International Journal of Scientific Engineering and Applied Science (IJSEAS) – Volume-3, Issue- 4, April 2017
ISSN: 2395-3470
www.ijseas.com

Comparative Study on Antibacterial Activity of *Jatropha curcas* Linn. Leaves Extract and Neomycin Sulfate Against *Staphylococcus aureus* ATCC 25923

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Abstract

The present study was carried out for comparing the antibacterial effect of *Jatropha curcas* leaves ethanol extract with neomycin sulfate against *Staphylococcus aureus* ATCC 25923. The extraction of *J. Caracas* leaves were prepared using a maceration method. The secondary metabolites of the extract were analyzed using standard method. The antibacterial activity of the extract and comparative analysis were tested using the agar diffusion method. While the determination of minimal inhibition concentration (MIC) test was conducted using macrodillution method, followed by subculturing the overnight incubation of the MIC test onto Mueller Hinton Agar medium surface, for determining the minimum bactericidal concentration (MBC) value. The results showed that the ethanol extract of *J. curcas* leaves had antibacterial activity against *S. aureus* with MIC/MBC ranged at 0,625-1,25%w/v. The comparative value of *J. curcas* extract against Neomycin sulfate on antibacterial effect was 1: 1413,83.

Keywords: *Jatropha curcas,* comparative, neomycin sulfate

Introduction

Staphylococcus aureus is a major human pathogen that causes a wide range of clinical infections [1]. Humans are a natural reservoir of *S aureus*, with 30 to 50% of healthy adults colonized, 10% to 20% persistently so [2,3]. Persons colonized with *S. aureus* are at increased risk for subsequent infections [4]. *Staphylococcus aureus* is a leading cause of keratitis worldwide [5,6]. Bacterial keratitis is an infection and inflammation of the cornea that cause pain, reduced vision, light sensitivity and tearing or discharge from the eye that can, in severe cases cause loss of vision. Bacterial keratitis progresses rapidly and corneal destruction may be complete in 24 - 48 hours with some of the more virulent bacteria. Bacterial keratitis is a sight-threatening process. Bacterial keratitis makes the cornea cloudy. It may also cause abscesses to develop in the stroma, which is located beneath the outer layer of the cornea. Interruption of an intact corneal epithelium and/or abnormal tear film permits entrance of microorganisms into the corneal stroma, where they may proliferate and cause ulceration [7].

Infectious keratitis generally requires antibacterial therapy to treat the infection. Prescribe neomycin, however, as a combination medication in adults, as researchers have yet to establish its safety in children. A research study mentioned that

the in vivo antibacterial effectiveness in the rabbit cornea of a number of commercially available ophthalmic antibiotic preparations was determined against a single strain of penicillinase producing *Staphylococcus aureus* isolated from a human corneal ulcer. Each antibiotic was instilled topically at hourly intervals, and the number of residual viable organisms in the cornea subsequently was ascertained. In vivo measurements demonstrated that five antibiotics—neomycin sulfate, gentamicin sulfate, erythromycin, tetracycline hydrochloride, and chlortetracycline hydrochloride—were equally effective in suppressing growth of the strain of *S. aureus* studied. It is a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections [8].

Staphylococcus aureus has a long history of evolving to more resistant states, and this trend is expected to continue [9, 10]. Therefore, new antibiotics are needed to manage future cases of *S. aureus*-induced keratitis. The use of natural materials based compounds can be used as a treatment option. One of the natural material as a potential antibacterial is leaves of *J. curcas*. The Jatropha leaves ethanol extracts known to contain several secondary metabolites that act as antibacterials, including saponins, tannins, glycosides, steroids, alkaloids and flavonoids [11]. With this knowledge, the present study was aimed to compare the antibacterial effect of crude extracts of *J. curcas* and neomycin sulfate as part of searching new bio-active compounds against *S. aureus* keratitis and other infection.

Materials and Methods

Material

The culture media that were used are *Mueller-Hinton Agar* (MHA-Oxoid), and *Mueller-Hinton Broth* (MHB-Oxoid). In this study, neomycin sulfate (Bernofarm) was used as a comparison substance. The chemicals used are distilled water, normal saline solution, barium chloride solution (Merck), sulfuric acid solution (Merck), n-butanol, ferric chloride reagent (Merck), Dragendorf reagents, Lieberman - Burchard reagent, Mayer reagent, technical toluene (Brataco), and vanillin (Merck).

Plant Material

The samples that were utilized in this study are *Jatropha curcas* leaves from Manoko Garden, Lembang, West Java, Indonesia. Plant sample was identified in Plant Taxonomy Laboratory of Biology Major, Faculty of Mathematics and Natural Science Padjadjaran University.

Methods

Preparation of Leaf Extracts

Jatropha leaves were cleaned and air dried at ambient temperature for several days until well dried. Of 6 Kg wet weights, were gained 1 Kg of dried simplisia. Then the dried leaves were chopped, and extracted by maceration during 3x24 h using ethanol 70% as the solvent. The extracts were evaporated using a rotary evaporator at 40-50 ^{O}C, then continued to evaporate on a water bath until dried extract with a constant weight was obtained. From 1 Kg dried simplisia, can obtain 228.92 g viscous extracts.

Phytochemical Screening of Secondary Metabolites

Phytochemical screening of secondary metabolites was using a standard method to determine the contains alkaloids, flavonoids, tannins, quinones, saponins, steroids, and triterpenoids, in both simplisia and ethanol extracts of Jatropha leaf [12].

Preparation of The Bacterial Suspension

At least three to five *S. aureus* colonies from an agar plate culture were taken with a loop by touching the top of each colony. The growth is transferred into a tube containing 4 to 5 ml of MHB medium. The broth culture is incubated at 37^0C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours). The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension containing approximately 1 x 10^8 CFU/ml for *S. aureus*. To perform this step properly, either a photometric device can be used or, if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines [13].

Antibacterial Activity Test

The antimicrobial activity of the extracts was done using the agar diffusion methods. The volume of 20 µl standardized cell suspension and 20 ml MHA media at 40-45 ^{0}C was poured in a sterile petri dish, then the mixture was homogenized and allowed to solidify. By utilizing perforation method, four holes were made in the agar. Extracts were solved on dimethyl sulfoxide with the comparison 1 g of extract was solved in 1 ml of dimethyl sulfoxide (100 %^{w}/v). Then the extract solution was diluted using dimethyl sulfoxide, until the variation of testing concentration as follows: 20, 40, 60, and 80% ^{w}/v were achieved. The volume of 50 µL of each concentration was poured into the hole. The plates were incubated aerobically at 37°C for 18-24 h. The diameter zones of inhibition were measured using a caliper. The tests were carried out in duplicate.

Determination of MIC and MBC Value

MIC value of the *J. curcas* leaf extract was determined using macrodillution broth. The concentration of extract ranges should be prepared one step higher than the final dilution range required. Then 1 ml extract with concentration 20% ^{w}/v was added into the first tube. Then the volume of 1 ml from the first tube was pipetted then added to the second tube, then the tubes mixed thoroughly and so on until the concentration of every tube was 10%; 5%; 2.5%; 1.25%; 0.625% and 0.3125 ^{w}/v. Then 10 µL of bacterial suspension was added to every tube. The liquid media, then were incubated at temperature 37ºC for 20 h. The MIC value was the lowest concentration (in µg/ml) of an extract that inhibits the growth of *S. aureus*. As MBC determination, the loop was dipped into the overnight incubation of MIC tube, then streaked it on to the agar surface. After that, the plates were incubated at temperature 37ºC for 20 h.

Comparison Analysis of Antibacterial Activity

The comparison analysis test procedure was made the same appeal with the antibacterial activity test procedure that was using the agar diffusion method. However, in this procedure, each of tested extract and neomycin sulfates as a comparator antibiotic was tested in the same plate. Then the plates were incubated at 37 ^{0}C for 18-24 h. The diameter of inhibition zones was observed, measured and compared. Data of diameter inhibition zones were plotting to curve inhibitory against log concentration.

Results and Discussion

Phytochemical Screening Result

The results of the phytochemical analysis revealed varying constituents of these extracts, as follows: tannins, flavonoids, steroids, and saponins**.** The result of

phytochemical screening can be seen in Table 1.

Table 1: Phytochemical screening

Secondary metabolites	Results	
	Simplisia	Extract
Alkaloids	-	-
Quinones	-	-
Tannins	+	+
Flavonoids	+	+
Saponins	+	+
Steroids/Triterpenoids	+	+

Note: (+) presence; (-) absence

In another study stated that the same phytochemical screening result of ethanolic extract of *Curcuma mangga*, that contained flavonoids, saponins, quinone and steroid, revealed strong antibacterial activity against *S. aureus* [14]. So, it can be concluded that the presence of these secondary metabolites may contribute also for antimicrobial activity of Jatropha leaves ethanol extracts against *S. aureus*.

Antibacterial Activity Result

The increasing of inhibition diameter, which produced by the antibacterial activity of ethanol extract of Jatropha leaves against *S. aureus*, which is directly proportional to the increasing concentration of the extract, showed that the extract was active as antibacterial. The diameter data can be seen in table 2.

Table 2: Antibacterial activity results

Concentration (%w/v)	Inhibitory zone diameter (mm)
20	12.267±0.169
40	13.300±0.216
60	14.033±0.047
80	14.733±0.094

Note: Perforator diameter = 6 mm

MIC and MBC Determination Result

Minimum inhibitory concentrations (MIC) refer to the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism. The result of MIC determination can be seen in Table 3.

Table 3: Minimum inhibitory concentration value

Extract concentration (%^{w}/v)	Bacterial growth
0.3125	+
0.625	+
1.25	-
2.5	-
5	-
10	-

Note: (+) = colony absence; (-) = colony presence

The extracts showed the value of MIC ranged between 0.625 and 1.25 %^{w}/v. The lower concentration of MIC of the extract has been proving its antibacterial capabilities.

Comparative Analysis Result

Comparative test was conducted to find the value of comparative antibacterial activity of ethanol extracts of Jatropha leaves to neomycin sulfate to generate the same inhibition diameter against *S. aureus*. The diameters of inhibition zone can be seen in Table 4. Each of these diameters of the inhibition was plotted into the equation in order to obtain the line using linear regression methods. The line equation of neomycin sulfates was y = 4.3185x + 4.6315; as for the tested extract was 3.7726x – 7.8488. If the concentration of 100 ppm neomycin sulfate was plotted into the line equation of neomycin sulfate, then drag the resulting diameter was 11.34 mm. If the diameter put into the equation line of the

extract, then to produce inhibitory diameter of 11.34 mm, the concentration of the extract which required was 141 383,445 ppm. Thus, the antibacterial comparative value of the Jatropha extract to neomycin sulfate was 1 : 1413,83.

Table 4: Comparison of inhibition zone diameter

Material	Concentration (ppm)	Inhibitory Zone Diameter (mm)
Extracts	200000	12.000 ± 0.000
	400000	13.350 ± 0.050
	600000	13.900 ± 0.500
Neomycin sulfate	20	11.550 ± 0.450
	40	12.200 ± 0.100
	60	13.800 ± 0.200

The results indicated that neomycin sulfate demonstrated greater antibacterial activity than the Jatropha extracts at the same concentration. The fact that the plant extract is only a crude extract may account for differences in activity.

Conclusion

Our results demonstrated that ethanol extracts of *Jatropha curcas* leaves have active antibacterial activity against *S. aureus* ATCC 25923. But when compared with neomycin sulfate, antibacterial activity of extracts of Jatropha still have to set the dose up to achieve an effective dose.

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