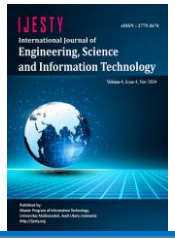
International Journal of Engineering, Science and InformationTechnology
Volume 4, No. 4 (2024) pp. 24-28
ISSN 2775-2674 (online)
Website: http://ijesty.org/index.php/ijesty
DOI: https://doi.org/10.52088/ijesty.v4i4.561
Research Paper, Short Communication, Review, Technical Paper



Determination of Total Phenolic Content of Ethanol Extract of Black Turmeric Rhizome (*Curcuma caesia* Roxb.) by Folin-Ciocalteu Method Spectrophotometrically

Zulfisa[1], Faizah Ulfa[1], Mhd Riza Marjoni[2*]

[1]*Department of Chemistry, Akademi Farmasi Dwi Farma Bukittinggi, Indonesia*
[2]*Department of Phytochemistry, Akademi Farmasi Dwi Farma Bukittinggi, Indonesia*

**Corresponding author Email: mhdriza.marjoni@gmail.com*

The manuscript was received on 1 March 2024, revised on 15 April 2024, and accepted on 10 September 2024, date of publication 1 October 2024

Abstract

Due to the high concentration of secondary metabolites present in nearly every part of the plant, black turmeric (Curcuma caesia Roxb.), a traditional medicinal plant, may provide a natural supply of antioxidants. The objective of this research is to determine the total phenolic content of the black turmeric rhizome ethanol extract using UV-Vis spectrophotometry. The extraction was performed by macerating the material in a solution consisting of 70% ethanol. We employed the Folin-Ciocalteu method to ascertain the total phenolic content, which was then represented as gallic acid equivalent (GAE). The research revealed that there were 192 mg GAE/g of total phenolic content in the ethanol extract of black turmeric rhizome. According to the metrics measuring total phenolic content, the black turmeric rhizome ethanol extract may have antioxidant properties. This study provides important information about black turmeric's antioxidant potential which can be used to develop herbal products based on this plant.

Keywords: *Curcuma Caesia, Total Phenolic, UV-Vis Spectrophotometry, Folin-Ciocalteu, Antioxidants.*

1. Introduction

Traditional medicinal plant use has grown in prominence as a therapeutic method in several nations, Indonesia included. Indonesian people have long utilized their natural wealth, including medicinal plants, as part of efforts to maintain health and treat diseases. These plants are believed to have benefits that can improve well-being without causing dangerous side effects like synthetic drugs [2]. In recent decades, the global trend that emphasizes the concept of returning to nature has further strengthened interest in herbal medicines. Many people are switching to natural ingredients because they are considered safer and have a low risk of side effects. People view herbal medicines as a healthier alternative, particularly for those seeking long-term health maintenance without reliance on synthetic chemicals [23]. One of the medicinal plants that is increasingly popular among researchers and health practitioners is black turmeric (Curcuma caesia Roxb.). Black turmeric is known for its bioactive compound content, which is believed to have the potential to treat various diseases. Researchers continue to explore the benefits of this plant, aiming to develop it into a more proven and effective herbal medicine.

The Zingiberaceae family includes the black turmeric (Curcuma caesia Roxb.), which has its roots in central and northeastern India. However, various tropical countries, including Indonesia, have successfully cultivated this plant over time. The uniqueness of black turmeric lies in its color and bioactive content, which are considered to have potential for traditional medicine. According to research by Hasri and Hasan (2023), the use of this plant is increasing, along with the growing interest in herbal medicine around the world. People have traditionally used black turmeric to treat a variety of health conditions, including abdominal pain, digestive disorders, asthma, cancer, and infectious diseases. This plant has become an important part of traditional medicine in several cultures because it is considered to have potentially strong anti-inflammatory, anticancer, and antimicrobial properties [10]. Those seeking natural solutions to various diseases often use black turmeric in the form of a potion or paste as an alternative treatment. Traditional herbal medicine in Indonesia also widely uses black turmeric. This herbal ingredient is known as a natural supplement to increase stamina, increase appetite,

and relieve coughs. According to research by Nuraeni the local herbal medicine culture has long included this plant, both in its fresh and processed forms, due to its beneficial properties for overall body health [17].

Numerous studies have demonstrated a close relationship between the pharmacological activity of black turmeric and its bioactive compound content, particularly phenolic compounds. Phenolic compounds contained in black turmeric are important components that provide various health benefits [21]. Plants widely contain phenolics, a group of compounds that fight disease through various biochemical mechanisms. Secondary metabolites with a wide variety of chemical structures make up phenolic compounds; these might be as simple as phenolic acids or as complex as tannins and lignins. Because of its chemical structure, this molecule has a wide range of therapeutic uses, including as an anti-inflammatory and anticancer agent [15]. Because of their structural variety, phenolics play a crucial role in preventing a wide range of biological harm to cells. Their capacity to operate as potent antioxidants is one of the main roles played by phenolic compounds. Antioxidants play a crucial role in protecting cells in the body from free radical oxidative damage [11]. Chronic diseases like cancer, heart disease, and neurological disorders can be averted with this practice. Therefore, black turmeric, with its high phenolic content, has great potential in the development of effective and safe herbal medicines.

Given the importance of phenolic compounds in plant pharmacological activity, determining total phenolic levels is a critical step in evaluating the potential of medicinal plants. Phenolic content acts as a primary indicator of biological activity, including the significant antioxidant capacity of plants. As a result, assessing total phenolics can provide valuable information on a plant's pharmacological potential in the context of herbal and natural medicine. One popular way to find the total phenolic levels is using the Folin-Ciocalteu method. The phenolic compounds and Folin-Ciocalteu reagents undergo an oxidation-reduction reaction, which is then followed by colorimetric detection, in this approach. This method can provide accurate results in measuring the amount of phenolics contained in the sample. The oxidation process that occurs between the reagent and phenolic compounds produces a color whose intensity can be measured to determine the sample's phenolic levels [14]. The Folin-Ciocalteu method benefits from its simplicity, high sensitivity, and versatility in handling various plant samples. This method not only finds widespread use in the laboratory but also plays a significant role in medicinal plant-based research. This method's flexibility and ease of use make it an effective tool for evaluating bioactive components in a variety of plants, including black turmeric [13].

Traditional medicine has long used black turmeric, but scientific research on its bioactive compound content, particularly phenolic compounds, is still relatively limited. To understand the pharmacological potential of black turmeric, one important step is to conduct in-depth research on its bioactive content. Because of their potent antioxidant action, phenolic chemicals play a significant role here. The purpose of this work is to determine the total phenolic content of black turmeric rhizome ethanol extract using UV-Vis spectrophotometry and the Folin-Ciocalteu reagent. The antioxidant activity of black turmeric can be better understood by determining its total phenolic content. Being familiar with the use of ethanol solvents in the extraction of bioactive plant components led us to select the ethanol extract of black turmeric rhizomes. Because of its extensive use and demonstrated sensitivity in studying phenolic compounds, the UV-Vis spectrophotometry method with the Folin-Ciocalteu reagent was chosen. This measurement will provide important data on the antioxidant potential of black turmeric, which can then be used as a natural antioxidant raw material. We expect the study's results to contribute scientifically to the understanding of black turmeric's potential as a natural antioxidant source. This data is useful not only in supporting claims for the use of black turmeric in traditional medicine but also as a basis for the development of herbal products based on this plant. The health and beauty industry's growing interest in natural ingredients presents a significant opportunity to develop black turmeric into high-value herbal products.

2. Research Method

Black turmeric rhizome (Curcuma caesia Roxb.), sourced from Bukittinggi, West Sumatra, was the primary material utilized in this investigation. To verify its legitimacy, the plant species was identified by the Andalas University Herbarium. Some of the chemicals used in this study included 70% ethanol (Merck), gallic acid (Sigma-Aldrich), Folin-Ciocalteu reagent (Merck), Na_2CO_3 (Merck), $FeCl_3$ (Merck), distilled water, and magnesium powder (Mg). The tools used included a UV-Vis spectrophotometer, rotary evaporator, analytical balance, blender, and standard laboratory glassware. The sample preparation process began by cleaning fresh black turmeric rhizome, slicing it thinly, and drying it in the sun for 3-5 days. Next, we ground the dried simplicia using a dry blender and proceeded with the maceration process, using 96% ethanol as the solvent. The maceration process took place over 24 hours, with a 1:10 ratio of simplicia to solvent, and the mixture underwent hourly stirring for the first 6 hours. We repeated this process twice, collected the obtained macerate, and evaporated it using a water bath at a temperature of less than 50°C. Screening of phenolic compounds was carried out by dissolving the ethanol extract in distilled water and adding 1% $FeCl_3$ to see the color change as a positive indicator. Total phenolic content was measured by the reaction method using Folin-Ciocalteu reagent and Na_2CO_3. We determined the maximum wavelength by measuring the absorbance of the gallic acid solution, and we determined the operating time based on the stability of the absorbance over a specific time interval. We calculated the total phenolic content results using the regression equation from the calibration curve and expressed them in percent w/w equivalent of gallic acid.

3. Result and Discussions

The black turmeric rhizome (Curcuma caesia Roxb.) from Cimahi City, West Java, served as the study's sample. We carried out sampling at the same location to ensure consistency of secondary metabolite content. This aims to minimize differences in active compound content that may occur due to different environmental factors. This study started with 50 grams of fresh black turmeric rhizome samples for further processing. The stages of making simplicia begin with a wet sorting process to separate unwanted parts from the sample. We then washed the sample clean to eliminate any remaining dirt. We then thinly sliced the clean rhizome to facilitate the drying process. We carefully carried out the drying process, avoiding direct sunlight, to maintain the stability of the active compound content in the black turmeric rhizome. We carried out dry sorting after drying to separate the unsuitable parts and then ground the dried rhizome into Simplicia powder. This Simplicia-making process yielded 15 grams of black turmeric Simplicia powder from 50 grams of the wet sample. The next stages of research, including the extraction and analysis of the active compound content, can utilize this herbal medicine.

In this study, we employed the maceration method to extract the black turmeric rhizome herbal medicine. We chose the maceration method due to its safety for thermolabile compounds like phenolic and flavonoid compounds, which are susceptible to damage from high temperatures [7]. This method guarantees the optimal extraction of the herbal medicine's active compound content without any degradation. 96% ethanol serves as the solvent in the maceration process. The decision to use 96% ethanol is based on several advantages. This ethanol is effective in extracting secondary metabolites, resists fungi and bacteria growth, is non-toxic, and has good absorption. In addition, 96% ethanol also requires a relatively short time in the concentration process, making it an efficient and safe solvent for processing herbal medicines [19]. In terms of health, 96% ethanol is also considered more beneficial and safer to use in the extraction process of herbal medicines (Indonesia, 2023). To keep the active components stable, we thicken the liquid ethanol extract using a basic water bath once the maceration process is finished. The temperature should not exceed 50°C. The procedure ended with an extract weight of 2.18 grams and a yield value of 4.36%. This yield demonstrates the extraction process's efficiency in obtaining active compounds from black turmeric rhizomes.

Using phytochemical screening to qualitatively identify the group of compounds contained in plants, researchers in this study concentrate on the phenolic compounds discovered in the ethanol extract of black turmeric rhizomes [12]. The screening process aims to early identify the potential bioactive compounds contained in plants. Based on the screening results, the ethanol extract of black turmeric rhizomes showed positive results in terms of phenolic and flavonoid compounds. Strong antioxidant properties of phenolic compounds are known to protect cells from damage by free radicals. This makes plants such as black turmeric, which contains phenolic compounds, a potential source of natural antioxidants. The $FeCl_3$ test was used to detect phenolic compounds in an ethanol extract of black turmeric rhizome, and the formation of a blackish-green color indicated a positive result for phenolic compounds. These phytochemical screening results are in line with what other studies have found: Curcuma caesia contains phenolic compounds, such as phenolic acids and flavonoids [5]. These findings bolster the evidence that black turmeric possesses a significant phenolic content, potentially serving as a natural antioxidant source for health applications.

For this purpose, we extracted the rhizomes of black turmeric from their roots and measured their total phenolic content using the Folin-Ciocalteu method. This method works by reducing phosphomolybdate-phosphotungstate by the aromatic nucleus of phenolic compounds found in black turmeric rhizomes [20]. This creates a blue molybdenum-tungsten complex. This process requires the reaction to take place in a basic environment, so it is necessary to add sodium carbonate to create these conditions. These basic conditions function to dissociate protons in phenolic compounds, which produce phenolate ions [24]. These phenolate ions are highly reactive and can contribute to the reduction process that occurs during analysis. In this context, the addition of sodium carbonate not only acts as a pH regulator but also ensures that the phenolic compounds are in an active form and can interact with the reagents used. The Folin-Ciocalteu method is well-known for determining total phenolic levels because of its accuracy and ability to detect various types of phenolic compounds. We anticipate that this method will provide clear information about the phenolic potential in the ethanol extract of black turmeric rhizome, which is critical for understanding the potential health benefits of this plant. The standard curve of gallic acid was made using a series of concentrations, namely 10, 20, 30, 40, and 50 µg/ml. As a phenolic chemical with a straightforward structure, excellent stability, and complete availability, gallic acid was selected as our standard [20]. A linear regression equation is produced using the ethanol extract of black turmeric rhizome using the gallic acid standard curve as a measuring point. We measured the gallic acid absorbance for 60 minutes at a maximum wavelength of 774 nm. Other research has shown that total phenolic analysis using the Folin-Ciocalteu method is most effective when conducted at wavelengths between 760 and 765 nm, albeit this maximum wavelength is marginally different from that [22]. This variance can be because the phenolic component makeup of the samples examined varied or because the analytical instruments used had different specifications. The importance of determining the maximum wavelength and using a standard curve is to ensure accuracy and consistency in determining total phenolic levels. Understanding the characteristics of the gallic acid standard curve ensures the measurement results are reliable and provide valid information about the potential of phenolic compounds in black turmeric extract, a source of natural antioxidants with potential health benefits.

We set the operating time for absorbance measurements at 60 minutes, at which point the absorbance reached stability. This duration is longer than some standard protocols that generally use an incubation time of 30 minutes. This longer incubation time may be needed to make sure that the reaction between the phenolic compounds in the sample and the Folin-Ciocalteu reagent works perfectly. A longer incubation time can increase the interaction between the phenolic compounds and the reagent, allowing the formation of a more stable complex and increasing measurement accuracy. We anticipate that the ethanol extract of black turmeric rhizomes will more faithfully represent the total phenolic levels if the correct reaction is carried out, which should improve the measurement results. Research including bioactive components, such as phenolic compounds with antioxidant potential, necessitates longer incubation times to ensure the validity of the results. Therefore, if you want more precise and trustworthy answers from your investigation, a longer incubation time would be a good place to start.

Figure 1 shows the gallic acid calibration curve, and Table 1 shows the absorbance of the gallic acid standard.

Table 1. Gallic Acid Absorbance Data

Concentration (µg/ml)	Average Absorbance
10	0,207
20	0,308
30	0,401
40	0,559
50	0,629

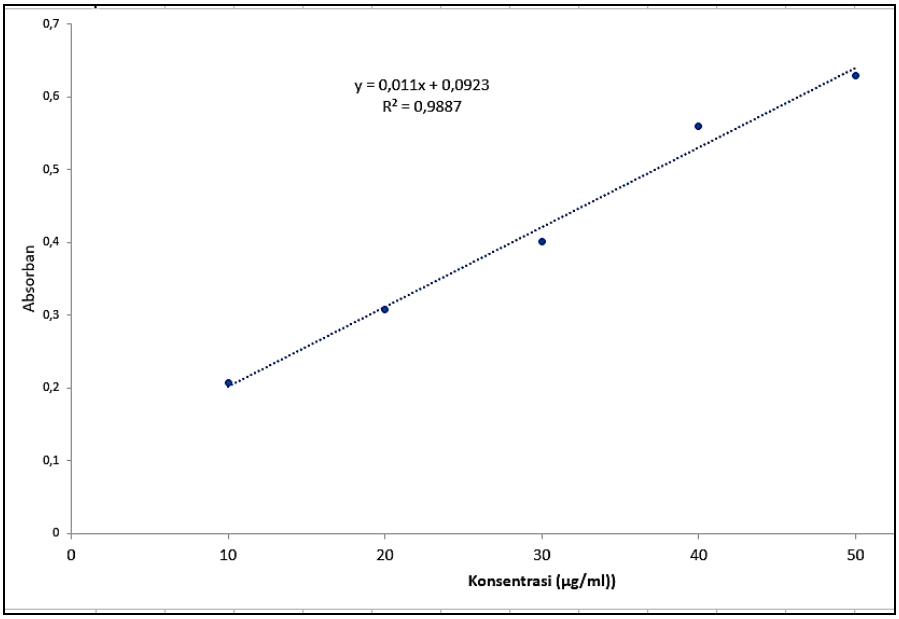


Fig 1. Calibration Curve of Gallic Acid

The gallic acid calibration curve showed good linearity, characterized by a regression equation (y = 0.0923 + 0.1095x) and a coefficient of determination (R2) of 0.9887. With an R-value of 0.9958, the link between gallic acid content and absorbance is strongly indicated. With an R^2 value above 0.99, these results are deemed acceptable, meeting the criteria for high linearity in the analysis procedure. This linear regression equation will allow us to perform additional analysis to ascertain the overall phenolic content of the sample of extract. By utilizing a UV-visible spectrophotometer to assess the extract sample's absorbance, we can ascertain the phenolic content with confidence thanks to prior calibrations. Because phenolic compounds may play a role in antioxidant activity and other plant biological processes, including those involving black turmeric rhizomes, accurately estimating the total phenolic content is of the utmost importance. The data obtained from this analysis can provide insight into the therapeutic value of black turmeric rhizomes and their applications in health product development. Due to its high phenolic component concentration, black turmeric rhizome extract shows promise as a natural antioxidant source. According to studies done by Aryal et al., there is a direct correlation between antioxidant activity and the total flavonoid and phenolic content [4]. This shows that flavonoids and other phenolic components are crucial in enhancing an extract's antioxidant capability. In addition, research by Johari and Khong also confirmed that the higher the total phenolic content in an extract, the higher the antioxidant activity. These findings establish a robust foundation for investigating the potential of black turmeric rhizomes in the creation of natural products, particularly in the health and nutrition sector, where antioxidant compounds are essential for combating free radicals and enhancing overall health [9]. Therefore, in addition to its therapeutic value, black turmeric rhizome extract can serve as a raw material for the production of more effective health products.

4. Conclusion

A total phenolic content of 192 mg GAE/g was produced by the ethanol extract of black turmeric rhizome, indicating that it possesses considerable antioxidant potential. A significant indication that black turmeric rhizome can operate as a source of natural antioxidants is the presence of substantial levels of phenolic chemicals. This confirms what other research has shown, which is that antioxidant activity is directly proportional to the overall flavonoid and phenolic concentration. This indicates that an extract's capacity to combat free radicals and offer protection to the body is directly proportional to the concentration of phenolic and flavonoid chemicals it contains. Additional research also indicates that an increase in total phenolic content is directly proportional to an increase in antioxidant activity. Therefore, the therapeutic potential of black turmeric rhizome extract is not only promising, but it can also serve as an alternative in the development of health products that prioritize antioxidant protection.

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