diploma Three Program, Department of Pharmacy, Akademi Farmasi Surabaya, Surabaya, East Java, 60232, Indonesia.
[2]Faculty of Pharmacy, Universitas Hang Tuah, Jl. Arief Rahman Hakim No. 150, Keputih, Sukolilo District, Surabaya, East Java 60111, Indonesia.

Submitted: 26-09-2024
Reviewed: 22-01-2025
Accepted: 21-04-2025

Keywords: chitosan, cholesterol, effervescent, Lieberman Burchard, mangrove crab shell.

Kata Kunci: cangkang kepiting bakau, kitosan, kolesterol, effervescent, Lieberman Burchard.

Correspondence:
Hilya Nur Imtihani
hilya.nur@hangtuah.ac.id

Abstract

Changes in human lifestyle—such as smoking, lack of physical activity, and poor dietary habits—have contributed significantly to the increasing incidence of hypercholesterolemia. Most cholesterol-lowering drugs currently available are chemically synthesized and may be associated with undesirable side effects. Consequently, there is growing interest in developing safer, natural alternatives. Indonesia produces a large volume of crab shell waste, which presents an opportunity for the sustainable production of chitosan, a biopolymer known for its cholesterol-lowering potential. However, chitosan has limited solubility in water, which can hinder its absorption and usability. To address this limitation, chitosan was formulated into an effervescent granule suspension to enhance solubility and improve patient compliance through easier consumption in liquid form. The effervescent granule suspensions were prepared using the wet granulation method. This study aimed to evaluate their cholesterol-lowering effects using the in vitro Lieberman–Burchard method, employing three different chitosan doses: F1 (45 mg), F2 (55 mg), and F3 (75 mg), in order to determine the optimal dosage. The average cholesterol reduction percentages were F1 = 14.66% ± 2.12, F2 = 22.39% ± 6.06, F3 = 13.37% ± 2.99, and simvastatin = 19.02% ± 0.74. Although F2 exhibited the highest cholesterol-lowering activity, the differences among the three formulations were not statistically significant (p = 0.156).

Abstrak

Pola hidup manusia yang telah berubah menjadi kebiasaan yang tidak sehat seperti merokok, jarang berolahraga, dan pola makan yang buruk. Hal ini dapat menyebabkan hiperkolesterolemia. Namun, obat penurun kolesterol yang beredar di pasaran berasal dari bahan kimia yang memiliki efek samping tertentu, oleh karena itu diperlukan alternatif lain dari bahan alam. Indonesia memiliki banyak limbah cangkang kepiting yang dapat disintesis menjadi kitosan. Kitosan memiliki efektivitas sebagai penurun kolesterol. Namun, kitosan memiliki sifat kelarutan yang agak sulit dalam air sehingga alternatif sediaan yang dipilih adalah dibuat menjadi suspensi granul *effervescent*. Suspensi granul *effervescent* dibuat dengan metode granulasi basah. Selanjutnya, suspensi tersebut dilakukan uji penurunan kolesterol secara *in-vitro* metode *Lieberman Burchard* dengan simvastatin sebagai kontrol positif. Pada penelitian ini digunakan 3 formula dengan dosis kitosan yang berbeda, yaitu F1 = 45 mg, F2 = 55 mg dan F3 = 75 mg. Rata-rata hasil persentase penurunan kolesterol yaitu F1 = 14,66% ± 2,12; F2 = 22,39% ± 6,06; F3 = 13,37% ± 2.99 dan simvastatin = 19,02% ± 0,74. Dari hasil yang diperoleh dapat diketahui bahwa dosis kitosan 55 mg (F2) memiliki nilai penurunan kolesterol paling tinggi dibandingkan dengan F1 dan F3. Meskipun demikian, tidak terdapat perbedaan yang signifikan antara hasil ketiga formula dengan nilai *p-value* yang diperoleh sebesar 0,156.

How to cite: (citation style AMA 11th Ed.)
Novyanti, ES, Andina, FR, Subekti, LA, Imtihani, HN. In-Vitro Cholesterol Reduction Using Three Dosages of Chitosan from Mangrove Crab Shells (*Scylla serrata*) in Effervescent Granules Suspension. *J. Ilm. Medicam.*, 2025:11(1), 58-66, DOI: 10.36733/medicamento.v11i1.9914

INTRODUCTION

The modern lifestyle of people in Indonesia has led to lower awareness of health, especially on diet, so that health problems often arise. Fast food that contains more saturated fat is preferred by the public. If consuming fast food continuously, not balanced with sufficient exercise, there will be a buildup of fat in the blood vessels and cause hypercholesterolemia. Changes in the ratio of Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) play a role in the pathogenesis of Coronary Heart Disease (CHD), so it is necessary to manage the amount of Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) to avoid preventing coronary heart disease due to the formation of atherosclerotic plaques in the coronary arteries.[1]

The diversity of biota that has not been maximally utilized is the shelled animals of the Crustacea group. Crustacean waste is generally a source of chitosan. Mangrove crabs (*Scylla serrata*) are one of the largest groups of animals from the crab community compared to king crabs. Chitin content in shrimp shells reaches 42%-57%, while in crab shells it reaches 50%-60%.[2]

Chitin obtained from demineralization and deproteination process is converted into chitosan by deacetylation process.[3] Chitosan compounds can degrade contaminants, separate wastewater from petroleum residues, serve as seed coatings, exhibit anti-cholesterol and anticoagulant properties, and absorb heavy metals.[4] Chitosan is biodegradable, not digested by the body and reduces fat content by functioning as an absorbent.

Chitosan has anti-cholesterol effectiveness, research by Agustina (2014),[5] which compares the administration of chitosan doses of 35 mg, 45 mg and 55 mg in rats fed high trans fatty acid feed, states that chitosan can reduce total cholesterol levels in vivo. In the previous research, mangrove crab shell chitosan extract (*Scylla serrata*) prepared into solid dispersion system that was able to reduce cholesterol levels in vitro test results from a 55 mg chitosan solid dispersion sample: PVP K-30 = 1: 2. which obtained the best result of 29.56%.[6] This research looked at the comparison of 3 doses of chitosan (45 mg, 55 mg, 75 mg) to find out the most optimal dose for lowering cholesterol.

In this study, chitosan was prepared in suspension dosage form specifically effervescent granule suspension because chitosan have a limit solubility in water so that suspension can be an alternative to water-insoluble active ingredients in liquid formulation.[7] Effervescent preparations are acid and base formulations that can produce gas when reacting with water. This dosage form was chosen because it provides a fresh sensation and are able to cover the bitter taste of active substances when consumed. This drink is in demand and sells well because of its delicious taste and easy way of serving.[8] The effervescent granule suspension was prepared by wet granulation method. The wet granulation method is commonly employed when the active pharmaceutical ingredient is resistant to moisture and heat, and exhibits relatively poor flowability and compressibility. The method of making effervescent granules with wet granulation is carried out by separating the acid and base components so that premature carbonation reactions do not occur.[7]

The in vitro test was carried out with the Liberman Burchard test method. The principle of the Liberman Burchard test to identify steroid class compounds, one of which is cholesterol.[9] Lieberman Burchard uses a mixed reagent of anhydrous acetate and concentrated sulfuric acid. Testing is done using a UV-Vis spectrophotometer at a wavelength of 200-800 nm.[10]

This study aims to compare the effectiveness of chitosan doses of 45 mg, 55 mg, 75 mg and the positive control was Simvastatin 10 mg in reducing cholesterol levels with in vitro tests of effervescent granule suspension of mangrove crab shell chitosan (*Scylla serrata*).

RESEARCH METHOD

Tools. The tools used in this study were analytical balance (OHAUS-PA214), Spectrophotometer UV-Vis (Thermo Genesys 102), Oven (Memmert), Centrifuge (PCL-03), Ultrasonic (GT Sonic), mortar, stamper, beaker glass, stir bar, parchment paper, watch glass, spatula, tweezers, measuring cup, porcelain cup, and erlenmeyer.

Materials. Chitosan was synthesized from discarded mangrove crab (Scylla serrata) shells obtained from Layar Restaurant located in Surabaya, Indonesia. Granule effervescent materials such as anhydrous lactose (pharmaceutical grade) was purchased from Pharma Chemical, citric acid (pharmaceutical grade) and tartaric acid (pharmaceutical grade) from Dwilab Indonesia, PVP K-30 (pharmaceutical grade) from Aloin Labora, sodium bicarbonate (pharmaceutical grade) from Mitra Wacana Media, Xanthan Gum (pharmaceutical grade) from Aloin Labora, PEG 6000 (pharmaceutical grade) from Merck, Tween 80 (pharmaceutical grade) from Muda Berkah Jogja, ethanol 70% (pharmaceutical grade) from Medika and Aquadest.

Formulation of Effervescent Granules Suspension

The effervescent granule suspension formulation of chitosan extract of mangrove crab shell (*Scylla serrata*) was made by wet granulation method. Each formula was made with a dose comparison of chitosan to see its effect on the effectiveness of the in vitro test in reducing cholesterol levels. The 3 formulas as listed in **Table 1**.

Table 1 Effervescent Granule Suspension Formula[7]

Material Name	Material Function	F1 (%)	F2 (%)	F3 (%)
Chitosan	Active Ingredients	45 mg	55 mg	75 mg
Citric Acid	Acid Source	10	10	10
Tartric Acid	Acid Source	20	20	20
Sodium Bicarbonate	Base Source	30	30	30
PVP K-30	Binder	3	3	3
Xanthan Gum	Suspending Agent	1	1	1
Lactose ad	Fillers/Sweeteners	100	100	100
70% Alcohol	Wetting Agent	qs	qs	qs

Preparation of Effervescent Granule Suspension

Citric acid and tartric acid was put into a mortar and then crushed until homogeneous into an acid mixture. The acid mixture add with PVP K-30, xanthan gum, lactose then stirred until homogeneous and dripped little by little with 70% alcohol to form a callous mass into an acid granule mass. The acid granule mass was sieved with mesh no. 12 sieve into acid granule. The acid granule was placed on a baking sheet and then placed in an oven at 50°C for 30 minutes to become acid granule. Acid granule is sieved with mesh sieve no. 16 until there is no granule left and then put into a container to become acid granule.[11]

Sodium bicarbonate, PVP K-30, xanthan gum, and lactose were put into a mortar and crushed until homogeneous into a base mixture. The base mixture was dripped with 70% alcohol to form a callous mass into a base granule mass. The base granule mass was sieved with mesh sieve no. 12 to become base granule. The base granule was placed on a baking tray and then placed in an oven at 50°C for 30 minutes to become base granule. Base granule was sieved with mesh sieve no. 16 until there was no granule left and then put into a container to become base granule. Acid granule, base granule, and chitosan according to all formula were put into a tumbling jar and tumbled until homogeneous.[11]

Chitosan Characterization

Organoleptic Test

Organoleptic test is a test that uses the senses including texture, smell, taste and color produced from chitosan. Then adjusted to international standards, namely the shape of flakes to powder, white or yellowish color. odorless and tasteless.[12]

Moisture content

Chitosan was weighed as much as 1 gram in a porcelain cup then heated in an oven at 105ºC for 30 minutes then cooled in a desiccator and then weighed, the process was repeated until the weight was constant.

The quality requirement of chitosan water content is ≤10%. Calculation of water content can be calculated by the following formula.[13]

$$\%\ moisture\ content = \frac{a-b}{c} \text{ x } 100\%$$

a= weight of container + wet sample (grams)
b= weight of container + dry sample (grams)
c= weight of wet sample

Ash Content Test

The ash content test was carried out by weighing the cup then inserting the sample into the cup. in the cup and then weighed again. Then the cup and sample are heated on over the flame of a burner, then froze in an electric furnace with occasional openings at a maximum temperature of 550°C until complete ashing. opened at a maximum temperature of 550°C until complete ashing. After that cooled in a desiccator, then weighed until a constant weight was obtained. Weighed the results of the cup and sample that has been in the furnace.[14]

$$\%\ ash\ content = \frac{(w1-w2)}{w} \text{ x } 100\%$$

w = Sample weight (g)
w1 = Weight of sample + cup after ashing (g)
w2 = Weight of empty cup (g)

Ninhydrin test

Chitosan was weighed as much as 250 mg and then dripped with enough ninhydrin solution and allowed to stand for 5 minutes. Observe the color change that occurs, if the sample changes color to purple, it is true that there is an amine group (NH2) in the sample.[15]

Deacetylation degree test

Chitosan was weighed as much as 250 mg then added 1% KBr and then pulverized. The sample is inserted into the infrared spectroscopy (FTIR) holder and observed the value of the number that appears and the % transmittance Calculation of the degree of deacetylation can be calculated by the following formula.[16]

$$\text{Deacetylation Degree (DD)} = 97{,}67 - (26{,}486 \times \left(\frac{A1655}{A3450}\right))$$

A1655 = wavelength of hydroxy/amine group (NH_2)
A3450 = wavelength of acetyl group (CH_3CO)

In Vitro Measurement of Cholesterol Levels

Preparation of 1000 ppm Cholesterol Standard Solution

Weighing 100 mg of cholesterol powder and then dissolved with 100 mL of chloroform stir until dissolved then stored in the refrigerator at 2-8ºC.[10]

Determination of Maximum Wavelength

The 1000 ppm cholesterol standard solution that has been made is taken 1 mL and then put into a volumetric flask and then added to 5 mL of chloroform so that it becomes a 100 ppm cholesterol solution. become 100 ppm cholesterol solution. Added 1 mL of acetic anhydrate and added 0.1 mL of concentrated H_2SO_4 into the measuring flask then vortexed for 30 seconds and then immersed in cold water and stored in a dark place for 90 minutes. Measured using a UV-Vis spectrophotometer at a wavelength of wavelength of 200-800 nm.[10,17]

Preparation of Cholesterol Standard Solution Concentration Series

Cholesterol standard solution with a concentration of 1000 ppm made seven concentration series, namely 40 ppm; 50 ppm; 60 ppm; 70 ppm; 80 ppm; 90 ppm; and 100 ppm added 5 mL chloroform. Added 1 mL of acetic anhydride solution to each concentration of solution then vortexed for 30 seconds and immersed in cold water and stored in a dark place for 30 minutes.

Added 0.1 mL of concentrated H_2SO_4 solution to each solution concentration then vortexed for 30 seconds and immersed in cold water and stored in a dark place for 90 minutes. In a dark place for 90 minutes. The absorbance was measured at the maximum λ length that has been determined.[10]

Cholesterol Level Measurement In Vitro

Prepared a suspension of effervescent granule formula I, formula II, and simvastatin (positive control) 10 mg then each added 5 mL of 100 ppm cholesterol solution, each mixture was vortexed for 30 seconds and incubated for 60 minutes at 37°C then centrifuged for 5 minutes at 4000 rpm then filtered. Each mixture was taken supernatant as much as 5 mL and then transferred into a closed test tube. Added 1 mL of acetic anhydrate to each mixture then vortexed for 30 seconds and immersed in cold water and stored in a dark place for 30 minutes. Added 0.1 mL of concentrated H_2SO_4 to each mixture then vortexed and immersed in cold water and stored in a dark place for 90 minutes. dark place for 90 minutes after storage, each mixture was filtered before being measured. before being measured. The absorbance was measured at the maximum λ length according to the results of previous measurements. previous measurement. Percent reduction in cholesterol levels was calculated by formula.[6]

$$\% \text{ Decreased cholesterol levels} = \frac{\text{Initial cholesterol level} - \text{Final cholesterol level}}{\text{Initial cholesterol level}} \times 100\%$$

Statistical analysis

The data obtained were processed using statistical tests in SPSS. First, a normality test was conducted to see whether the data was normally distributed or not using the Shapiro Wilk test. Then a homogeneity test was conducted using the Levene Test. If the significance value ≥ 0.05 is said to be normal and homogeneous, then a parametric test can be conducted using one-way ANOVA. If the research data is not normally distributed and homogeneous, a nonparametric test Kruskal wallies is carried out.[18]

RESULT AND DISCUSSION

The results of chitosan evaluation which includes organoleptic test, moisture content test, ash content test, ninhydrin test, and deacetylation degree test can be seen in the **Table 2**.

Table 2. Evaluation results of mangrove crab (*Scylla serrata*) shell chitosan

Evaluation	Specifications	Results
Organoleptic		
- Shape	Powder	Powder
- Color	Yellowish white	Yellowish white
- Odor	Odorless	Odorless
- Taste	Flavorless	Flavorless
Moisture content (%)	≤10	9,33± 0,5773
Ash content (%)	≤36	5,93 ± 0,4509
Ninhydrin	Purple	Purple
Deacetylation degree (%)	≥70	81 ± 9,4749

From the results of the chitosan evaluation that has been carried out, it can be seen that all tests carried out meet the existing specifications starting from the organoleptic test (shape, color, smell, and taste) in the form of powder, yellowish white, odorless, and tasteless,[12] the moisture content results obtained 9.33% (≤10), the ash content results obtained 5.93% (≤36), the ninhydrin shows a color change to purple,[15] and the deacetylation degree results obtained 81% (≥70).[19]

This research begins by measuring the maximum wavelength with a concentration of 100 ppm in the range of 300-500 nm. The maximum wavelength obtained was 412 nm. According to the reference the maximum wavelength of cholesterol is at 410 nm.[10] Therefore, the maximum wavelength was chosen around 400 nm, namely 412 nm which shows the highest absorbance value.

After getting the maximum wavelength, the next step is to make a standard curve of cholesterol solution with seven variations. This standard curve can later be used to find a linear regression equation. The

linear regression equation obtained will be used to calculate the levels that have known absorbance. The absorbance of the standard curve shown in the **Table 3**.

Table 3 Absorbance Results of Concentration Series

Concenstration (ppm)	Absorbance
40	0,206
50	0,314
60	0,349
70	0,438
80	0,491
90	0,574
100	0,665

Based on the results of the concentration series curve it can be seen that the curve formed is in accordance with Lambert Beer's law, with the results of the value of a = -0.0759, b = 0.0073, and r2 = 0.995, so that the linear regression equation obtained is y = 0.0073x-0.0759. Standard curve can be seen in **Figure 1**.

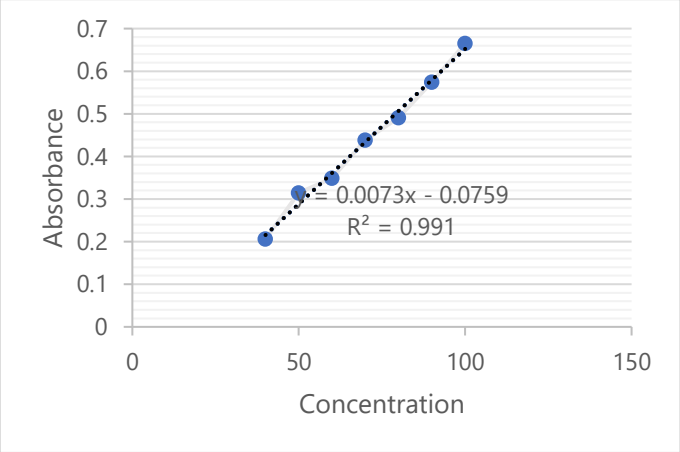

Figure 1. Concentration Series Absorbance Graph

From the correlation coefficient value obtained, it is stated that the correlation coefficient gives linear results because it meets the acceptable criteria of 0.99 or close to 1.00.[20] Measurement of cholesterol levels tested in vitro using the Lieberman-Burchard method. Then the results obtained are calculated the final cholesterol level using the linear regression equation that has been obtained. After obtaining the final cholesterol level, the percentage reduction in cholesterol levels of each sample and positive control was calculated. From the observations and calculations that have been made, the absorbance results and the percent reduction in cholesterol levels by chitosan are shown in **Table 4**.

Table 4. Results of in vitro test to reduce cholesterol levels of mangrove crab (*Scylla serrata*) effervescent granule suspension

Sample	Initial cholesterol level	Final cholesterol level		Reduction of cholesterol level		Average Reduction (%)
		Rep 1 (ppm)	Rep 2 (ppm)	Rep 1 (%)	Rep 2 (%)	
F1	100 ppm	86,84	83,82	13,16	16,16	14,66 ± 2,12
F2		73,32	81,90	26,68	18,1	22,39 ± 6,06
F3		84,51	88,75	15,49	11,25	13,37 ± 2,99
Simvastatin 10 mg		81,77	80,12	18,23	19,8	19,02 ± 0,74

From this research obtained that result of average reduction cholesterol level of F2 = 22.39 ± 6.06% is higher than F1 = 14.66% ± 2.12 and F3 = 13.37± 2.99 and positive control Simvastatin = 19.02 ± 0.74%. Thus F2 with a dose of 55 mg is better than F1 (45 mg) and F3 (75 mg) to reduce cholesterol levels. This can be caused by the high concentration of hydroxyl groups contained in it, so that it is linear to bind heavy metals

and cations from organic substances such as fats, this is in accordance with research conducted by Agustina (2014) which compared chitosan doses of 35 mg, 45 mg and 55 mg in rats fed high trans fatty acid feed, stating that chitosan 55 mg was proven effective in reducing total cholesterol levels in vivo in rats in the first two weeks of observation with a cholesterol level reduction value of 64.35% compared to chitosan 35 mg with a value of 59.15% and chitosan 45 mg with a value of 61.15%.[5] In addition, research conducted by Agustina (2014) also said that the maximum dose of chitosan to reduce cholesterol is 55 mg.[5] This is in accordance with the results obtained in F3 with a dose of 75 mg, where the results obtained lower cholesterol reduction when compared to doses of 45mg and 55mg.

Based on research by Imtihani et al. (2021)[6] the solid dispersion system of raw crab shell chitosan extract is able to reduce cholesterol levels in vitro test results from the 55 mg chitosan solid dispersion sample: PVP K-30 = 1: 2 which obtained the best result of 29.56% while in the sample of chitosan solid dispersion system 55 mg: PVP K-30 = 1:1 obtained a result of 18.44%. The cholesterol reduction level obtained in the solid dispersion system is higher than the effervescent granule suspension because solid dispersion formulation can improve their solubility by reduction of particle size so that can provide a better effect on lowering cholesterol levels.[21]

Chitosan is able to reduce total cholesterol levels because chitosan plays a role in inhibiting fat absorption. Chitosan has a hypocholesterolemic effect by increasing the excretion of neutral sterols and reducing cholesterol. excretion and reducing cholesterol. Chitosan has polycationic properties. The presence of hydroxyl and amino groups along the polymer chain polymer chain makes chitosan very effective in binding heavy metal ion cations and cations from organic substances (proteins and fats).[15] The main mechanism in chitosan that is thought to reduce cholesterol is by increasing bile acid excretion and reducing cholesterol absorption, similar to how cholysteramine drugs work.[22]

Subsequent to the results obtained of the percent reduction in cholesterol levels, the data that has been obtained is then processed using SPSS. The first stage is the normality test using the shapiro wilk method, the results obtained are the data obtained are not normally distributed. After the normality test, the homogeneity test was then carried out using the Levene test method, the results obtained were that the data was not homogeneously distributed. Because the data is not normally distributed and homogeneous, thus the Kruskal Wallis test was applied. And the results obtained were no significant difference between the sample and the positive control simvastatin 10 mg. The result of statistic analysis shown in the **Table 5**.

Table 5 Statistical Test Results

Statistics Test	Sig Requirements	Sig Results	Conclusions
Normality Test	≥ 0,05	0,00	Data is not normally distributed
Homogenity Test	≥ 0,05	0,00	Data is not homogeneously distributed
Kruskal Wallis	< 0,05	0,156	No significant difference

Conduct a normality test to see whether the data is normally distributed or not using the shapiro wilk test. If the significance value is ≥ 0.05, the data is normally distributed, while if the significance value is < 0.05, the data is not normally distributed. The normality test results obtained in FI, F2, F3 and positive control are 0.00 ≤ 0.05, so it can be concluded that the data is not normally distributed. The homogeneity test uses the Levene Test if the significance value ≥ 0.05 then the sample has almost the same variant (homogeneous) while if the significance value < 0.05 then the sample has an inhomogeneous variant. The homogeneity test results obtained are sig 0.00 ≤ 0.05, it can be concluded that the data is not homogeneously distributed[18]. Because the data were not normally distributed and homogeneous, the Kruskal Wallies statistical test was conducted. Kruskal wallies test obtained sig results 0.156 > 0.05 which means there is no significant difference between F1, F2, F3 and positive control so it can be concluded that the different doses of chitosan in F1, F2 and F3 have no significant effect on reducing cholesterol levels.

CONCLUSION

The in vitro test of cholesterol reduction using effervescent granule suspensions containing chitosan extracted from mangrove crab shells (*Scylla serrata*) demonstrated that the 55 mg chitosan dose achieved the highest average percentage reduction in cholesterol levels. Different concentrations of Chitosan, 45 mg, 55 mg, and 75 mg exhibited differences of cholesterol reduction level, however no significant differences were observed among the three formulas, as indicated by a p-value of 0.139.

ACKNOWLEDGEMENT

The authors would like to thank for financial support from Akademi Farmasi Surabaya for internal research grants agreement No. 013/AKFAR-SBY/PPPM/50.02/III/2024, and also thank for all facilities from Akademi Farmasi Surabaya.

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding this manuscript.

REFERENCES

1. Meidayanti D. Manfaat Likopen Dalam Tomat Sebagai Pencegahan Terhadap Timbulnya Aterosklerosis. J Med Hutama. 2021;02(03):2-6.
2. Dali S, Safitri NRD, Fawwaz M. Isolasi Kitosan Dari Limbah Cangkang Kepiting Bakau (Scylla serrata) dan Aplikasinya Terhadap Penyerapan Trigliserida. J Ilm As-Syifaa. 2016;8(2):20-27. doi:10.33096/jifa.v8i2.200
3. Agusta I. Ekstraksi Kitosan Dari Limbah Kulit Udang dengan Proses Deasetilasi. J Chem Eng. 2021;2(2):38-43.
4. Younes I, Rinaudo M, Harding D, Sashiwa H. Chitin and Chitosan Preparation from Marine Sources. Structure, Properties and Applications. Mar Drugs 2015, Vol 13, Pages 1133-1174. 2015;13(3):1133-1174. doi:10.3390/MD13031133
5. Agustina. Pengaruh Pemberian Kitosan Terhadap Kadar Kolesterol Total Tikus (Sprague-dawley) yang Diberi Pakan Tinggi Asam Lemak Trans. Inst Pertan Bogor. Published online 2014:1-31.
6. Imtihani HN, Permatasari SN, Prasetya RA. In Vitro Evaluation of Cholesterol-Reducing Ability of Chitosan from Mangrove Crab (Scylla serrata) Shell Solid Dispersion using PVP K-30 as a Carrier. J Farm Galen. 2021;7(2):99-109. doi:10.22487/j24428744.2021.v7.i2.15597
7. Rani KC, Parfati N, Muarofah D, Sacharia SN. Formulasi Granul Effervescent Herba Meniran (Phyllanthus niruri L.) dengan Variasi Suspending Agent Xanthan Gum, CMC-Na, dan Kombinasi CMC-Na-Mikrokristalin Selulosa RC- 591. J Sains Farm Klin. 2020;7(1):39. doi:10.25077/jsfk.7.1.39-51.2020
8. Pratama R, Saputro MR, Sani AR, Robiatul RS, Awaliyah. Pengaruh eksipien terhadap sifat fisik granul effervescent : Review. An-Najat J Ilmu Farm dan Kesehat. 2024;2(1):137-154.
9. Sahriwati, Sumarlin, Wahyuni S. Validasi Metode dan Penetapan Kadar Kolesterol Ayam Broiler. Lutjanus. Published online 2020:31-40.
10. Adu JK, Amengor CDK, Kabiri N, et al. Validation of a Simple and Robust Liebermann – Burchard Colorimetric Method for the Assay of Cholesterol in Selected Milk Products in Ghana. Published online 2019:1-7. doi:https://doi.org/10.1155/2019/9045938
11. Abdullah HS, Imtihani HN. Formulasi dan Evaluasi Granul Dispersi Padat Ekstrak Kitosan Cangkang Kepiting Bakau (Scylla serrata) Dengan Perbandingan Kitosan:PVP K-30 1:2. J Kefarmasian Akfarindo. 2022;7(1):45-51. doi:10.37089/jofar.vi0.119
12. Amelia RN, Aryati F, Sastyarina Y. Isolasi dan Karakterisasi Kitosan dari Limbah Cangkang Kerang Asia (Corbicula fluminea). Proceeding Mulawarman Pharm Conf. 2021;14:267-271. doi:10.25026/mpc.v14i1.583
13. Aguirre-Loredo RY, Rodríguez-Hernández AI, Morales-Sánchez E, Gómez-Aldapa CA, Velazquez G. Effect of equilibrium moisture content on barrier, mechanical and thermal properties of chitosan films. Food Chem. 2016;196:560-566. doi:10.1016/J.FOODCHEM.2015.09.065
14. Suresh HN, Mahalingam CA, Priyadharshini P. Physico - chemical Characteristics of Chitosan Extracted from Silkworm Pupae. Madras Agric J. 2013;100(10-12):883-886.
15. Agustina S, Swantara I made D, Suartha IN. Isolasi Kitin, Karakterisasi, dan Sintesis Kitosan Dari Kulit Udang. J Kim. 2015;9(2):271-278.

16. Heidari F, Razavi M, Bahrololoom ME, et al. Preparation of natural chitosan from shrimp shell with different deacetylation degree. Mater Res Innov. 2018;22(3):177-181. doi:10.1080/14328917.2016.1271591
17. Kenny AP. The determination of cholesterol by the Liebermann-Burchard reaction. Biochem J. 1952;52(4):611-619. doi:10.1042/bj0520611
18. Qurnia Sari A, Sukestiyarno Y, Agoestanto A. Batasan Prasyarat Uji Normalitas dan Uji Homogenitas pada Model Regresi Linear. Unnes J Math. 2017;6(2):168-177.
19. Imtihani HN, Permatasari SN. Sintesis dan Karakterisasi Kitosan dari Limbah Kulit Udang Kaki Putih (Litopenaeus vannamei). Simbiosa. 2020;9(2):129. doi:10.33373/sim-bio.v9i2.2699
20. Gupta A, Stead TS, Ganti L. Determining a Meaningful R-squared Value in Clinical Medicine. Acad Med Surg. Published online October 27, 2024. doi:10.62186/001C.125154
21. Imtihani HN, Prasetya RA, Permatasari SN. Preparation of Solid Dispersion Systems for Natural Chitosan from Mangrove Crab (Scylla serrata) Shell: Physical Characterization and In Vitro Cholesterol-Binding Evaluation. Res J Pharm Technol. 2024;17(3):1386-1392. doi:10.52711/0974-360X.2024.00219
22. Mmed GX, Huang Bmed X, Mmed LQ, Mmed JW, Bmed YH. Mechanism study of chitosan on lipid metabolism in hyperlipidemic rats. Asia Pac J Clin Nutr. 2007;16(1):313-317.