Functional properties of long pepper (*Piper retrofractum* Vahl) aqueous extracts

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Abstract. Long Pepper (*Piper retrofractum* Vahl) fruit is an herbal plant that is often used as a raw material for herbal medicine. Long pepper in Indonesia is referred to as Javanese chili. The use of Javanese chilies traditionally uses extraction using water as a solvent. However, there is no identification of Javanese chili compounds for aqueous extracts. The purpose of the study was to identify the Javanese chili compound extracted using water using several methods. The research methods used are infusion, maceration, microwave, and high pressure. Research parameters are phytochemical screening, antioxidant activity, total phenolic content, and antimicrobial. The results showed that the Javanese chili extract from the infusion method contained alkaloids, tannins, saponins, and flavonoids, while Javanese chili extract using the microwave method only contained flavonoids. The antioxidant also results also showed that the Javanese chili extract with the high-pressure method gave the greatest inhibitory value, while the long pepper extract with the maceration method gave the lowest inhibitory value. The highest total phenolics are produced at the high-pressure extraction and lowest treatments in microwave extraction. All extracts have antimicrobial properties on gram-positive and negative bacteria. The highest inhibiting properties in gram-negative bacteria are produced by the high-pressure method of water extract. While the highest inhibiting properties for gram-positive bacteria are produced by the infusion method. Javanese chili extract using water with the infusion method and high pressure has compounds that have high antioxidant and antibacterial properties.

1 Introduction

Long Pepper plant *(Piper retrofractum* Vahl) is a spice and phytopharmaka plant from Indonesia that is widely spread in East Java and Lampung which is used as a traditional medicine for the community or for the food, beverage, herbal medicine, and medicine industry [1]. Long pepper in Indonesia is referred to as Javanese chili. Javanese chili plants contain alkaloids, saponins, tannins, flavonoids, steroids, triferpenoids, glycosides, piperine (about 4-6%), piperlonguminine, slvatine, tiltilime, sitosterol, citrate and linalool scattered throughout the leaf organ, stem, fruit and roots of the Javanese chili plant. From the content, Javanese chili have many health benefits including asthma, headaches, stomach spasms, bacterial infections and colds. Javanese chili extract also has antibacterial properties inhibiting the growth of pathogen microorganisms [2]. The many contents and benefits of the plant make Javanese chili plant widely used as an herbal medicine plant or traditional herbs, especially in the Madura area. With the development of technology, some manufacturers began to develop Javanese chili products into liquid Javanese chili drinks [3].

Traditional brewing that has been done so far using Javanese chili extract that is only extracted using water. Although there is no scientific evidence that shows that the extract provides health benefits, it is believed to provide health effects. Therefore, it needs to be proven by doing research to determine whether the extract has health effects as has been believed so far. The product development that has been carried out by [3] also uses water solvent extract but there has been no research on phytochemical composition and health benefits. The use of water solvents will facilitate application in the production process because the solvent's properties are cheap and not harmful to health and do not need to be separated.

The method of extraction of bioactive compounds from herbal plants that have been widely done is using infusion and maceration methods. In this study also developed extraction methods using microwaves and high pressure. The solvent used is a water solvent to facilitate applications in the industry. The purpose of the study was to qualitatively analyses phytochemical compounds, total phenols, and antioxidant activity of extracts.

2 Method

2.1 Material

Javanese chili obtained from Sumenep Madura, aquadest, Ethanol, methanol, HCl.

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2.2 Sample preparation

Dried Javanese chili are milled and sifted with 100 mesh sieves. Furthermore, the material is stored for further extraction.

2.3 Extraction

A total of 50 grams of the sample is weighed and then dissolved in water as much as 500 mL (1:10). Next the sample is extracted using the infusion method (heated for 10 minutes at a temperature of 90 C), pressure liquid extraction for 10 minutes, microwave for 10 minutes, maceration 2 days. The extraction results are then filtered and condensed into a viscous extract using a rotary evaporator. Condensed extracts are then analysed.

2.4 Phytochemical screening

Screening Phytochemical is the initial test to find out the number or absence of phytochemical compounds in the material. Phytochemical screening is an alkaloid test, tannin, saponin test, and flavonoid test.

2.4.1 Alkaloid

Dragendorff's test. 1mL of extract was taken and placed into a test tube. Then 1mL of potassium bismuth iodide solution (Dragendorff's reagent) was added and shaken. An orange red precipitate formed indicates the presence of alkaloids.

2.4.2 Flavonoid test

1mL of extract was taken and placed into a test tube. Then few drops of sodium hydroxide solution were added and shaken. Emergence of intense yellow color that turns to colourless after adding dilute acid implies the existence of flavonoids.

2.5 Total phenolic content

Total phenolic contents in the extracts were determined by the modified Folin-Ciocalteu method described by (4). An aliquot of the extract was mixed with 2 mL Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 2 mL (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 20 min at 25 °C for color development. Absorbance was then measured at 760 nm UV-spectrophotometer (Shimadzu, USA). Samples of extract were evaluated at a final concentration of 0.1 and 0.15 mg/mL. Total phenolic contents were expressed in terms of gallic acid equivalent, GAE (standard curve equation: $y = 0.091\times + 0.167$, $R2 = 0.994$), mg of GA/g of dry extract. The experiment was repeated three times at each concentration [5].

2.6 Antioxidant activity

Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay as described by [6]. The hydrogen atom donating ability of the plant extractives was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/ purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants. A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of extract in methanol at different concentrations (12.5–150 μg/mL). The reaction mixture was vortexed thoroughly and left in the dark at RT for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. BHT was used as reference. Percentage DPPH radical scavenging activity was calculated by the following equation: where A0 is the absorbance of the control, and A1 is the absorbance of the extractives/standard. Then % of inhibition was plotted against concentration, and from the graph IC50 was calculated. The experiment was repeated three times at each concentration.

3 Result and discussion

3.1 Phytochemical screening

Javanese chili extract contains several bioactive compounds, including alkaloids, saponins, tannins, and flavonoids. The results of phytochemical compound screening can be seen in Table 1.

Tabel 1. Results of phytochemical screening of Javanese chili extract.

Sample	Alkaloid	Saponin	Tanin	Flavonoid
Maceration	+	-	+	+
High pressure	+	-	+	+
Microwave	-	-	-	+
Infusion	+	+	+	+

Ket : + = detected, - = not detected.

The results of the study showed that the extract used the infusion method indicates contains all phytochemical compounds, while the extract using the microwave method contains only flavonoids. Extracts from maceration and high pressure contain alkaloids, tannins and flavonoids but do not contain saponins.

The results showed that extraction using the microwave method produced fewer phytochemical compounds than other methods. Optimal conditions for microwave, such as specific power levels, extraction times, and solvent concentrations, significantly influence the yield and quality of the extracts [7-8].

3.2 Antioxidant activity

The antioxidant activity measured in this study was radical scavenger activity (RSA). The RSA value of Javanese chili extract can be seen in Fig. 1.

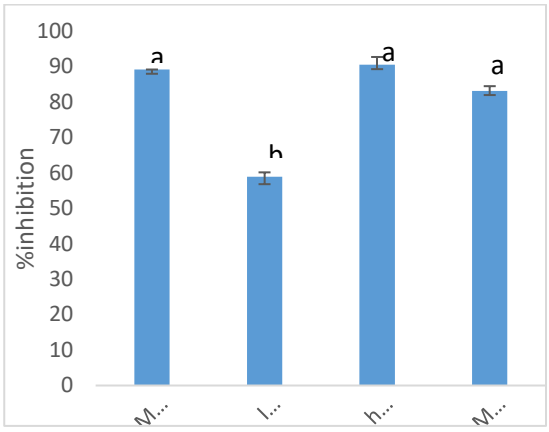

Fig. 1. Antioxidant activity of Javanese chili extract from various methods.

The results showed that all three methods provided the same percentage of inhibition statistically, while the infuse method provided the smallest percentage of RSA. The percentage of RSA from Javanese Chili extract ranges from 58.9% - 90.5% with the highest percentage of RSA obtained from Javanese Chili extract high pressure method. Extract from infusion method had the lowest RSA. It is suitable for extracting water-soluble compounds but may not be as effective for other types of phytochemicals [9]. Extraction using high pressure gives the highest %RSA indicating the extraction process can run effectively. The high-pressure extraction method has high effectiveness because it can extract oil and other bioactive compounds [10]. Green tea extracts extracted using the high-pressure method have the highest antioxidant activity compared to other methods [11]. Extracts by the maceration method also produce a high %RSA. Several studies show that extracts from the maceration method have high antioxidant activity, namely green pepper [12] and Falcaria vulgaris [13]. There is a strong correlation between high antioxidant activity and high total phenolic content in extracts [12].

Microwave and high-pressure extraction methods generally provide higher antioxidant activities compared to maceration. Microwave extraction is particularly noted for its efficiency and high yield of bioactive compounds, while high-pressure extraction excels in extracting potent antioxidants in a short time. Maceration, although less efficient, still produces extracts with significant antioxidant activities due to high phenolic content [12].

Extraction using the infusion method provides the least antioxidant activity. Factors that affect the antioxidant activity for the infusion method are temperature and boiling time. Research by [14] showed that a temperature of 100 ° C and a boiling time of 15 minutes resulted in the highest antioxidant activity of tea.

3.3 Total phenolic content

Total phenolic content is the total phenolic compound in the extract. Total phenolic content is an indicator of antioxidant activity. The total phenolic content of the extract is presented in Fig. 2.

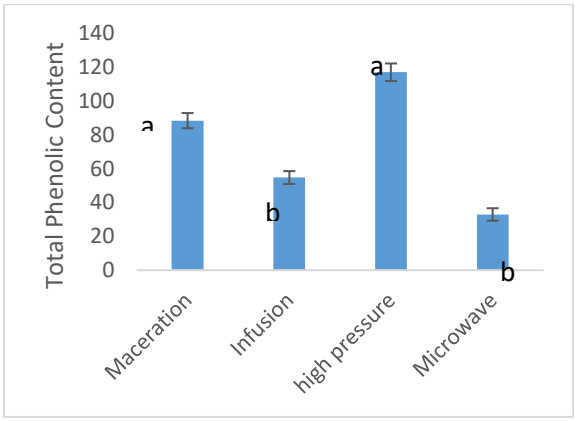

Fig. 2. Total phenolic content of Javanese chili extract from various methods.

The results showed that the extracts produced from the high-pressure method had the highest total phenolic content, while the extracts that had the lowest total phenolic content were extracts from the infusion and microwave methods. The total phenolic content of the Javanese chili extract produced ranges from 32.95 – 117.097 mg GAE/mL. Extraction using the high-pressure method yields the highest polyphenol in the green tea extract [11]. Maceration also results in a high total phenol

Infusion typically involves steeping plant material in hot water, which may not be as effective in breaking down cell walls and releasing phenolic compounds compared to more aggressive method. The efficiency of phenolic extraction in infusions is highly dependent on water temperature and steeping time, with higher temperatures and shorter times generally being more effective [14]. Research by [15] showed that a 20-minute infusion treatment for red tea yielded the highest polyphenols.

3.4 Antibacterial activity

The antibacterial activity of the extract was measured using the disc method. The bacteria tested consisted of gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli*). The inhibiting properties of Javanese chili extract can be presented in Fig. 3.

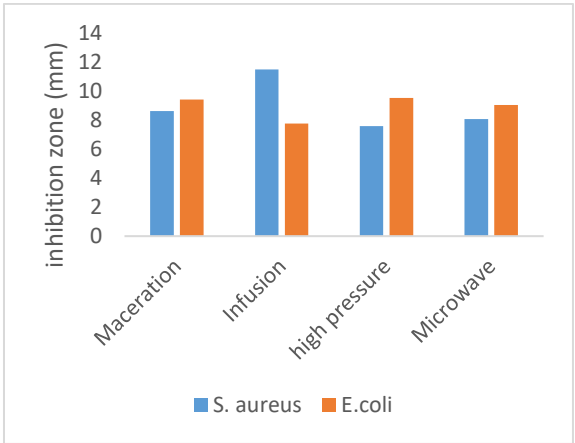

Fig. 3. Antibacterial activity of Javanese chili extract from various methods.

The antibacterial properties of the extract were tested on two bacteria, namely *S.aureus* and *E.coli*. Both bacteria were chosen because they represent gram-positive bacteria and gram-negative bacteria. The highest inhibition for *S. aureus* bacteria is extracted from the infusion method and the lowest is the high-pressure method. While the greatest inhibition for *E. coli* bacteria is produced in the high-pressure extract, and the lowest is produced from the infusion method. This shows that extracts from the effective infusion method inhibit gram-positive bacteria but are not effective in inhibiting gram-negative bacteria. On the other hand, extracts from the high-pressure method are effective in inhibiting gram-negative bacteria but are not effective in inhibiting gram-positive bacteria

The inhibition for *S. aureus* bacteria ranges from 7.6 – 11.51 mm and *E. coli* ranges from 7.76 – 9.54 mm. The inhibitory capacity of the extract against *E. coli* is classified as moderate resistance because all range from 5 – 10 mm. Meanwhile, the inhibition of extracts against *S. aureus* is classified as moderate inhibition except for extracts from the infusion method. Extracts from the infusion method are included in the strong inhibition because they have an inhibition of between 10 – 15 mm. Rossella extract using the infusion method provides the lowest bacterial inhibition value compared to other methods [16]. Bilberry leaf extract from the high-pressure method can inhibit gram-positive and gram-negative bacteria higher than conventional methods [17].

This study presents data that only extracts the infuse method is more effective at inhibiting gram-positive bacteria. While extracts with other methods produce the opposite. Some studies show otherwise. As research by [18] shows all extracts are more effective at inhibiting gram-positive bacteria. The phenolic compound content of the extract has been proven to be antibacterial. Hydroxyl groups have a strong affinity to proteins thus inhibiting bacterial enzymes and increasing interactions with the cytoplasmic membrane [19]. The total phenolic content of the extract of the infusion method is indeed low, so compounds that may play a role in the inhibition of gram-negative bacteria are alkaloids and saponins. Alkaloids can inhibit bacterial cell wall synthesis, alter cell membrane permeability, inhibit bacterial metabolism, and interfere with nucleic acid and protein synthesis (20).

4 Conclusion

Extracts from the high-pressure method have the highest total phenolic and antioxidant activity compared to other methods. However, extracts from the infusion method have the highest gram-positive bacterial inhibition properties compared to others. Javanese chili water extract with the high-pressure method has the potential to be developed as a functional drink that has antioxidant and antibacterial effects, especially gram-negative bacteria.

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