ANTIBACTERIAL EFFECT OF CRUDE ALCOHOLIC AND AQUEOUS EXTRACTS OF SIX MEDICINAL PLANTS AGAINST *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI*

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ABSTRACT: Antibacterial activity of twelve crude alcoholic and aqueous extracts from six medicinal plants against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 was studied. The medicinal plants included *Alstonia macrophylla* Wall., *Bixa orellana* L., *Blumea balsamifera* (L.) D.C., *Azadirachta indica* A. Juss., *Arcangelisia flava* (L.) Merr., and *Leea rubra* Blume. Crude ethanolic extracts from leaves of *Bixa orellana* L. and bark of *Alstonia macrophylla* Wall. showed potential antibacterial effect against *S. aureus* with minimal inhibitory concentration (MIC) of 62.5 µg/ml. However, none of the crude aqueous and ethanolic extracts showed antibacterial effect against *E. coli.*

Keywords: antibacterial effect, medicinal plants, *Staphylococcus aureus*, *Escherichia coli*

INTRODUCTION: Antibiotic resistance has long been a significant problem in treatment of bacterial infection since several types of bacteria have been found to be able to resist more than one type of antibiotics. This results in the need of higher dose use with increased risk of drug toxicity or consideration to change the regimen. Moreover, excessive budget is currently spent on import of antibiotics manufactured abroad. Therefore, antibacterial activity of local medicinal plants should be studied to provide alternative and locally available antibacterial regimens.

Six medicinal plants, which have been used as folk medicine for gastrointestinal diseases, were selected for this research to study antibacterial activity of their crude alcoholic and aqueous extracts against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. These plants included *Alstonia macrophylla* Wall., *Bixa orellana* L., *Blumea balsamifera* (L.) D.C., *Azadirachta indica* A. Juss., *Arcangelisia flava* (L.) Merr., and *Leea rubra* Blume.

MATERIALS AND METHODS:

Antibacterial activity test using disk diffusion technique[1)]

Crude extracts from six medicinal plants; including bark of *Alstonia macrophylla* Wall., leaves of *Bixa orellana* L., leaves of *Blumea balsamifera* (L.) D.C., bark of *Azadirachta indica* A. Juss., vines of *Arcangelisia flava* (L.) Merr., and roots of *Leea rubra* Blume; were diluted in aqueous and alcoholic solvents and prepared in three concentrations; 100, 50, and 10 mg/ml. 16.5 µl of each preparation was dropped on disk of 6 mm in diameter and the concentrations became 1.65, 0.825, and 0.165 mg/disk respectively. One of two divided parts of the disk was exposed to dry at 55 ºC for 18-24 hours. The others were immediately used. The technique used for testing antibacterial activity is disk diffusion test[1)]. Inocula of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, which their turbidities were prepared to 0.5 McFarland, were spread on Mueller Hinton Agar (MHA). Inocula were incubated at 37 Cº for 18-24 hours. Inhibition zone were measured using vernier caliper. The tests were undertaken twice and standard antibiotics, including penicillin G for *S. aureus* and ampicillin for *E. coli*, were used as controls.

Standard antibiotic sensitivity test

Inocula of *S. aureus* and *E. coli*, which their turbidities were prepared to 0.5 McFarland, were spread throughout MHA before placing standard antibiotics. Penicillin G, gentamicin, vancomycin, methicillin, and cefotaxine were placed on to the dish with *S. aureus* while ampicillin, tetracycline, gentamicin, streptomycin, and amikacin were placed onto the dish with *E. coli.* After incubation at 37 Cº for 18-24 hours, diameters of inhibition zones were measured using vernier caliper. This antibiotic sensitivity test of each standard antibiotic was undertaken twice.

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Measurement of minimal inhibition concentration (MIC) using broth dilution technique[2]

Each extract with antibacterial effect shown in the disk diffusion test was further diluted with aqueous solvent, ethanol, and 10% aqueous dimethylsulfoxide (DMSO) to 2,000 µg/ml and serial two fold dilution was performed in 10 tubes of the dilute extract using MHB as diluent. Final volume after dilution was 0.5 ml and the concentrations were 1,000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.906, and 1.953 µg/ml. 0.5 ml of *S. aureus*, which its turbidity was prepared to 0.5 McFarland and diluted 1:200 fold, was put into each tube. All the tubes were incubated at 37 Cº for 24 hours. Positive control was prepared by serial two fold dilution of 50 µg/ml of penicillin G in 10 tubes with 0.5 ml content in each. 0.5 ml of broth was mixed with content in the last tube before 0.5 ml of the blend was removed. 0.5 ml of *S. aureus* was finally put in each tube.

Two tubes with different contents used as negative controls were also prepared. The content of the first negative control composed of DMSO or ethanol, water, bacteria, and broth while the others composed only of bacteria and broth. Negative controls were used as references in measuring MIC and minimal bactericidal concentration (MBC). The concentration of the last tube with clear content is the minimal inhibition concentration. The tubes with clear content were selected and the content in each of these tubes was streaked on nutrient agar. The MBC is the concentration of the content from the last tube which was streaked on nutrient agar and there was no bacteria found after cultivation.

Measurement of minimal inhibition concentration (MIC) using agar dilution technique

Each extract with antibacterial effect shown in the disk diffusion test was further diluted. Crude aqueous extracts were diluted to 10, 5, 2.5, 1.25, 0.625, and 0.3125 mg/ml. Crude ethanolic extracts were diluted with ethanol or 10% DMSO to 100, 50, 25, 12.5, 6.25, and 3.125 mg/ml respectively. 600 µl of each diluted crude aqueous extracts was mixed with 5.4 ml of melted MHA while 60 µl of each diluted ethanolic extracts was mixed with 6 ml of melted MHA and then plated to 6 cm sterile Petri dish. Each dilution was prepared in duplication. The concentrations of the extracts became 1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/ml respectively. Each concentration was prepared for 2 dishes. Inoculum of *S. aureus* was prepared to 0.5 McFarland and diluted 1:10,000 fold. 1 µl of the inoculum was dropped onto previously prepared MHA. DMSO and water were used as substitutes of the extracts in controls. All specimens were incubated at 37 Cº for 18-24 hours. MIC was measured from the specimen with the lowest concentration which was sufficient to inhibit growth of bacteria.

RESULTS: From antibacterial activity test and standard antibiotic sensitivity test, *S. aureus* was sensitive to penicillin G, gentamicin, vancomycin, methicillin, and cefotaxime while *E. coli* was sensitive to ampicillin, tetracycline, gentamicin, streptomycin, and amikacin.

Crude aqueous extracts from leaves of *Bixa orellana* L., bark of *Azadirachta indica* A. Juss., and bark of *Alstonia macrophylla* Wall. inhibited growth of *S. aureus*. The crude aqueous extract from bark of *Azadirachta indica* A. Juss.; at concentrations of 1.65 and 0.825 mg/disk in moist condition and 1.65, 0.825, and 0.165 mg/disk in dry condition; showed widest average inhibition zone which indicated the most anti-bacterial effect against *S. aureus*. Crude ethanolic extracts from vines of *Arcangelisia flava* (L.) Merr., bark of *Azadirachta indica* A. Juss., and bark of *Alstonia macrophylla* Wall. inhibited the growth of *S. aureus*. The crude ethanolic extract from bark of *Alstonia macrophylla* Wall.; at concentrations of 1.65 and 0.825 mg/disk in both dry and moist conditions; showed the widest average inhibition zone and this also indicated the most anti-bacterial effect against *S. aureus*. Nevertheless, none of the crude aqueous and ethanolic extracts showed antibacterial activity against *E. coli*.

Measured MICs of all the extracts which showed antibacterial effect against *S. aureus* were in the range of 62.5-500 µg/ml and the crude ethanolic extracts showed the greatest anti-bacterial effect. The two most effective crude ethanolic extracts, from leaves of *Bixa orellana* L.

and bark of *Alstonia macrophylla* Wall., showed equal MIC of 62.5 µg/ml. However, the measured MBC of the extract from leaves of *Bixa orellana* L. was lower, indicating the greater bactericidal effect. The crude aqueous extracts had greater MICs, indicating lower antibacterial activity, compared to the crude ethanolic extracts. Crude aqueous extracts from bark of *Azadirachta indica* A. Juss. and bark of *Alstonia macrophylla* Wall. showed relatively equal MIC of 250 µg/ml. Nevertheless, the MBC of the crude aqueous extract from *Azadirachta indica* A. Juss. (500 µg/ml) was lower than that of the extract from *Alstonia macrophylla* Wall. which showed bactericidal effect at the concentration above 1,000 µg/ml. The MIC of penicillin G used as standard antibiotic was 0.0976 µg/ml.

From the measurement using agar diffusion technique, none of the extracts showed antibacterial effect against *E. coli*. However, all the extracts showed antibacterial activity against *S. aureus* with MICs in the range of 0.0625-0.50 µg/ml. The extracts from leaves of *Bixa orellana* L. and bark of *Alstonia macrophylla* Wall. showed the greatest antibacterial effect against *S. aureus* and had equal MIC of 0.0625 µg/ml. The crude ethanolic extracts from bark of *Azadirachta indica* A. Juss. and vines of *Arcangelisia flava* (L.) Merr. showed the least antibacterial activity agains *S. aureus* with the MICs of 0.125 and 0.25 µg/ml respectively. The crude aqueous extracts showed lesser antibacterial activity against *S. aureus* than that of the crude ethanolic ones. The crude aqueous extracts which showed significant effect were extracts from bark of *Azadirachta indica* A. Juss. and bark of *Alstonia macrophylla* Wall. with equal MIC of 0.25 mg/ml. The crude aqueous extracts from leaves of *Bixa orellana* L. and vines of *Arcangelisia flava* (L.) Merr. showed the least effect with MIC of 0.50 mg/ml.

According to the control, *S. aureus* was sensitive to penicillin G while *E. coli* was sensitive to ampicillin with equal MIC of 12.50 µg/ml.

DISCUSSION: The result of antibacterial activity test using disk diffusion technique showed minute average inhibition zone which may be caused by limited diffusion of the extracts through agar. However, in broth dilution technique, the result showed better antibacterial activity as the extracts could directly exposed to the microbes.

The inhibition zones found in dry condition of disk diffusion test were greater in size than those in the moist condition. This might be due to the use of distilled water as solvent in moist condition. Distilled water might alter pH of agar which limited diffusion of the extracts through agar. Therefore, pH of distilled water must be measured prior to its use to evidently identify the mentioned effect.

The crude aqueous extract from bark of *Azadirachta indica* A. Juss. showed greater antibacterial activity than that of the crude ethanolic ones in both moist and dry conditions. However, the crude ethanolic extract showed greater antibacterial activity indicated by lower MIC and MBC. The result is explained as the consequence of greater solubility of the extract in the ethanolic solvent which influenced the antibacterial activity. This contrastive results found in different techniques may be explained that the crude ethanolic extract might have limited diffusion through agar and result in smaller inhibition zone while the extract could efficiently access to the bacteria in the agar and broth dilution techniques.

The crude aqueous extract from leaves of *Bixa orellana* L. showed marked antibacterial activity while the crude ethanolic ones did not show any effect. This may be due to the fact that the extracts from leaves of *Bixa orellana* L. has greater solubility in aqueous solvent. In this study, the crude aqueous extract from the leaves of *Bixa orellana* L. showed antibacterial effect only against the gram positive bacteria. However, the methanolic extract from the bark of *Bixa orellana*, L. showed antibacterial effect against both gram positive and negative bacteria[3]. Marked antifungal effect, inhibition of conidia germination, against *Dreschslera oryzae* of aqueous extract from the leaves of *Bixa orellana* L. has also been reported[4].

The crude ethanolic extracts from the leaves of *Bixa orellana* L. and the bark of *Alstonia macrophylla* Wall. showed the greatest antibacterial activity against *S. aureus* in the tests using broth and agar dilution techniques. However, the extract with greater bactericidal effect against *S. aureus* was the ethanolic ones from the leaves of *Bixa orellana* L. as its MBC is lower than that of the others.

According to a previous study, (2R,3R)-7,5'-di methoxy-3,5',2'-trihydroxyflavanone could be extracted from leaves of *Blumea balsamifera*, (L.) D.C. using chloroform as a solvent[5] while morphological and physiological characteristics of *Leea rubra* Blume. have been widely studied[6]. However, from this study, the crude aqueous and ethanolic extracts from the leaves of *Blumea balsamifera*, (L.) D.C. and the roots of *Leea rubra* Blume. did not show any antibacterial effect.

Although antibacterial activity of *Alstonia macrophylla* Wall. has seldom been studied and has not yet identified, the crude aqueous and ethanolic extracts from the bark of *Alstonia macrophylla* Wall. showed marked antibacterial effect in this study. Previous studies about *Alstonia macrophylla* Wall. involved mostly in identifying its chemical compositions and extracting its alkaloids. For instances, alstomacrophylline and alstomacroline are alkaloid extracted from its cork while alstonerine, alsotophylline, yohimbine, and betayohimbine are alkaloids found in its fruits[7]. Moreover, *Alstonia* spp. was found to have marked antimalarial effect and antipyresis. It was also found to inhibit tumoriogenesis[8].

Extracts from many parts of *Azadirachta indica* A. Juss.; including its leaves, bark, fruits and roots; were found to have antimicrobial activity. Further study should be undertaken to understand its antimicrobial effect and apply accordingly.

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