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Cite as: AIP Conference Proceedings **2231**, 030005 (2020); https://doi.org/10.1063/5.0002490
Published Online: 22 April 2020

Bayyinatul Muchtaromah, Evika Sandi Safitri, Prilya Dewi Fitriasari, and Jujuk Istiwandhani

Antibacterial Activities of *Curcuma mangga* Val. Extract in Some Solvents to *Staphylococcus aureus* and *Escherichia coli*

Bayyinatul Muchtaromah[a)], Evika Sandi Safitri[b)], Prilya Dewi Fitriasari[c)], and Jujuk Istiwandhani[d)]

Department of Biology, Faculty of Science and Technology, The State Islamic University of Maulana Malik Ibrahim Malang, Jl Gajayana 50, Malang, Indonesia

[a)] Corresponding author: bayyinatul@bio.uin-malang.ac.id
[b)] evikasandi@yahoo.co.id
[c)] prilyadewi@bio.uin-malang.ac.id
[d)] jujukisti20@gmail.com

Abstract. *Curcuma mangga* is one of the traditional medicines that have the effect dealing with infertility problems. *Staphylococcus aureus* and *Escherichia coli* are bacteria often found as the cause of the female reproductive tract infection. This study aimed to determine the antibacterial activities of several solvents of *C. mangga* extract against *S.aureus* and *E.coli*. *C. mangga* rhizome was extracted by a maceration method using ethanol (polar), chloroform (semi-polar), and n-hexane (non-polar) solvents. The antibacterial activity test against *S.aureus* and *E.coli* using Kirby Bauer with the extract various concentrations of (%) 50, 25,12.5, 6.25, 3.13, 1.56, 0.78, and 0.39. The highest inhibition zone diameter for *S.aureus* was obtained by ethanol (10.11 m), chloroform (9.21 mm), n-hexane (6.05 mm) extract while for *E. coli* , the highest inhibition zone diameter was respectively achieved by ethanol extract (8.06 mm), n-hexane (5.88 mm) and, chloroform (4.19 mm). The MIC value of ethanol extract to *S. aureus* was found at concentrations of 3.13% and MBC at 6.25%. The MIC value of ethanol extract on *E. coli* was attained at concentration of 6.25% and MBC at 12.50%. Ethanol extract of *C. mangga* rhizome produced the most antibacterial activities than chloroform and n-hexane.

INTRODUCTION

One medicinal plant widely used by Indonesian people is the *Curcuma mangga* Val. It is one of the ingredients for the herbal medicine "Jamu Subur Kandungan" that has been used for generations by Maduranese community, Indonesia. *C. mangga* Val. is effective in neutralizing toxins, relieving joint pain, reducing blood cholesterol levels, antibacterial, and being a natural antioxidant to prevent dangerous free radical compounds [1-3]. Chemical substances found in *C. mangga* Val. that have potential as an antibacterial are essential oils, alkaloids, flavonoids, tannins, curcuminoids, and terpenoids [4]. Research that has been carried out [5, 6] found that the phytochemical test of the extract of the mango rhizome (*C. mangga Val.*) in ethanol, chloroform, and n-hexane solvents contained antifungal compounds that varied according to their level of polarity. The extract of the *C. mangga* Val. in some solvents had antifungal activity with various spectrum. The inhibition zones of the ethanol, n-hexane, and chloroform extract of *C. mangga* Val. were, respectively, 5.172 mm, 3.434 mm, and 1.780 mm against *Candida albicans* [6].

The potential of the *C. mangga* Val. as an antimicrobial can be used to treat reproductive tract infections. *Staphylococcus aureus* and *Escherichia coli* cause infection reproductive tract infections called aerobic vaginitis (AV) [7]. *S. aureus* cause infection of the reproductive tract about 18.6% [8] while attacks of *E. coli* infection is 25.4% isolated from the vaginal organs with reproductive tract infections [9]. Extraction is the process to get bioactive compound of secondary metabolism in plants. The process uses three types of solvents with different polarity levels; namely, ethanol is polar, chloroform is semipolar, and n-hexane is nonpolar. The different types of solvents will affect the characteristics of the bioactive compounds found in *C. mangga* rhizome. This study aimed to find out the

International Conference on Life Sciences and Technology (ICoLiST)
AIP Conf. Proc. 2231, 030005-1–030005-6; https://doi.org/10.1063/5.0002490
Published by AIP Publishing. 978-0-7354-1990-2/$30.00

antibacterial activities, the values of the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC) of several solvents of *C. mangga* Val. against *S. aureus* and *E. coli*.

EXPERIMENTAL DETAILS

S. aureus and *E. coli* as test bacteria were subcultured on 5 mL of Nutrient Agar (NA) (Himedia) medium. Incubation was carried out for 24 hours, at 37 ºC. Bacterial colonies that grew were then taken one loop and added with 0.9% NaCl sterile as much as 5 mL, homogenized and compared with turbidity with Mc Farland 0.5 solution (10^8 CFU/mL) [10]. The antibacterial activity test was carried out through the diffusion method using paper discs (6 mm diameter) [11]. The 200 µL bacterial inoculum was grown on the Mueller-Hinton Agar (MHA) (Himedia) medium by the pour plate method [12]. On top of the MHA medium, sterile disc paper was placed soaked with extracts of the *C. mangga* Val. using ethanol, chloroform, and n-hexane with 100% concentration for 60 minutes. A positive control was carried out by immersing disc paper in clindamycin and negative control using PEG 400 solvent. This negative control was used to ensure that the inhibited zone produced did not originate from the solvent. Incubation was undertaken at 37 ºC for 24 hours [11]. The presence of clear areas around disc paper indicated the presence of antibacterial activity. The inhibition zone area was calculated by Equation 1 [13].

$$\text{Inhibitory zone diameter} = \text{Clear zone diameter} - \text{Paper disc diameter} \quad (1)$$

Determination of MIC and MBC was done by calculating the total bacterial colonies (total plate count) that could grow on media by the drop plate method. MIC was indicated with a minimum concentration that could inhibit bacteria while MBC was determined with a minimum concentration that could kill bacteria. The series of *C. mangga* Val. extract concentrations were 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78%, and 0.39% [14] while the method of determining the MIC and MBC values carried out referred to research [15] on antibacterial super empot herbal medicine (Jamu Super Empot) against bacteria *S. saprophyticus* and *E. coli*. The bacterial suspension used was 10^6 CFU/mL with Nutrient Broth (NB) (Himedia) medium. Furthermore, the mixture between the extract and the bacteria was incubated for 24 hours. After 24 hours, the number of colonies from each treatment was calculated. The MBC value was indicated by no bacterial colonies growing on petri dishes [16]. Data obtained from the antibacterial test were the amount of inhibition zone, the MIC value, the KBM value, and the total bacterial colony. Inhibitory zones indicate the ability of extracts as antibacterial.

RESULTS AND DISCUSSION

Inhibitory zones of *Curcuma mangga* Val. Extract in Several Solvents Against *S. aureus* and *E. coli*

The antibacterial activity test of the extract in ethanol, chloroform, and n-hexane solvents concentration 100% with three repetitions showed the formation of various inhibition zone diameters (Table 1 and Table 2). The inhibition zone is the zone where the bacteria do not show growth activity. In this study, the growth of *S. aureus* and *E. coli* bacteria was inhibited by the extracts of *C. mangga* Val.

TABLE 1. Inhibition Zones of *C. mangga* Val. extract in Several Solvents against *S.aureus*

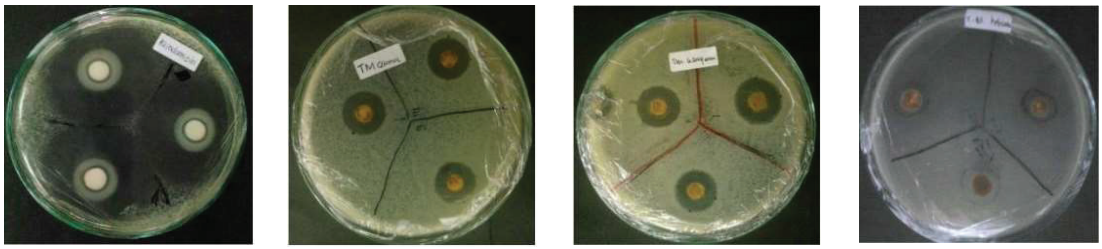
Treatment	Inhibitory zone diameter (mm) ± SD	Inhibition zone category [17]
Ethanol Extract (P1)	10.11 ± 0.434	Strong
Chloroform extract (P2)	9.21 ± 0.403	Strong
N-hexane extract (P3)	6.05 ± 1.165	Strong
Clindamycin (K +)	37.54 ± 0.564	Strong
PEG 400 (K-) solvent	0.00	No activity

TABLE 2. Inhibition Zones of *C. mangga* Val. extract in Several Solvents against *E. coli*

Treatment	Inhibitory zone diameter (mm) ± SD	Inhibition zone category [17]
Ethanol Extract (P1)	8.06 ± 0.400	Strong
Chloroform extract (P2)	4.19 ± 0.847	Average
N-hexane extract (P3)	5.88 ± 1.531	Average
Clindamycin (K +)	30.32 ± 2.385	Strong
PEG 400 (K-) solvent	0.00	No activity

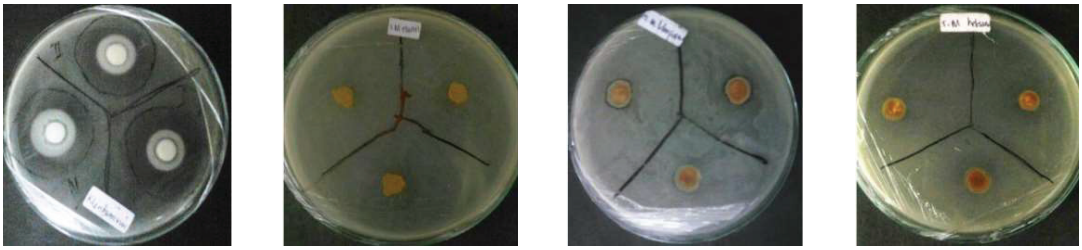
Based on Table 1 and Table 2, the difference in the extraction of solvent treatment influences the mean diameter of the bacterial inhibitory zone formed. The inhibition zone of the extract of the of *C. mangga* Val. was smaller than clindamycin (positive control). The results of the measurement of the inhibition zone diameter showed that the *C. mangga* Val. extract affected bacterial growth and potential as an antibacterial. Test on *S. aureus* bacteria producing inhibition zones with diameters from the largest to the smallest sequentially were *C. mangga* Val. extracts in 10.11 mm of ethanol solvent, 9.21 mm of chloroform, and 6.05 mm of n-hexane. In *E. coli,* the bacteria produced inhibitory zones in order *C. mangga* Val. extracts were 8.06 mm of ethanol solvent, 5.88 mm of n-hexane, and 4.19 mm of chloroform. Ethanol extract of *C. mangga* Val. had the highest inhibitory zone diameter compared to chloroform extract and n-hexane extract against *S. aureus* and *E. coli* because antibacterial compounds tended to be distributed in polar solvents. *C. mangga* Val. extract in ethanol solvent has flavonoid and triterpenoid active compounds [18, 19]. Ethanol extract of *C. mangga* Val. has the best antimicrobial activity against the fungus *Candida albicans* compared with chloroform and n-hexane solvents [15].

In the positive control, the inhibition zone formed was in a strong category with inhibition zone diameter in *S. aureus* at 37.54 mm and in *E. coli* at 30.32 mm. Clindamycin is a type of broad-spectrum antibiotic indicated for treating diseases caused by Gram-positive and Gram-negative aerobic bacterial infections [20]. The working mechanism of clindamycin antibacterial inhibits the growth or reproduction of bacteria by inhibiting protein synthesis. The action of clindamycin involves cutting the elongation of the peptide chain, blocking site A on the ribosome, reading errors in the genetic code or preventing attachment of the oligosaccharide chain to the glycoprotein [21].



(A) **(B)** **(C)** **(D)**

FIGURE 1. Clindamycin inhibitory zone (A), *C. mangga* Val. extract in ethanol (B), chloroform (C), and n-hexane (D) against *S. aureus*



(A) **(B)** **(C)** **(D)**

FIGURE 2. Clindamycin inhibitory zone (A), *C. mangga* Val. extract in ethanol (B), chloroform (C), and n-hexane (D) against *E. coli*

The inhibitory zone of the *C. mangga* Val. extract is the radical inhibitory zone while the inhibitory zone of clindamycin is the irradical inhibitory zone (Fig. 1 and 2). The irradical zone is an area around the disc paper where bacterial growth is inhibited by antibacterial but not killed. The irradical zone is characterized by the presence of bacteria around the inhibitory zone, while the radical zone is not found at all bacterial growth in the inhibitory zone area [22]. Although the inhibitory zone formed is large, the ability of clindamycin to kill *S. aureus* and *E. coli* is categorized as bacteriostatic. *C. mangga* Val. extract is included in the radical zone because there are no bacteria at all in the clear zone. The extract of the *C. mangga* Val. is bactericidal (an antibacterial that can kill bacteria). Antibiotics have bacteriostatic properties; some are bactericidal [23]. Antibacterial should have bactericidal properties and not bacteriostatic properties [22].

The difference in inhibition zone diameter is influenced not only by the content of the active compound of an extract but also the differences in the ability of the extract to diffuse on disc paper and agar media. There are variations in inhibition zones in the extract of *C. mangga* Val, *Curcuma zedoaria* (Christm) (Roscoe) and *Kaempferia rotunda* L. against *E. coli* and *Salmonella thyposa*. The diffusion ability of active substances that affect inhibition zone is also influenced by the thickness of the medium, the reaction between the active ingredient and the medium, the viscosity of the medium, and the incubation temperature [24].

Based on the test results of inhibition zone of *C. mangga* Val. extract in ethanol solvent, the diameter of *S. aureus* (Gram-positive) was more significant than *E. coli* (Gram-negative). These two bacteria have different cell wall structures, which cause differences in sensitivity to certain antibacterial compounds. The structure of the cell wall of Gram-positive is a single layer with low lipid content (1-4%). It has teacoic acid making bioactive material more easily enter the cell. Teacoic acid is a polar water-soluble polymer that functions as transport of positive ions to get in or out of cells. This water solubility indicates that the cell walls of Gram-positive bacteria are more polar than Gram-negative cell walls. The Gram-negative bacterial cell wall has a complex structure. Three-layer outside the lipoprotein, the middle layer of lipopolysaccharides which acts as a barrier to the entry of antibacterial bioactive material, and the inner layer is peptidoglycan with a high lipid content (11-22%) [22]. The active flavonoid compound contained in the *C. mangga* Val. extract can penetrate the polar peptidoglycan layer than the non-polar lipid layer [25].

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Extract of *Curcuma mangga* Val. in Several Solvents against *S. aureus* and *E. coli*

The results of inhibition zones formed showed that the ethanol extract of the *C. mangga* Val. had the best inhibition zone against *S. aureus* and *E. coli* bacteria compared to other solvents. The results of the calculation of colonies growing in NA media of ethanol extract of the *C. mangga* Val. against *S. aureus* and *E. coli* which showed minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are presented in Table 3.

TABLE 3. Test Results of MIC and MBC Ethanol Extract of Mango Ginger Rhizome (*C. mangga* Val.) against *S. aureus* and *E. coli*

Test sample	Total amount of average colony (CFU/mL)	
	Staphylococcus aureus	*Escherichia coli*
Microbial Control	15.86×10^{13}	16.74×10^{17}
Extract 0.39%	14.13×10^{13}	20.43×10^{13}
Extract 0.78%	11.86×10^{13}	18.06×10^{13}
Extract 1.56%	15.30×10^{12}	13.23×10^{13}
Extract 3.13%	59.33×10^{9} **)	67.00×10^{11}
Extract 6.25%	0*)	99.00×10^{8} **)
Extract 12.50%	0	0*)
Extract 25.00%	0	0
Extract 50.00%	0	0
Material Control	0	0

Note: **) MIC (Minimum Inhibitory Concentration)
*) MBC (Minimum Bactericidal Concentration)

Based on Table 3, it is known that microbial control contains the number of bacteria, namely 158.66 × 1012 CFU/mL on *S. aureus* and 167.40 × 1016 CFU/mL on *E. coli*. The results of the test treatment at the lowest extract concentration (0.39%) showed the number of colonies was less than the control, i.e., 14.13 × 1013 CFU/mL at *S. aureus* and 20.43 × 1013 CFU/mL. The higher concentration of *C. mangga* Val. Extract showed a smaller number of bacterial colonies. The higher the concentration of the extract, the more antibacterial active compounds contained, so that the ability to inhibit bacterial growth was higher [22, 26].

The results showed that the MIC value of the ethanol extract of the *C. mangga* Val. against *S. aureus* was at a concentration of 3.13% while the *E. coli* concentration was 6.25%. The concentration of the ethanol extract of the *C. mangga* Val. extract that could kill the growth of *S. aureus* was a concentration of 6.25%. In *E. coli*, the minimum kill concentration value was at a concentration of 12.50%. This result is supported by research [27] finding that the MIC and MBC values of fresh *Curcuma xanthorriza* extract had a MIC of 12.5% and a MBC of 25% so that the extract of *C. xanthorriza* at a concentration of low was bacteriostatic and at high concentrations was bactericidal.

The content of active compounds flavonoids and triterpenoids of ethanol extract of *C. mangga* Val. cause the antibacterial mechanism against *S. aureus* and *E. coli*. Both of these compounds form a mechanism to attack bacteria. Triterpenoids will react with porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming strong polymeric bonds that cause damage to the porin [28]. After porin is damaged, flavonoids as phenol compounds enter the cell and damage the cytoplasmic membrane. H + ions from phenol compounds and their derivatives bind to polar groups (phosphate groups) so that phospholipid molecules will break down into glycerol, carboxylic acids, and phosphoric acids. The phospholipids cannot maintain the cytoplasmic shape so that the cytoplasmic membrane becomes leaky that causes the transport of substances into the cell and out of the cell to become uncontrolled. Substances in cells, such as enzymes, amino acids, and nutrients can leave the cell. The unavailability of nutrients is disrupted by bacterial metabolism [29]. The results of this study indicated a decrease in the number of colonies. The death of *S. aureus* and *E. coli* is along with an increase in the concentration of the ethanol solvents *C. mangga* Val. Extract. The conclusion is the extract of the *C. mangga* Val. has been proven to be used as an antibacterial compound against *S. aureus* and *E. coli*.

SUMMARY

C. mangga Val. extract in several solvents has antibacterial activities against *S. aureus*, i.e., 10.11 mm of ethanol solvent, 9.21 mm of chloroform, 6.05 mm of n-hexane and *E. coli*, i.e., 8.06 mm of ethanol solvent, 5.88 mm of n-hexane, 4.19 mm of chloroform. The MIC value of the ethanol extract of the *C. mangga* Val. against *S. aureus* was 3.13%, and *E. coli* was 6.25%. MBC value on *S. aureus* was 6.25%, and *E. coli* was 12.50%. *C. mangga* Val. extract has the potential as an antibacterial compound for *S. aureus* and *E. coli*.

ACKNOWLEDGMENTS

This research was supported by Department ofBiology, Faculty of Science and Technology, The State Islamic University of Maulana Malik Ibrahim Malang. The authors thank all parties involved in this research.

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