

Characterization of Bila Tea as a Natural Antioxidant in *Usada*-Based Product Development

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Abstract

Background: Bila Tea is a *Usada*-based herbal preparation formulated from amla (*Phyllanthus emblica* L.) fruit powder and avocado (*Persea americana* Mill.) seed powder in a 1:1 ratio, each 2 g tea bag containing 1 g of each component; both materials are used empirically in Bali, amla as *loloh* or decoction for fever, cough, and intestinal complaints and avocado seed as an astringent for digestive complaints.

Objective: This study aimed to characterize its compound profile and evaluate its *in vitro* antioxidant activity.

Methods: Volatile constituents were identified by gas chromatography–mass spectrometry (GC-MS), antioxidant activity was measured using the DPPH radical scavenging assay, and total phenolic, flavonoid, and tannin contents were determined spectrophotometrically, each reported as mean ± SD of three replicates.

Results: GC-MS detected twelve compound peaks in the Bila Tea extract; the largest relative peak areas corresponded to gamma-gurjunene (23.875%), 1H-cycloprop(e)azulene (14.440%), and delta-guaiene (11.988%). IC₅₀ values were 5.74 ppm for amla, 51.93 ppm for avocado seed, and 17.24 ppm for Bila Tea, corresponding to antioxidant activity indices (AAI) of 6.9653, 0.7702, and 2.3196, respectively. Bila Tea contained 2860.08 ± 0.27 mg GAE/100 g total phenolics, 354.47 ± 0.10 mg QE/100 g total flavonoids, and 18312.06 ± 6.50 mg TAE/100 g total tannins. These results indicate that Bila Tea exhibits very strong DPPH radical scavenging activity under the tested conditions. However, its activity was lower than that of amla alone and lower than the value predicted by dose additivity (combination index 1.67), providing no evidence of synergy between the two components.

Conclusion: The findings establish a preliminary chemical and antioxidant profile of Bila Tea; standardization, stability, safety, and *in vivo* evaluation remain necessary.

INTRODUCTION

Oxidative stress occurs when the formation of reactive oxygen species exceeds the capacity of the antioxidant system and contributes to various pathological processes. In Indonesia the burden of degenerative disease, notably stroke, cancer, diabetes mellitus and hypertension, has risen steeply over the past decade, and free-radical-mediated oxidative damage is one of the mechanisms implicated in its development. The practical problem this creates is twofold: long-term reliance on synthetic antioxidants and synthetic drugs carries a risk of adverse effects, while the plant-based preparations that communities already use as an alternative are, in most cases, still unsupported by chemical and pharmacological evidence. Antioxidants can reduce the reactivity of free radicals through electron or hydrogen atom donation mechanisms, so measuring antioxidant activity *in vitro* is widely used as an initial step in characterizing natural products.^{1–3}

The use of medicinal plants is part of the traditional Balinese healing practice known as *Usada*, a body of knowledge recorded in palm-leaf manuscripts such as the *Lontar Taru Pramana* and transmitted across generations, in which leaves, flowers, fruits, seeds, stems and roots are prepared as *loloh* (fresh juice), *jamu*, herbal tea and *boreh* and are believed to carry therapeutic value. One of the plants used in traditional medicine is amla (*Phyllanthus emblica* L.), known in Bali as *kalimoko* or *kalimaka*. Empirically the fruit is taken as *loloh* or as a decoction (*dekokta*) to relieve fever, cough, thirst, hemorrhoids and intestinal complaints and to restore general vitality, and within the Ayurvedic framework that underlies much *Usada* practice it is described as carrying all six tastes (*sad rasa*) with sour predominating and as

particularly suited to disorders of *pitta* (heat). Earlier work on Balinese aqueous preparations recorded an antioxidant capacity of 100 mg/100 mL (IC₅₀ 706 µg/mL) for amla *loloh* and 164 mg/100 mL (IC₅₀ 691.1 µg/mL) for the decoction, so the traditional water-based forms retain measurable activity. Chemically this activity is attributed to a material-specific set of marker metabolites rather than to phenolic compounds in general: ascorbic acid, gallic and ellagic acid, and the hydrolysable tannins emblicanin A and B, punigluconin, pedunculagin and corilagin. Recent studies have shown that *P. emblica* has a complex phytochemical profile and prominent antioxidant activity in various experimental models.^{4–9}

Avocado seeds (*Persea americana* Mill.) also contain secondary metabolites, but their phenolics, are of a chemically distinct type. In Balinese and wider Indonesian empirical practice, the seed decoction is drunk as an astringent for digestive complaints and to help moderate the rate of intestinal glucose absorption. In contrast to amla, the phenolic fraction of the seed is dominated by the flavan-3-ol monomers (+)-catechin and (–)-epicatechin together with condensed tannins (procyanidins B1 and B2 and their higher oligomers) and hydroxycinnamic acids such as 3-*O*-caffeoylquinic (chlorogenic) acid, accompanied by a substantial lipid fraction. Studies on avocado seed extracts and oils have demonstrated radical scavenging activity and measurable phenolic and flavonoid content. Because avocado seeds are often wasted, their use as a raw material for herbal preparations offers both product development value and the potential for utilizing byproducts.^{10–16}

Bila Tea was developed by combining amla fruit and avocado seed as a Usada-based herbal tea. The pairing was deliberate rather than arbitrary. Polyherbal formulation is itself a principle of Usada and Ayurvedic practice, in which materials with complementary *rasa* and complementary chemistry are blended so that the preparation is broader in action and more acceptable to drink than a single ingredient. Amla and avocado seed were selected because (i) their antioxidant constituents belong to different chemical classes, hydrolysable tannins with ascorbic and gallic acid on one side and condensed tannins with flavan-3-ols on the other, so that the blend draws on two mechanistically distinct pools of hydrogen-atom donors; (ii) their sensory profiles are complementary, the amla infusion being markedly sour and the avocado seed infusion bland, so that an equal-mass blend yields a neutral, tea-like brew (Table 1); and (iii) the combination pairs a high-value medicinal fruit with a processing by-product, which is directly relevant to the product-development aim of this study. Although both materials have individually been reported to contain phenolic compounds and exhibit antioxidant activity, information regarding the chemical profile and antioxidant characteristics of their combination in an herbal tea formulation remains limited. To our knowledge no published study has characterized the amla-avocado seed combination as a single herbal tea preparation, and none has reported its volatile constituent profile, its DPPH radical-scavenging activity and its phenolic, flavonoid and tannin contents together. That is the specific knowledge gap addressed here. Furthermore, antioxidant interaction between the two ingredients cannot be inferred solely from IC₅₀ values without a specifically designed combination analysis. Therefore, characterization of the formulation is necessary to provide preliminary scientific data regarding its phytochemical profile and in vitro antioxidant activity.^{13,14,17}

This study aimed to characterize the chemical profile of Bila Tea extract using GC-MS, evaluate its DPPH radical-scavenging activity, and determine its total phenolic, flavonoid, and tannin contents. The study was accordingly designed to answer three questions that follow directly from that gap: which constituents can be detected in the preparation, how strong its radical-scavenging activity is, and how the activity of the blend compares with that of each single component. Establishing this chemical and in vitro antioxidant baseline is the necessary first stage of a staged development pathway for a Usada-based product: it defines both the constituents present and the level of activity against which subsequent standardization, stability, safety and in vivo studies can be measured, and it supplies the preliminary scientific evidence required before a traditional preparation can be advanced towards a standardized herbal product.^{18–21}

METHODS

This study employed a descriptive-exploratory design and a laboratory experimental approach. The study was conducted from July to December 2025. Sample preparation was carried out at the Ayurvedic Health Laboratory, Faculty of Health, Universitas Hindu Indonesia. The DPPH assay and the total phenolic, flavonoid, and tannin analyses were performed at the Laboratory of the Faculty of Food Technology, Universitas Udayana, whereas GC-MS analysis was performed at the Laboratory of Pharmaceutical Chemistry, Universitas Mahasaraswati Denpasar.

Plant material, botanical determination and formulation

Amla fruit and avocado seeds were dried without direct sunlight, ground, and then formulated in a 1:1 ratio. Amla fruit was collected in Buleleng Regency and avocado seeds in Denpasar City, Bali, Indonesia, between July and August 2025. To ensure that the material analyzed corresponded to the intended species, voucher specimens of both raw materials were submitted for taxonomic determination before processing. Determination confirmed the identity of the fruit as *Phyllanthus emblica* L. (Phyllanthaceae) and of the seed as *Persea americana* Mill. Materials were selected while fresh and undamaged, air-dried in the shade without direct sunlight, milled, and sieved; from 20 kg of amla fruit 5 kg of dried simplicia was obtained, and from 4 kg of avocado seeds 1.35 kg of powder was obtained. The two powders were then formulated in a 1:1 (w/w) ratio. Each tea bag contained 2 g of powder, consisting of 1 g of amla powder and 1 g of avocado seed powder. This ratio was fixed a priori on three grounds. First, raw-material yield: the drying yields obtained (25% for amla fruit and 34% for avocado seed) make an equal-mass blend readily reproducible at pilot scale from locally available material. Second, dosing and palatability: a 2 g sachet is the conventional unit for a single-serve herbal tea bag, an equal split makes the dose of each component transparent to the user, and organoleptic screening (Table 1) showed that equal masses give a neutral, tea-like brew whereas amla-dominant blends retain the strongly sour character of the fruit. Third, comparability: an equal-mass blend can be compared directly against each single component at the same total mass, which is the arrangement required for the dose-additivity calculation reported below. Only this one ratio was prepared, so the comparison of combinations reported here is restricted to the 1:1 blend versus each single component and versus the Loewe dose-additivity prediction; a multi-ratio design (for example 3:1, 2:1, 1:1, 1:2 and 1:3 w/w) analyzed by the Chou-Talalay method would be required before any ratio could be described as optimal. For GC-MS analysis, 500 g of each powder (amla, avocado seed, and the 1:1 Bila Tea blend) was macerated in 500 mL of 96% ethanol for 3 × 24 h, the solvent being renewed every 24 h; the pooled filtrates were concentrated under reduced pressure in a rotary evaporator, yielding 8 g of amla extract and 13 g of avocado seed extract. For the DPPH assay and the determination of phenolics, flavonoids, and tannins, an aqueous infusion was prepared by steeping one 2 g tea bag in sterile water that had been boiled and allowed to cool to 80 °C, following standard laboratory procedures.²²

GC-MS analysis

GC-MS was selected as the first-line characterization technique for three reasons: it offers high resolving power and sensitivity for thermally stable constituents present at low concentration, it separates complex mixtures without prior fractionation, and reference mass-spectral libraries permit tentative identification when authentic standards are not available. Its analytical window must, however, be stated explicitly. The constituents that GC-MS resolves in these matrices are volatile and semi-volatile compounds, namely sesquiterpene hydrocarbons and oxygenated sesquiterpenes together with free fatty acids and their methyl esters. The constituents that dominate an aqueous infusion of these two materials, namely ascorbic acid, gallic and ellagic acid, hydrolysable tannins and proanthocyanidins, are non-volatile, thermolabile and highly polar; they are therefore not amenable to GC-MS without prior derivatization and do not appear in the chromatograms reported here. GC-MS was accordingly used to describe the volatile fingerprint of the preparation, while the non-volatile fraction was addressed separately by the spectrophotometric determinations of total phenolics, flavonoids and tannins. This division of labor is what makes the two data sets complementary rather than redundant.

GC-MS analysis was performed on a Shimadzu GC-MS QP-2010 system (Shimadzu, Kyoto, Japan) fitted with a DB-5MS fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). Ultra-high-purity helium was used as the carrier gas. The oven was held at 60 °C for 5 min and then ramped at 10 °C/min to 325 °C. Compounds were identified by matching their mass-fragmentation patterns against the NIST and Wiley 7 spectral libraries, and only peaks with a similarity index (SI) ≥ 90 were reported. Results are expressed as retention time (RT), peak area, relative area (%), and SI. Because no authentic reference standards were co-injected and no retention indices were calculated, all identifications are tentative.²⁰

Antioxidant activity and determination of bioactive contents

Antioxidant activity was determined using 0.1 mM DPPH (40 ppm) with a sample:DPPH ratio of 1:1 and readings at a maximum wavelength of 515.5 nm. Each sample and control were measured three times. The concentration range based on actual test data was 1-5 ppm for amla infusion and 10-50 ppm for avocado seed infusion and Bila Tea. The percentage inhibition was calculated from the change in absorbance compared to the control. The IC₅₀ was obtained

from the linear regression $y = bx + a$, where y is the percentage inhibition and x the sample concentration, as $IC_{50} = (50 - a)/b$. The antioxidant activity index was calculated as $AAI = [DPPH]/IC_{50}$ with $[DPPH] = 40$ ppm, and interpreted as poor ($AAI < 0.5$), moderate ($0.5-1.0$), strong ($1.0-2.0$), and very strong (> 2.0). All concentrations are expressed in ppm, where $1 \text{ ppm} = 1 \mu\text{g/mL} = 0.001 \text{ mg/mL}$. No synthetic positive control (ascorbic acid, Trolox, or BHT) was run in parallel; the IC_{50} values reported here are therefore internally comparable but not directly transferable between laboratories.^{3,23,24}

Total phenols were analyzed with the Folin-Ciocalteu reagent using gallic acid as a standard and read at 760 nm. Total flavonoids were analyzed using quercetin and $AlCl_3$ at 415 nm, while total tannins were analyzed using the Folin-Denis reagent with tannic acid as a standard at 725 nm. The calibration curves were $y = 0.0085x + 0.0025$ ($R^2 = 0.9997$) for gallic acid over 0–100 mg/L, $y = 0.0388x - 0.0050$ ($R^2 = 0.9978$) for quercetin over 0–25 mg/L, and $y = 0.0047x + 0.1148$ ($R^2 = 0.9980$) for tannic acid over 0–100 mg/L. Each analysis was performed three times. Values for each replicate were calculated using the calibration curve equation and dilution factor, then reported as the mean $\pm$ standard deviation (SD; $n=3$). In the data audit, phenol counts were recalculated from the three individual absorbances using the average value after blank correction before conversion through the calibration equation. Phenolic contents are expressed per 100 mL of infusion (mg GAE/100 mL) and flavonoid and tannin contents per 100 g of preparation (mg QE/100 g and mg TAE/100 g); these units are used consistently throughout the manuscript.^{25–27}

The analysis was descriptive. No inferential testing or formal synergy analysis was performed because the study design did not include the dose combination design required to calculate the combination index. Therefore, interpretation was limited to comparisons of the activity of each sample and in vitro characterization.

RESULT AND DISCUSSION

The actual formulation of Bila Tea analyzed in this study was 1:1, i.e. 1 g of amla powder and 1 g of avocado seed powder in every 2 g tea bag. This ratio was used consistently for interpretation of the results. Organoleptic screening of the three infusions showed that all were liquid, brownish-yellow and free of sediment but differed in taste and aroma: the amla infusion was distinctly sour with the characteristic aroma of the fruit, the avocado seed infusion was bland and neutral in aroma, and the equal-mass blend was neutral in taste with an herbal-tea aroma. This observation is the practical basis for the 1:1 ratio set out in the Methods, since the seed matrix moderates the pronounced sourness of amla and yields a brew acceptable as a beverage.

Compound characterization was performed by identifying active compounds using chromatography. Characterization was performed on each ingredient: amla fruit extract, avocado seeds, and Bila tea. The characterization results and chromatogram for amla fruit are presented in **Table 1** and **Figure 1**.

Table 1. Characterization of Active Compounds from Amla Fruit

Peak	RT	Area	Area (%)	Compound	SI
1	10.66	9980362	7.066	4,7- Methanoazulene1,2,3,4,5,6,7,8-1,4,9,9-tetramethyl	92
2	11.143	8430181	5.969	Trans-Caryophyllene	92
3	11.332	20486210	14.505	Azulene	96
4	11.610	23440016	16.596	Seychellene (CAS) 1,6-Methanonaphthalene	90
5	11.760	10547176	7.468	1H-3a,7-Methanoazulene	92
6	11.796	1614030	1.143	1H-Cycloprop(e)Azulene	90
7	12.240	15508224	10.980	Delta-Guaiene	96
8	13.314	1497913	1.061	(-)- caryophyllene oxide	92
9	14.268	2788303	1.974	Epiglobulol	90
10	14.538	20988912	14.861	Patchouli alcohol	93
11	17.131	10586847	7.496	Hexadecanoic acid	97
12	18.933	4334067	3.069	9-Octadecenoic acid	97
13	19.175	1965579	1.392	Octadecanoic acid, methyl ester	97

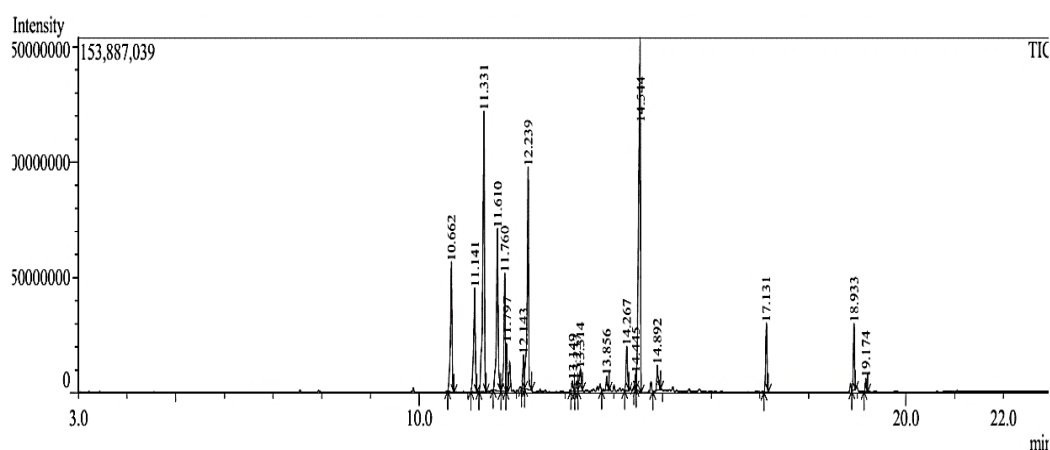


Figure 1. Chromatogram of Amla Fruit (*Phyllanthus emblica* L.)

In addition to amla fruit, characterization was also conducted on another component of Bila Tea, namely avocado seeds. The results of the avocado seed characterization and its chromatogram are presented in **Table 2** and **Figure 2**.

Table 2. Characterization of Active Compounds in Avocado Seeds (*Persea americana* Mill)

Peak	RT	Area	Area (%)	Compound	SI
1	10.657	7879383	6.463	1H-Cycloprop(e) azulene	92
2	11.139	6669024	5.470	Trans-Caryophyllene	96
3	11.325	16914259	13.873	Alpha-Guaiene	95
4	11.603	20949531	17.183	Seychellene (CAS) 1,6-Methanonaphthalene	93
5	11.756	9753660	8.000	1H-3a,7-Methanoazulene	91
6	11.856	1056754	0.867	Aromadendrene	93
7	12.234	13471160	11.049	Delta-Guaiene	96
8	13.311	1282199	1.052	(-)- Caryophyllene oxide	91
9	14.536	24939437	20.455	Patchouli alcohol	95
10	17.126	5427010	4.451	Hexadecanoic acid	97
11	18.925	1340430	1.099	9-Octadecenoic acid, methyl ester	97
12	19.171	861641	0.707	Octadecanoic acid, methyl ester (CAS) Methyl stearate	97

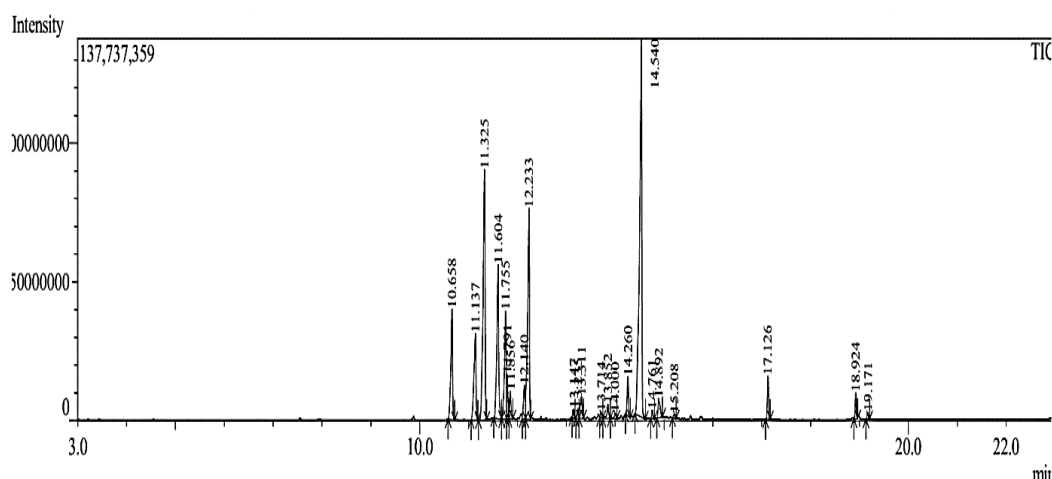
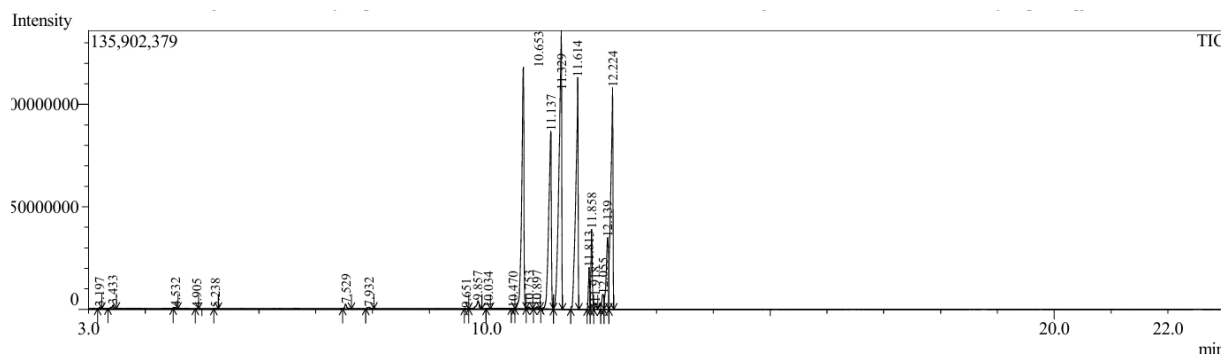


Figure 2. Avocado Seed Chromatogram (*Persea americana* Mill)

GC-MS analysis of the Bila Tea extract yielded 12 tentatively identified peaks (**Table 3, Figure 3**). The peaks with the largest relative areas included gamma-gurjunene (23.875%), 1H-cycloprop(e)azulene (14.440%), delta-guaiene (11.988%), alloaromadendrene (2.357%), and bicyclo (5,3,0) decane (1.769%). All twelve are volatile or semi-volatile terpenoid or fatty-acid-derived constituents, which is consistent with the analytical window of the technique as set out in the Methods; no phenolic acid, flavonoid or tannin appears in this list, and their absence reflects the non-volatility of those compound classes rather than their absence from the preparation. GC-MS identification in this study was tentative based on spectral match with the library; therefore, compound identity was not treated as absolute structural confirmation.²⁰

Table 3. GC-MS profile of Bila Tea Extract

Peak	RT (min)	Area	Area (%)	Compound	SI
1	7.529	606804	0.418	Menthol	98
2	9.651	130311	0.090	Decanoic acid, methyl ester (methyl caprate)	94
3	9.857	1492991	1.028	Cyclohexene	96
4	10.652	20975503	14.440	1H-Cycloprop(e)azulene	91
5	10.755	148880	0.102	4,7-Methanoazulene	93
6	10.897	175787	0.121	Trans-caryophyllene	96
7	11.609	34680893	23.875	Gamma-gurjunene	90
8	11.812	1597115	1.099	Seychellene	90
9	11.860	3424473	2.357	Alloaromadendrene	90
10	11.920	1739257	1.197	Gamma-gurjunene	94
11	12.055	2569120	1.769	Bicyclo(5,3,0)decane	91
12	12.225	17413855	11.988	Delta-guaiene	96


Figure 3. GC-MS Chromatogram of Bila Tea Extract

The DPPH data showed an increase in the percentage of inhibition with increasing concentration in all three samples (**Table 4**). Linear regression yielded IC_{50} values of 5.74 ppm for amla, 51.93 ppm for avocado seed, and 17.24 ppm for Bila Tea (**Table 5**). The AAI values were 6.9653, 0.7702, and 2.3196, respectively. Thus, Bila Tea exhibited very strong DPPH radical-scavenging activity according to the AAI classification used, but its activity remained lower than that of amla alone based on IC_{50} . These findings do not prove a synergistic effect and should not be interpreted as a synergistic interaction. The amla and avocado-seed IC_{50} estimates are slightly above the highest tested concentrations (5 and 50 ppm, respectively) and therefore represent limited extrapolations from the corresponding linear regressions.^{3,20,23}

Table 4. DPPH inhibition data at each concentration

Amla concentration (ppm)	Inhibition (%)	Avocado concentration (ppm)	Inhibition (%)	Bila Tea concentration (ppm)	Inhibition (%)
1	16.56	10	14.53	10	41.09
2	24.52	20	23.73	20	54.11
3	29.30	30	32.72	30	63.24
4	36.85	40	40.58	40	76.26
5	45.77	50	47.55	50	83.41

Table 5. Regression, IC_{50} , and Antioxidant Activity Index (AAI)

Sample	Regression equation	R^2	IC_{50} (ppm)	AAI	AAI Interpretation
Amla Fruit	$y = 7.0741x + 9.3754$	0.9913	5.74	6.9653	Very strong
Avocado seed	$y = 0.8289x + 6.9547$	0.9967	51.93	0.7702	Moderate
Bila Tea	$y = 1.0678x + 31.5872$	0.9920	17.24	2.3196	Very strong

The results of the determination of bioactive compounds after calculating three replicates showed that the total phenols of amla were 3486.43 ± 2.32 mg GAE/100 mL, avocado seeds 838.90 ± 0.54 mg GAE/100 mL, and Bila Tea 2860.08 ± 0.27 mg GAE/100 mL. The total flavonoids were 474.08 ± 0.20 ; 103.57 ± 0.07 ; and 354.47 ± 0.10 mg QE/100 g, respectively. The total tannins were 26211.35 ± 13.00 ; 1741.84 ± 4.91 ; and 18312.06 ± 6.50 mg TAE/100 g, respectively.

The rank order of DPPH activity mirrored the rank order of phenolic content: amla infusion (IC₅₀ 5.74 ppm; 3486.43 mg GAE/100 mL) was the most active, followed by Bila Tea (17.24 ppm; 2860.08 mg GAE/100 mL) and avocado seed infusion (51.93 ppm; 838.90 mg GAE/100 mL). Because the 1:1 formulation replaces half of the amla with a markedly less active matrix, a lower activity than amla alone is the arithmetically expected outcome and does not by itself indicate an unfavorable interaction. The relevant question is whether the mixture performs better or worse than simple dose additivity predicts. Under the Loewe criterion, a purely additive 1:1 blend of the two infusions would give $IC_{50} = 1/(0.5/5.74 + 0.5/51.93) = 10.34$ ppm; the observed value of 17.24 ppm corresponds to a combination index of 1.67 at the 50% effect level. The combination is therefore sub-additive (mildly antagonistic) rather than synergistic, and the claim of synergy made in earlier drafts of this work is not supported.

The direction of this deviation is worth examining critically. Amla is dominated by the hydrolysable tannins emblicanin A and B, punigluconin, pedunculagin and corilagin, together with gallic acid derivatives and ascorbic acid, whose radical-scavenging capacity is high, but which are also strongly susceptible to complexation. Avocado seed, by contrast, is rich in the flavan-3-ol monomers (+)-catechin and (-)-epicatechin and in condensed tannins such as procyanidins B1 and B2, together with protein-binding constituents.²⁸ Competitive interaction between these two tannin classes, and adsorption of gallate esters onto the avocado-seed matrix, offer a plausible chemical explanation for a mixture that scavenges DPPH less efficiently than dose additivity would predict. Notably, the measured composition of Bila Tea was higher than the additive prediction for all three parameters (phenolics 2860.08 vs 2162.67 mg GAE/100 mL; flavonoids 354.47 vs 288.83 mg QE/100 g; tannins 18312.06 vs 13976.60 mg TAE/100 g), while its radical-scavenging activity was lower than the additive prediction. Folin-Ciocalteu and Folin-Denis reagents respond to total reducing capacity rather than to functionally available antioxidants, so a higher colorimetric reading does not necessarily translate into a stronger DPPH response; this divergence should nonetheless be resolved by compound-specific quantification (HPLC or LC-MS/MS) before the formulation is developed further.

The GC-MS profile does not explain the observed activity and should not be read as if it did. The Bila Tea extract was dominated by sesquiterpene hydrocarbons (gamma-gurjunene, delta-guaiene, 1H-cycloprop(e)azulene), which lack phenolic hydroxyl groups and are therefore weak hydrogen-atom donors in the DPPH system. Moreover, the GC-MS profile was obtained from a 96% ethanolic macerate, whereas activity and phenolic content were measured on the aqueous infusion; the hydrolysable tannins and gallic acid derivatives that most plausibly drive the measured activity are non-volatile and escape GC-MS detection without derivatization. The chemical characterization and the activity data are therefore complementary rather than causally linked, and future work should quantify marker phenolics directly in the infusion.

Determining which constituents actually contribute to the observed activity requires a targeted rather than a descriptive approach, and an *in silico* stage is a logical and low-cost next step alongside compound-specific quantification. Two complementary computational strategies are appropriate here. First, quantum-chemical descriptors of antioxidant capacity, namely bond-dissociation enthalpy for the hydrogen-atom-transfer pathway and ionization potential and proton affinity for the single-electron-transfer and sequential proton-loss pathways, can be calculated by density functional theory for each identified constituent, allowing the sesquiterpenes detected by GC-MS and the phenolic markers expected in the infusion to be ranked on a common scale of hydrogen-donating ability.^{29,30} Second, molecular docking against targets in the oxidative-stress network, such as the Keap1-Nrf2 interface, xanthine oxidase and NADPH oxidase, can prioritize candidate compounds for experimental confirmation and extend the interpretation beyond direct radical scavenging. Such predictions cannot replace assay data, but they would allow the compounds most likely to be responsible for the activity of Bila Tea to be selected rationally before further wet-laboratory work, and they are proposed here as the immediate continuation of this study.

Placed alongside the literature, the IC₅₀ obtained here for amla infusion is of the same order as values reported for polar *P. emblica* fruit extracts, whereas the value for avocado seed infusion is consistent with reports that avocado seed is an appreciable but comparatively modest source of radical-scavenging polyphenols. In practical terms, the 1:1 ratio evaluated here should be regarded as a yield- and palatability-driven compromise rather than an activity-optimized formulation. Ratios enriched in amla, and a multi-ratio design permitting a full Chou-Talalay isobologram, are the logical next step if antioxidant potency is the primary development objective.

Limitation

This study has limitations that bound its conclusions. The design was *in vitro* and single-laboratory, and the material characterized was not the material tested: GC-MS profiling used a 96% ethanolic macerate whereas the DPPH

assay and the phenolic, flavonoid and tannin determinations used the aqueous infusion, so the volatile sesquiterpenes reported cannot be assumed to drive the measured activity, while the non-volatile tannins and gallic acid derivatives that dominate the infusion escape GC-MS detection. Compound identities rest on library matching without authentic standards and are therefore tentative; activity was assessed with a single radical system and without a positive control, so the IC₅₀ values are internally comparable but not transferable between laboratories; and the interaction analysis derives from one fixed 1:1 ratio, making it indicative rather than definitive. The triplicate readings were analytical replicates from a single batch of one harvest, so the reported SD reflects instrumental repeatability rather than batch, cultivar or seasonal variability, and the preparation was not standardized against a marker compound. No safety, microbiological, heavy metal, stability, toxicity, bio accessibility, in vivo or clinical data were generated; no inference about safety, efficacy, regulatory compliance or disease prevention is therefore warranted. Future work should test multiple mass ratios for a full Chou–Talalay analysis, add complementary assays such as ABTS, FRAP and a cell-based model, quantify marker phenolics in the infusion by HPLC or LC-MS/MS, apply in silico screening (density-functional reactivity descriptors and molecular docking) to prioritize the constituents responsible for the activity, and proceed to standardization, safety testing and in vivo evaluation.

CONCLUSION

Bila Tea, formulated at a 1:1 (w/w) ratio of amla fruit and avocado seed powder, met both objectives of this study. With respect to the first objective, GC-MS characterization resolved twelve tentatively identified peaks forming a predominantly sesquiterpenoid volatile profile in which gamma-gurjunene (23.875%), 1H-cycloprop(e)azulene (14.440%) and delta-guaiene (11.988%) were the principal constituents, and no phenolic compound was detected within the analytical window of the technique, consistent with the non-volatile nature of the phenolics that dominate the aqueous infusion. With respect to the second objective, the aqueous infusion of Bila Tea gave an IC₅₀ of 17.24 ppm with an antioxidant activity index of 2.32, which places it in the very strong category of DPPH radical-scavenging activity; on the same scale the amla infusion was very strong (IC₅₀ 5.74 ppm; AAI 6.97) and the avocado seed infusion moderate (IC₅₀ 51.93 ppm; AAI 0.77), and the blend contained 2860.08 mg GAE/100 mL total phenolics, 354.47 mg QE/100 g total flavonoids and 18312.06 mg TAE/100 g total tannins. The activity of the blend was nevertheless weaker than that of amla alone and weaker than dose additivity predicts (combination index 1.67), so the data provides no evidence of synergy between the two components. Bila Tea is therefore established here as a chemically characterized, very strongly radical-scavenging *Usada*-based herbal preparation, while its safety, stability, standardization and in vivo efficacy remain to be demonstrated.

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Generative AI assistance (GPT-5.2; Anthropic, Claude Opus 5, 2026) was used to enhance the structural clarity and English phrasing of this paper. All AI-assisted drafts were critically evaluated and corrected by the authors. The researchers hold total responsibility for the integrity of the data and the conclusions presented herein.

AUTHOR CONTRIBUTION STATEMENT

Putu Lakustini Cahyaningrum: conceptualization, methodology, supervision, project administration, formal analysis, writing-original draft, writing-review & editing, funding acquisition. **Anak Agung Ayu Sauca Sunia Widyantari:** methodology, investigation, GC-MS analysis support, writing-review & editing. **Putut Dewantha Jenar:** investigation,

sample preparation, writing-review & editing. **Ni Kadek Citra Lestari** and **I Kadek Tresna Adi Sentana**: investigation and sample preparation. All authors reviewed and approved the final manuscript.

CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest. This research was funded by the 2025 Internal Research Grant of Universitas Hindu Indonesia; the funder had no role in the design of the study, in the collection, analysis or interpretation of the data, in the writing of the manuscript, or in the decision to submit it for publication. The authors hold no patent, license, royalty arrangement or commercial interest relating to the Bila Tea preparation described in this article, and no author has any financial, personal or professional relationship that could reasonably be perceived to have influenced the work reported

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