Determination of Total Phenol Content With Spectrophotometer and GC-MS Analysis of Javanese Long Pepper Fruits Dried Using Two Different Methods

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Abstract
Javanese long pepper (Piper retrofractum Vahl.) contain kavicin, palmatic acid, tetrahydropiperic acid, piperidine, essential oils, sesamin, and the pungent substance piperine. This fruit also contains alkaloids, flavonoids, terpenoids, steroids, tannins, and polyphenols. This study is to determine the total phenol content and phytochemicals remaining in the material after the drying process which analyzed by GC-MS. The first drying method was aerated (method I), while the second was then boiled for a few minutes and dried in direct sunlight (method II). The dry powder then macerated with ethanol 96 % in 24 hours and then continued with twice remaceration. The total phenol concentrations obtained were 23.64 mg GAE/g extract and 15.61 mg GAE/g for method I and II, respectively. The GC-MS chromatogram showed that 20 peaks were detected in method I, while 24 peaks in Method II. Compounds from the ethanol extract of Javanese long pepper in Method I were Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (Z, Z)-, and (2E,4E,14E)-N-Isobutylicosa-2,4,14-trienamide. While the compounds from Method II which had the highest peaks, namely Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (Z, Z)-, and 8-Heptadecene. The highest compound contained in the ethanol extract of Javanese long pepper in both two methods is piperidine.

***Keywords*:** *Piper retrofractum Vahl., GC-MS, TPC, Drying Method*.

Abstrak
Cabe Jawa (Piper retrofractum Vahl.) mengandung kavicin, asam palmatik, asam tetrahidropiperik, piperidin, minyak atsiri, sesamin, dan zat pedas piperin. Buah ini juga mengandung alkaloid, flavonoid, terpenoid, steroid, tannin, dan polifenol. Penelitian ini bertujuan untuk menentukan kadar fenol total dan senyawa fitokimia yang masih ada dalam bahan setelah proses pengeringan, yang dianalisis dengan GC-MS. Metode pengeringan pertama adalah dengan penjemuran (metode I), sementara yang kedua direbus

sebentar dan dikeringkan di bawah sinar matahari langsung (metode II). Serbuk kering kemudian dimaserasi dengan etanol 96% selama 24 jam, diikuti dengan dua kali remaserasi. Konsentrasi fenol total yang diperoleh adalah 23,64 mg GAE/g ekstrak dan 15,61 mg GAE/g untuk metode I dan II, secara berturut-turut. Kromatogram GC-MS menunjukkan bahwa terdapat 20 puncak yang terdeteksi dalam Metode I, sementara 24 puncak dalam Metode II. Senyawa dari ekstrak etanol Cabe Jawa dengan metode I memiliki puncak tertinggi adalah Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (Z, Z)-, and (2E,4E,14E)-N-Isobutylicosa-2,4,14-trienamide. Sementara pada Metode II puncak tertinggi adalah Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (Z, Z)-, and 8-Heptadecene. Senyawa tertinggi yang terkandung dalam ekstrak etanol Cabe Jawa dalam kedua metode adalah piperidin.

Keywords**:** *Piper retrofractum Vahl., GC-MS, TPC, Metode Pengeringan*.

1. Introduction

Javanese long pepper (*Piper retrofractum* Vahl.) were detected as one of the potential traditional medicinal ingredients. This fruit contains alkaloids, flavonoids, terpenoids, steroids tannins, and polyphenols (Mahaldar *et al.*, 2020) (Syafitri, Suryandari and Martha, 2023) . Apart from that, it also contains kavicine, palmitic acid, tetrahydropiperic acid, piperidine, essential oil, sesamin, and the pungent compound piperine (Hasanah, 2013). The piperine content is influenced by the moisture content. The higher the moisture content, the lower the piperine content. To maintain the piperine content in javanese long pepper without damaging other phytochemical compounds, a drying process is performed (Wulandari, 2020).

There are several simple drying methods, including air drying and direct sunlight drying. Air-drying is believed to prevent damage to thermolabile phytochemical compounds but requires a relatively longer time, usually ranging from 2-4 weeks. Therefore, farmers in Madura prefer to dry javanese long pepper by sun drying after a special pre-treatment. Mature fruits are washed and then boiled in boiling water for about 7 minutes. This pretreatment is referred to as blanching. Blanching is a rapid soaking process at high temperatures, typically 90-100°C for 0.6-9 minutes (Nunik, 2020). After draining, the fruits are sun-dried. This drying process typically takes only 3-5 days. However, direct exposure to ultraviolet rays from the sun during drying is a concern as it may cause damage to the chemical content of the dried material (Winangsih, 2017). The drying process can affect the content of phytochemical compounds, including total phenol content (Yuan *et al.*, 2015). Therefore, in this experiment, the total phenol content will be determined using a spectrophotometer. Apart from that, analysis will also be carried out using GC-MS so that we can find out the compound content in more detail.

2. Method

Preparation of Ethanol Extract from Javanese Long Pepper Fruit

The javanese long pepper fruit that the colour is red, is thoroughly washed with running water. The sample is then divided into two groups. The first sample was dried by the air-dry method, hereafter referred to as Method I. The second sample is immersed in boiling water for 7 minutes (blanching pretreatment) and then dried under the sun, hereafter referred to as Method II. The dried samples are ground into a powder. A total of 200 grams of dry powder from each group is macerated using 600 mL ethanol 96 % for 24 hours, followed by two re-macerations. The obtained total filtrate is then concentrated using a rotary vacuum evaporator at 40°C. The concentrated extracts from each group are subsequently used for the determination of total phenol content with spectrophotometer and chromatogram analysis using GC-MS.

Determination of Total Phenol Content

The determination of total phenol content is carried out using UV-Vis spectroscopy (Thermoscientyfic, Genesis10s UV-Vis type). The determination of the maximum wavelength is done by pipetting 0.2 mL of 300 ppm gallic acid solution and adding 15.8 mL of distilled water, followed by adding 1 mL of Folin-Ciocalteu reagent. After 8 minutes, 3 mL of sodium carbonate 20 % solution is added. Incubation is done for 70 minutes. Scanning of the wavelength is performed at 600-800 nm to determine the maximum wavelength.

Next, the absorbance of the standard gallic acid solution with concentrations of 200, 300, 400, 500, and 600 ppm is determined. 0.2 ml of each solution was pipetted and then 15.8 ml of distilled water was added, along with 1 mL of Folin-Ciocalteu reagent. After 8 minutes, 3 ml of sodium carbonate 20 % is added. Incubation is done for 70 minutes, and then absorbance is read at the maximum wavelength. The linear regression equation is then calculated as $y = bx+a$.

The determination of total phenol content is performed by weighing 200 mg of extract from each group. It is then dissolved in 96% ethanol and added to a 10 mL volumetric flask up to the mark. Each sample solution is taken at 0.2 mL and added to 15.8 mL of distilled water, along with 1 mL of Folin-Ciocalteu reagent. After 8 minutes, 3 mL of 20% sodium carbonate is added. Incubation is done for 70 minutes, and then absorbance is read at the maximum wavelength. Each sample is replicated three times. The results obtained are analyzed using independent-samples t-test with a significance level of $p < 0.05$. The software used is PASW Statistics 18.

Analysis of Ethanol Extract Using GC-MS

The gas chromatography (GC) setup was configured as follows: using an HP-5MS (30 m × 0.25 mm × 0.25 μm); employing a splitless injection mode; setting the injection port temperature to 250 °C; injecting a volume of 0.5 μL; implementing a solvent delay of 4 minutes; helium with a purity of 99.999% as the carrier gas at a flow rate of 1 mL/ minute. The temperature program included starting at an initial column temperature of 40 °C, maintaining it for 5 minutes, then ramping up to 220 °C at a rate of 4 °C/minute, maintaining that

temperature for 5 minutes, further increasing to 300 °C at a rate of 10 °C/minute, and holding it steady for 5 minutes.

For the mass spectrometry (MS) conditions: setting the electron impact energy to 70 electron volts (eV); maintaining the ion source temperature at 230 °C; keeping the transmission line temperature at 300 °C; and scanning within the range of 30 to 400 atomic mass units (amu) (Wang et al., 2022).

3. Result and Discussion

In this research, gallic acid was used as a reference with its maximum wavelength located at 757 nm. The calibration curve equation for the standard gallic acid obtained is y = 0.0012 x + 0.0216 with a correlation coefficient (r) value of 0.9878. The calibration graph for the standard gallic acid is shown in Figure 1.

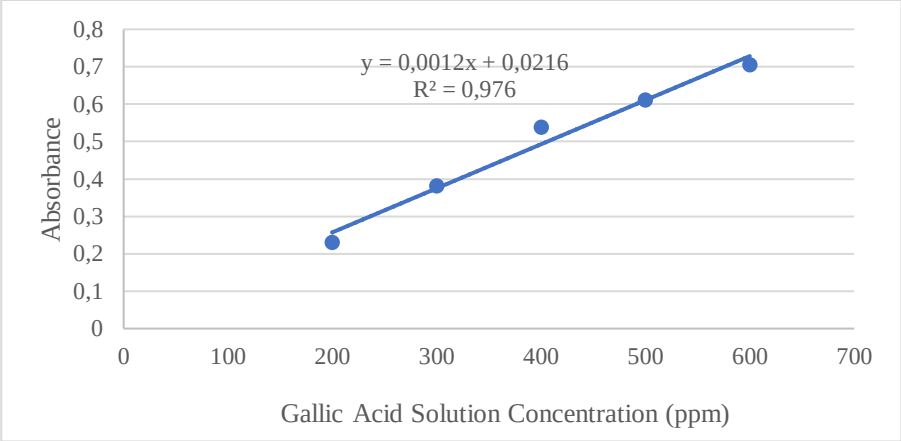

Figure 1. Standard Gallic Acid Calibration Curve

Next, the total phenolic content (TPC) of both samples is calculated based on the linear regression equation that has been generated. The results of TPC from both groups are listed in Table 1.

Table 1. Total Phenolic Content

Group	Average of Total Phenolic Content (mg GAE/g extract)
Method I	23.64 ± 0,64*
Method II	15.61 ± 0,53

Note : (*) Significantly different to Method II ($p < 0.05$)

Among the two groups, the highest TPC is found in Method I, with a value of 23.64 ± 0.64 mg GAE (Gallic Acid Equivalent) per gram of extract, followed by Method II with 15.61 ± 0.53 mg GAE per gram of extract. The statistical analysis results show a significant difference between the two groups. The low TPC in method II is in line with the research conducted by Duarte et al. (2017), which showed that the total phenolic content decreased compared to the control group. This could be due to the leaching of polyphenols during soaking as a result of hot water extraction (Duarte et al., 2017). Additionally, the lower TPC was observed in the sun-dried samples due to degradation caused by the oxidation of phenolic compounds (Benjamin *et al.*, 2022).

The concentrated extracts are then analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS analysis results show that there are 20 peaks in Method I and 24 peaks in Method II, each containing 4 major peaks is shown Table 2. Fragmentation of the gas chromatography mass spectrometer components of both methods are listed in Figures 2 and 3.

Table 2. Major Peaks of GC-MS Analysis of Method I and II

Method	Compound	Concentration (%)
I	Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (Z,Z)-	45.50
	(2E,4E,14E)-N-Isobutylicosa-2,4,14-trienamide	2.78
	(E)-5-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)pent-2-en-1-one	1.56
	(2E,4E,10E)-N-Isobutylhexadeca-2,4,10-trienamide	1.43
II	Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (Z,Z)-	46.15
	8-Heptadecene	4.72
	(2E,4E,14E)-N-Isobutylicosa-2,4,14-trienamide	3.74
	(2E,4E,10E)-N-Isobutylhexadeca-2,4,10-trienamide	3.08

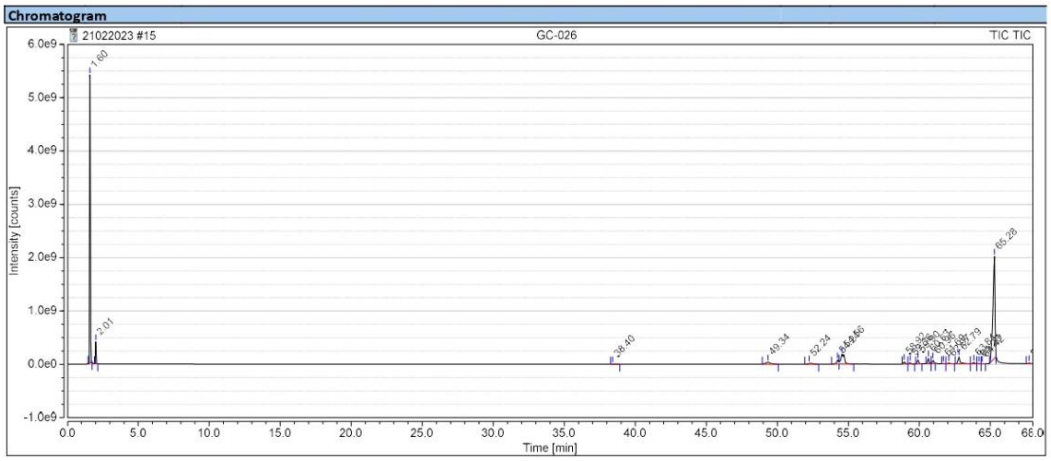

Figure 2. GC-MS Chromatogram of Method I

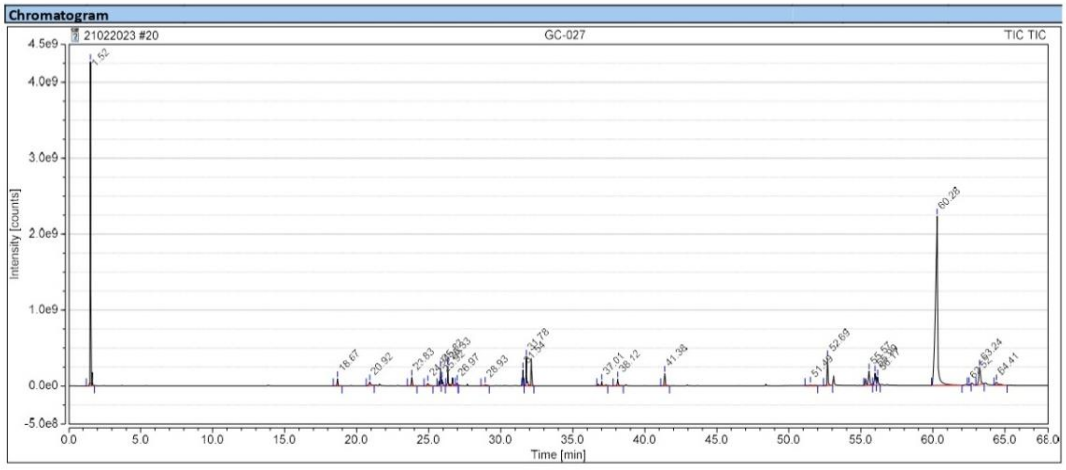

Figure 3. GC-MS Chromatogram of Method II

Browning reaction is the process of forming yellow-colored pigments that quickly turn dark brown, both enzymatic and non-enzymatic browning. The blanching pretreatment in Method II can prevent the browning reaction. Blanching serves to deactivate enzymatic systems responsible for sensory and vitamin alterations, thereby restricting oxidation. Moreover, it enhances the colors of plants, improving their presentation. Additionally, it is also intended to reduce the presence of microbes in the product and can help accelerate the drying process by altering the permeability of cell membranes, thereby preventing a decrease in quality (Ioannou and Ghoul, 2013) (Syafitri et al., 2023). However, blanching also carries drawbacks, including nutrient losses and reduced product weight.

The analysis of chemical compounds present in the ethanol extract of javanese long pepper using GC-MS, the chromatogram comparison of both methods can be seen in Figure 2 and Figure 3. From the chromatogram data, it can be observed that the types of chemical compounds identified in the ethanol extract of javanese long Pepper using Method I had 20 peaks (compounds), whereas in Method II, there were 24 peaks. Some peaks that were lost in Method I are suspected to have evaporated during the drying process, resulting in fewer types of compounds compared to Method II. Mass spectrometry data shows the molecular mass of each compound along with its fragmentation pattern. The compounds comprising the ethanol extract of javanese long pepper are interpreted based on their fragmentation patterns and similarity percentages with the database (>90%) (Supriyanto, 2012).

Through GC-MS chromatogram analysis, it is known that both samples contain the main component alkylamide which is known to have pharmacological activity as antifungal, antibacterial, antioxidant, and treats skin disorders (Elufioye, Habtemariam and Adejare, 2020). The most abundant compound in both Method I and Method II extracts of javanese long pepper is piperidine. Piperidine belongs to the alkaloid group and has antipyretic, analgesic, anti-inflammatory, and central nervous system-suppressing activities (Hotimah, 2015).

4. Conclusion

Based on the conducted research, it can be concluded that the total phenol content in Method I is significantly higher at 23.64 mg GAE/g extract compared to Method II, which has a content of only 15.61 mg GAE/g extract. Additionally, differences are also observed in the GC-MS profiles of the two extracts, with Method I showing 20 identified peaks, while Method II has 24 detected peaks.

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