ANTIBACTERIAL CURCUMA CAESIA

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ANTIBACTERIAL POTENTIAL OF *Curcuma caesia Roxb* ETHANOL EXTRACT AGAINST NOSOCOMIAL INFECTIONS

Abstract

Nosocomial infection is one of the infections that occurs in the hospital environment that can cause several diseases, but nosocomial infection can also cause the sufferer to experience sepsis to death if they do not get treatment. The purpose of this study was to determine the active compound of black turmeric extract (*Curcuma caesia Roxb*) and measure the inhibitory ability of several bacteria that cause nosocomial infections. The method used in this study was *Kirby bauer* disk diffusion method by measuring the diameter of the inhibition zone. The results showed that black turmeric extract has the potential as an antibacterial against Gram-positive and Gram-negative bacteria. The highest results were obtained at highest concentration (80%) against *Staphylococus aureus* bacteria with 15.10 ± 6.95 mm inhibition zone. Based on the results of this study, it can be stated that black turmeric extract (*C. caesia Roxb)* has the potential to be an antibacterial control of nosocomial infections.

Keywords: *Antimicrobial, Curcuma caesia Roxb, black turmeric, nosocomial infections, pathogenic bacteria*

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 (Tolera *et al*., 2018). The World Health Organization (WHO) conducted epidemiological studies conducted in 14 countries around the world and found that the overall prevalence of nosocomial infections was 8.7% (ranging from 5.0% in North America and Europe to 40.0% in Asia, Latin America, and Sub-Saharan Africa) (Ghashghaee *et al*., 2018).

Nosocomial infections can be caused by Bacteria, viruses, parasites and fungi however, bacteria are the main causative agents of most cases of infection resulting in death (Vonberg *et al*., 2011). 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 (Burke, 2003; Khan *et al*., 2015; Mssillou *et al*., 2021)

Antibiotics have been applied to reduce morbidity and mortality caused by bacterial infections (Brandt *et al*., 2015; Su *et al*., 2020). 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 (Su *et al*., 2020). The increasing resistance of bacteria to existing antibiotics, encourages important efforts to find alternatives by administering infection-preventing antibiotics from natural ingredients (He *et al*., 2019; Homem andSantos, 2011)

Black turmeric (*C. caesia Roxb*) which is a family of zingiberaceae is used as a traditional medicine herbal ingredients in India, Pakistan, and Turkey (Jose and Thomas, 2014; Pandey and Gupta, 2014). Black turmeric (*C. caesia Roxb*) has the potential as a medicinal plant efficacious because it contains bioactive compounds such as flavonoids, phenols, curcuminoids, proteins, amino acids, alkaloids, and essential oils responsible for antibacterial activi (Baghel *et al*., 2013). Therefore, this study aims to evaluate the antibacterial potential of *C. caesia* against Gram positive and Gram negative pathogenic bacteria.

Materials and methods

2.1 Materials

The ingredients used in this study were black turmeric, ethanol, Disk antibiotic (chloramphenicol), Muller Hilton Agar (MHA), aqueous solution, Physiologist NaCl and phytochemical test materials .

2.2 Data collection procedures

1. **Plant material collection**

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

2. **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. he dry powder of black turmeric was extracted with 70% ethanol for 5 days. The liquid extract obtained is evaporated using a *rotary vacuum evaporator* at a temperature of 70 ° C until a thick extract is obtained (Novaryatiin and Ardhany, 2020)

3. **Microorganisms used for testing**

   This research was carried out with bacterial strains obtained from the Abdurrab University Laboratory Pekanbaru Indonesia. The bacterium 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 (Pandey and Gupta, 2014)

4. **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 37^0C in a rotary shake incubator for 16-18 hours. The inoculum size of each bacterial strain was standardized by adjusting the optical density of nutrient broth to dredge corresponding to0.5 at 620 nm using a spectrophotometer equivalent to 108 cfu/ml 12 (Pandey and Gupta, 2014)

5. **Analysis active compounds of Black Turmeric Extract**

   1. **Phytochemical Test**

      Crude extracts of black turmeric samples were tested for active compound content through phytochemical tests as described by Trease and Evans (1983) and Harbone (1998).

   2. **Total Phenolic Content (TFC)**

      The total phenolic content of the sample extract was carried out according to the method (Taga, Miller, & Pratt, 1984) with minor modifications. In this analysis, gallic acid was used as the standard and the sample was replaced with ethanol (70%) for control. A standard curve of gallic acid is created to determine the phenolic content in terms of the gallic acid equivalent (lg/ml) of the extract. In short, 1 ml of each sample is added to 2 ml of concentrated Na CO 1: 3. Then the mixture is left to settle for 2 minutes for incubation at room temperature. In the next step, 0.1 ml of 50% Folin-Ciacalteu phenol reagent is added to the mixture. The mixture is incubated at room temperature in dark conditions for 30 minutes. Finally, the absorbance was read at a wavelength of 765 nm using a spectrophotometer (Shidmazu UV-1800, UK). The anti-bacterial potential is calculated from the standard curve of gallic acid and expressed as the Gallic Acid Equivalent (mg GAE / g), calculated by the following formula:

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

3. Total Flavonoid Content(TPC)

The flavonoid content of the sample was determined according to the method (Özkök, D'arcy, & Sorkun, 2010). Quercetin is used as a standard. In summary, 1 ml of standard / sample is added with 3 ml of 95% ethanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. The mixture is stirred for 30 seconds. Then, the mixture is left at room temperature for 30 minutes Finally, the absorbance is read at 415 nm using a spectrophotometer (Shidmazu UV-1800, UK). The total flavonoid content of the sample (mg / ml) is calculated from the standard curve of quercetin and expressed as Quercetin Equivalent (QE) calculated with the formula below:

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

6. Antibacterial Activity Test

The antibacterial activity test is carried out by the method of diffusion of discs. Petri dishes are prepared with 20 ml of sterile MHA medium. The test culture is applied on the top of a solid medium and left to dry for 10 minutes. Paper discs (diameter 6 mm, Whatman filter paper no. 1) containing different concentrations (20%, 40%, 60%, and 80%) viscous extracts of *C caesia Roxb* with three replays are dried and placed aseptically on a agar medium with the help of a sterile swab. The loaded disc is placed on the surface of the substrate and left for 30 minutes at room temperature for diffusion of the compound. The negative control is prepared using ethanol solvent. The antibiotic chloramphenicol is used as a positive control. The plate was incubated for 24 hours at 37°C. The results were recorded by measuring the growth inhibition zone around the disc and each experiment was repeated three times (Jose andThomas, 2014)

2.3 Data analysis

The data of 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 and discussion

1. Result of Yield Extract

In this study, black turmeric was used as much as 3 kg. black turmeric was washed and then dried under a fairly stable weather. 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 being overgrown with mold during the research process. Samples that have been dried are then extracted by maceration (Wulan *et al*., 2019). 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 dried powder and filtered with a 100 mesh filter. Approximately 500 Grams of dried powder obtained, then black turmeric dried powder is macerated for 5x24 hours after which it is filtered and obtained a brown black turmeric filtrate. 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, soaking black turmeric dried powder (*C. caesia Roxb*) with ethanol solvent concentration of 70%. This solvent can bind polar compounds so that it can penetrate the cell wall and enter the cell cavity containing the active substance. Maceration is carried out for 3 days which every day carries out the stirring process at room temperature. To maximize the extraction yield, black turmeric ethanol extract is filtered and evaporated in the rotary evaporator until a viscous extract is obtained (Amin *et al*., 2021). The results of the research showed that the evaporation extract using a *rotary evaporator* produced 13 grams of brown viscous extract with a paste-shaped texture. The viscous extract obtained was tested for the effectiveness of black turmeric ethanol extract (*C. caesia Roxb*) against several types of bacteria.

2. 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 that 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 of 2.35% while total flavonoids are 0.61%.

Table 1. Phytochemical Test Results of Black Turmeric Extract (*C. caesia Roxb*)

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

1. Antimicrobial activity

The study was conducted to evaluate the antibacterial activity of black turmeric ethanol extract (*C. caesia Roxb*) against several types of Gram-positive and Gram-negative bacteria. Chloramphenicol is used as a positive control because it is known as one of the broad-spectrum antibiotics used for treatment. In this study (Table 2) it was seen that the diameter of the inhibition zone formed was different, namely in the Gram-positive battery group, the higher the concentration, the higher the inhibition zone formed, while in the Gram negative bacteria group the higher the concentration, the smaller the diameter of the inhibition zone formed.

In general, the size of the block zone diameter tends to be comparable to the concentration

Tabel.2 : Mean zone of growth inhibition of 70 % ethanol extract of *C. caesia Roxb*

	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
Kloramfenichol (+)	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 ; standar error mean ; conc = concentration ; SL = *Salmonella* ; EC = *Escherichia coli* ; PA = *Pseudomonas aeruginosa* ; SA = *Staphylococus aureus* ; SE = *Staphylococus epdermidis* ; SM = *Streptococus mutan* ; KM = *Klorampenichol* ; ET = Ethanol

of the extract, but in this study there was a decrease in the diameter of the inhibition zone at a larger concentration. According to Elifah in (Ariyanti *et al*., 2012) 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 so that it 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 inhibition zone will decrease and tend to be constant (Sarjono, 2007). 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 *Staphylococcus epidermidis, Staphylococcus aureus*, *Escherchia coli,* and *Pseudomonas aeruginosa*. In the literature, the concentration that effectively forms the inhibitory zone is a concentration of 45%, 60%, 75% and the higher the concentration, the lower the diameter of the inhibition zone produced. 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%, 40%, the results of the study showed that at a concentration of 5% worth 11.05 mm while a concentration of 40% only produces an inhibitory zone of 4.825 mm. 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.

According to (Jawetz *et al*., 1996) antibacterial activity can be influenced by four factors including extract concentration, metabolite compound content, diffusion power of extract, and the type of bacteria inhibited. *Salmonella typhi* is a gram-negative bacterium where according to (Sudarmi *et al*., 2017), 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 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 in antibacterial activity

The highest antibacterial activity is produced by black turmeric ethanol extract at a concentration of 80% against S. aureus which is 15.10 ± 6.95mm. This study showed that black turmeric ethanol extract against all test bacteria whose resistance zones are in the range of 6.3 ± 0.33 - 15.10 ± 6.95 (Table. 2). Antimicrobial activity can be classified into three levels, weak activity (inhibition zone lower than 12 mm), moderate activity (inhibition zone between 12 and 20 mm), and strong activity (inhibition zone more than 20 mm). it can be said that black turmeric ethanol extract has a strong activity against *S. 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 black turmeric ethanol 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 alcohol groups in flavonoids, so that cell walls are damaged and cause these compounds to enter the nucleus of bacterial cells (Mentari, 2016).

The mechanism of saponins by lysing bacterial cells by making hydrogen bonds that cause the permeability of bacterial cells to be unbalanced, then bacterial cells will lysis. The mechanism of action of tannin compounds as antibacterial substances is by shrinking cell walls or cell membranes that have been lysis. Tannin compounds have the ability to inactivate microbial adhesins, transport proteins and enzymes on cell membranes (Riyanto and Suhartati, 2019).

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 (Zaini, 2021). The mechanism of steroids as antibacterial is related to lipid membranes and sensitivity to steroid components that cause leaks in liposomes. Steroids can interact with cell phospholipid membranes that are permeable to lipophilic compounds, causing membrane integrity to decrease and cell membrane morphology to change which causes cells to be brittle and lysis (Sudarmi *et al*., 2017)

In disc paper containing PA ethanol as a negative control there is no inhibition zone. The selection of solvents using PA 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 PA ethanol solvent. As for positive control, it uses chloramphenicol antibiotics which are broad-spectrum antibiotics that are able to inhibit the growth of gram-positive or gram-negative bacteria. Chloramphenicol is highly efficient in inhibiting the growth of *Staphylococcus aureus* bacteria. (Prayoga, 2013)

This test is carried out with three repetitions, each repetition has not obtained 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 should use a suspension that is 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 (Zeniusa *et al*., 2019).

Conclusion

The results showed that black turmeric ethanol extract (*C. 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 (*C. caesia Roxb*) has a different inhibitory activity in each test bacteria. The highest inhibition occurred in Gram-positive bacterial species, namely *S. aureus* species of 15.10 $\pm$ 6.95 mm with a highest inhibitory activity. Based on this, black turmeric extract (*C. caesia Roxb*) has the potential to be antibacterial in the future.

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10

Conflict of interest

The authors do not have any conflicts of interest regarding the content of the present work

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