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Antibacterial Activity Of Ethanol Extract Of Jatropha Leaves Against *Pseudomonas aeruginosa* ATCC 27853.

Sri Agung Fitri Kusuma[1]*, Irma Erika H.[2] , Galih Annisa P[2]

[1]Department of Pharmaceutical Biology, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia 45363
[2]Department of Pharmacy, Al-Ghifari University, Cisaranteun Kulon, Bandung, Indonesia

ABSTRACT

Pseudomonas aeruginosa can be commonly found on the skin, especially in the axillary and anogenital regions. However, healthy people do not normally develop Pseudomonas infection. Infection of the skin by *P. aeruginosa* tends to be serious and complex because these bacteria are invasive, toxigenic and often antibiotic resistant. This prompted the need for an excellent source of natural antibacterial for skin infection disease. Empirically, leaves of Jatropha (*Jatropha curcas* L.) are used to treat skin infections such as boils and itching. The objectives of this study were to determine the content of secondary metabolites of Jatropha leaves ethanol extracts, the antibacterial activity, determined the minimum inhibitory concentration (MIC) against *P. aeruginosa* ATCC 27853, and compared the activity with neomycin sulfates. The antibacterial activity test and its comparative test were assessed using the agar diffusion method, while determining MIC was carried out using macrodillution method. The results demonstrated that ethanol extract of Jatropha leaves contained secondary metabolites as follows: saponins, steroids, tannins and flavonoids. These data supported the ability of test extract as an antibacterial agent against *P. aeruginosa*. The MIC value of the extract was ranged 2.5–5%$^{w}/_{v}$. In a comparative analysis of the extracts with neomycin sulfate, indicated that neomycin sulfate demonstrated greater antibacterial activity than the extract at the same concentration. Based on the inhibition diameter category, the extracts showed active antibacterial activity. In conclusion, *Jatropha curcas* offers potential antibacterial property which validates it uses for skin infection treatment in traditional medicine, especially pseudomonas infection.

Keywords: *Pseudomonas aeruginosa*, skin, *Jatropha curcas* L, antibacterial.

Corresponding author

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INTRODUCTION

Almost 50 years ago, *Pseudomonas aeruginosa* was rarely considered as a real pathogen. In the 1970s, it was recognized as the microorganism associated with bacteremia in the neutropenia host. Nowadays, it is among the most common pathogens involved in nosocomial infections [1]. The effect of skin infections caused by *P. aeruginosa* can be both local and systemic [2]. *P. aeruginosa* will continue to belong among the major virulent pathogens implicated in the following major syndromes: pneumonia, cystic fibrosis, bacteremia, endocarditis, malignant external otitis in diabetics, nosocomial meningitis, endophthalmitis, and osteomyelitis [3,4,5].

The kind of available antibiotics with antipseudomonal activity includes the aminoglycosides, ticarcillin, ureidopenicillins, ceftazidime, cefepime, aztreonam, the carbapenems and ciprofloxacin [6]. However, there is no evidence that newer antipseudomonal antibiotics active against multidrug-resistant clones will appear in the future [7]. In addition, the resistance of *P. aeruginosa*, to different antimicrobial agent, especially β-lactam and carbapenem, has been reported with increasing frequency worldwide. A research study reported that clinical isolates of *P. aeruginosa* showing resistance to cefotaxime, ceftizoxime, amikacin and imipenem [8]. Patients infected with *P. aeruginosa* in the new millennium should survive given the existing antibiotics [1]. Therefore, it is necessary to search for new antibacterial. This can be done by using natural materials such as *Jatropha curcas*.

Nowadays, utilization of Jatropha trees (*Jatropha curcas* L.) just as a plant fence or barrier fields because uneconomical. Leaves and fruits of Jatropha were only used as animal feed [9]. After having found a way to extract the fruit of Jatropha into oil, Jatropha seen as attractive as a source of biodiesel because of its oil content is high [10]. In the health sector, traditionally, people use the leaves to treat various health problems such as chronic wounds [11]. *Jatropha curcas* variously known as physic nut, purging nut or pignut [12,13] is used in folklore remedies for treatment of various ailments such as skin infections, gonorrhea, jaundice and fever [14].

Utilization of Jatropha leaf as anti infection against *P. aeruginosa* can be supported with its chemical content. The chemical contents of the Jatropha leaves are as follows: n-l-triakontanol, alpha-amini, kampesterol, stigmast-5-ene-3 beta, alpha 7-diol, stigmasterol, betasitosterol, iso-viteksin, viteksin, and 7-keto-betasitosterol. Such compounds can act as putative antibacterial against *P. aeruginosa* [15]. Scientific proof of the benefits of the leaf of the distance of the fence as a antiinfection against *P. aeruginosa* can provide positive contributions, especially in the field of health. The antibacterial activity of the methanolic extract of the leaves of *J. curcas* has been investigated against 13 bacterial species, including *Escherichia coli*, *P. aeruginosa* and *Staphylococcus aureus* [14]. The aim of this study was to further evaluate the antibacterial activity of the ethanolic extracts of the leaves of *J. curcas* against *P. aeruginosa* ATCC 27853.

MATERIALS AND METHODS

Materials

The samples that were utilized in this study are *Jatropha curcas* leaves. The bacteria that were used is *P. aeruginosa* ATCC 27853. 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).

Sample collection and Identification

Leaf samples of *Jatropha curcas* were collected from Manoko (Drug Experiment Garden), Lembang, Indonesia. The leaves were sent for proper identification**.** Leaf samples were identified in Plant Taxonomy Laboratory at School of Biological Sciences and Technology, Bandung of Institute Technology, Indonesia, were shown in Figure 1.

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Figure 1: Leaf samples of Jatropha curcas

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 done 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 [16]**.**

Preparation of The Bacterial Suspension

The standard most commonly used in the clinical microbiology laboratory is the 0.5 McFarland standard, which is prescribed for antimicrobial susceptibility testing and culture media performance testing [17]. The bacterial suspension was prepared by transferring 1-2 isolated colonies from slant agar into sterile normal saline in a sterile tube. The turbidity was adjusted to McFarland turbidity standard tube No. 0.5 by adding sterile normal saline [18]. A McFarland standard is a chemical solution of barium chloride and sulfuric acid; the reaction between these two chemicals results in the production of a fine precipitate, barium sulfate. When shook well, the turbidity of 0.5 McFarland standards is visually comparable to a bacterial suspension of approximately 1 x 10^8 bacterial cells /mL [19].

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 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration test was done using macrodilution method. The volume of 1 ml *Mueller-Hinton Broth* was added to every sterilized tube. Then 1 ml extract with concentration 40% $^{w}/_{v}$ was added into the first tube. Then the volume of 1 ml from the first tube was pipetted then added to the second tube, and so on until the concentration of every tube was 20%; 10%; 5%; 2.5%; 1.25%; and 0.625% $^{w}/_{v}$. Then 10 µL of bacterial suspension was added to every tube. The liquid media, then were incubated at temperature 37^{o}C for 20 h. The MIC value was determined from the smallest concentration which did not show any turbidity. As cell growth confirmation, the loop was dipped into the MIC tube, then streaked it on to the agar surface. After that, the plates were incubated at temperature 37^{o}C for 20 h.

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Comparison Analysis of Antibacterial Activity

The comparison analysis test procedure was done 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

Ethanol extracts of Jatropha leaves had shown the presence of tannins, flavonoids, steroids, and saponins**.** The result of phytochemical screening can be seen in Table 1.

Table 1: Phytochemical screening

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

Notes: (+) = detected; (-) = not detected

In another study stated that the same phytochemical screening result of all parts of *P. pinnata*, that contained alkaloid, flavonoids, saponins, sterol, tannins and terpenoids, revealed that the ethanol extract of *P. pinnata* possesses antimicrobial activity against *P. aeruginosa* [20]. So, it can be concluded that the presence of these secondary metabolites may contribute for antimicrobial activity of Jatropha leaves ethanol extracts against *P. aeruginosa* ATCC 27853.

Antibacterial Activity Result

The antibacterial activity test was done using the agar diffusion method with perforation technique. Ethanol extract of Jatropha leaves had showed antimicrobial activity against *P. aeruginosa* ATCC 27853. The result of the antibacterial activity test can be seen in Table 2.

Table 2: Antibacterial activity of the ethanol extracts of *Jatropha curcas* leaves

Concentration (%$^w/_v$)	Inhibitory zone diameter (mm)
20	8.30 ± 0.10
40	9.65 ± 0.15
60	10.20 ± 0.00
80	12.15 ± 0.12

Note: Perforator diameter = 6 mm

The evaluation of inhibition can be classified into three categories based on the diameter of zones of inhibition; very active (above 11 mm), medium activity (active) (between 6-11 mm), while non-active (6 mm). According to this criterion, the ethanol extract of Jatropha leaves was active since the diameter of zones of inhibition was between 6-11 mm [21]. The conclusion is also supported by ANOVA statistics. Based on the ANOVA calculation, can be seen that with the significant level α = 5%, H_0 for F-count (1.011534) is smaller than F-table (4.07). This data showed that with *95%* confidences, antibacterial of Jatropha leaves extracts against *P. aeruginosa* ATCC 27853 was given effect to the greater the inhibition zone produced.

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Minimum Inhibitory Concentration 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
0.625	(+)
1.25	(+)
2.5	(+)
5	(-)
10	(-)
20	(-)

Note : (+) = no growth inhibition; (-) = minimal growth inhibition

The extracts showed the value of MIC ranged between 2.5 and 5 %$^{w}/_{v}$. The lower concentration of MIC of the extract has been proving its antibacterial capabilities.

Comparison of Antibacterial Activity 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 *P. aeruginosa*. The diameters of inhibition zone can be seen in Fig. Table 4. Each of these diameters of the inhibition was plotted into the equation in order to obtain the line using linear regression method. The line equation of neomycin sulfates was y = 3,756x + 3.435; as for the tested extract was y = 3,290x - 9.0055. If the concentration of 100 ppm neomycin sulfate was plotted into the line equation of neomycin sulfate, then drag the resulting diameter was 10.95 mm. If the diameter put into the equation line of the extract, then to produce inhibitory diameter of 10.95 mm, the concentration of the extract which required was 1,161,532 ppm. Thus, the antibacterial comparative value of the Jatropha extract to neomycin sulfate was 1: 11615.32.

Table 4: Comparative of inhibitory zone diameter

Material	Concentration (ppm)	Inhibitory Zone Diameter (mm)
Extracts	400000	9.45 ± 0.10
	600000	9.95 ± 0.30
	800000	10.45 ± 0.50
neomycin sulfate	40	9.50 ± 0.65
	60	10.00 ± 0.60
	80	10.65 ± 0.30

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. The comparison of the activity of the plant extract with conventional antibiotics confirmed reports by other researches [22]. Emeruwa (1982) reported that conventional antibiotics are more active than plant extracts [23].

CONCLUSION

Our results demonstrated that ethanol extracts of *Jatropha curcas* leaves have active antibacterial activity against *P. aeruginosa* ATCC 27853. This finding is significant because *P. aeruginosa* have been reported to be completely resistant to the action of most antibacterial drugs available in Indonesia. Further study needs to be carried out on the isolation of bioactive components of the tested plants and their in vivo effect.

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REFERENCES

[1] Helen G. J. Antimicrob. Chemother 2002; 49 (2): 229-233.
[2] Entjang, I. Mikrobiologi dan Parasitologi Untuk Akademi Keperawatan dan Sekolah Tenaga Kesehatan Yang Sederajat (2nd ed.). Citra Aditya Bakti. Bandung, 2003. pp. 99-100.
[3] Pollack, M. *Pseudomonas aeruginosa*. In Principles and Practice of Infectious Diseases (4th ed). Churchill Livingstone, London, UK, 1995. pp. 1980–2003.
[4] Fagon JY, Chastre J, Vuagnat A, Trouillet JI, Novara A & Gibert C. JAMA 1996; 275: 866–9.
[5] Vidal F, Mensa JM, Martinez JA. Eur J Clin Microbiol Infect Dis 1999; 18: 473–477.
[6] Giamarellou H, Antoniadou A. Med Clin North Am 2001; 85: 19–42.
[7] Cohen ML. Epidemiology of drug resistance: implications for a post-antimicrobial era. Science 1992; 257: 1050–1055.
[8] Merina Paul Das. Res J Pharm Biol Chem Sci 2013; 4(1): 686-92.
[9] Hambali E, Suryani A, Dadang, Yadi H, Hanafie H, Reksowardojo I, et al, Jarak Pagar Tanaman Penghasil Biodisel. Penebar Swadaya, Jakarta, 2007.
[10] Prihandana R, Hambali E, Mujdalipah S, Hendroko R. Meraup Untung dari Jarak Pagar. Agromedia Pustaka, Jakarta, 2008.
[11] Hariana A. Tumbuhan Obat dan Khasiatnya. Niaga Swadaya. Jakarta, 2007.
[12] Uche FI, Aprioku JS. J. Appl. Sci. Environ. Manage 2008;12(4): 99-102
[13] Igbinosa OO, Igbinosa EO, Aiyegoro OA. Afr. J. Pharm. Pharmacol 2009; 3(2): 058-062
[14] Akinpelu DA, Aiyegoro OA, Okoh A I. Afr. J. Biotechnol 2009; 8(8): 1660-1664
[15] Dalimartha S. Atlas Tumbuhan Obat (5th ed). Pustaka Bunda, Jakarta, 1999 pp. 55-57.
[16] Harborne JB. Phytochemical methods (3th ed). Chapman and Hall Int., New York, 1998 pp 12.
[17] NCCLS. M22-A2 Quality assurance of commercially prepared microbiological culture media (2nd ed). Wayne, PA: NCCLS, 1996.
[18] Ekundayo EO, Ekekwe JN. Afr. J. Microbiol. Res 2013; 7(44): 5097-5100.
[19] McFarland J. J Am Med Assoc 1907; 14:1176-1178.
[20] Rinu K, Gupta AK. Res J Pharm Biol Chem Sci 2015; 6(2): 577-582.
[21] Nurliana, Sudarwanto M, Sudirman L, Sanjaya AW. The prospect of Acehnese traditional foods as a healthy food: the initial detection of antimicrobial activity of plieku oil and plieku crude extracts. Dissertation 2009. Veterinary science study program. Bogor Agricultural Institute.
[22] Awe S, Omajosala PF. Ethnobotanical leaflets 2009; 13: 1197-1204.
[23] Emeruwa AL. Jnatprod 1982; 45: 123-127.