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Effect of Ethanol Concentration and Extraction Time on Piperine Yield from Piper retrofractum Vahl.

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ABSTRACT

Piperine, the primary bioactive compound in Piper retrofractum Vahl or Javanese long pepper, offers various pharmacological benefits, including anti-inflammatory, anticancer properties, and the enhancement of drug bioavailability. The need for efficient piperine extraction has grown as ongoing research emphasizes its potential as a key component in the pharmaceutical and nutraceutical industries. This study aims to evaluate the impact of ethanol concentration and maceration time on the yield of piperine from the plant Piper retrofractum Vahl, commonly known as Javanese long pepper. Piperine is the primary active compound responsible for the pharmacological properties and the pungent taste characteristic of the plant. An ethanol-based extraction method was chosen due to its perceived environmental friendliness and cost-effectiveness. In this study, extraction was carried out using various ethanol concentrations (75%, 80%, 85%, 90%, and 95%) and different maceration durations (14, 16, 18, 20 and 22 hours). The results showed that increasing ethanol concentration and extending maceration time significantly enhanced the piperine yield, with the optimal piperine concentration obtained using 95% ethanol and a maceration time of 20 hours. The extraction product from this operating condition yielded about 35.02%, with a piperine content of 3.245%.

Keywords: Ethanol; Extract; Herbal; Javanese long pepper; Piperine

INTRODUCTION

Piper retrofractum Vahl is a type of agricultural product frequently found across various regions in Indonesia, such as Java, Sumatra, Kalimantan, Nusa Tenggara, and Bali (Sudarmaji, Hayati and Rahayu, 2019). This commodity holds significant economic potential, with an Free On Board value of approximately USD 1,733,662. This is evident from the high demand in 2023, with about 135.33 tons of Javanese long pepper being exported to markets such as India, the United States, China, Japan, Nepal, and Bangladesh (Badan Pusat Statistik, 2024).

Piper retrofractum Vahl possesses promising nutritional and phytochemical properties that enhance its potential for pharmaceutical and other applications. It contains carbohydrates (63.4%), crude protein (11.4%), total ash (4.29%), dietary fiber (28.8%), and total fat (2.97%). Additionally, it includes calcium (414.97 ± 5.85 mg/100 g), copper (0.91 ± 0.04 mg/100 g), iron (5.12 ± 0.06 mg/100 g), magnesium (166.4 ± 0.8 mg/100 g), phosphorus (203.30 ± 0.26 mg/100 g), sodium (9.41 ± 0.32 mg/100 g), and zinc (0.93 ± 0.08 mg/100 g). Among these components, piperine is the primary alkaloid present in this genus (Jadid *et al.*, 2018).

Numerous studies have shown that piperine have various biological functions, including hepatoprotective, antidepressant, and anticancer effects (Jadid *et al.*, 2018). Other

research has also identified additional benefits of this substance, including its role as an alternative energy booster in sports activities and its aphrodisiac properties (Pradipta, Kusumawardhana and Herlambang, 2018). Numerous studies have shown that piperine has various biological functions, including liver protection, antidepressant effects, and anticancer properties (Gutierrez, Gonzalez and Hoyo-Vadillo, 2013).

In several previous studies, the extraction of Javanese long pepper has been carried out using various solvents, including hexane, methanol, and ethanol. The result of using hexane as a solvent showed piperine with a yield of 0.265%±0.008% (Jadid *et al.*, 2018). On the other hand, when methanol was used as the solvent, piperine in the Javanese long pepper extract was only detectable qualitatively, and the yield was extremely low. The extraction with 95% ethanol as the solvent produced the highest yield, reaching 25.89±0.09 (Musthapa and Gumilar, 2017).

In a previous study, extraction was performed using a 1:10 ratio of simplicia mass to solvent volume, with a maceration time of 18 hours. The Javanese long pepper used were sourced from various regions, including Bogor, Central Lampung, and Madura. The findings indicated that Javanese long pepper from Central Lampung had the highest yield and piperine content among the samples, with a yield of 25.89±0.09 and a piperine content of 1.54±0.02 (Hikmawanti *et al.*, 2021).

In a separate study, the Pulsed Electric Field (PEF) extraction method was used, where Javanese long pepper was exposed to an electric voltage of 4000 to 6000 volts for 240 to 480 seconds. Afterward, The simplicia underwent a 24-hour maceration process in 96% ethanol, utilizing a solvent-to-simplicia ratio of 5:1. The study reported an optimum yield of 11.80±0.30% (Rahman *et al.*, 2023).

The piperine content from the Piper genus was successfully analyzed using a UV-Vis spectrophotometer. This method is well-suited for the purpose as it is fast, simple, accurate, sensitive, and reproducible (Chauhan *et al.*, 1998).

Based on the aforementioned explanation, this study investigates the extraction of piperine from Javanese long pepper (*Piper retrofractum Vahl*.), whose utilization in Indonesia remains largely limited to the sale of dried or fresh fruits. Enhancing the value of this commodity through piperine extraction is therefore of considerable interest, particularly for its potential applications in the pharmaceutical industry.

This study contributes by providing a systematic experimental evaluation of key extraction parameters, namely ethanol concentration and extraction time, for Javanese long pepper sourced from Mojokerto, a material that has received limited attention in previous studies. This study aims to generate baseline experimental data and to identify observable trends in piperine yield and content across a defined range of extraction conditions.

Specifically, ethanol concentrations of 75%, 80%, 85%, 90%, and 95%, along with extraction times of 14, 16, 18, 20, and 22 h, were investigated to examine their influence on extraction performance. The results are expected to provide practical insights and preliminary reference data for future studies and potential process development and scale-up.

Several studies have reported the significant impact of various factors on the outcomes of piperine extraction. The table 1 summarizes the key findings from these studies, allowing us to explore the potential for more optimal operating conditions.

Table 1. Summary of Piperine Extraction from Javanese Long Pepper

No	Method	Analysis	Optimum result	Reference
1	Method : Maceration Solvent : Methanol Ratio simplicia to solvent : - Time : - Origin region: -	2D NMR	Yield : 1.5% Concentration : -	(Musthapa and Gumilar, 2017)
2	Method : Maceration Solvent : n-Hexane Ratio simplicia to solvent : - Time : - Origin region: Sumenep	-	Yield : 0.265±0.008% Concentration : -	(Jadid *et al.*, 2018)
3	Method : Maceration Solvent : Ethanol 95% Ratio simplicia to Solvent : 1:10 Time : 18 hour Origin region: Central Lampung	spektrofotometer UV-Vis	Yield : 25,89±0,09% Concentration : 1,54±0,02%	(Hikmawanti *et al.*, 2021)
4	Method : Maceration Solvent : Ethanol 95% Ratio simplicia to Solvent : 1:10 Time : 18 hour Origin region: Madura	spektrofotometer UV-Vis	Yield : 22,08±0,12% Concentration : 1,44±0,02%	(Hikmawanti *et al.*, 2021)
5	Method : Maceration Solvent : Ethanol 95% Ratio simplicia to solvent : 1:10 Time : 18 hour Origin region: Bogor	spektrofotometer UV-Vis	Yield : 19,84±0,14% Concentration : 1,41±0,02%	(Hikmawanti *et al.*, 2021)
6	Method : Soxhlet Solvent : Ethanol 96% Ratio simplicia to solvent : - Time : 58 hour Origin region: Wonogiri	HPLC (High Performance Liquid Chromatography)	Yield : 23% Concentration : 6.8%	(Cahyono *et al.*, 2019)
7	Method : PEF extraction Solvent : Ethanol 96% Ratio simplicia to solvent : 1:5 Time : 24 hour Origin region: Pamekasan	Thin Layer Chromatography (TLC)	Yield : 11.80±0.30% Concentration : 40.38±2.44%	(Rahman *et al.*, 2023)
8	Method : Thermal extraction Solvent : Ethanol 99.8% Ratio simplicia to solvent : - Time : - Origin region: Pasuruan	spektrofotometer UV-Vis	Yield : - Concentration : 2.42+0.01%	(Hawa *et al.*, 2021)

The optimum extraction method for piperine from *Piper retrofractum Vahl*, commonly known as Javanese long pepper, is maceration using 95% ethanol. This method yielded the highest concentration of 25.89% when applied to samples sourced from Central Lampung over a period of 18 hours. Its combination of extraction efficiency and simplicity makes it suitable for various applications. Additionally, other methods, such as Soxhlet extraction with 96% ethanol, also demonstrated promising results, yielding 23%. These findings highlight that both the type of solvent and the extraction time are critical factors in maximizing piperine recovery.

In comparison, TLC's rapid and straightforward process allows for the simultaneous analysis of multiple samples, minimizing degradation and providing clearer results. Furthermore, the visibility of piperine spots under UV light enhances quantification

accuracy. Although 2D NMR provides detailed structural information, it is often less sensitive for concentration measurements compared to TLC. These characteristics position TLC as a particularly valuable method for assessing piperine concentrations in *Piper retrofractum Vahl* and similar samples.

RESEARCH METHOD

The materials used are Javanese long pepper from Mojokerto Regency - East Java, ethanol (concentrations of 75%, 80%, 85%, 90%, and 95%), $CHCl_3$, filter paper, and piperine standard. The equipment used includes a balance, Erlenmeyer flask, beaker glass, stirring rod, funnel, UV-Vis spectrophotometer, measuring glass, vacuum rotary evaporator (Buchi), hot plate, blender, ultrasonic, shaker, and timer.

In this research, two stages were carried out. The first stage aimed to determine the effect of solvent concentration variation on the yield and piperine content produced during the extraction process with a maceration time of 18 hours. In the second stage, the extraction process was conducted using the optimum concentration from the first stage, and different maceration times were used to obtain the optimum time for the process, with the same dependent variables: yield and piperine content.

In the first stage, dried Javanese long pepper was cleaned of impurities and then blended until fine. Each sample of 150g of simplicia was macerated using 1.5L of ethanol at 75%, 80%, 85%, 90%, and 95% concentrations (1:10). During the maceration process, shaking was performed using a shaker. The soaking process was carried out for 18 hours.

In the second stage, the Javanese long pepper samples were cleaned and prepared, with each 20g of simplicia being macerated using 200mL of 95% ethanol (1:10). During the maceration process, shaking was performed using a shaker. The soaking process was carried out for 14, 16, 18, 20, and 22 hours.

The extraction results from both the first and second stages underwent the same process, where the macerates were gathered and concentrated with a vacuum rotary evaporator at 50°C for 90 minutes, resulting in a thick extract. The yield of Java long pepper extract was calculated as shown in Equation (1).

$$\text{Yield } (\%) = \frac{\text{Extract weight}}{\text{Simplicial weight}} \times 100\% \quad (1)$$

Measurement of piperine content is performed using the following steps: Weigh 125 mg of piperine extract and 25 mg of piperine standard. Dilute the extract in a 25 mL volumetric flask with $CHCl_3$. Homogenize the solution by sonication for 30 minutes. Pipette 1.0 mL of this solution and dilute it in a 50 mL volumetric flask. Prepare the piperine standard solution by diluting it with $CHCl_3$ in a 100 mL volumetric flask. Measure the absorbance of both the sample solution and the piperine standard using a UV-Vis spectrophotometer at a wavelength of 343 nm (Kementerian Kesehatan RI, 2017). The calculation of piperine shown content in Equation (2).

$$\% \text{ Piperin} = \frac{\text{Abs spl} \times \text{W std} \times \text{F spl}}{\text{Abs std} \times \text{W spl}} \times 100 \quad (2)$$

In which Abs spl is the absorbance of the sample, W std is the weight of the piperine standard, F spl is the dilution factor of the sample, Abs std is the absorbance of the standard, and W spl is the weight of the sample used.

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RESULT AND DISCUSSION

The piperine yield obtained under various extraction conditions is presented in Table 2. The data show the measured yields for each experimental run, providing an overview of the influence of the studied extraction parameters on piperine recovery. It should be noted that all values reported were obtained from single (non-replicated) analyses.

Table 2. Effect of Extraction Conditions on Piperine Yield

(a) Effect of Ethanol Concentration	
Ethanol Concentration (%)	**Piperine Yield (%)**
75	11.12
80	14.76
85	20.65
90	28.19
95	33.36
(b) Effect of Extraction Time	
Extraction Time (h)	**Piperine Yield (%)**
14	31.08
16	32.18
18	33.36
20	35.02
22	33.39

The results indicate that increasing ethanol concentration leads to a substantial increase in piperine yield, rising from 11.12% at 75% ethanol to 33.36% at higher concentrations. A similar trend is observed with increasing maceration time, where the yield improves from 31.08% at 14 h and reaches a maximum of 35.02% at 20 h, followed by a slight decline at longer extraction time. These results suggest that both solvent concentration and extraction time play important roles in determining piperine recovery.

The highest yields obtained in this study were 33.36% for ethanol concentration variation and 35.02% for extraction time variation, indicating the presence of optimal operating conditions. The increase in yield with higher ethanol concentration can be attributed to improved solubility of piperine, while the effect of extraction time is associated with enhanced mass transfer up to an equilibrium point, beyond which saturation or possible degradation may occur.

Ethanol concentration significantly influences both the yield and piperine content in extracts from Javanese long pepper (*Piper retrofractum Vahl*.). As the ethanol concentration increases, the yield also increases, as shown in Figure 1, along with the piperine content depicted in Figure 2.

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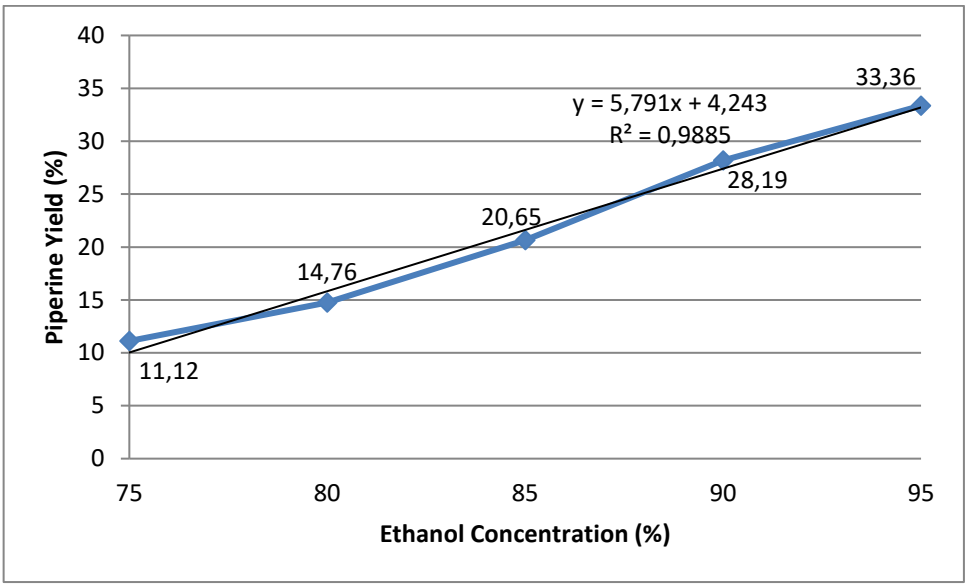

Figure 1. Relation between ethanol concentration and piperine yield

Piperine is a relatively nonpolar organic compound, enabling it to dissolve in solvents with low to moderate polarity. This property makes piperine particularly suitable for extraction processes using such solvents. Its unique chemical structure contributes to its solubility, which is essential for effectively isolating the compound from natural sources like black pepper and Javanese long pepper.

As ethanol concentration increases, the overall polarity of the solvent system decreases, which enhances its affinity toward moderately nonpolar compounds such as piperine. Piperine, an alkaloid with limited polarity, exhibits higher solubility in less polar organic solvents; therefore, increasing ethanol concentration promotes its dissolution and improves extraction efficiency. This observation is consistent with the principle of "like dissolves like," where solvent polarity plays a key role in determining solute solubility (Chemat, Vian and Cravotto, 2012; Azwanida, 2015).

In addition to solubility effects, the extraction process in maceration is governed by mass transfer mechanisms, particularly diffusion of solute from plant matrices into the solvent. Higher ethanol concentrations can enhance the diffusion rate by improving solvent penetration into plant tissues and reducing intermolecular interactions between piperine and the matrix. As described by Fick's law, the driving force for diffusion increases with concentration gradients, facilitating the release of target compounds over time (Cacace and Mazza, 2003). Therefore, both solvent polarity and diffusion kinetics contribute synergistically to the observed increase in piperine yield with higher ethanol concentration.

In this study, maceration times of 14, 16, 18, 20, and 22 h were applied with 2-hour intervals to capture the progression of extraction efficiency. After extraction, the samples were concentrated using a vacuum rotary evaporator at 50 °C to obtain a viscous extract, followed by yield and piperine content analysis.

As shown in Figure 2, the yield increased progressively with extraction time and reached a maximum at 20 h, after which a slight decline was observed at 22 h. This behavior suggests that the extraction process approached an equilibrium condition at prolonged contact time. The decrease in yield beyond the optimum point may be associated with possible degradation or oxidation of piperine due to extended exposure to oxygen and processing conditions. (Damanik *et al.*, 2020). In another study, evidence was reinforced for the decrease in piperine yield resulting from its transformation into Piperonal and 3,4-

Methylenedioxycinnamaldehyde through co-oxidation reactions, with a reaction time exceeding 20 hours at a temperature of 30°C and a pH of 7 (Krahe *et al.*, 2021).

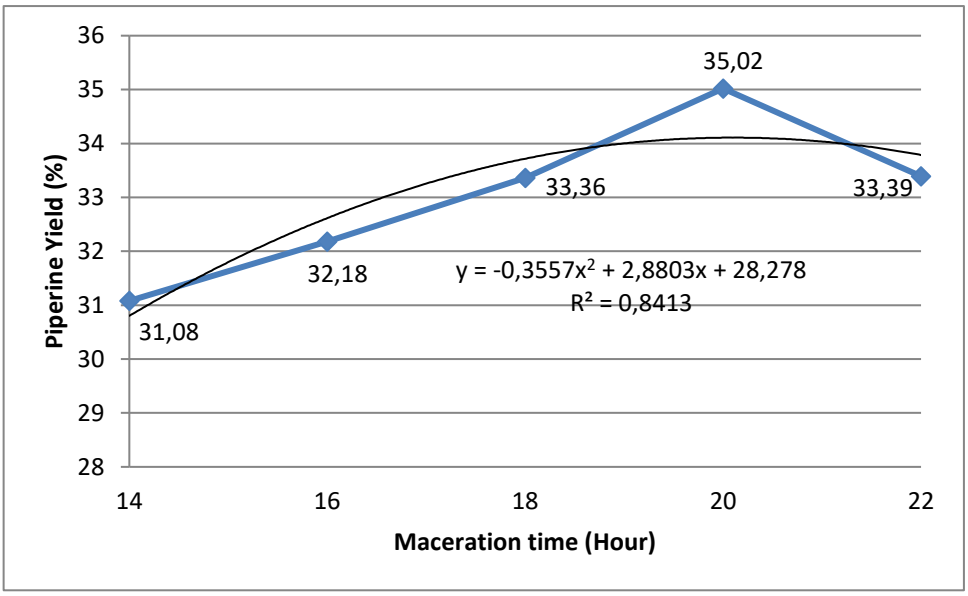

Figure 2. Relation between maceration time and piperine yield

Based on Table 1, Thin Layer Chromatography (TLC) offers a simple and rapid approach for compound analysis, making it suitable for preliminary screening and qualitative separation. However, compared with UV–Vis spectrophotometry, High-Performance Liquid Chromatography (HPLC), and 2D NMR, TLC generally exhibits lower accuracy and sensitivity for quantitative analysis. Among these techniques, HPLC provides the highest accuracy and sensitivity due to its superior resolution and reliable quantification capabilities, while 2D NMR is primarily employed for structural elucidation rather than routine quantification.

UV–Vis spectrophotometry, although potentially limited by overlapping absorbance spectra, remains a practical and widely accepted method for quantitative analysis when appropriate calibration and sample preparation are applied. In contrast, TLC is more appropriately utilized as a complementary technique for confirming compound separation rather than as a primary quantitative method.

In the context of this study, UV–Vis spectrophotometry was selected as the primary analytical method due to its simplicity, cost-effectiveness, and suitability for routine analysis. Nevertheless, incorporating TLC as a supporting technique could enhance analytical reliability by providing additional qualitative confirmation. Furthermore, the use of HPLC as a reference or validation method in future studies may further improve the accuracy and robustness of piperine quantification in Piper retrofractum Vahl.

CONCLUSION

According to the research findings, 95% ethanol with a maceration time of 20 hours is the most effective for extracting piperine from Javanese long pepper (*Piper retrofractum Vahl*.), resulting in a yield of 35.02% and a piperine content of 3.245%. The yield of piperine from long pepper harvested in Mojokerto is higher compared to several other regions such as Central Lampung, Madura, Wonogiri, Pasuruan and Bogor.

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