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Antibacterial potential of *Curcuma caesia Roxb* ethanol extract against nosocomial infections

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ABSTRACT

Introduction: Nosocomial infection is one of the infections that occurs in the hospital environment and can cause several diseases, but nosocomial infection can also cause the sufferer to experience sepsis and even die if they do not get treatment. This study aims to determine the active compound of black turmeric extract (*Curcuma caesia Roxb*) and measure the inhibitory ability of several bacteria that cause nosocomial infections.

Methods: The diameter of the inhibitory zone was evaluated using the Kirby-Bauer disk diffusion method in this investigation.

Results: The results showed that black turmeric extract has the potential to act as an antibacterial against both gram-positive and gram-negative bacteria. The highest results were obtained at the highest concentration (80%) against *Staphylococcus aureus* bacteria with a 15.10 ± 6.95 mm inhibition zone.

Conclusion: Based on the results of this study, it can be stated that black turmeric extract (*Curcuma caesia Roxb*) has the potential to be an antibacterial control for nosocomial infections.

Keywords: antimicrobial, black turmeric, *Curcuma caesia Roxb*, nosocomial infections, pathogenic bacteria.
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INTRODUCTION

Nosocomial infection is a local or systemic infection obtained from the hospital environment. This infection develops within 48 hours after admission or after discharge from the hospital.[1] The World Health Organization (WHO) estimated that there was an 8.7% global prevalence of nosocomial infections, with rates ranging from 5.0% in North America and Europe to 40.0% in Asia, Latin America, and Sub-Saharan Africa.[2] Prevention of nosocomial infections can be done by increasing the knowledge of nurses about the application of universal precautions, while control can be achieved using antibacterial.[3]

Nosocomial infections can be caused by bacteria, viruses, parasites, and fungi. However, bacteria are the main causative agents in most cases of infection resulting in death.[2] Infection can be controlled with the use of antibiotics, but excessive use of antibiotics with improper prescribing causes antibiotics to be increasingly ineffective so that microorganisms are resistant to one or more classes of antibiotics.[4]

The use of antibiotics has helped to lower bacterial illnesses' morbidity and fatality rates.[5,6] Antibiotic therapy in infections proved progressive for a certain period of time, but the effect of bacterial prevention was reduced due to the development of antibiotic resistance, which led to a reduction in the efficacy of the drug against infection by such bacteria.[5] The increasing resistance of bacteria to existing antibiotics encourages important efforts to find alternatives by administering infection-preventing antibiotics from natural ingredients.[7,8]

Black turmeric (*Curcuma caesia Roxb*), which is a family of *zingiberaceae*, is used as a traditional medicine herbal ingredient in India, Pakistan, and Turkey.[9,10] Black turmeric has the potential to be an effective medicinal plant because it contains bioactive compounds such as flavonoids, phenols, curcuminoids, proteins, amino acids, alkaloids, and essential oils responsible for antibacterial activity.[11] Therefore, this study aims to assess *Curcuma caesia Roxb*'s antibacterial ability against pathogenic gram-positive and gram-negative bacteria.

MATERIALS AND METHODS

Materials

The ingredients used in this study were black turmeric, ethanol, disk antibiotic (chloramphenicol), Muller Hilton Agar (MHA), aqueous solution, physiological NaCl, and

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phytochemical test materials.

Plant material collection

Curcuma caesia Roxb was obtained from Pekanbaru, Riau, Indonesia, and was approved by Prof. Dr. Fitmawati, M.Si. (Riau University), and according to the voucher, the specimen is stored in the herbarium of the same institution with the number rab053.

Extract Preparation

Part of the rhizome of black turmeric was used in the study. Rhizomes are cleaned with tap water, dried in the sun, mashed using a blender, and stored. The dry powder of black turmeric was extracted with 70% ethanol for 5 days. A rotary vacuum evaporator is used to evaporate the obtained liquid extract at a temperature of 70 °C till a thick extract is produced.

Microorganisms used for testing

This research was carried out with bacterial strains obtained from the Abdurrab University Laboratory in Pekanbaru, Indonesia. The bacteria strains used for antibacterial screening are *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans, Pseudomonas aeruginosa, Salmonella typhi*, and *Escherichia coli*. Bacterial strains are retained on agar nutrient media for gram-positive and Mac Conkey for gram-negative and then stored in the refrigerator for further use.

Preparation inoculum

A full loop of bacterial culture grown overnight was inoculated in 3/4 tubes of 10 ml of nutrient broth at a temperature of 37 °C in a rotary shake incubator for 16–18 hours. By changing the optical density of nutritional broth to a level equating to 0.5 at 620 nm using a spectrophotometer equivalent to 108 cfu/mL, the inoculum size of each bacterial strain was standardized.

Phytochemical test

Black turmeric crude extract samples were tested for active compound content through phytochemical tests as described by Trease and Evans (1983) and Harbone (1998).[12,13]

Total phenolic content

The method of Taga, Miller, and Pratt (1984) was used to determine the sample extract's total phenolic content (TPC), with a few minor adjustments.[14] Gallic acid was employed as the reference in this study, while 70% ethanol served as the sample replacement. The gallic acid equivalent (lg/ml) of the extract is used to calculate the phenolic content using a standard curve for gallic acid. In essence, 2 ml of concentrated Na CO 1: 3 are added to 1 ml of each sample. The mixture is then given two minutes to settle before being incubated at room temperature. The combination is then given 0.1 ml of 50% Folin-Ciacalténe phenol reagent. For 30 minutes, the mixture is incubated at room temperature in the dark. Finally, a spectrophotometer was used to read the absorbance at a wavelength of 765 nm (Shidmazu UV-1800, UK). The gallic acid equivalent (mg GAE/g), which is determined using the gallic acid standard curve, is used to express the anti-bacterial potential.

TPC (mg GAE/g) = [GAE (mg/ml) x extract volume (ml)] / sample weight (g)

Total flavonoid content

The total flavonoid content (TFC) of the sample was determined according to the method by Özkök, D'arcy, and Sorkun (2010).[15] As a reference, quercetin is utilized. 5.6 ml of distilled water, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate, and 1 ml of standard are combined with 1 ml of standard per sample. Thirty seconds are spent stirring the mixture. The mixture is then permitted to rest for 30 minutes at room temperature. Finally, a spectrophotometer is used to read the absorbance at 415 nm (Shidmazu UV-1800, UK). The sample's total flavonoid concentration (in mg/ml) is determined using the quercetin standard curve and expressed as quercetin equivalent (QE), which is determined using the formula below.

TFC (mg QE/g) = [QE (mg/ml) x volume (ml)] / sample weight (g)

Antibacterial activity test

The method of disc diffusion is used to conduct the antibacterial activity test. Twenty milliliters of sterile MHA medium are produced for petri dishes. A solid medium is covered with the test culture, which is then given 10 minutes to dry. With the use of a sterile swab, dried paper discs (diameter 6 mm, Whatman filter paper no. 1) containing various concentrations (20%, 40%, 60%, and 80%) of viscous extracts of *Curcuma caesia Roxb* are deposited aseptically on an agar medium. The loaded disc is set down on the substrate's surface and left at room temperature for 30 minutes to allow the compound to diffuse. The ethanol solvent is used to prepare the negative control. As a positive control, chloramphenicol, an antibiotic, is utilized. At 37°C, the plate was incubated for 24 hours. Each experiment was carried out three times, and the findings were documented by measuring the growth inhibition zone surrounding the disc.

Data analysis

The data from the research results are presented in the form of a table, then a descriptive analysis is carried out and compared with the literature related to the discussion.

RESULTS

Result of Yield Extract

In this study, black turmeric was used in amounts up to 3 kg. Black turmeric was washed and then dried under fairly stable weather conditions. Drying is carried out in order to reduce the moisture content contained in the plant. In addition, the drying process aims to prevent the sample from becoming overgrown with mold during the research process. Samples that have been dried are then extracted by maceration. The color of black turmeric before drying is blue-black, and after drying, the color of black turmeric changes to brown. The dried black turmeric is then blended to form a dried powder and filtered through a 100-mesh filter. Approximately 500 grams of dried powder are obtained, and then black turmeric dried powder is macerated for 5x24 hours, after which it is filtered and a brown black turmeric filtrate is obtained. The maceration method was chosen because it aims to attract efficacious substances that are resistant to heating as well as those that do not withstand heating. This maceration process involves

Table 1. Phytochemical test of black turmeric extract

No	Secondary metabolites	Reagent	Extract	Reaction
1	Alkaloid	Mayer	-	No white deposits are formed
		Bourchard	+	Formed brown precipitate
		Wagner	+	Formed brown precipitate
		Dragondorf	+	Snapped orange deposits
2	Flavonoid		+	Formed orange color
3	Saponin		+	Formed foam
4	Tanin		+	Formed in black / blackish green
5	Terpenoid		+	Formed blackish/purple ring

Table 2. Mean zone of growth inhibition of 70 % ethanol extract of black turmeric extract

	Mean zone of growth inhibition (mm) ± SEM					
Conc	SL	EC	PA	SA	SE	SM
20%	9.19 ± 0.09	14.3 ± 0.06	8.5 ± 0.5	10.04 ± 1.86	9.3 ± 0.67	NA
40%	8.2 ± 0.04	8.36 ± 0.09	7.16 ± 0.17	11.55 ± 3.44	10 ± 0.58	8.2 ± 0.04
60%	7.45 ± 0.05	7.43 ± 0.12	7.16 ± 0.17	13.83 ± 5.58	10.16 ± 0.73	8.5 ± 0.5
80%	7.05 ± 0.05	7.16 ± 0.92	7.6 ± 0.33	15.10 ± 6.95	11.33 ± 0.33	9.3 ± 0.67
Chloramphenicol (+)	36.5 ± 0.01	36.57 ± 0.01	11.66 ± 0.60	30.68 ± 2.68	29.33 ± 0.44	29.66 ± 0.33
Ethanol (-)	NA	NA	NA	NA	NA	NA

Zona of growth inhibition = diameter of well plus zone of growth inhibition; NA= not activity; SEM = standard error mean; conc = concentration; SL = *Salmonella*; EC = *Escherichia coli* ; PA = *Pseudomonas aeruginosa*; SA = *Staphylococcus aureus;* SE = *Staphylococcus epidermidis;* SM = *Streptococcus mutans*

soaking black turmeric dried powder in 70% ethanol solvent concentration. This solvent can bind polar compounds so that It can break through the cell wall and enter the cavity that houses the active substance. Maceration lasts three days, with stirring at room temperature taking place every day. To maximize the extraction yield, black turmeric ethanol extract is filtered and evaporated in the rotary evaporator until a viscous extract is obtained. The results of the research showed that the evaporation extract using a rotary evaporator produced 13 grams of brown viscous extract with a paste-like texture. The viscous extract obtained was tested for the effectiveness of black turmeric ethanol extract against several types of bacteria.

Content of active compounds

In this study, the identification of active compounds (qualitative) contained in black turmeric extract was carried out through phytochemical tests (Table 1). Based on the results obtained, black turmeric extract has active compounds of alkaloids, flavonoids, saponins, tannins, and terpenoids. Based on the quantitative test results, black turmeric ethanol extract has a total phenolic content of 2.35%, while total flavonoids are 0.61%.

Antimicrobial activity

The goal of the study was to assess the black turmeric ethanol extract's antibacterial effectiveness against various gram-positive and gram-negative microorganisms. Since chloramphenicol is one of the widely-used antibiotics for therapy, it is employed as a positive control. According to this study's results (Table 2), the diameter of the inhibition zone formed varied depending on the type of bacteria used. For example, the inhibition zone formed by gram-positive bacteria increased with concentration, whereas it shrank with concentration in the case of gram-negative bacteria.

DISCUSSION

In general, the size of the block zone diameter tends to be comparable to the concentration of the extract, but in this study, there was a decrease in the diameter of the inhibition zone at a higher concentration. The diameter of the inhibitory zone does not always rise in proportion to the increase in the concentration of antibacterial substances. This can occur due to the possible difference in the diffusion speed of different antibacterial compounds, which can cause the diameter of the inhibitory zone at the smallest concentration to be greater than the larger concentration. The antibacterial activity of an extract will initially increase until a maximum inhibition zone is obtained at a certain concentration. If the concentration is increased again, the resistance zone will decrease. The higher the concentration, the more the inhibition zone will decrease and tend to be constant.[16,17]

A large incidence of the diameter of the inhibitory zone that is not proportional to the magnitude of the concentration also occurred in research (Asdedi et al., 2016), which stated that black turmeric rhizome extract has active substances that can inhibit the growth of bacteria such as *Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*. In the literature, the concentration that effectively forms the inhibitory zone is a concentration of 45%, 60%, or 75%, and the higher the concentration, the smaller the diameter of the inhibition zone produced.[18] Furthermore, Fatoni et al. (2017) also obtained a similar thing, namely, in the test of antibacterial activity of extracting ethanol stem tabat barito (*Ficus deltoideajack*) against *Streptococcus pyogenes* bacteria using extract concentrations of 5%, 10%, 20%, and 40%. The results of the study

showed that at a concentration of 5%, the inhibitory zone was 11.05 mm, while a concentration of 40% only produced a 4.825 mm.[19] Therefore, it is very likely that the diameter of the inhibitory zone that is not proportional to the magnitude of the extract concentration may occur because it is influenced by the diffusion ability of an extract and can also be caused by several factors that affect antibacterial activity.

The antibacterial activity can be influenced by four factors, including the extract concentration, metabolite compound content, diffusion power of the extract, and the type of bacteria inhibited. The gram-negative cell wall has a thinner peptidoglycan layer than gram-positive bacteria but has a more complex additional outer membrane layer, as a result of which it will generally be more difficult to penetrate the cell wall of gram-negative bacteria than gram-positive bacteria. Other factors can also influence antibacterial activity, such as pH, temperature, environment, age of microbes, and several other factors that are considered sensitive to antibacterial activity.[20]

Black turmeric ethanol extract, which has an antibacterial activity of 80% against *Staphylococcus aureus*, which measures 15.10 + 6.95 mm, produces the maximum antibacterial activity. Three levels of antimicrobial activity can be distinguished: mild activity (inhibition zone less than 12 mm), moderate activity (inhibition zone between 12 and 20 mm), and strong activity (inhibition zone greater than 20 mm) (inhibition zone more than 20 mm). It is clear that the ethanol extract of black turmeric is highly effective against *Staphylococcus aureus*.

The large diameter of the inhibitory zone produced by black turmeric ethanol extract is due to the presence of chemical compounds contained in the extract, namely flavonoids, saponins, tannins, phenols, and steroids. The biological activity of flavonoid compounds works by damaging the cell walls of bacteria. This mechanism can occur due to the reaction between lipid compounds and amino acids with the alcohol groups in flavonoids, so that cell walls are damaged and cause these compounds to enter the nucleus of bacterial cells.

The mechanism of saponins involves lysing bacterial cells by making hydrogen bonds that cause the permeability of bacterial cells to be unbalanced, and then bacterial cells will lyse. The mechanism of action of tannin compounds as antibacterial substances is by shrinking cell walls or cell membranes that have been lysed. Tannin compounds have the ability to inactivate microbial adhesins and transport proteins and enzymes on cell membranes.[21] The mechanism of action of phenol compounds can also cause protein denaturation contained in cell walls, so that it can damage the arrangement and change the permeability mechanism of microsomes, lysosomes, and cell walls.[22] Steroid components that produce leaks in liposomes are related to lipid membrane sensitivity and the mechanism through which steroids act as antibacterial agents. Steroids can interact with lipophilic compound-permeable cell phospholipid membranes, resulting in decreased membrane integrity and altered cell membrane shape, which makes cells brittle and subject to lysis.[20]

In disc paper containing ethanol as a negative control, there is no inhibition zone. The selection of solvents using ethanol takes into account that ethanol is easy to obtain, selective, and does not affect efficacious substances. In each repetition, there was no inhibition zone, which corroborated that there was no effect of the ethanol solvent. As for positive control, it uses chloramphenicol antibiotics, which are broad-spectrum antibiotics that are able to inhibit the development of gram-positive or gram-negative bacteria. Chloramphenicol is highly efficient in preventing the development of *Staphylococcus aureus* bacteria.[23]

This test is carried out with three repetitions, and each repetition does not obtain optimal results until the last repetition. This is due to several factors that affect the diameter of the inhibition zone, namely the turbidity of the suspension, which should be measured using a spectrophotometer so that the turbidity of the bacterial suspension is more accurate when compared to the turbidity of the MC Farland 0.5. In addition, the thickness of the agar medium can also be one of the factors that affect the inhibition zone of bacterial growth. The effective agar thickness is 4 mm.[24] This study has limitations because it was unable to identify specifically which of the active compounds in *Curcuma caesia Roxb* is most effective at preventing nosocomial infections.

CONCLUSION

The results showed that black turmeric ethanol extract (*Curcuma caesia Roxb*) has active compounds of alkaloids, flavonoids, saponins, tannins, and terpenoids. Based on the test results of antibacterial activity, it can be stated that black turmeric extract has different inhibitory activity in each test bacteria. The highest inhibition occurred in gram-positive bacterial species, namely *Staphylococcus aureus* species (15.10 $\pm$ 6.95 mm), with the highest inhibitory activity. Based on this, black turmeric extract has the potential to be antibacterial in the future.

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ETHICAL CONSIDERATIONS

This study has received ethical approval from Abdurrab University Indonesia and Tun Hussein Onn Malaysia University.

CONFLICT OF INTEREST

There are no conflicts of interest between the writers and the subject matter of the current study.

AUTHOR CONTRIBUTIONS

All authors contributed the same amount of work to this study.

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