Determination of Piperine Content in *Peperomia pellucida* (L.) Kunth and *Peperomia obtusifolia* (L.) A. Dietr

Syumillah Saepudin[1*], Ita Inayah[1], Shinta Dewi Kumalasari[1], Putri Gina Rotul Huda[1]

[1] Department of Pharmacy, Science and Math Faculty, Al Ghifari University, Bandung, Indonesia

*Email: symillas1221@gmail.com

Abstract. Piperine is an alkaloid compound found in plants of the Piperaceae family and is known to exhibit pharmacological effects such as anti-arthritic, anti-aging, and anticancer activities. *Peperomia pellucida* (L.) Kunth and *Peperomia obtusifolia* (L.) A. Dietr are members of the Piperaceae family with potential to contain piperine. This study aimed to analyze the piperine content in the extracts and fractions of both plants using UV-Visible spectrophotometry. Extraction was performed by maceration using 70% ethanol, followed by liquid-liquid extraction with water, ethyl acetate, and n-hexane as solvents. Quantification of piperine was conducted using UV-Visible spectrophotometry with methanol as the blank, and measurement was performed at a wavelength of 343.60 nm. The results showed that the ethyl acetate fractions of *Peperomia pellucida* (L.) Kunth and *Peperomia obtusifolia* (L.) A. Dietr contained the highest piperine concentrations, at 52.981 mg PE/g and 44.413 mg PE/g, respectively

Keywords: Piperine, Piperaceae, *Peperomia pellucida* (L.) Kunth, *Peperomia obtusifolia* (L.) A. Dietr, UV-Vis spectrophotometry

Introduction

Peperomia pellucida (L.) Kunth and *Peperomia obtusifolia* (L.) A. Dietr are species belonging to the genus *Peperomia*, a member of the *Piperaceae* family. These plants are native to Central and South America, Mexico, and tropical regions of Asia (Kartika et al., 2016; Ware et al., 2022). Traditionally, both species have been used in ethnomedicine for the treatment of various conditions, including pruritus, inflammation, hypercholesterolemia, skin disorders, insect and snake bites, and burn wounds (Ware et al., 2022). Phytochemical studies have revealed that P. pellucida (L.) Kunth and P. obtusifolia (L.) A. Dietr contain a diverse range of secondary metabolites, such as tannins, saponins, flavonoids, steroids, triterpenoids, chromenes, lignans, and alkaloids (Kartika et al., 2016; Saepudin & Susilawati, 2022; Ware et al., 2022).

Piperine is a prominent alkaloid compound characteristic of plants in the Piperaceae family**.** It contains a nitrogen atom, appears as a yellow crystalline solid, has a molecular weight of 285.33 g·mol^{-1}, and a melting point of 128–130 °C. Piperine was first isolated from the fruit of black pepper (*Piper nigrum*), where it contributes to the plant's pungent taste (Tripathi et al., 2022). The compound is readily soluble in ether and alcohol, but poorly soluble in water (Vasavirama & Upender, 2014). A wide range of pharmacological activities have been attributed to piperine, including antiproliferative, antitumor, antiangiogenic, antioxidant, antidiabetic, anti-obesity, cardioprotective, antimicrobial, anti-aging, hepatoprotective, antiallergic, anti-inflammatory, neuroprotective, and immunomodulatory effects (Aswad et al., 2023). Despite these reported benefits, studies investigating the piperine content in Piperaceae—particularly within the *Peperomia* genus—remain limited.

The quantification of piperine can be carried out using a UV-Visible spectrophotometer. This method is relatively simple and offers high selectivity (Shintawati, 2019). UV-Visible spectrophotometry is an analytical technique that utilizes ultraviolet light in the wavelength range of 100–400 nm and visible light in the range of 400–750 nm (Suhartati, 2017).

This study aimed to determine the piperine content in the 70% ethanol extract as well as the *n*-hexane, ethyl acetate, and aqueous fractions of *Peperomia pellucida* (L.) Kunth and *Peperomia obtusifolia* (L.) A. Dietr using UV-Vis spectrophotometry.

Methods

Materials

P. pellucida (L.) Kunth herbs were collected from from Kebun Percobaan Manoko, Cikahuripan, Lembang, Kabupaten Bandung Barat, Jawa Barat. While P. obtusifolia (L.) A. Dietr leaves were collected from Kampung Pasir Kunci, Bandung, West Java. The plants were identified at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor, West Java, with number authentication 48/HB/01/2025 and 114/HB/01/2020, which claimed that the plant used was *Peperomia pellucida* (L.) Kunth and *Peperomia obtusifolia* (L.) A. Dietr. Piperine were analytical grade and bought from Sigma-Aldrich.

Shrinkage drying

The determination of shrinkage drying in the simplicia was carried out using an oven. An empty crucible was first weighed, followed by weighing 5 grams of dried simplicia, which was then placed into the crucible. The crucible containing the simplicia was placed in an oven and dried at 105°C until a constant weight was achieved (Kemenkes, 2017)

Moisture content

Moisture content determination was carried out using a Moisture Analyzer. A total of 5 g of the sample was evenly spread on the aluminum dish of the instrument. The device was then set to a temperature of 100°C for 10 minutes. The moisture content displayed on the Moisture Analyzer was recorded (Saepudin et al., 2024).

Identifying the substances that dissolve in particular solutions

Each 8 grams of powdered simplicia (herbs of P. pellucida (L.) Kunth and leaves of P. obtusifolia (L.) A. Dietr) were divided equally to obtain two samples named Extract A and Extract B. Extract A macerated in a closed container with 100 mL chloroform-saturated water for 24 hours, and Extract B in 100 mL 96% ethanol under identical conditions. After filtration, 20 mL of the filtrate was evaporated. The dish containing the filtrate was evaporated to dryness and then heated at 105°C until a constant weight was obtained (Herawati & Saepudin, 2025)

Phytochemical screening

The phytochemical screening was carried out to test for the presence of alkaloids using various reagents. A total of 1 grams of the sample was placed in a test tube, followed by the addition of 4 mL of 10% ammonia and 4 mL of chloroform, then shaken. The chloroform layer was pipetted and transferred into a new test tube, and 8 mL of 2N hydrochloric acid was added and shaken until two layers formed. The acidic layer was pipetted and divided into four test tubes (Saepudin et al., 2024), followed by the following tests:

Dragendorff's Test

Three drops of Dragendorff's reagent were added to the test tube containing the sample. A positive result for alkaloids is indicated by the formation of an orange to red precipitate (Saepudin et al., 2024).

Wagner's Test

Three drops of Wagner's reagent were added to the test tube containing the sample. A positive result for alkaloids is indicated by the formation of a brown or reddish precipitate (Saepudin et al., 2024).

Hager's Test

Three drops of 2% picric acid were added to the test tube containing the sample. A positive result for alkaloids is indicated by the formation of a yellow precipitate (Saepudin et al., 2024).

Extraction

The herbs of P. pellucida (L.) Kunth and leaves of P. obtusifolia (L.) A. Dietr were macerated using 70% ethanol as the solvent for 3x24 h. During the maceration, the solvent was replaced every 24 hours. The

macerate was filtered and then concentrated using a rotary evaporator, followed by further thickening of the extract using a water bath (Saepudin et al., 2024).

Fractionation

Fractionation was performed using the liquid-liquid extraction (LLE) method. A total of 10 grams of the concentrated extract was diluted with 50 mL of distilled water and transferred into a separatory funnel, followed by the addition of 50 mL of n-hexane. The mixture was thoroughly shaken and allowed to stand until two distinct phases separated. The n-hexane layer was collected into a beaker. This partitioning process was repeated five times to maximize extraction. The remaining aqueous phase was subsequently partitioned with 50 mL of ethyl acetate using the same procedure. After shaking and allowing phase separation, the ethyl acetate layer was collected. This step was also repeated five times. The obtained all fractions were then concentrated to dryness for further analysis (Peratiwi et al., 2023; Saputra et al., 2023)

Determination of Piperine

A total of 10 mg of piperine was dissolved in a 10 mL volumetric flask using methanol. The stock solution was then diluted to obtain five different concentrations: 1, 2, 3, 4, and 5 mg/L, each prepared in a 10 mL volumetric flask using methanol as the solvent. The absorbance of each solution was measured at 343.60 nm. A calibration curve was then constructed by plotting concentration (x-axis) against absorbance (y-axis). For determinate samples, 10 mg of each sample was dissolved in a 10 mL volumetric flask using an appropriate solvent. Then, 1 mL of each sample solution was pipetted and diluted with methanol in a 10 mL volumetric flask. The absorbance of each solution was measured maximum wavelength of piperine. Each sample was measured in triplicate (Cahyono et al., 2019; Hikmawanti et al., 2021). The piperine content was expressed in mg PE/g using the following formula 1 (Hanifah et al., 2021):

$$TPC\ (mg\ PE/g)\ = \frac{c\ x\ v\ x\ fp}{g} \qquad (1)$$

Description:
TPC : Total Piperine Content (mg PE/g)
c : concentration of piperine compound (mg/L)
v : volume of the sample used (L)
fp : dilution factor
g : weight of the sample (g)

Result and Discussion

Maceration is an extraction technique in which plant materials are soaked in a suitable solvent for the targeted compounds without the application of heat, thereby preserving the integrity of thermolabile active constituents (Chairunnisa et al., 2019; Saepudin et al., 2024). Liquid–liquid extraction (LLE) is a fractionation method that utilizes two immiscible solvents. This process involves the transfer of analytes from an aqueous phase into a non-polar or slightly polar organic solvent (Herdiana & Aji, 2020). The results and yields of the fractionation process are presented in the table 1.

Tabel 1: The yields of extract and fractions of P. pellucida (L.) Kunth and P. obtusifolia (L.) A. Dietr

Plants	Samples	Weight (gram)	Yield (%)
P. pellucida (L.) Kunth	Ethanol extract	18.36	20.43
	Aqueous fraction	9.31	93.10
	Ethyl acetate fraction	0.022	2.20
	n-Hexane fraction	0.018	1.80
P. obtusifolia (L.) A. Dietr	Ethanol extract	20.08	22.31
	Aqueous fraction	7.61	76.10
	Ethyl acetate fraction	1.10	11.00
	n-Hexane fraction	0.80	8.00

The determination of shrinkage drying aims to establish the maximum allowable limit of compounds lost during the drying process. The purpose of moisture content analysis is to define the acceptable amount of water present in the simplicia, in order to prevent microbial growth during storage. The parameters of water-soluble and ethanol-soluble extractives are intended to quantify the amount of compounds dissolved in water (polar) and ethanol (semi-polar), respectively (Kemenkes, 2017; Saepudin et al., 2024). The results of drying loss, moisture content, water-soluble extractive, and ethanol-soluble extractive analyses are presented in Table 2

Tabel 2: The result of shrinkage drying, moisture content, and water-ethanol soluble compounds of simplicia P. pellucida (L.) Kunth and P. obtusifolia (L.) A. Dietr

Parameters	Samples		Requirement (Kemenkes, 2017)
	P. pellucida (L.) Kunth	P. obtusifolia (L.) A. Dietr	
Shrinkage drying (%)	10.00	10.00	no more than 10.00%
Moisture content (%)	8.80	5.00	no more than 10.00%
Water soluble compounds (%)	18.75	23.00	not less than 5.00%
Ethanol soluble compounds (%)	11.25	13.00	not less than 6.40%

Shrinkage drying, moisture content, and water-ethanol soluble compounds of both simplicia comply with the requirements of the Indonesian Herbal Pharmacopeia.

The phytochemical screening for alkaloid compounds was conducted on the simplicia, extract, and fractions of P. pellucida (L.) Kunth and P. obtusifolia (L.) A. Dietr. This test was performed to identify the presence of alkaloid compounds in the samples. Specific changes observed in the chemical reaction after the addition of reagents indicated a positive result for alkaloid compounds (Herawati & Saepudin, 2025). The result of alkaloid compound identification from all samples are presented in Table 3.

Tabel 3: The result of alkaloid compounds identification of P. pellucida (L.) Kunth and P. obtusifolia (L.) A. Dietr

Plants	Samples	Reagents		
		Dragendorff	Wagner	Hager
P. pellucida (L.) Kunth	Simplicia	-	-	+
	Ethanol extract	-	-	+
	Aqueous fraction	-	-	+
	Ethyl acetate fraction	-	-	+
	n-Hexane fraction	-	-	-
P. obtusifolia (L.) A. Dietr	Simplicia	-	-	+
	Ethanol extract	+	+	+
	Aqueous fraction	-	-	-
	Ethyl acetate fraction	-	-	+
	n-Hexane fraction	-	-	+

*(+) = positive test (exist); (-) = negative test (not exist)

The principle of the alkaloid identification method is based on a precipitation reaction resulting from ligand exchange. The nitrogen atom in alkaloids, which possesses a lone pair of electrons, can replace the iodo ion in the reagents. In this study, the samples were tested using three reagents, including Dragendorff's reagent, Wagner's reagent, and Hager's reagent.

Dragendorff's reagent contains bismuth nitrate and potassium iodide in glacial acetic acid solution (forming potassium tetraiodobismuthate (III)). A positive result for alkaloids in the Dragendorff test is indicated by the formation of a light brown to yellow precipitate. This precipitate is a potassium-alkaloid complex. The alkaloid test using Wagner's reagent gives a positive result if a brown to yellow precipitate is formed. Wagner's reagent consists of potassium iodide (KI) and iodine (I_2), which react to form triiodide ions (I_3^-) that appear brown in color. The precipitate is a potassium-alkaloid complex, resulting from the coordinate covalent bond formed between the K^+ ion and the nitrogen atom in the alkaloid. Hager's reagent contains saturated picric acid, which can form complex bonds with alkaloid compounds under acidic conditions, resulting in an ionic complex. The structure of alkaloid compounds (containing a pyridine ring) undergoes protonation at the pyridine nitrogen in an acidic medium, producing a positively charged ion. The ionized

picric acid ($C_6H_2(NO_2)_3O^-$) forms an ionic bond with the protonated alkaloid compound, resulting in a stable complex (Khafid et al., 2023; Saepudin et al., 2024).

Determination of piperine was conduct using UV-Vis Spectrophotometer. The maximum wavelength of piperine was obtained at 343.60 nm (Figure 1). The result of the maximum wavelength is consistent with the findings reported by (Cahyono et al., 2019). The piperine standard curve was obtained from the absorbance readings of various piperine concentrations, plotted as a graph of concentration versus absorbance. The resulting linear regression equation was y = 0.1423x – 0.0006, with a correlation coefficient (r) = 0.9994.

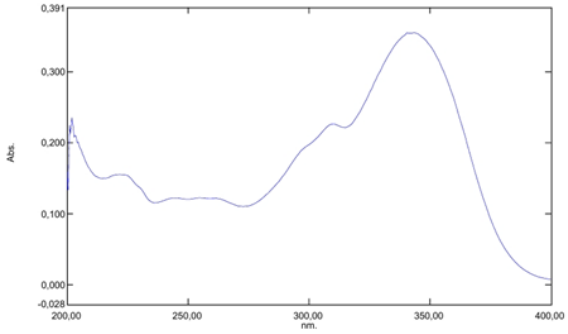

Figure 1. Maximum wavelength of piperine (343.60 nm)

Tabel 4: The result of absorbance of piperine standard

Concentration (mg/L)	Absorbance
1	0.137
2	0.269
3	0.415
4	0.570
5	0.707

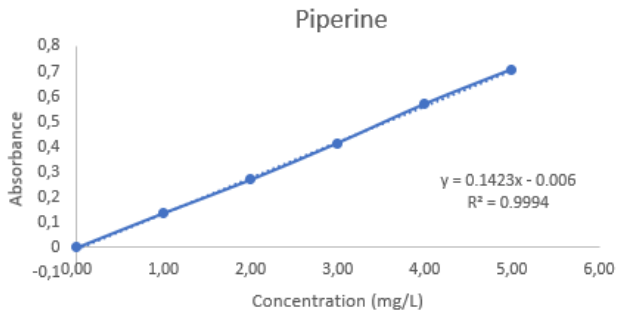

Figure 2. Standard curve of piperine

Determination of piperine from ethanol extract and fractions of P. pellucida (L.) Kunth and P. obtusifolia (L.) A. Dietr was performed by measuring the absorbance at a wavelength of 343.60 nm. The calculated piperine content (mg PE/g) is presented in Table 5.

Tabel 5: Piperine content of extract and fractions of P. pellucida (L.) Kunth and P. obtusifolia (L.) A. Dietr

Plants	Samples	Piperine content (mg PE/g)
P. pellucida (L.) Kunth	Ethanol extract	21.455 ± 0.041
	Aqueous fraction	17.042 ± 0.001
	Ethyl acetate fraction	52.981 ± 0.041
	n-Hexane fraction	6.291 ± 0.041
P. obtusifolia (L.) A. Dietr	Ethanol extract	25.399 ± 0.226
	Aqueous fraction	18.615 ± 0.041
	Ethyl acetate fraction	44.413 ± 0.108
	n-Hexane fraction	6.009 ± 0.285

Based on the data presented in Table 5, the determination of piperine content showed that the ethyl acetate fractions of both plants had the highest piperine concentrations, with 52.981 mg PE/g for the ethyl acetate fraction of P. pellucida (L.) Kunth and 44.413 mg PE/g for the ethyl acetate fraction of P. obtusifolia (L.) A. Dietr. According to Muthia et al (2024), ethyl acetate is a semi-polar solvent, which makes it suitable for extracting semi-polar compounds such as piperine. The piperine content obtained in this study was lower than that reported by Cahyono et al (2019). This difference may be attributed to several influencing factors, including the extraction method and the type of solvent used. The low piperine content in the *n*-hexane fraction indicates that both highly polar and non-polar solvents are not effective for dissolving piperine (Alzanando et al., 2022).

Piperine is an alkaloid compound with the chemical structure of piperoylpiperidine, having the molecular formula $C_{17}H_{19}NO_3$ and the IUPAC name 1-(5-[1,3-benzodioxol-5-yl]-1-oxo-2,4-pentadienyl) piperidine. Piperine is classified as a semi-polar compound due to the presence of a polar amide group in its structure, along with a long non-polar carbon chain, which reduces its overall polarity in polar solvents (Gorgani et al., 2017). Several studies have reported that piperine exhibits various pharmacological effects, including anti-inflammatory, anti-arthritic, anti-aging, and anticancer activities (Tiwari et al., 2020).

Conclusion

The ethyl acetate fractions of *Peperomia pellucida* (L.) Kunth and *Peperomia obtusifolia* (L.) A. Dietr contained the highest piperine concentrations, at 52.981 mg PE/g and 44.413 mg PE/g, respectively. Indicating these species as potential alternative sources of piperine within the Piperaceae family.

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