



Solvent Variation Effects on Secondary Metabolites Content of Suruhan Herbs and Antioxidant Activity Using DPPH Method

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Article Info	ABSTRACT
Received: 29-05-2026 Revised: 15-06-2026 Accepted: 25-06-2026 *Corresponding author: Muhammad Sa'ad Email: muhammads@stikesnas.ac.id	Background: Suruhan herb (<i>Peperomia pellucida</i> (L.) Kunth) had great potential as a natural antioxidant source due to its secondary metabolite content. Comparative studies evaluating the effects of varying solvent polarities on its specific secondary metabolite profiles and radical scavenging efficiency remain limited, creating a critical gap in optimizing its herbal extraction process. A research study needs to be conducted to determine which solvent yields an extract with the highest antioxidant activity. Objective: This study aimed to determine the effect of solvent polarity (n-hexane, ethyl acetate, and distilled water) on the secondary metabolite profiles through phytochemical screening and antioxidant activity of suruhan herb extracts using the DPPH method. Method: Samples were extracted via maceration at a 1:10 ratio. Ethyl acetate and n-hexane extracts were concentrated using a rotary evaporator, while the distilled water extract was dried using a freeze dryer. Results: Phytochemical screening showed that all extracts contained alkaloids, flavonoids, tannins, and phenols. N-hexane and ethyl acetate extracts contained steroids/terpenoids and essential oils, while the distilled water extract contained saponins. One-way ANOVA showed highly significant differences in antioxidant activity. All extracts were categorized as very strong, with the lowest IC₅₀ produced by ethyl acetate (29.37 µg/mL), followed by n-hexane (30.60 µg/mL) and distilled water (30.91 µg/mL). For comparison, Vitamin C had an IC₅₀ of 4.08 µg/mL. Conclusion: Solvent polarity directly influenced the secondary metabolite profiles and antioxidant activity of the extracts. All variations exhibited very strong antioxidant activity (IC₅₀ < 50 µg/mL), with statistically significant differences among the groups and the positive control. Each solvent yielded a significantly different antioxidant potency, ranking: ethyl acetate, n-hexane, and distilled water. Statistical analysis confirmed that ethyl acetate was the most effective solvent for extracting antioxidant compounds. Keywords: <i>Peperomia pellucida</i>; Antioxidant; Solvent variation; Phytochemical; Chinese betel

INTRODUCTION

Indonesia possesses highly abundant biodiversity, which has long been utilized as a source of traditional medicine. Natural product-based traditional medicines remain highly favored by the public due to their perceived efficacy, safety, and affordability

(Kencana *et al.*, 2022; Adiyasa & Meiyanti, 2021; Sari *et al.*, 2025). Furthermore, the World Health Organization (WHO) encourages the utilization of traditional medicines for health maintenance, disease prevention, and treatment (Sudirman & Hamdila, 2020).

The extraction efficiency of secondary metabolites from a plant is significantly influenced by the type of solvent used and its respective polarity. The selection of solvents with varying polarities polar, semi-polar, and non-polar determines the type and yield of the extracted bioactive compounds, thereby affecting their phytochemical profiles and biological activities (Besan *et al.*, 2024) One such plant with notable potential is the suruhan herb (*Peperomia pellucida* (L.) Kunth) from the *Piperaceae* family, which contains various secondary metabolites, including flavonoids, alkaloids, tannins, and terpenoids, that act as antioxidants (Pratiwi *et al.*, 2021). The extraction efficacy of these compounds is strictly contingent upon the type of solvent and the extraction method employed.

Previous studies have demonstrated significant variations in the antioxidant activity of *P. pellucida*. Polar solvents, such as 70% methanol, have been reported to exhibit highly potent antioxidant activity with an IC_{50} value of 7.0 $\mu\text{g/mL}$, whereas non-polar solvents, such as n-hexane extracted via hydrodistillation, demonstrated substantially weaker activity with an IC_{50} value of 2,830.0 $\mu\text{g/mL}$ (Widyantari & Armita Sari, 2023). Interestingly, (Shilpa *et al.*, 2021) reported that the aqueous extract of *P. pellucida* obtained through maceration also possessed very strong antioxidant activity with an IC_{50} value of 16 $\mu\text{g/mL}$, indicating that certain polar solvents can effectively extract antioxidant compounds. These findings are corroborated by (Besan *et al.*, 2024), who fractionated the 70% ethanolic extract of *P. pellucida*; their study revealed that the ethyl acetate (semi-polar) fraction exhibited the highest antioxidant activity with an IC_{50} value of 16.98 $\mu\text{g/mL}$, outperforming both the n-hexane (non-polar) and aqueous (polar) fractions.

Despite these findings, comparative studies specifically investigating the effects of varying solvent polarities on the secondary metabolite profiles and antioxidant activities of *P. pellucida* remain limited. Therefore, this study aims to determine the effect of using distilled water (polar), ethyl acetate (semi-polar), and n-hexane (non-polar) solvents via the maceration method on the secondary metabolite content of *Peperomia pellucida* (L.) Kunth, as well as to evaluate its antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

METHODS

Materials

The primary instruments used in this study included an analytical balance (Ohaus), laboratory glassware, maceration vessels, a water bath (Memmert), a UV-Vis spectrophotometer (Shimadzu) equipped with a 1-cm cuvette (Hellma Analytics), micropipettes (Eppendorf), a rotary evaporator (IKA), and a freeze dryer. The primary plant material used in this study was the suruhan herb (*Peperomia pellucida* (L.) Kunth), obtained from Bangsalsari District, Jember Regency, East Java. Botanical identification was performed at the Biology Laboratory of the Universitas Muhammadiyah Surakarta. The samples consisted of the herbage (leaves, stems, and young roots) meeting a minimum age criterion of 4 months. Fresh herbs (11.6 kg) were washed and dried using a dehydrator followed by an oven until a crude drug (simplicia) weight of 895.3 g was obtained. The crude drug was then ground into a fine powder. The chemical reagents used included 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Aldrich), Vitamin C (Merck), analytical grade (p.a.) ethanol, distilled water, ethyl acetate, and n-hexane. For phytochemical screening, the reagents used were Mayer's,

Dragendorff's, and Wagner's reagents, as well as sulfuric acid (H_2SO_4), magnesium powder, chloroform, ammonia, hydrochloric acid (HCl), iron (III) chloride (FeCl_3), and Sudan III solution.

Methods

Extraction and Drying

The powdered sample (150 g) was macerated in 1500 mL of solvent (1:10 w/v ratio) for 72 hours at room temperature with daily stirring, followed by filtration (Susilowati *et al.*, 2024). The ethyl acetate and n-hexane extracts were concentrated using a rotary evaporator and a water bath (Nur Khofifah & Indah Amelia, 2025). The aqueous extract was subsequently freeze-dried (lyophilized).

Phytochemical Screening

The identification of secondary metabolites was conducted using standard qualitative colorimetric and precipitation tests (Annisa & Najib, 2022; Yanti *et al.*, 2023; Togelang *et al.*, 2023)

1. Alkaloid: The extract was treated with chloroform, ammonia, and H_2SO_4 . The presence of alkaloids was confirmed using Mayer's (white precipitate), Wagner's (brown precipitate), and Dragendorff's (orange precipitate) reagents.
2. Flavonoid: The extract was heated in ethanol, followed by the addition of concentrated HCl and Mg powder. A positive result yielded a dark red, yellow, or orange color.
3. Saponin: The extract was boiled in distilled water and vigorously shaken. The formation of a stable froth indicated a positive result.
4. Tannin: The addition of 1% FeCl_3 to the ethanolic extract produced a bluish-black or green color.
5. Steroid/Terpenoid Test: The extract was mixed with glacial acetic acid and concentrated H_2SO_4 , yielding a red, orange, or purple color.

6. Phenol: The addition of 5% FeCl_3 produced a bluish-green, blue, or purple color.

7. Essential Oil: The extract was treated with Sudan III, indicated by a color change to red.

Antioxidant Activity

The DPPH method described by Pratiwi *et al.* (2021) was employed to evaluate free radical scavenging capacity. Initially, a 100 ppm DPPH working solution was formulated using analytical-grade ethanol. For the assay, 3.2 mL of either the extract samples (20–40 ppm) or the Vitamin C reference standard (1–5 ppm) were mixed with 1.8 mL of the prepared DPPH solution. Following thorough homogenization, the mixtures were subjected to dark incubation for an optimal duration of 17 to 20 minutes. Finally, absorbance readings were recorded against an ethanol blank at a peak wavelength (λ_{max}) of 515 nm using a UV-Vis spectrophotometer.

To calculate the percentage of antiradical activity (inhibition), the following formula was applied:

$$\% \text{ Inhibisi} = \frac{A_{\text{control}} - (A_{\text{sample}})}{A_{\text{control}}} \times 100$$

Description:

A_{control} = DPPH absorption value

A_{sample} = DPPH absorption value after sample addition

The IC_{50} value indicating the precise concentration needed to neutralize 50% of the DPPH free radicals was determined through linear regression analysis ($Y = bX + a$). This model was generated by plotting the sample concentrations against their corresponding inhibition percentages (Utami *et al.*, 2025).

RESULTS AND DISCUSSION

The overall process of this study involved the morphological authentication of fresh *Peperomia pellucida* leaves, followed by extraction using solvents of

varying polarities. The macroscopic appearance of the fresh plant specimen and the resulting concentrated extracts (ethyl

acetate, n-hexane, and aqueous) are visually presented in Figure 1.

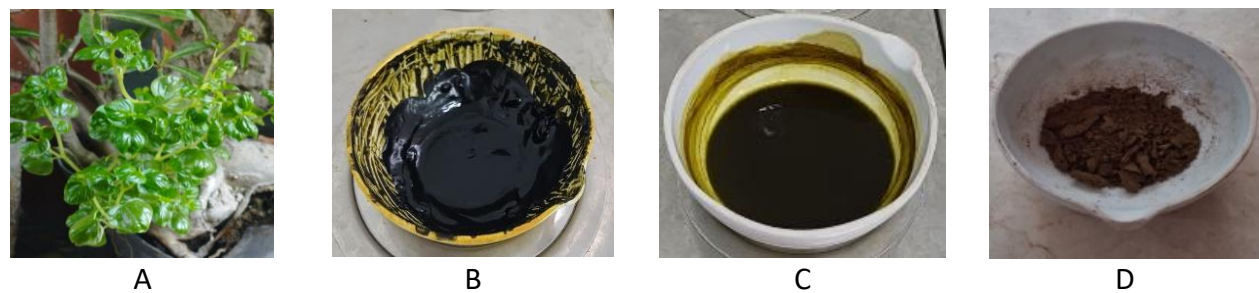


Figure 1. Visual representation of the *Peperomia pellucida* sample and its extraction results. (A) Fresh *Peperomia pellucida* plant; (B) Concentrated ethyl acetate extract; (C) Concentrated n-hexane extract; (D) Aqueous extract.

Plant Authentication

Botanical determination was conducted at the Biology Laboratory of Universitas Muhammadiyah Surakarta to ensure the authenticity of the biological sample and prevent taxonomic errors. The evaluation confirmed that the sample obtained from Bangsalsari District, Jember, was authentically *Peperomia pellucida* (L.) Kunth, a member of the *Piperaceae* family.

Extraction Yield

The extraction of *P. pellucida* was carried out via maceration applying three solvents of distinct polarities. The ethyl acetate and n-hexane extracts underwent concentration in a rotary evaporator set to 50°C. Meanwhile, the aqueous extract was processed through freeze-drying

(lyophilization), applying a rapid freezing step at -43.5°C followed by sublimation between -50.0°C and -53.0°C. The extraction yields are presented in Table 1.

Phytochemical Screening

Qualitative analysis was conducted to identify the distribution profile of secondary metabolites across the three extract variations based on specific reagent reactions. The screening results (Table 2) indicated that alkaloids, flavonoids, tannins, phenols, and essential oils were detected in all solvent variations. Saponins were exclusively detected in the aqueous extract, whereas steroids/terpenoids were only found in the n-hexane and ethyl acetate extracts.

Table 1. Extraction Yield of *P. pellucida* Extracts

Parameter	n-Hexane Extract	Ethyl Acetate Extract	Aqueous Extract
Crude Drug Weight (g)	150 gram	150 gram	150 gram
Extract Weight (g)	4,95 gram	2,25 gram	10,60 gram
Yield (%)	3,00 %	1,50 %	4,06 %

Table 2. Phytochemical Screening of *P. pellucida* Extracts

Secondary Metabolites	Reagents	n-Hexane	Test Results Ethyl Acetate	Aqueous	Observation Results (+) Detected
Alkaloids	Mayer	+	+	+	White precipitate formed
	Wagner	+	+	+	Brown precipitate

Secondary Metabolites	Reagents	n-Hexane	Test Results Ethyl Acetate	Aqueous	Observation Results (+) Detected
	Dragendorff	+	+	+	Orange precipitate
Flavonoids	Ethanol + conc. HCl + Mg	+	+	+	Brick-red color (aqueous), yellow color (n-hexane, ethyl acetate)
Saponins	Distilled water (shaking)	-	-	+	Formation of stable froth
Tannins	FeCl ₃	+	+	+	Appearance of black color
Steroids/ Terpenoids	Liebermann-Burchard	+	+	-	Color change to yellow
Phenols	FeCl ₃	+	+	+	Color change from green to blackish-green
Essential Oils	Sudan III solution	+	+	+	Red coloration

Antioxidant Activity and Statistical Analysis

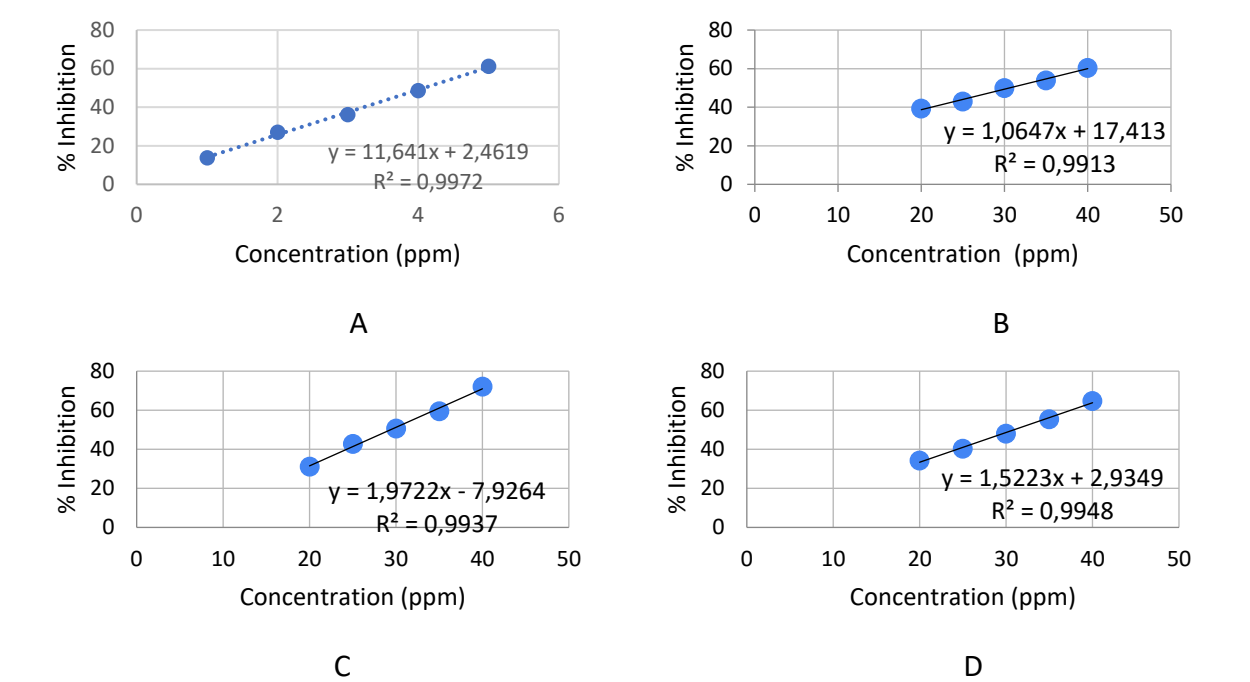


Figure 2. Antioxidant activity regression curves. (A) Vitamin C standard curve; (B) Ethyl acetate extract; (C) n-Hexane extract; (D) Aqueous extract.

Table 3. DPPH Scavenging Activity of *P. pellucida* Extracts and Vitamin C

Sample / Standard	Linear Regression Equation	R ²	IC ₅₀ (µg/mL)	Antioxidant Category
Vitamin C (Standard)	$y = 11.641x + 2.4619$	0.9972	4.08	Very Strong
Ethyl Acetate Extract	$y = 1.0647x + 17.413$	0.9913	29.37	Very Strong
n-Hexane Extract	$y = 1.9722x - 7.9264$	0.9937	30.60	Very Strong
Aqueous Extract	$y = 1.5223x + 2.9349$	0.9948	30.91	Very Strong

Optimum operating time (OT) recorded between 17 and 20 minutes. Vitamin C, used as the positive control, exhibited a highly potent antioxidant activity with a linear regression equation of $Y =$

$11.641X + 2.4619$ ($R^2 = 0.9972$) and an IC₅₀ value of 4.08 µg/mL.

The IC₅₀ values for the *P. pellucida* extracts were calculated based on their dose-response relationships at

concentrations ranging from 20 to 40 ppm. The results demonstrated that all extracts possessed very strong antioxidant activity ($IC_{50} < 50 \mu\text{g/mL}$). The ethyl acetate extract exhibited the lowest IC_{50} ($29.37 \mu\text{g/mL}$, $R^2 = 0.9937$), followed by n-hexane ($30.60 \mu\text{g/mL}$, $R^2 = 0.9913$) and the aqueous extract ($30.91 \mu\text{g/mL}$, $R^2 = 0.9948$).

Before executing the One-way ANOVA, the dataset was checked to confirm a normal distribution (Shapiro-Wilk, $p > 0.05$) and equal variances (Levene's test, $p = 0.757$). Even though the initial ANOVA indicated notable differences in the average IC_{50} results among the groups ($p < 0.001$), further post-hoc testing showed that the different solvents were statistically comparable. Despite the ethyl acetate extract producing the strongest antioxidant activity in absolute numbers, statistical analysis found no meaningful distinction among the ethyl acetate, n-hexane, and aqueous extract.

The extraction yields demonstrated significant variation based on the choice of solvent. Notably, the aqueous extract yielded the highest percentage (4.06%), suggesting that the predominant chemical constituents within *P. Pellucida* such as phenolics, glycosides, minerals, and carbohydrates are highly polar. For this polar extract, freeze-drying was specifically employed. As supported by (Souza et al., 2025) and (Zubia et al., 2023), lyophilization effectively concentrates extracts without compromising the stability of thermolabile polar compounds, minimizing the loss of phenolic and antioxidant contents compared to conventional hot-air drying (Kittibunchakul et al., 2023; Li et al., 2025)

Conversely, the n-hexane extract yielded 3.00%, which was higher than that of ethyl acetate (1.50%). This indicates a substantial extraction of non-polar compounds, such as chlorophyll, lipids, and

waxes, adhering to the "like dissolves like" principle. This finding aligns with (Ho et al., 2022), who reported that the n-hexane fraction of *P. pellucida* yielded a higher mass than its ethyl acetate counterpart.

The phytochemical distribution distinctly reflects the influence of solvent polarity. Saponins were exclusively extracted by distilled water due to their highly polar nature. The formation of stable froth during the saponin screening is driven by the presence of hydrophilic hydroxyl groups and lipophilic carbon chains within the saponin structure, which allows these organic compounds to act as active surface agents in aqueous solutions (Oktaviani et al., 2024) In contrast, lipophilic compounds such as steroids and terpenoids were only detected in the n-hexane and ethyl acetate extracts (Korany et al., 2025). Ethyl acetate proved to be the most comprehensive solvent, capable of extracting a broad spectrum of compounds due to its semi-polar characteristics, which allow for the solvation of both polar and non-polar moieties (Putri et al., 2023; Subandrate et al., 2024)

Interestingly, essential oils tested positive across all solvents. In the n-hexane and ethyl acetate extracts, their presence is associated with the free or aglycone forms, which are volatile and non-polar. However, the positive result in the aqueous extract suggests that essential oil components may be bound in glycosidic forms, where the sugar molecular bonds enhance their solubility in polar solvents (Fajaryanti et al., 2025), allowing them to be optimally extracted. Furthermore, the presence of tannins and phenols in all extracts was confirmed by the formation of dark complex compounds upon the addition of FeCl_3 , wherein the hydroxyl groups of these compounds react with Fe^{3+} ions (Ratnasari et al., 2023).

The DPPH assay evaluated the extracts' capacity to neutralize free radicals via hydrogen donation. Vitamin C demonstrated superior activity (IC_{50} 4.08 $\mu\text{g/mL}$) due to its ascorbic acid structure containing labile enediol hydroxyl groups, which rapidly donate hydrogen atoms. As a pure compound, its antioxidant capacity is kinetically faster and more concentrated than complex botanical extracts (Angeli *et al.*, 2023; Chavan *et al.*, 2025; Szerlauth *et al.*, 2019). Based on the established DPPH radical scavenging evaluation for botanical extracts, samples exhibiting an IC_{50} value below 50 $\mu\text{g/mL}$ are classified as very strong antioxidants (Halimah & Sa'ad, 2025). In alignment with this classification, although all three extract variations fell into the "very strong" category ($IC_{50} < 50 \mu\text{g/mL}$) according to (Asyraf *et al.*, 2025 ; Halimah & Sa'ad, 2025), statistical analysis indicated an equivalence in antioxidant capacity across the solvents, with no significant difference observed.

Although all three extract variations fell into the "very strong" classification ($IC_{50} < 50 \mu\text{g/mL}$) according to (Asyraf *et al.*, 2025), statistical analysis indicated an equivalence in antioxidant capacity across the solvents, with no significant difference observed. Numerically, the ethyl acetate extract exhibited the lowest IC_{50} (29.37 $\mu\text{g/mL}$), followed by n-hexane (30.60 $\mu\text{g/mL}$) and the aqueous extract (30.91 $\mu\text{g/mL}$). The numerical superiority of the ethyl acetate extract is attributed to its semi-polar nature, which comprehensively extracts potent hydrogen-donating compounds containing both polar and non-polar groups, such as flavonoid aglycones and steroids (Putri *et al.*, 2023; Subandrate *et al.*, 2024; Haroen *et al.*, 2025). Similarly, the strong numerical antioxidant capacity of the n-hexane extract is supported by the successful extraction of essential oils and lipophilic terpenoids via the "like dissolves like" principle (Korany *et*

al., 2025). Conversely, the slightly higher numerical IC_{50} of the aqueous extract aligns with its typical limitation in extracting lipophilic metabolites such as terpenoids and certain flavonoid aglycones (Hussen & Endalew, 2023).

However, the statistical equivalence across all solvents suggests that *P. pellucida* possesses a robust and diverse array of antioxidant metabolites that remain highly effective regardless of the extraction medium's polarity. Consequently, although the polar solvent (distilled water) did not exhibit the lowest numerical IC_{50} , the findings highlight a critical practical advantage. The aqueous extract provides a comparable, very strong antioxidant efficacy while offering a significantly safer, more economical, and environmentally friendly extraction alternative to organic solvents, which is highly beneficial for future clinical or pharmaceutical applications.

CONCLUSION

According to the results of this research, the polarity of the solvents used influenced the secondary metabolite profiles and antioxidant activity of *Peperomia pellucida* (L.) Kunth. Specifically, solvent polarity contributed to selective variations in the phytochemical composition, particularly in the extraction of saponins and terpenoids. Despite these compositional variations, all three extract variations exhibited very strong antioxidant activity ($IC_{50} < 50 \mu\text{g/mL}$), with statistically significant differences in their mean IC_{50} values ($p < 0.001$) when compared both to the positive control (Vitamin C) and among the extract groups. Each solvent exerted a significantly different effect on the antioxidant activity, ranked in the following order of potency: ethyl acetate (IC_{50} 29.37 $\mu\text{g/mL}$), n-hexane (30.60 $\mu\text{g/mL}$), and distilled water (30.91 $\mu\text{g/mL}$). Although ethyl acetate produced the highest numerical antioxidant activity, statistical analysis

revealed its efficacy to be equivalent to the other tested solvents.

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CONFLICT OF INTEREST

No potential conflicts of interest have been reported by the authors.

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