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Phytochemical Screening and Total Flavonoid Content of the Combined Ethanol Extract of Turmeric (*Curcuma longa*) Rhizome and Ginseng (*Panax ginseng*) Root

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Abstract: Turmeric (*Curcuma longa*) and ginseng (*Panax ginseng*) are medicinal plants with high antioxidant potential due to their rich phytochemical constituents; however, the synergistic effect of their combined extraction has not been fully explored. This study aimed to identify the secondary metabolite profile and determine the total flavonoid content of the combined ethanolic extract of turmeric rhizome and ginseng root at a 1:1 ratio. Extraction was performed using the maceration method with 96% ethanol. Qualitative phytochemical screening was conducted to identify major secondary metabolites, while total flavonoid content was measured using the aluminium chloride ($AlCl_3$) colourimetric method by UV-Vis spectrophotometry at 415 nm with quercetin as the standard. All measurements were carried out in triplicate and analyzed using One-Way ANOVA followed by Tukey HSD post hoc test. The phytochemical screening showed positive results for flavonoids, saponins, tannins/phenolics, and triterpenoids. The combined extract exhibited a total flavonoid content of 48.65 ± 1.2 mg QE/g extract, which was significantly higher ($p < 0.05$) than the single turmeric extract (41.05 ± 0.9 mg QE/g) and the single ginseng extract (27.59 ± 0.7 mg QE/g). These findings indicate a synergistic interaction during co-maceration that enhanced flavonoid yield. In conclusion, the combined formulation improved the extraction efficiency of bioactive compounds and provides a scientific basis for the development of standardized phytopharmaceutical raw materials. Further in vivo studies are recommended to evaluate its therapeutic and neuroprotective potential.

Keywords: *Curcuma longa*; *Panax ginseng*; Phytochemicals; Spectrophotometry; Total Flavonoids.

Introduction

Indonesia is known as a megabiodiversity country with extraordinary floral wealth, where thousands of plant species have been utilized for generations as traditional medicines. The utilization of these herbal plants is driven by strong traditional knowledge and the abundant availability of raw materials across various regions of the archipelago [1]. Along with the global "back to nature" trend, the exploration of the therapeutic potential of plant secondary metabolites is being intensified to discover safer chemopreventive and curative agents for humans [2].

One plant with a long history of medical use is turmeric (*Curcuma longa).* Turmeric rhizome contains major active compounds in the form of curcuminoids, which consist of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. These compounds have been scientifically proven to possess a broad spectrum of biological activities, ranging from antioxidant and anti-inflammatory effects to effective hepatoprotective activity, shielding body cells from oxidative damage caused by free radicals [3]. Besides turmeric, ginseng (*Panax ginseng*) root has also become one of the most widely researched medicinal plants in the world due to its adaptogenic properties. Ginseng is known to enhance the body's stamina, reduce fatigue, and improve cognitive function. Although its main active components are ginsenosides, ginseng also contains significant levels of phenolics and flavonoids, which work synergistically to support the immune system and combat environmental stress at the cellular level [4].

The current development of herbal products is shifting from single-use applications toward the combinatorial formulation of several plant extracts. This combination strategy aims to achieve a synergistic effect, in which interactions among active compounds in the mixture can provide higher therapeutic efficacy than single extracts [5]. Furthermore, combining extracts is often able to minimize side effects or toxicity by lowering the individual dose of each active ingredient without compromising its clinical benefits [6]. In terms of chemical composition, flavonoids are the largest group of phenolic compounds in turmeric and ginseng and play a crucial role as natural antioxidants. Flavonoids work by donating hydrogen atoms to free radicals or by chelating metals, thereby preventing the chain reactions that damage lipids, proteins, and DNA. Therefore, determining the total flavonoid content in an extract preparation is an essential parameter for evaluating the quality and bioactivity potential of these natural materials [7].

The extraction process plays an important role in determining the efficacy of extracting flavonoid compounds from the plant matrix. A 96% ethanol solvent is frequently chosen in phytochemical research due to its universal nature

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and its optimal capability to extract both polar and semi-polar compounds. In addition, ethanol is a relatively safe, low-toxicity solvent, making it highly suitable for the development of herbal medicinal raw materials or healthcare products intended for human use [8]. Although the potential of turmeric and ginseng has been extensively studied separately, quality standardisation through phytochemical screening and determination of flavonoid levels in their combination still requires further investigation. Phytochemical screening provides an initial qualitative overview of the secondary metabolite groups present, while quantitative determination of content using UV-Vis spectrophotometry provides accurate concentration data for active compounds. These data are highly necessary as a scientific foundation in the development of standardized preparation formulations [9].

Although numerous studies have reported the antioxidant, anti-inflammatory, and pharmacological activities of *Curcuma longa* and *Panax ginseng* individually, research investigating the synergistic phytochemical interactions between these two medicinal plants remains limited. Recent studies have mainly focused on single-extract bioactivity evaluation, antioxidant assays, or isolated compound characterization, while quantitative investigations regarding the enhancement of total flavonoid yield in combined extracts are still rarely reported [4]. In particular, there is a lack of scientific evidence on whether co-maceration of turmeric and ginseng can improve flavonoid extraction efficiency through synergistic interactions among secondary metabolites such as curcuminoids and ginsenosides [6]. Previous reports published emphasized the importance of multicomponent herbal formulations for increasing phytochemical stability and bioactivity; however, studies specifically evaluating quantitative flavonoid synergism in turmeric–ginseng combined extracts are still unavailable [7]. Therefore, this study was conducted to address this research gap by evaluating, both qualitatively and quantitatively, the phytochemical profile and total flavonoid content of the combined ethanolic extract of *Curcuma longa* and *Panax ginseng*.

This study aims to conduct phytochemical screening and determine the total flavonoid content in the combined extract of turmeric rhizome and ginseng root at a 1:1 ratio. The justification for selecting this dose and combination ratio is entirely based on literature reviews of previous studies demonstrating the effectiveness of synergy in this composition. Thus, this study focuses on strengthening the phytochemical data to support the use of this combination of two plants as a natural antioxidant source in pharmaceutical preparations, without requiring additional preliminary in vitro tests [10].

Research Methods

This study used a completely randomized design (CRD) with three treatment groups: single turmeric extract, single ginseng extract, and combined turmeric–ginseng extract (1:1). All analyses were conducted in triplicate to ensure reliability and reproducibility. The total flavonoid assay using the UV-Vis spectrophotometric aluminum chloride method was validated through linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) parameters. The quercetin calibration curve showed excellent linearity ($R^2 = 0.998$), and precision and accuracy were evaluated via repeated measurements and recovery analysis to ensure method reliability.

Time and Location of Research

This research was conducted from October to December 2025. The sample preparation, extraction, and qualitative phytochemical testing processes were carried out at the Clinical and Community Pharmacy Laboratory, Bali Institute of Technology and Health. The quantitative analysis of total flavonoid content was conducted using a UV-Vis spectrophotometer at the Integrated Laboratory of the Bali Institute of Technology and Health.

Tools and Materials

The instruments used in this study included a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan), analytical balance (Ohaus Pioneer, USA), rotary evaporator (IKA RV 10 Digital, Germany), water bath (Memmert WNB Series, Germany), blender (Philips HR2115, Netherlands), 40-mesh sieve (Retsch, Germany), spot plate, Buchner funnel (Pyrex, USA), test tubes (Iwaki, Japan), measuring cylinders, volumetric flasks, beakers, and other standard laboratory glassware (Pyrex, USA). The plant materials used were turmeric (*Curcuma longa*) rhizomes and ginseng (*Panax ginseng*) roots obtained from a local herbal supplier in Indonesia. The chemicals used for extraction and phytochemical analysis included technical grade 96% ethanol (Brataco Chemical, Indonesia), analytical grade methanol p.a. (Merck, Germany), distilled water, magnesium (Mg) powder (Merck, Germany), concentrated hydrochloric acid (HCl) (Merck, Germany), 10% aluminum chloride ($AlCl_3$) solution (Sigma-Aldrich, USA), 1 M potassium acetate (Merck, Germany), ferric chloride ($FeCl_3$) (Merck, Germany), and quercetin standard (Sigma-Aldrich, USA).

Plant Identification and Sample Preparation

Samples of turmeric rhizomes and ginseng roots were obtained from the Badung Market in Denpasar, Bali. Plant identification and determination were carried out at the UPT Balai Konservasi Tumbuhan Kebun Raya "Eka Karya" Bali – LIPI (BRIN) to ensure the botanical authenticity of the species under investigation. The turmeric rhizomes and ginseng roots were cleaned of dirt with running water, then thinly sliced to increase the surface area for evaporation. The samples were air-dried at room temperature without direct sunlight exposure to maintain the stability of thermolabile compounds. The dried simplicia was then ground into a fine powder and sifted using a 40-mesh sieve.

Extraction Process

The extract was prepared using the maceration method. The simplicia powders of turmeric rhizome and ginseng root were mixed at a 1:1 ratio. The determination of this combination ratio was based on previous literature studies indicating an optimal synergistic effect at this composition. A total of 500 grams of the powder mixture was placed in a maceration vessel and soaked in 96% ethanol for

3 × 24 hours. The extraction solvent was replaced every 24 hours (remaceration). The collected macerate was filtered through a Buchner funnel, concentrated on a rotary evaporator at 50°C, and then evaporated over a water bath to obtain a thick ethanol extract of the turmeric-ginseng combination.

Phytochemical Screening (Wilstatter Test)

The qualitative test for flavonoids was conducted to confirm the presence of a benzopyrone backbone. A total of 10 mg of the extract was dissolved in 2 mL of methanol in a test tube. A small amount of magnesium powder and 3-5 drops of concentrated HCl were added to the solution. A positive result was indicated by a color change in the solution to orange, red, or pink.

Determination of Total Flavonoid Content

Preparation of Quercetin Standard Solution

A total of 25 mg of quercetin powder was dissolved in p.a. ethanol to a final volume of 25 mL (1000 ppm). This stock solution was then diluted into a concentration series of 10, 20, 30, 40, and 50 ppm.

Determination of Maximum Wavelength

1 mL of the quercetin standard solution was taken, mixed with 1 mL of 10% AlCl3 and 1 mL of 1 M potassium acetate, and then diluted to 10 mL with distilled water. The mixture was incubated for 30 minutes (operating time). The absorbance was measured using a UV-Vis spectrophotometer over the wavelength range of 400-450 nm to determine the maximum absorbance.

Sample Measurement

A total of 10 mg of the combined ethanol extract was dissolved in 10 mL of p.a ethanol. From this solution, 1 mL was taken and mixed with 1 mL of 10% AlCl3, 1 mL of 1 M potassium acetate, and distilled water to a final volume of 10 mL. After a 30-minute incubation, the sample's absorbance was measured at 415 nm. The test was conducted in triplicate.

Data Analysis

Statistical tests comprising the normality test (Shapiro-Wilk) and homogeneity of variance test (Levene's test) were performed prior to the main analysis using the parametric One-Way Analysis of Variance (ANOVA) test with a significance level of 95% (alpha = 0.05). If the ANOVA results indicated a statistically significant difference ($p < 0.05$), the analysis was followed by the Tukey HSD (Honestly Significant Difference) post hoc test to identify which treatment groups differed in content.

Results and Discussion

Extraction

The extraction of bioactive compounds from turmeric rhizome (*Curcuma longa*) and ginseng root (*Panax ginseng*) was performed by maceration using 96% ethanol as the solvent. Maceration was selected because it is a cold-extraction technique highly effective at isolating natural compounds without degrading thermolabile constituents during heat exposure. The use of 96% ethanol was based on its polarity, which enables optimal extraction of polar to semi-polar compounds, including flavonoids and other polyphenolic constituents. Evaporation of the macerate produced a concentrated ethanolic extract with an adequate yield for further analysis.

Phytochemical Screening: Flavonoid Test

Phytochemical screening was conducted to provide a qualitative overview of the diversity of secondary metabolites present in the combined ethanolic extract. The results of phytochemical screening are presented in Table 1.

Table 1. Phytochemical Screening Results of the Combined Turmeric–Ginseng Ethanolic Extract

Compound Class	Reagent / Method	Observation Result	Conclusion
Flavonoids	Wilstatter Test (Mg + HCl)	Reddish orange	(+)
Saponins	Foam Test (Shaking)	Stable foam ±1.5 cm	(+)
Tannins/Phenolics	5% $FeCl_3$ Solution	Dark greenish-black	(+)
Triterpenoids	Liebermann-Burchard	Brownish-red ring	(+)
Alkaloids	Mayer / Dragendorff	No precipitate	(-)

The results in Table 1 indicate that the combined extract was rich in phenolic compounds, flavonoids, saponins, and triterpenoids. The dominant presence of triterpenoid saponins was mainly attributed to the characteristic ginsenosides found in ginseng root, whereas the phenolic and flavonoid contents were synergistically contributed by both plants, particularly curcuminoids from turmeric rhizome.

Total Flavonoid Content

Quantitative determination of total flavonoid content was performed using the aluminium chloride ($AlCl_3$) colorimetric method and measured by UV-Vis spectrophotometry. This method is based on the formation of a stable complex between aluminium chloride and the C4 keto group, as well as the hydroxyl groups at C3 or C5 in flavone and flavonol structures. This reaction results in a bathochromic shift that can be measured spectrophotometrically.

Quercetin was used as the standard compound because it belongs to the flavonol group and possesses functional groups reactive toward the $AlCl_3$ reagent. The absorbance values of standard quercetin solutions measured at the maximum wavelength of 415 nm are shown in Table 2.

Table 2. Absorbance Data of Standard Quercetin Calibration Curve

Quercetin Concentration (ppm)	Absorbance (λ = 415 nm)
10	0.066
20	0.119
30	0.170
40	0.223
50	0.274

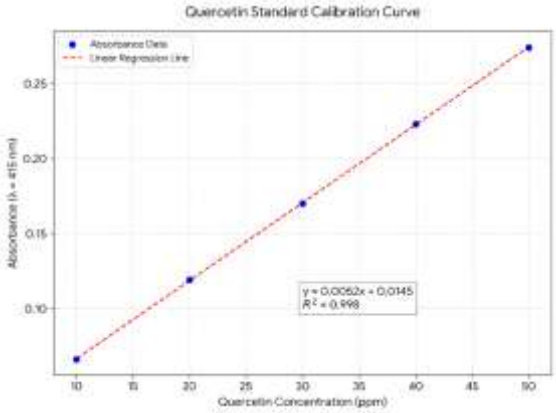

Figure 1. Quercetin Standard Calibration Curve

Total Flavonoid Content of Samples

Measurements were conducted on single extracts and the combined extract (1:1 ratio) to evaluate the effect of combining raw materials on the total bioactive compounds. The results are presented in Table 3.

Table 3. Total Flavonoid Content Determination Results (Mean ± SD)

Sample Group	Mean Absorbance	Flavonoid Content (mg QE/g)
Single Turmeric Extract	0.228	41.05 ± 0.9a
Single Ginseng Extract	0.158	27.59 ± 0.7b
Combined Extract	0.267	48.65 ± 1.2c

Note: Different superscript letters within the same column indicate significant differences ($p < 0.05$) based on the Tukey HSD test.

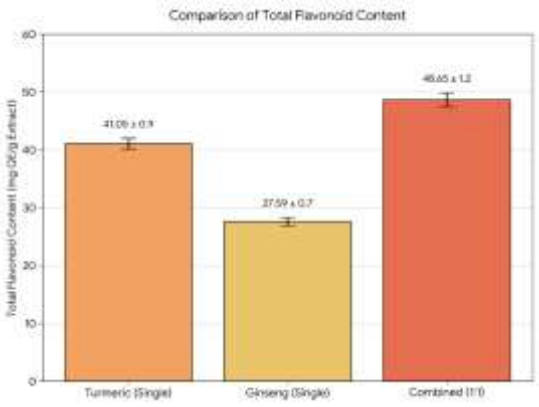

Figure 2. Comparison of Total Flavonoid Content

One-Way ANOVA analysis showed a highly significant difference among treatment groups ($p = 0.000$). Tukey HSD post hoc analysis confirmed that the total flavonoid content of the combined extract (48.65 mg QE/g) was significantly higher than that of either the turmeric or the ginseng single extract.

The increased flavonoid content in the combined preparation provides scientific evidence of synergistic interaction. The selection of a 1:1 ratio in this study was based on prior literature indicating that a balanced ratio of turmeric curcuminoids to ginseng saponins may enhance solubility and improve the extraction efficiency of total phenolic compounds. This combination not only increased the yield of secondary metabolites but may also strengthen antioxidant activity and physiological protection through the diversity of flavonoids extracted by 96% ethanol. These findings support the utilization of this combination as a standardized raw material for health products. The extraction of bioactive compounds from turmeric rhizome (*Curcuma longa*) and ginseng root (*Panax ginseng*) was performed using cold extraction by maceration. This method was selected because it is highly effective for extracting natural materials containing thermolabile secondary metabolites, such as flavonoids and phenolics, which are susceptible to degradation at elevated temperatures. Furthermore, immersion for 3×24 hours using remaceration (solvent replacement) allowed maximum contact time between the solvent and powdered simplicia, thereby maintaining the concentration gradient and optimizing cellular diffusion of compounds from the plant matrix into the solvent [11].

The selection of 96% ethanol as the extraction solvent was based on its universal polarity characteristics. Ethanol has a dielectric constant that enables it to dissolve compounds across a broad polarity range, from highly polar substances such as flavonoid glycosides and tannins to semi-polar compounds such as flavonoid aglycones, triterpenoid saponins, and curcuminoids. In addition to its high extraction efficiency, 96% ethanol is recognized as a safe solvent with very low toxicity, making its extracts ideal for further application as raw materials for traditional medicine [12]. Macroscopically, evaporation of the macerate using a rotary evaporator produced a dark reddish-brown viscous extract with a characteristic aromatic odor resulting from the combination of turmeric and ginseng. The intense color of the extract served as an initial visual indicator of the high polyphenolic pigment content, particularly curcuminoid derivatives from turmeric rhizome. The extract yield obtained was relatively high, reflecting the solvent's efficiency in disrupting cell walls and extracting most secondary metabolites from the simplicia matrix [13].

Qualitative phytochemical screening was conducted as an initial step to profile the diversity of extracted secondary metabolites. This phytochemical profile is important for predicting the potential spectrum of pharmacological activity of the preparation [14]. The screening results demonstrated that the combined extract showed strong positive reactions for flavonoids, saponins, tannins/phenolics, and triterpenoids, confirming that the combination of both simplicia contributed complementary phytochemical diversity [15]. In the qualitative flavonoid test using the Wilstätter method, the sample solution changed from yellowish-brown to reddish-orange. This reaction was triggered by the addition of magnesium powder and concentrated hydrochloric acid, generating nascent hydrogen gas. The hydrogen reduced the benzopyrone ring, which is the fundamental structure of flavonoids, resulting in the

formation of flavilium salt or anthocyanidin complexes that produce an intense orange to red coloration [16].

In addition to flavonoids, the combined extract tested positive for saponins and triterpenoids. The formation of a stable foam approximately 1.5 cm high after shaking and the addition of hot water indicated the presence of saponins with both hydrophilic and lipophilic groups (natural surfactants). This finding is reasonable, given that ginseng root is a major source of ginsenosides, which are structurally complex triterpenoid saponins that contribute significantly to the plant's adaptogenic properties [17]. Testing for tannins and other polyphenols using 5% ferric chloride ($FeCl_3$) also produced a dark greenish-black color. This reaction confirmed the presence of free phenolic groups forming coordination complexes with Fe^{3+} ions [18]. In contrast, the extract gave negative results in the alkaloid test using Mayer and Dragendorff reagents. The absence of precipitate is consistent with chemotaxonomic data indicating that turmeric rhizome and ginseng root are not major producers of nitrogen-containing alkaloid metabolites [19].

For quantitative analysis, total flavonoid content was determined spectrophotometrically using the aluminium chloride ($AlCl_3$) colorimetric method. The basic principle involves complex formation between Al^{3+} ions and functional groups in flavonoid molecules, specifically the C-4 ketone and the C-3 or C-5 hydroxyl groups. This complex formation causes a bathochromic shift toward longer wavelengths, allowing accurate measurement at 415 nm [20].

Quercetin was selected as the calibration standard because it is representative of the flavonol subclass, which is widely distributed in nature. Structurally, quercetin has an ideal conformation that is highly reactive in forming stable coordination complexes with aluminium chloride compared with many other flavonoids. Therefore, the quantitative results were expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g) [21]. The validity of the analytical method was assessed using a standard quercetin calibration curve. The linear regression equation obtained was $y = 0.0052x + 0.0145$, with a coefficient of determination (R^2) of 0.998. This value, being very close to 1, demonstrated excellent linearity. Thus, 99.8% of the absorbance changes were linearly proportional to increasing quercetin concentration, indicating that the instrument was highly reliable for converting sample absorbance values into concentration data [22].

Based on quantitative measurements, the total flavonoid content of the single turmeric extract was 41.05 ± 0.9 mg QE/g, whereas the single ginseng extract contained 27.59 ± 0.7 mg QE/g. The high phenolic content in turmeric rhizome was mainly contributed by curcuminoid compounds and their polyphenolic derivatives. Meanwhile, although ginseng root is dominated by ginsenosides, it still contains sufficient flavonoids to support its health-promoting bioactivity [23].

The most notable finding of this study was observed in the combined extract preparation (1:1 ratio), in which the total flavonoid content reached the highest value of 48.65 ± 1.2 mg QE/g. This value was not merely the average of its two constituents; it indicated an increase in overall phytochemical yield. This phenomenon suggests that combined maceration facilitated the dissolution of active compounds more efficiently than individual extraction [24]. To scientifically validate the significance of these findings, inferential statistical analysis using One-Way ANOVA was applied. The statistical results showed a p-value of 0.000 ($p < 0.05$), indicating a statistically significant difference among the sample groups. Further Tukey HSD analysis specifically confirmed that the flavonoid content of the combined extract was significantly higher than that of the single extracts [25].

The significantly higher total flavonoid content observed in the combined turmeric–ginseng extract (48.65 mg QE/g) compared with the single turmeric extract (41.05 mg QE/g) and the single ginseng extract (27.59 mg QE/g) quantitatively demonstrates phytochemical synergism during co-maceration. The approximately 18.5% increase over turmeric extract alone suggests that the interaction between curcuminoids and ginsenosides may enhance the solubility and extraction efficiency of phenolic compounds in ethanol. This finding is consistent with recent studies reporting that multicomponent herbal formulations can improve phytochemical stability, extraction yield, and antioxidant capacity through intermolecular interactions among secondary metabolites. Unlike previous studies that mainly focused on individual plant extracts, the present study provides quantitative evidence that combined extraction significantly enhances flavonoid recovery. Furthermore, the graphical results and statistical analysis confirmed that the increase was not only visually apparent but also statistically significant ($p < 0.05$), supporting the main objective of evaluating synergistic flavonoid enhancement. These findings suggest that combining *Curcuma longa* and *Panax ginseng* may offer greater potential for the development of standardized phytopharmaceutical formulations with improved antioxidant and therapeutic properties.

The significant increase in flavonoid content can be explained by the theory of phytochemical synergism in pharmacognosy. It is strongly suspected that saponin molecules from ginseng root acted as natural co-solvents or surfactant agents that modified solubility during extraction. This interaction may have increased ethanol penetration into turmeric tissue, allowing curcuminoids and polar polyphenols to be extracted more efficiently and generating a richer profile of bioactive compounds in the multicomponent preparation [26].

The high flavonoid content of the combined preparation carries promising therapeutic implications. Flavonoids can donate hydrogen atoms to stabilize free radical species, thereby interrupting chain reactions of cellular damage. With a flavonoid level of 48.65 mg QE/g, the combination of *Curcuma longa* and *Panax ginseng* is highly feasible and rational for further development as an active ingredient in plant biotechnology-based therapeutic products [27].

Conclusion

Based on the results of this study, it can be concluded that the combined ethanolic extract of turmeric rhizome (Curcuma longa) and ginseng root (Panax ginseng) qualitatively contained secondary metabolites belonging to the flavonoid, saponin, tannin/phenolic, and triterpenoid groups. Quantitative determination using the UV-Vis spectrophotometric method showed that the combined extract (1:1 ratio) possessed a high total flavonoid content of

48.65 ± 1.2 mg QE/g extract. This elevated total flavonoid content indicates a synergistic interaction that significantly enhances the extraction efficiency of bioactive compounds compared with the individual extracts of each plant.

Author's Contribution

A.A.I.M. Padmiswari: Designed the research framework, conducted the research, analysed the data, and prepared the results and discussion. N.T. Wulansari: Contributed to data analysis. K.B. Harditya: Contributed to data analysis.

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