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Research Article

Determination of Total Phenolic Levels of Ethyl Acetate Fraction of Clove Leaves (*Syzygium aromaticum* (L) Merr)

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ABSTRACT: Clove is a spice plant used in the kretek cigarette industry, food, beverage, and medicine. Clove leaves contain chemical compounds, namely saponins, flavonoids, tannins, and essential oils. The exploration of clove leaves has not been maximally carried out. Therefore, this study aims to determine the total phenolic content in the ethyl acetate fraction of clove leaves by using gallic acid as a comparison. Clove leaves were powdered and then macerated by ethanol. The ethanol extract was dissolved in distilled water and it was fractionated with ethyl acetate on a separating funnel. The method used in this assay is the UV-Vis spectrophotometry technique. The qualitative analysis was performed by adding the $FeCl_3$ reagent. The results showed that the ethyl acetate fraction of clove leaves contained a total phenolic compound of 65.52 mg gallic acid equivalent per gram of extract (GAE/g). Thus, the ethyl acetate fraction of clove leaves is feasible to be developed as a source of phenolic compound.

KEYWORDS: Gallic acid; clove; spectrophotometry; phenolic total.

1. INTRODUCTION

Cloves are plants that are widely distributed from plantations/industries in eastern Indonesia, such as in North Sulawesi. It is known that this clove plant grows in lowland areas at an altitude of 200-900 above sea level. Clove plants have a height of 5-10 m. Clove leaves have a greenish and reddish color and are also egg-shaped or oval in shape. This plant has the characteristic that all parts of this tree contain oil, starting from the roots, stems, leaves, and flowers (Rorong, 2008).

Natural plant antioxidant compounds are phenolic or polyphenolic compounds, namely flavonoids, tocopherols, and polyfunctional acids. Phenolic compounds are compounds that have one or more hydroxyl groups attached to an aromatic ring (Vermerris & Nicholson, 2006). Clove leaves contain chemical compounds, namely saponins, flavonoids, tannins, and essential oils (Rorong, 2008).

According to Wungkana *et al* (2013), it is known that the phenolic content in the corncob sample is more in the ethyl acetate fraction compared to other fractions such as butanol, 70% ethanol, and n-hexane fractions. The ethyl acetate fraction also has high free radical scavenging activity. Previous studies suggested that the ethyl acetate fraction was able to attract phenolic compounds, therefore a study was conducted to determine the total phenolic compound content in clove leaves (*Syzygium aromaticum* (L.) Merr) using the ethyl acetate fraction (Wungkana, 2013).

2. EXPERIMENTAL SECTION

2.1. General

Ethanol, gallic acid, Folin-Ciocalteau reagent, sodium bicarbonate solution, ethyl acetate, filter paper and sterile distilled water. A standard solution of 1000 ppm gallic acid was prepared by weighing 10 mg of gallic acid dissolved in ethanol to a volume of 10 mL. From the stock solution, a 2.5 mL pipette was diluted with ethanol to a volume of 25 ml to produce a concentration of 100 ppm. From this solution, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 mL pipettes were pipetted and added to 10 mL of ethanol, resulting in concentrations of 4, 5, 6, 7, 8, and 9 ppm (Fawwaz, 2017).

Blender (Cosmos), micropipette (Huaweai), rotary evaporator (BUCHI-R-210), UV-VIS spectrophotometer, analytical weigher (Ohaus), stir bar, porcelain dish, horn spoon, tube rack, volume pipette, test tube and vial.

2.2. Sample collection

Clove leaf sampling is done by picking, so it is very important to pay attention to picking skills. Skills are needed to obtain the correct and appropriate simplicia (for example, if young leaves are needed, old leaves and twigs are not picked) and do not damage the parent plant. After the sample was taken, it was cleaned of dirt attached to the leaves and then dried by aerating and not exposed to direct sunlight. After drying, the clove leaves are blended until they become powder, stored in a container, and then ready to be extracted (Fawwaz, 2017).

2.3. Extraction and fractionation

Clove leaves that have been powdered and then macerated weighed as much as 170 g. Ethanol was added slowly into the maceration vessel. It was left for 3 days so that the liquid filter soaked the simplicia powder, and also stirred occasionally. Re-maceration is done so that it becomes clear. After that, it was filtered in a new container to obtain a liquid extract. The extraction results from the extract were evaporated using a rotary evaporator below the boiling point until a thick extract was obtained (Fawwaz, 2017).

Ethanol extract 2 g was dissolved in 20 mL of distilled water. After that, it was fractionated with 20 mL of ethyl acetate solvent on a separating funnel, repeated 6 times. After that, the ethyl acetate extract was evaporated to obtain a thick ethyl acetate extract (Ritna, 2016).

2.4. Qualitative analysis

Clove leaf ethyl acetate extract was put into a test tube, added with an $FeCl_3$ reagent. If it is green to blue, it indicates that it is positive for phenol (Alfian & Susanti, 2012)

2.5. Quantitative analysis

2.5.1. Determination of maximum wavelength

A total of 100 µL of gallic acid solution with a concentration of 4 ppm added 0.4 mL of Folin-Ciocalteau reagent (1:10), shaken, and allowed to stand for 3 minutes. Then, 7% Na_2CO_3 solution was added, as much as 4.0 mL, then shaken homogeneously, and allowed to stand at room temperature, the absorbance was measured at a wavelength of 700 – 750 nm (Fawwaz *et al*, 2017).

2.5.2. Concentration series of standard gallic acid

A total of 100 µL for each concentration of 4, 5, 6, 7, 8, and 9 ppm was added 0.4 mL of Folin-Ciocalteau reagent, shaken and left for 4-8 minutes, added 4.0 mL of 7% Na2CO3 solution, shaken until homogeneous. Added distilled water up to 10 mL and allowed to stand for 2 hours at room temperature. Measurement of absorbance at a maximum wavelength of 735 nm, a calibration curve was made for the relationship between gallic acid concentration (ppm) and absorbance (Fawwaz *et al*, 2017).

2.5.3. Determination of total phenolic content of ethyl acetate fraction

A total of 10 mg of the extract of the ethyl acetate fraction was weighed, dissolved with 10 mL of ethanol to produce a concentration of 1000 ppm, and pipetted as much as 1 mL was supplemented with 10 mL of ethanol to produce a concentration of 100 ppm. A pipette of 100 µL was put into a test tube, then 0.4 mL of Folin-Ciocalteau reagent was added. After that, the mixture was vortexed for 2 minutes, then 4.0 mL of Na_2CO_3 solution was added. The mixture was incubated in the dark for 30 minutes. It was measured using a spectrophotometer at a maximum absorbance of 735 nm wavelength. Performed 3 repetitions. The total phenol content was expressed as gallic acid equivalents in the extract (Wungkana, 2013).

2.6. Data analysis

A calibration standard curve was obtained by running on UV-Vis spectrophotometry and then plotting peak areas against concentrations. For the curve, the best fit of the line was calculated by the equation of a line. Linearity was evaluated through the correlation coefficient (R^2). The correlation coefficient, intercept, and slope of the calibration curve was calculated. The best fit of data was determined by linear regression using the following equation: Y= bx + a, where:

Y = Peak area
b = Slope
x = Concentration
a = Intercept.

3. RESULTS AND DISCUSSION

Phenolics are compounds that are found in many plants. It is known that phenolics have an aromatic ring with one or more hydroxyl groups (OH) as well as other accompanying groups. All plants have different phenolic components. It is known that there are phenolic components that have a large number of OH groups and also phenolic components that have small amounts of OH groups. The OH group plays a role in the electron transfer process, to stabilize and also absorb free radicals. There are several studies that say that these phenolic compounds are known to have various biological effects, namely having antioxidant activity through reducing mechanisms, capturing free radicals, chelating metals, reducing the formation of oxygen shorts, and donating electrons (Indrawati, 2013). Phenol compounds can be used as antiseptics, disinfectants, medicinal raw materials, and also as plastic confounders (Astarini *et al*, 2010).

Clove is a spice plant used in the kretek cigarette industry, food, beverage and medicine. The efficacy of the clove plant for the treatment of cholera, measles, toothache, aches and pains, darkens the eyebrows, increases the heart rate, and warms the body (Argomedia, 2008). Therefore, the aim of this study is to find out how much phenolic content is contained in clove leaves by using UV-Vis spectrophotometry method. The sample found in Bulukumba Regency, South Sulawesi. The method of collection is by picking the leaves. After the sample was taken, it was cleaned of dirt attached to the leaves and then dried by aerating and stored in a drying cabinet. After drying, the clove leaves are blended until they become powder, stored in a container and then ready for extraction. The powdered sample of clove leaf was weighed 170 grams and dissolved in 1500 mL of 96% ethanol as solvent. 96% ethanol solvent is used because it is more selective, mold is difficult to grow in ethanol 20% and above, non-toxic, neutral, good absorbance, ethanol can be mixed with water in all ratios, requires less heat for the concentration process and limited soluble disturbing substances (Wahyulianingsih *et al*, 2016).

The extraction method used in this research is the maceration method, because the extraction method is cold and the simplest because it does not require special equipment. The ethanol extract obtained was 29.5277 grams with a yield of 17.36% w/w which can be seen in **Table 1**. Then proceed with the ethyl acetate fraction, the ethanol extract that has

been obtained is weighed as much as 2 grams to be extracted with ethyl acetate solvent by liquid-liquid partitioning. Liquid-liquid partition is used because the ethanol extract of clove leaves is soluble in water. The thick extract of clove leaves was dissolved using 20 mL of distilled water and then put in a separating funnel and partitioned with 20 mL of ethyl acetate. so that two layers are formed, namely, the top layer is ethyl acetate solvent, and the bottom layer is distilled water, it is known because the density of ethyl acetate (0.66 g/mL) is smaller than the density of aquadest (1 g/mL). Re-partitioned with 20 mL of ethyl acetate six times so that it was clear. The ethyl acetate layer was collected and evaporated so that a thick ethyl acetate fraction was obtained, and the percent yield of the clove leaf ethyl acetate fraction was calculated to obtain the percentage yield of the fraction which can be seen in **Table 1**.

Table 1. The percent yield of clove leaf ethanol extract and ethyl acetate fraction

Sample	Weight of sample (g)	Weight of extract / fraction (g)	Yield (%)
Clove leaves	170	29.53	17.36
Clove leaves extract	2.54	0.79	30.98

The qualitative test aims to identify a compound in the sample being tested. In a qualitative test using the tube method on the sample, when $FeCl_3$ is added, the color changes from green to blackish green which indicates that it contains phenolic positively. The color change occurs when the addition of $FeCl_3$ reacts with one of the hydroxyl groups present in polyphenol compounds (Aini, 2014).

In determining the total phenolic content, the first step that needs to be done is to determine the maximum wavelength by running a standard solution of 4 ppm gallic acid at a wavelength of 400-800 nm and in this study, a maximum wavelength of 735 nm was obtained. Standard solutions of gallic acid with concentrations of 4, 5, 6, 7, 8, and 9 ppm were then measured at a maximum wavelength of 735 nm. Measurement of standard solutions was carried out in order to obtain a calibration curve to obtain a linear regression equation by plotting between concentration and absorbance. After obtaining the absorbance of each concentration of the standard solution and obtaining a linear regression equation, it is possible to determine the total phenolic content of the clove leaf ethyl acetate fraction (**Table 2**).

Table 2. Concentration series of gallic acid

Concentration (ppm)	Absorbance (735 nm)
4	0.225
5	0.325
6	0.456
7	0.539
8	0.638
9	0.779

Based on the absorbance data of the standard solution from each concentration, the regression equation y= 0.108x – 0.210 was obtained with the correlation coefficient (r) = 0.997 which can be seen in the **Figure 1**.

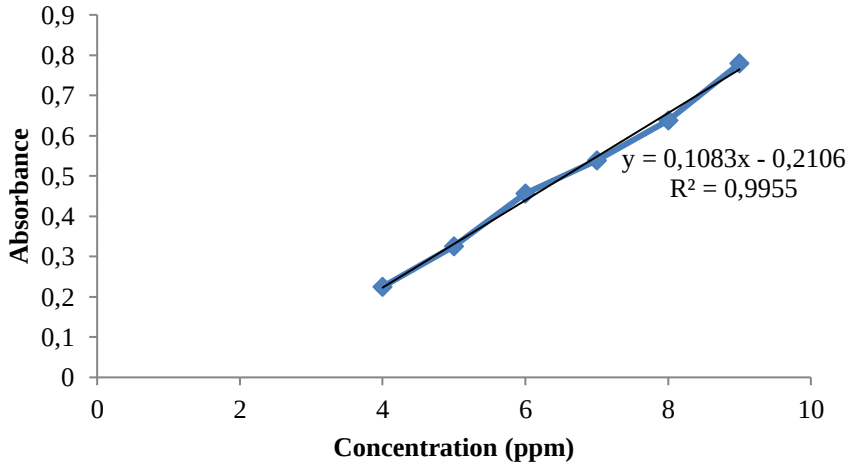

Figure 1. Gallic acid calibration curve at a maximum wavelength of 735 nm

In determining the total phenolic content, gallic acid was used as a standard solution. Gallic acid is a phenolic compound derived from hydroxybenzoic acid which is a simple phenolic acid. In addition, gallic acid is a natural phenol that is stable and relatively inexpensive. Gallic acid is also an option as a standard for the availability of a stable and pure substance (Ahmad *et al*, 2016). Folin-Ciocalteau reagent is used because phenolic compounds can react with Folin which can form a colored solution whose absorbance can be measured. The principle of the Folin-Ciocalteau method is the form

of a blue complex compound. This reagent oxidizes phenolics (alkali salts) or phenolic-hydroxy groups reducing heteropoly acids (phosphomolybdate-phosphotungstic) in the Folin-Ciocalteau reagent to form molybdenum-tungsten complexes. It is known that phenolic compounds react with Folin-Ciocalteau's reagent under alkaline conditions to cause the dissociation of protons in phenolic compounds into phenolic ions. To create alkaline conditions used 7% Na_2CO_3. During the reaction, the hydroxyl groups in phenolic compounds react with the Folin-Ciocelteau reagent, forming a blue molybdenum-tungsten complex with an unknown structure that can be detected by a spectrophotometer. The blue color formed will be equivalent to the concentration of phenolic ions formed, which means that the greater the concentration, the more phenolic ions that will reduce heteropoly acids into tungsten molybdenum complexes so that the blue color formed is more intense (Alfian, 2012).

Determination of the total phenolic content of the ethyl acetate fraction by UV-Vis spectrophotometric method. The maximum wavelength used in spectrophotometric measurements because it has a maximum sensitivity where the change in each concentration unit is the largest. The maximum wavelength for this absorbance curve complies with the Lambert-Beer law.

Table 3. Total phenolic content of the ethyl acetate fraction of clove leaf extract.

Replication	Absorbance (Y)	Initial phenolic content (mg/mL)	Phenolic content (mgGAE/g extract)	Average (mgGAE/g extract)	Average (%)
I	0.473	0.006324	62.61		
II	0.477	0.006361	60.58	62.52	6.25
III	0.499	0.006564	64.35		

The results of the determination of the total phenolic content of the ethyl acetate fraction of clove leaf had an average total phenol content of 3 replications, which was 62.52 mgGAE/g, meaning that in each gram of extract it was equivalent to 62.52 mg of gallic acid.

4. CONCLUSION

From the results of the research that has been carried out, it can be concluded that clove leaves contain phenolic compounds. The phenolic content in the ethyl acetate fraction of clove leaves was 62.52 mg GAE/g extract with a total phenolic content percentage of 6.25%.

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