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DIFFERENCE IN SOLVENT TYPE ON TOTAL PHENOLIC CONTENT OF CLOVE LEAF EXTRACT BY UV-VIS SPECTROPHOTOMETRY

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ABSTRACT

Background: Clove is one of the natural ingredients rich inphenolic compounds have several pharmacological activities, such as antioxidant, antimicrobial, anticancer, anti-inflammatory, cardio-protective, neuroprotective, endothelial protective, and hormonal modulation. The content of active compounds in cloves leaf extract is highly dependent on the ability of the solvent to extract secondary metabolites during the extraction process. This research aims to study the effect of solvent type on the total phenolic content (TPC) of cloves leaf extract using three different solvents. **Methods:**This study isquasi-experimental research to evaluate the impact of different solvents, namely ethyl acetate, 96% ethanol, and distilled water, on the TPC in cloves leaf extract.Analysis of TPC was carried out by UV-Vis. spectrophotometry using the Folin-CiocalteuMethod, a widely used technique for quantifying TPC in plant extracts. The difference between the three types of solvents in Total Phenolic Content was analyzed statistically using a one-way ANOVA test. **Results:**The distilled water extract showed the highest yield of 22%, followed by 96% ethanol extract with a 21% yield, and ethyl acetate extract had the lowest yield of 15.1%. The results showed that the extract using distilled water had the highest Total Phenolic Content of 668.54±2.68 mg/GAE/g. The 96% ethanol extract has a 634.09±2.72 mg/GAE/g phenol, while the ethyl acetate extract has the lowest phenol content, 600.84±3.48 mg/GAE/g. **Conclusion:** There is a significant difference in the use of solvent types on Total Phenolic Content, as indicated by the results of the one-way ANOVA test and Post hoc test. The obtained p-value of <0.05 (0.00) suggests that the difference is not due to random chance but rather due to the choice of solvent.

Keywords: Cloves leaf, Extract, Total phenolic content

Introduction

Phenols and polyphenols are a large group of chemical compounds produced through plants' secondary metabolic processes characterized by an aromatic structure with one or more hydroxyl group substituents (OH). Phenol and polyphenol compounds have a reasonably high distribution and abundance in various types of plants, making it easier to obtain materials and study them as potential medicinal substances. Most phenol and polyphenol compounds are bound to other compound molecules, such as sugar, so they have a reasonably high solubility in polar solvents such as water. Various studies prove that polyphenolic compounds have an extensive range of pharmacological activities, including as antioxidants, antimicrobials, anticancer, anti-inflammatory, cardio-protective, neuro-protective, endothelial-protective, to hormonal modulation(Andrés et al., 2023; Bertelli et al., 2021; Ebrahimi & Lante, 2021; Habibah, 2024; Habibah et al., 2023; Habibah & Ratih, 2023; Upadhyay & Dixit, 2015; Zhang et al., 2022).

The content of phenol and polyphenol compounds in a material is closely related to its bioactivity as an antioxidant (Ferreira et al., 2016; Habibah et al., 2023; Hossain & Rahman, 2011). The characteristics of phenol and polyphenol compounds that have hydroxyl groups in their ring structure allow hydrogen donors to free radical molecules, which is one of the main mechanisms of suppression of free radical compounds by antioxidant compounds (Azizah dkk., 2019; Rohmanna dkk., 2023; Ardani et al., 2023). Some previous studies prove that the relationship of antioxidant activity is positively correlated with the content of total phenol compounds, where the greater Total Phenolic Content will increase antioxidant activity(Lushaini dkk., 2015).

Exposure to free radicals is one of the risk factors for increased oxidative stress in the body that can trigger the formation and development of various degenerative diseases such as cardiovascular disease, hypertension, stroke, aging, liver damage to cancer (Ardani et al., 2023; Sharifi-Rad et al., 2020; Zehiroglu & Ozturk Sarikaya, 2019). In recent decades, degenerative diseases have increased in incidence and have become globally widespread and potentially the leading cause of death. The potential impact of this research on global health is significant, with global data reporting that by 2020, 73% of deaths worldwide were caused by degenerative diseases(Mokalu dkk., 2023). Increased exposure to risk factors and lifestyle changes have caused a shift in the pattern of degenerative diseases, which were initially experienced by the elderly and are now found at a younger age(Hamdin & Muliasari, 2020; Habibah et al., 2024).

This phenomenon increases the importance of various efforts to prevent and deal with the increasing prevalence of degenerative diseases. One of the efforts is developing and utilizing potential natural materials rich in bioactive compounds such as phenols and polyphenols. Cloves are one of the interesting natural ingredients that benefit our health. Cloves are one of the natural sources of phenolic compounds. Clove contains eugenol, eugenol acetate, and gallic acid compounds potentially developed in the pharmaceutical, cosmetic, and food fields. Some research proves that clove plants have higher antimicrobial and antioxidant abilities than other vegetables, fruits, and spices (Cortés dkk., 2014). The cloves leaf is one part that has a lot of potential and has not been widely utilized.

The utilization of potential natural materials is carried out through several stages, including extraction to isolate bioactive compounds contained in a material. The extraction process is important in the quality and quantity of bioactive compounds extracted. Several factors can affect the effectiveness of extraction, including solvent polarity. The extraction process occurs through the principle

of like dissolved like, where the compound will be dissolved in a solvent with the same solubility properties (Lenny, 2016). The more bioactive compounds extracted, the stronger the pharmacological effect. Therefore, the choice of solvent is a significant factor in the effectiveness of extraction, which depends on extracting these compounds during the extraction process.

Based on this background, this study was conducted to determine the effect of solvent type on Total Phenolic Content in clove leaves. This study used three types of solvents with different polarities to extract clove leaves: distilled water, 96% ethanol, and ethyl acetate. Furthermore, the Total Phenolic Content was determined using the Folin-Ciocalteu Method using UV-Vis spectrophotometry. The results of this study are expected to provide more information about the effect of solvent type on Total Phenolic Content (TPC) and help in determining the most effective solvent for extracting phenol compounds from clove leaves.

Method

In this study, the method used was a quasi-experiment with a one-group posttest-only design. The research was conducted at the Center for Post-Harvest Processing of Medicinal Plants of the Bali Provincial Health Office and the Basic and Applied Chemistry Laboratory of the PoltekkesKemenkes Denpasar. The samples were clove leaves from Pupuan Village, Tabanan Regency, Bali. Solvents for extraction include distilled water, 96% ethanol, and ethyl acetate. The Total Phenolic Content was measured using the Folin-Ciocalteu method, and measurements were made using UV-Vis spectrophotometry. The data were then subjected to a thorough statistical analysis. If the data were normally distributed, statistical analysis was performed using the Oneway ANOVA test. If the data were not normally distributed, statistical analysis was performed using the Kruskal-Wallis nonparametric test, ensuring the validity of the results.

The Process of Cloves Leaf Extract

Cloves leaves were obtained from Pupuan Village, Tabanan, Bali. The selected leaves were washed thoroughly with running water, then the leaves were air-dried to remove residual water from washing. The drying process is a crucial step in our research methodology, as it ensures the preservation of the leaves' properties. The leaves were then dried using an oven at a temperature of 40 ± 1˚C for approximately 20-40 hours until completely dry. After drying, the leaves were sorted to separate them from the stems and other parts that were involved in drying. Leaves that had passed the dry sorting stage were then crushed using a blender and sieved to obtain simplicia powder of relatively the same size(Habibah et al., 2023).

The extraction process was performed by weighing 400 g of dried leaves, which are then dissolved in aquades, 96% ethanol and ethyl acetate at a ratio of 1:5. The re-maceration process was carried out twice, to increase the effectiveness of the extraction process. In addition, agitation was performed for 15 minutes each day so that the ethanol could reach all parts of the leaf powder. The obtained extract was filtered with filter paper. The obtained filtrate was concentrated in the rotary evaporator vacuum at 30˚C(Habibah et al., 2023). The extract yields obtained based on the mass of simplicia powder were 22%, 21% and 15.1% for distilled water, 96% ethanol and ethyl acetate extracts, respectively.

Total Phenolic Content analysis

The Total Phenolic Content (TPC) test on distilled water, 96% ethanol, and ethyl acetate extracts was carried out using the Folin-Ciocalteu method. The process involves several sequential steps. First, one milliliter of the diluted extract is added to a test tube, followed by the addition of one milliliter of Folin-Ciocalteu reagent. The mixture is then incubated in a dark room for eight minutes.

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Next, 4 ml of sodium carbonate (Na_2CO_3), 75%, is added, the solution is gently shaken (homogenized), and then incubated for 30 minutes in a dark room. The TPC value is then determined as mgGAE/g through absorbance measurement at a wavelength of 768 nm using a spectrophotometer(Habibah, Nugroho, et al., 2023). TPC levels are determined based on the following equation and expressed in mg GAE/g.

$$TPC = \frac{sample\ concentration\ (\frac{mg}{L})\ x\ sample\ volume\ (mL)\ x\ dillution\ factor}{sample\ weight\ (g)}$$

Result

The yield of cloves leaf extract obtained through the maceration method in various types of solvents is presented in Table 1.

Table 1 Yield of Clove Leaf Extract

Solvent Type	Weight of Simplicia (g)	Weight of Extract (g)	Yield (%)	Color
Distilled water	400	88.62	22	Brownish green
96% Ethanol	400	83.97	21	Intense green
Ethyl Acetate	400	60.59	15.1	Intense green

As shown in Table 1, cloves leaf extract in distilled water solvent produces more yield compared to 96% ethanol and ethyl acetate solvents.

Determination of Total Phenolic Content in this study was carried out using gallic acid as the standard solution. Based on the absorbance measurements of a series of gallic acid solutions at a concentration of 12, 16, 20, 24, and 30 ppm yielded a standard curve as presented in Figure 4.

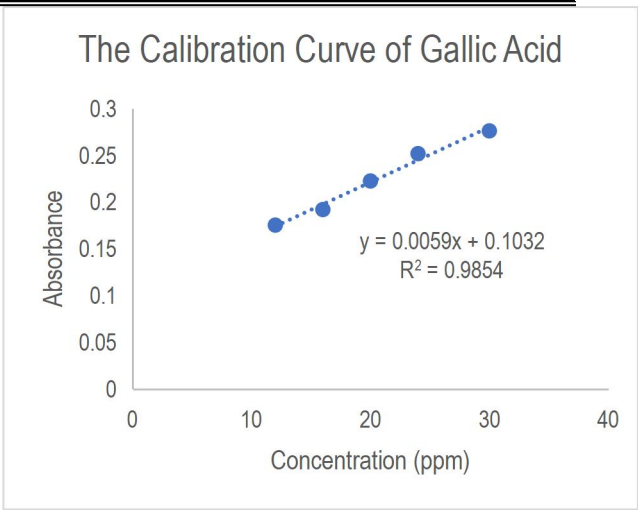

Figure 1. Calibration Curve of Gallic Acid Standard Solution

The calibration curve is used to determine the Total Phenolic Content in the sample extract by entering the measured absorbance of the sample into the linear regression equation of the calibration curve obtained. Furthermore, the Total Phenolic Content was expressed in mg GAE/g using the following equation.

$$TPC = \frac{sample\ concentration\ (\frac{mg}{L})\ x\ sample\ volume\ (mL)\ x\ dillution\ factor}{sample\ weight\ (g)}$$

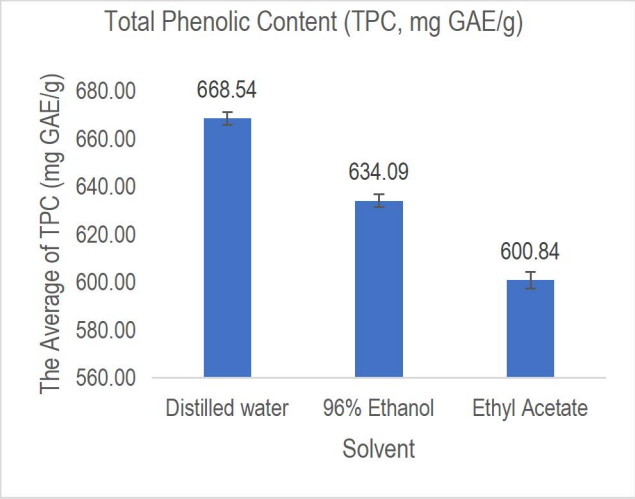

Figure 2. Total Phenolic Content

The Total Phenolic Content in the distilled water extract was higher than the 96% ethanol and ethyl acetate extracts; as seen in Figure 2, the distilled water extract reached 668.54±2.68mg/GAE/g. The 96% ethanol extract has a 634.09±2.72mg/GAE/g phenol, while the ethyl acetate extract has the lowest phenol content, 600.84±3.48 mg/GAE/g.

Table 2.Oneway ANOVA Test of Total Phenolic Content

Variable	r	TPC (mg GAE/g)	*p*
Distilled water	3	671,45	0,000
		667,97	
		666,19	
96% Ethanol	3	637,05	0,000
		633,52	
		631,71	
Ethyl Acetate	3	604,36	0,000
		600,76	
		597,41	

Based on the data presented in Table 2, it is proved that there is a significant difference between the treatments using distilled water, ethyl acetate, and 96% ethanol ($p < 0.05$).

Table 3. Duncan's Post Hoc Test for Total Phenolic Content

Variable	Distilled water	96% Ethanol	Ethyl Acetate
Distilled water	1,000	0,000	0,000
96% Ethanol	0,000	1,000	0,000
Ethyl Acetate	0,000	0,000	1,000

Table 3 shows a significant difference between the solvents used, namely distilled water, 96% ethanol, and ethyl acetate, on Total Phenolic Content with Sig. 0.000, which indicates a p-value <0.05.

Discussion

The extraction process in the study was carried out using the maceration method. The maceration method has advantages over other extraction methods because it requires relatively simple equipment, is more efficient, and does not require excessive heating, so the samples are not susceptible to degradation or damage(Atun, 2014; Habibah et al., 2023; Susanty & Bachmid, 2016). This study carried out the remaseration process three times using a new solvent. The remaseration process is carried out to increase the extraction efficiency so that the bioactive compounds contained in the sample are optimally extracted.

After extraction, the solvent was separated from the extract using a rotary evaporator at 50°C, resulting in a more concentrated extract. The concentrated extract obtained was then weighed, and the yield, which is the ratio between the weight of the sample used and the amount of metabolites obtained after extraction, was calculated. The distilled water extract showed the highest yield of 22%, followed by 96% ethanol extract with a 21% yield, and ethyl acetate extract had the lowest yield of 15.1%. The extract yield can be used to qualitatively determine the amount of bioactive compounds contained in the extracted material (Utami et al., 2020). The yield results indicate that the bioactive compounds in clove leaves are more soluble in polar solvents than semi-polar solvent. This study's results align with research(Rahmi dkk., 2021), which showed that distilled water solvent gave the highest yield, while ethyl acetate gave the lowest yield. The yield is

considered good if it exceeds 10%, so the extract yields in this study are considered satisfactory because they exceed this standard. Several factors such as solubility of the solvent, temperature, extraction time, and the level of solubility of the extracted material can affect the yield of extracts obtained.

The Folin-Ciocalteu Method was used to measure the Total Phenolic Content. This method is based on the principle of phenolic group oxidation reaction. The Total Phenolic Content in the sample was determined using gallic acid as the standard solution. The Folin-Ciocalteu reagent will oxidize the phenolic group and reduce the hetero-poly acids (phosphomolybdic-phosphotungstic) into molybdenum-tungsten complexes. The phenolic hydroxyl group will react with the Folin-Ciocalteu reagent during the reaction process to form a blue molybdenum-tungsten complex. Furthermore, the intensity of the blue color formed was measured spectrophotometrically at a wavelength of 760 nm. The intensity of the blue color is proportional to the total phenolic compound in the sample. The blue color formed will be more intense, equivalent to the concentration of phenolic ions formed, meaning that the greater the concentration of phenolic compounds, the more phenolic ions will reduce heteropoly-acids (phosphomolybdate-phosphotungstic) into molybdenum-tungsten complexes so that the color produced is more intense(Ardila, 2020; Habibah et al., 2023; Hossain & Rahman, 2011).

Gallic acid is a standard solution for determining Total Phenolic Content using the Folin-Ciocalteu method. Gallic acid is an effective standard solution for determining Total Phenolic Content due to its unique properties and established methodology. Gallic acid is widely recognized in methods such as the Folin-Ciocalteu assay, where TPC is expressed as gallic acid equivalents. Gallic acid is advantageous as a standard solution for determining Total Phenolic Content due to its wide availability, well-known properties, and established methods for isolation and analysis(Rizvi et al., 2023; Wianowska & Olszowy-Tomczyk, 2023). Recent advances have shown that gallic acid can be effectively detected, which provides a cheap and efficient means of analysis in complex matrices, offering reassurance in challenging research scenarios (Zanoni, et. Al., 2024). Gallic acid is a standard for phenolic content and has significant antioxidant properties, making it relevant in health-related studies and applications(Wianowska & Olszowy-Tomczyk, 2023)

The determination of Total Phenolic Content begins with determining the maximum wavelength. Determination of the maximum wavelength was carried out using the gallic acid standard solution at a concentration of 30 µg/ml. Based on the measurement results, it is known that the maximum wavelength in this study is 768nm. When compared with the standard method, it is known that there is a shift in the maximum wavelength to a higher wavelength. The maximum wavelength shift in the Folin-Ciocalteu method can be caused by reaction conditions, especially pH and matrix interference(Rizvi et al., 2023).

Furthermore, a standard curve of gallic acid with a concentration series of 12, 16, 20, 24, and 30 ppm was made. The calibration curve of the gallic acid standard solution presented in Figure 1 shows a linear relationship between the concentration and absorbance of the standard solution with R^2 of 0.9875. The linear regression equation obtained is Y = 0.0058x + 0.1046. Subsequently, this calibration curve is used to determine the Total Phenolic Content in the sample extract by entering the measured absorbance of the sample into the linear regression equation of the calibration curve obtained. Furthermore, the Total Phenolic Content was expressed in mg GAE/g using the following equation.

$$TPC = \frac{sample\ concentration\ (\frac{mg}{L})\ x\ sample\ volume\ (mL)\ x\ dillution\ factor}{sample\ weight\ (g)}$$

Based on the results, it is known that the highest Total Phenolic Content was obtained in distilled water extract, which amounted to 668.54 mg GAE/g, followed by 96% ethanol extract at 634.09 mg GAE/g, and ethyl acetate extract with the lowest Total Phenolic Content of 600.84 mg GAE/g. Based on these results, it can be seen that thepolarity of the solvent in the extraction process greatly affects the Total Phenolic Content (TPC). Some previous research shows that different solvents produce different levels of phenol compounds, which are important for their antioxidant properties. Other studies have shown that distilled water often yields the highest TPC. For example, in the extraction of *Micromeriagraeca*, water extracts yielded 360 mg GAE/g dry weight. Ethyl acetate generally extracts fewer phenolic compounds than water and ethanol, as seen in the *Micromeriagraeca* study, which showed the lowest antioxidant activity (El Kamari et al., 2024). In the case of *Euphorbia resinifiera*, distilled water also gave the highest phenolic content (Aghoutane et al., 2023). Phenol and polyphenol compounds are a class of secondary metabolite compounds that are more soluble in polar solvents (Habibah, 2024). Therefore, solvent selection is critical, with polar solvents such as distilled water and ethanol more effective for extracting phenolic compounds. However, the specific context of the plant material and extraction method can also influence outcomes.

Conclusion

Based on the results, it can be concluded that solvent selection is a significant factor in the extraction process of bioactive compounds. The choice of solvent type affects the extract yield and Total Phenolic Content of cloves leaf extract. Cloves leaf extract in distilled water solvent yields more than 96% ethanol and ethyl acetate solvents. In addition, polar solvents like distilled water and ethanol are more effective for extracting phenolic compounds than less polar solvents like ethyl acetate. Total Phenolic Content in cloves leaf extract was highest in distilled water extract at 668.54±2.68mgGAE/g, followed by 96% ethanol extract at 634.09±2.72mgGAE/g and ethyl acetate extract at 600.84±3.48mgGAE/g. The results of the one-way ANOVA test and Post hoc test showed significant differences in Total Phenolic Content with a p-value of 0.00 ($p < 0.05$).

Conflict Of Interest

There is no conflict of interest related to this research andpublication.

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