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Determination of Chemical Compounds and Antibacterial Activity in Turmeric Leaves (*Curcuma Longa Linn*)

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Abstract. The use of synthetic antibiotics in medicine has encountered widespread resistance, prompting the development of new drugs from herbal plants. Herbal plants have the potential to become a new alternative to antibiotics. Turmeric is a plant that is widely used as a traditional medicine. Turmeric contains many secondary metabolites such as alkaloids, triterpenoids, flavonoids, tannins, and polyphenols, making it a potential traditional medicine, especially as an antibiotic. This research method aims to determine the chemical content of turmeric and determine its antibacterial activity. The research method involved extracting turmeric leaves with methanol, identifying the compounds using TLC, FT-IR, and GC-MS, and then testing their antibacterial activity. The results of TLC, FT-IR, and GC-MS measurements showed that the most abundant chemical compound in turmeric leaves was phytol. The results of the antibacterial activity inhibition of turmeric leaf extract showed that the methanol extract of turmeric leaves inhibited S. aureus and E. coli bacteria with values of 7.07 mm and 6.93 mm, respectively. This experiment indicates that the methanol extract of turmeric leaves has moderate antibacterial potential against S. aureus and E. coli bacteria.

Keywords : Turmeric leaves, extraction, traditional medicine, antibacterial

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Introduction

Lifestyle changes and environmental pollution have caused many new health problems in society. Lifestyle changes that cause a decline in health are mostly due to the consumption of fast food, lack of exercise, frequent late nights, and smoking. Other factors, such as environmental pollution, also have a negative impact on human health. Some of the diseases that greatly affect human health are those caused by bacteria. These bacteria can originate from unhygienic food and contaminated environments such as water, soil, and air [1]. Various diseases caused by bacteria include diarrhea, boils, skin infections, and meningitis. These bacterial diseases can generally be cured with synthetic antibiotics. However, the continuous use of synthetic antibiotics can lead to drug resistance against diseases [2]. Therefore, it is necessary to develop traditional medicine as an alternative treatment for these various diseases. One medicinal plant with the potential to replace synthetic antibacterial drugs is turmeric.

The ginger family (*Zingiberaceae*) includes the herbaceous plant turmeric, which has rhizomes. Turmeric plant native to Asia and has now spread to subtropical regions around the world. The historic belief that turmeric plants can heal a variety of ailments has led to their widespread cultivation in modern times. Turmeric powder is used in traditional Chinese medicine to cure rheumatism, biliary disorders, inflammation, parasite infections, and skin conditions. Curcumin ($C_{21}H_{20}O_5$), a phenolic molecule that is a member of the phenol group, is the chemical component found in turmeric rhizomes [3]. Turmeric has been utilised to enhance appetite, heal wounds, alleviate itching, reduce swelling, address shortness of breath, manage diarrhoea, and promote flatulence. Turmeric is utilised as a beauty cleanser and cosmetic product. Furthermore, turmeric rhizomes serve as a colouring agent in food and beverages, as well as a culinary spice [4]. A multitude of research have empirically evaluated the advantages of turmeric. Turmeric comprises curcumin, which possesses anti-inflammatory qualities, biological antioxidants, anticarcinogenic effects, antimutagenic characteristics, anticoagulant effects, among others [5]. Turmeric rhizomes have leaves that are commonly used as a cooking spice. In addition, the leaves have also been studied for use in soap formulations as an antiseptic [6]. However, detailed research exploring turmeric leaves as an antibacterial agent is still limited.

Antibacterials are agents that can eradicate or inhibit the proliferation or reproduction of bacteria [7]. Antibacterials are categorised as antimicrobials that can suppress bacterial proliferation. The process by which active substances impede bacterial development involves destroying the cell wall, affecting cell permeability, modifying protein molecules and nucleic acids, reducing enzyme activity, and obstructing the creation of nucleic acids and bacterial proteins [8]. Therefore, this study aims to determine the chemical content of turmeric aduin and determine its antibacterial activity. The research method involved extracting turmeric leaves with methanol, identifying the compounds using TLC, FT-IR, and GC-MS, and then testing their antibacterial activity.

Experimental

Preparation and extraction of turmeric leaves. Turmeric leaves are cleaned and finely chopped, then weighed to make 10 grams of powder. The turmeric leaf powder is extracted by maceration using methanol as a solvent for 2 x 24 hours. The methanol extract obtained is filtered and evaporated using a rotary evaporator to obtain a concentrated extract. The concentrated extract is then weighed, and the percentage yield is calculated using the formula :

$$\%\ \text{percentage yield} = \frac{weight\ of\ extract\ obtained\ (g)}{weight\ of\ crude\ drug\ powder\ extracted(g)} x100\%$$

Chemical Compound Identification Test on Extracts. Turmeric leaf extract was dissolved in methanol and then identified using thin layer chromatography (TLC) using a combination of n-hexane and ethyl acetate solvents in appropriate ratios. Turmeric leaf extract was also identified using Fourier Transform Infrared (FTIR) test results to analyze the functional groups of its chemical compounds and using Gas chromatography–mass spectrometry (GC-MS) to determine the structure of its chemical compounds.

Antibacterial Test. This antibacterial test began with the preparation of a Nutrient Agar (NA) medium. The NA medium was prepared by dissolving 30 grams of NA in 1 liter of distilled water in an

Erlenmeyer flask. The medium is then heated to boiling and sterilized using an autoclave for 15 minutes at a temperature of 121°C and a pressure of 1 atm. The prepared agar medium is inoculated with suspensions of Escherichia coli and Staphylococcus aureus bacteria. A 100 µl suspension of bacteria is streaked in a zigzag pattern using a loop until the bacterial suspension is evenly distributed throughout the medium [9].

Antimicrobial activity was determined using the Kirby-Bauer method or the paper disk method. The paper disks were sterilized before being used for antimicrobial activity testing. Sterile paper discs were placed into each vial containing the turmeric extract solution prepared at a concentration of 40,000 ppm. The paper discs were then placed on an NA medium containing Escherichia coli and Staphylococcus aureus bacteria and incubated at 37°C for 24 hours. This experiment was repeated three times, and the inhibitory activity was observed after 24 hours. In this study, tetracycline was used as the positive control and DMSO as the negative control. The inhibition zones were measured using a Vernier caliper and compared between treatments.

Result and Discussion

The extraction method used in this study was maceration. This method's advantage is that it avoids damage to active substances due to excessive heating [9]. The results of macerating turmeric leaves were dark green extracts, indicating that binding had occurred with the compounds in the turmeric leaves. The concentrated turmeric leaf extract was analyzed to determine the percentage yield, which indicates the amount of secondary metabolites obtained. The percentage yield of the turmeric leaf extract was found to be 3.06%. The thickness of the cell walls and cell membranes highly determines the extraction results in plants using the maceration method. The maceration method is carried out by soaking plant material in a specific solvent, causing pressure differences between the inside and outside of the cells, which leads to the dissolution of secondary metabolites from the cytoplasm. This pressure difference causes the cell walls and membranes to rupture. This is what determines the yield obtained in a maceration extraction process.

Results of Chemical Compound Identification in Extracts

Thin layer chromatography (TLC) was used to qualitatively determine the content of compounds in turmeric leaves. The principle of TLC is to separate chemical components based on the principle of absorbance and partition determined by the stationary phase (adsorbent) and mobile phase (eluent). The stationary phase used is silica gel GF254 TLC plates, which are polar, and the mobile phase consists of a combination of solvents with different polarities. The optimal solvent combination used in this study was n-hexane and ethyl acetate with a ratio of 6:4. A good spot pattern can separate compounds with different polarities .

The data obtained from this TLC test were the retention factor (Rf) values as a result of TLC plate elution. These results provide information about the potential compounds contained in turmeric leaf methanol extract. The results of observing the spots of turmeric leaf extract with a solvent combination of n-hexane and ethyl acetate in a 6:4 ratio showed Rf values of 0.12, 0.57, 0.73, 0.82, and 0.96. The results of the test on the TLC plate were identified under UV light at 365 nm and 254 nm. Qualitative tests were conducted to determine the content of compounds in turmeric leaves using thin layer chromatography (TLC). More polar compounds are strongly retained in the stationary phase because the stationary phase is polar, resulting in low Rf values. Small Rf values in polar and nonpolar eluents indicate that the compounds in the spots are more strongly absorbed by the silica gel, causing the spots to appear at the bottom. This indicates that the spots have greater polarity due to hydrogen bonds between the compounds and the silica gel. High Rf values indicate that nonpolar compounds will continue to move upward in accordance with their degree of non-polarity [10]. Secondary metabolites that are predominantly polar include flavonoids, alkaloids, and tannins. On the other hand, secondary metabolites that are predominantly nonpolar include steroids and triterpenoids.

Fourier Transform Infrared (FTIR) Test Results. FTIR spectroscopy is a fast, simple, and non-destructive analysis technique. The results of the analysis using FTIR describe the functional groups present in the chemical compounds contained in turmeric leaf extract. The results of testing turmeric leaf extract using FTIR can be seen in Figure 1.

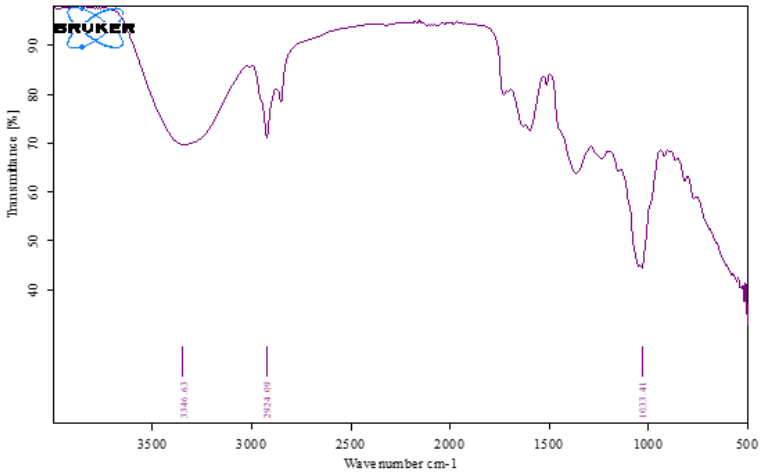

Figure 1. Spectroscopy results of turmeric leaf extract

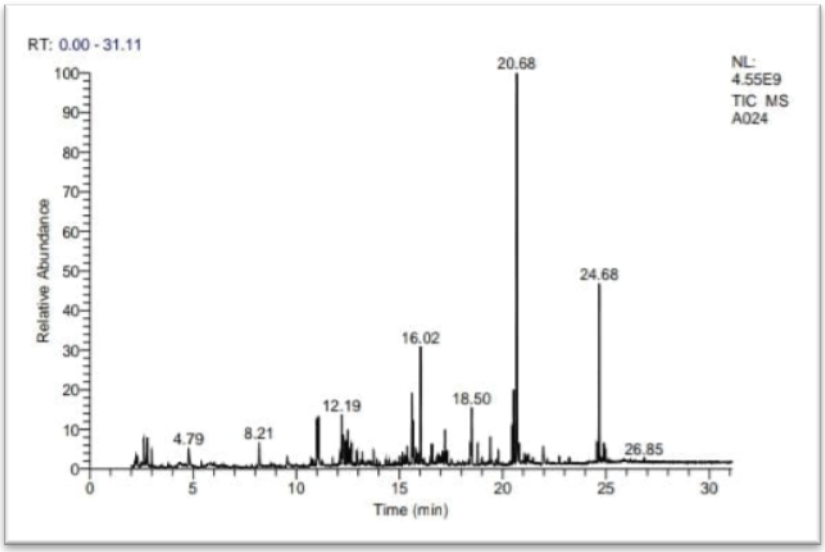

Figure 2. GC-MS analysis results of methanol extract

Based on the FT-IR test results in Figure 1, it can be seen that the FT-IR spectra for turmeric leaves show the presence of O-H group stretching vibrations at a wavelength of 3347.20 cm-1, C-H groups at a wavelength of 2922.35 cm-1 and 1361.15 cm-1, C=C groups at a wavelength of 1600.38 cm-1, and C-O groups at a wavelength of 1035.30 cm-1. These FTIR test results indicate that the functional groups present in turmeric leaves are compounds belonging to the alcohol and alkene groups.

Gas chromatography–mass spectrometry (GC-MS) results. Chemical compound analysis of turmeric leaf methanol extract using GC-MS, which is a combination of GC and MS, results in data on the possible molecular structure and the amount of compounds contained in the extract. In GC, the mixture of compounds is separated into individual compounds, which are then detected by MS based on their fragmentation patterns [11]. The GC-MS analysis results obtained from the methanol extract can be seen in Figure 2.

The GC-MS results of turmeric leaf methanol extract showed a variety of different chemical compounds. The separation of compounds in the column occurred where nonpolar compounds were retained longer in the column with longer retention times compared to polar compounds. The potential of polar metabolites in turmeric leaf extract includes phenolic compounds (RT 11.00) and alcohol groups (RT 18.50) (RT 20.68). Meanwhile, the potential of nonpolar secondary metabolites present in turmeric leaf extract includes sesquiterpenes (RT 15.60), (RT 15.65), and hexane (RT 16.02). Some chemical compounds from turmeric leaf extract can be seen in Table 1. The structures of compounds with polar and nonpolar properties can be seen in **Figure 3**.

Table 1. Rf values obtained from GC-MS analysis of compounds in turmeric leaf extract

Rf	Compouds	%Area
11.00	2-Methoxy-4-vinylphenol ($C_9H_{10}O_2$)	3.17
15.60	Ar-tumerone ($C_{15}H_{20}O$)	4.07
15.65	Tumerone ($C_{15}H_{22}O$)	2.94
16.02	Curlone ($C_{15}H_{22}O$)	7.09
18.50	Hexadecanoic acid, methyl ester($C_{17}H_{34}O$)	5.55
20.68	Phytol ($C_{20}H_{40}O$)	26.92
24.68	Diisooctyl phthalate ($C_{24}H_{38}O_4$)	11.34

Table 2. Measurement results of the inhibition zone diameter of turmeric leaf extract

Code Of Sample	Diameter of the inhibition zone (mm)		Control +	Control -
	S. aureus	*E. Coli*		
1	7.10	6.71	20.38	-
2	7.09	6.72	20.39	-
3	7.12	6.73	20.44	-
Average	7.10	6.72	20.40	-

Antibacterial Test Results

The results of the antibacterial activity test of turmeric leaf extract using the Kirby-Bauer method or paper disc method. The antibacterial test was conducted against the growth of Escherichia coli and Staphylococcus aureus bacteria. The inhibition zone values against these bacteria can be seen in **Table 2**. In this study, tetracycline was used as the positive control, while Dimethyl Sulfoxide (DMSO) was used as the negative control. DMSO is a good solvent for extracts without affecting the inhibitory activity against the test bacteria, so it was used as the negative control in this test. The measurement of the inhibition zone diameter can be seen from the diameter of the inhibition zone, which is the clear area around the paper disk [12].

The inhibition zone formed is less than ≤ 5 mm, so the inhibition zone response is categorized as weak. If the inhibition zone formed is 6–10 mm, the inhibition zone response falls into the moderate category, while 11–20 mm falls into the strong category, and ≥ 20 mm falls into the very strong category [13]. The results of the inhibition zone measurements in Table 2 indicate that the application of turmeric leaf extract at a concentration of 400 µg/disc produces an inhibition zone response classified as moderate against the growth of S. Aerus and E. coli. Turmeric leaf extract exhibits inhibitory activity against S. aureus with a value of 7.10 mm and an inhibition zone against E. coli of 6.72 mm. The positive control had an inhibition zone > 20 mm, very strong category. The antibacterial activity of turmeric leaf extract may be due to the presence of secondary metabolites with antibacterial properties in the extract. Potential antibacterial compounds in turmeric leaf extract were identified from the chemical components of the GC-MS results, namely alcohol or phenol content and terpenoid groups. The mechanism of alcohol and phenol content in inhibiting bacteria involves alcohol derivatives and phenol derivatives acting as antiseptics by denaturing and coagulating bacterial cell proteins. Alcohol compounds can cause the denaturation of bacterial cell proteins. Phenol derivatives interact with bacterial cells through absorption processes involving hydrogen bonding. At low concentrations, weak protein-phenol complexes form and quickly break down, followed by phenol penetration into the cell, causing precipitation and protein dena-

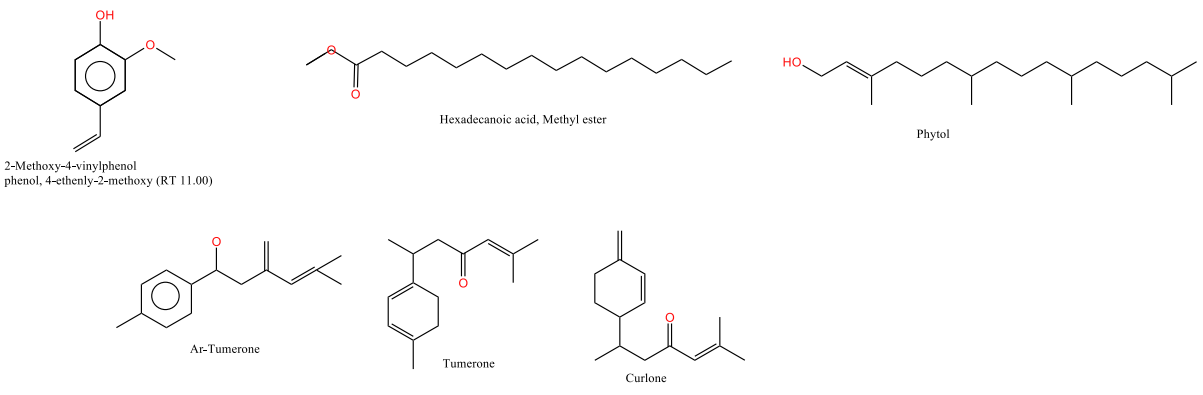

Figure 3. Structure of compounds in turmeric leaf extract

turation. At high concentrations, phenol causes protein coagulation and cell membrane lysis [14].

Conclusion

Turmeric leaves (*Curcuma longa Linn*) have the potential to contain chemical compounds consisting of polar and nonpolar molecules. Based on FTIR testing, turmeric leaf extract contains O-H group stretching vibrations, C-H groups, C=C groups, and C-O groups, indicating that the compounds contained may belong to the alcohol and alkene groups. The GC-MS results of turmeric leaf methanol extract showed the potential of polar metabolites in turmeric leaf extract, including phenolic compounds (RT 11.00) and alcohol groups (RT 18.50) (RT 20.68). Meanwhile, the potential of nonpolar secondary metabolites present in turmeric leaf extract includes sesquiterpenes (RT 15.60), (RT 15.65), and hexane (RT 16.02). The results of inhibition zone measurements show that an inhibition zone response classified as moderate against the growth of *S. Aerus* and *E. coli*. Turmeric leaf extract exhibits inhibitory activity against *S. Aureus* with a value of 7.10 mm and an inhibition zone against *E. coli* of 6.72 mm. The antibacterial activity of turmeric leaf extract may be due to the presence of secondary metabolites with antibacterial properties in the extract.

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