

Original Research Article

Phytochemical Screening and Antimicrobial Activity of Leaf Extract of *Jatropha curcas*

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ABSTRACT

The study investigated the antibacterial activity of *Jatropha curcas* using aqueous, methanol and ethanolic extracts that were obtained from the leaf against growth inhibition of *Escherichia coli*, *Staphylococcus aureus*, *Proteus spp*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. Tannins, saponins, flavonoids, alkaloids, oxalates and cyanogenic glycosides were the phytochemicals present. All test microorganisms were significantly inhibited by the leaf extracts but the presence of oxalates and cyanogenic glycosides in the leaf could present potential toxicity and therefore reduces the application of this plant to topical but not systemic use. Clearly, additional pharmacology and safety studies are warranted to investigate clinical potential of *Jatropha curcas* as a topical antibiotic for use against susceptible pathogens.

Keywords: *Jatropha curcas*, Phytochemical, antimicrobial

1. INTRODUCTION

Microbial infections are major public health problems in the developed countries. Due to general use of commercial antibiotics, the relative incidence of multiple antibiotic resistances in human pathogens is not only large but also growing. [1] Intractable problems of resistance to antibiotics by microorganisms previously thought to have been brought under control have led to the resurface of interest in herbal products as source of potential compounds to suppress or possibly eradicate the ever increasing problems of emergence of newer diseases. [2, 3] The World Health Organization estimated that about 80% of people still rely mainly on traditional remedies such as

herbs for their medicines. [4] Most of the plants used by the rural residential areas have biologically active compounds that have been proven by generations to be potent against specific disorders. [5-7] The prevention and treatment of diseases by the use of available and accessible medicinal plants in a particular locality will continue to play important roles in medical health care implementation in the developing countries as plants make up the primary source of new pharmaceuticals and health care products. [8] Natural products are therefore gaining attention as an alternative for antimicrobial agents. [9]

There have been major role played by *Jatropha curcas* in the treatment of different kinds of diseases, ranging from bacterial to fungal infections. [10] Glycosides, tannins, phytosterol, flavonoids and steroidal sapogenins are contained in different parts of the plants (leaves, fruits, latex and bark); a feature which enables it to demonstrate vast range of medicinal properties. [11-15] Also, the presence of ansovitexin, apigenin and vitexin in the leaf gives it the ability to serve as a cure for rheumatism and muscular pains. [16-18] Antibiotic activities of *Jatropha curcas* seed have been demonstrated against *Staphylococcus aureus* and *Escherichia coli*. [10] With the wealth of medicinal claims surrounding the use of *Jatropha curcas* leaves, [19] there is need for investigation of antibiotic potential of the leaves against some common pathogenic microorganisms. This research therefore investigates the growth inhibition of *E. coli*, *S. aureus*, *Proteus spp*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* by methanolic, ethanolic and aqueous leaf extracts of *Jatropha curcas*.

2. MATERIALS AND METHODS

2.1. Media Used: Nutrient media (Micro master) and MacConkey agar (Lab M).

2.2. Test Organisms: While carrying out this work, the following bacteria were used as the test organisms: *Escherichia coli*, *Staphylococcus aureus*, *Proteus spp*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. These organisms were obtained from the Microbiology Laboratory of Abia State University, Nigeria.

2.3. Leave Extracts: Fresh leaves of *Jatropha curcas* were collected from farm land in Agbani, Enugu State, Nigeria. The plant was identified and authenticated by the Department of Botany, University of Uyo, Nigeria. Voucher sample was prepared and deposited in the Herbarium for reference. The leaves of *Jatropha curcas* were allowed to air-dry at room temperature for fourteen (14) days until the leaves became brittle, and ground into fine powder in a clean mortar using a clean pestle. Twenty grams of the leaves powder were weighed and

62 poured into three (3) conical flasks; 100ml of distilled water, 70% ethanol, and 70% methanol.
63 Ethanol and methanol used were of analytical grade. The preparations were allowed to stand
64 undisturbed, but with occasional agitation, for 24 hours and filtered using Whatman No. 1 filter
65 paper. The residues were discarded and the filtrates (extracts) were divided into two portions.
66 One portion was refrigerated at 4°C until needed. The other was evaporated to dryness in a water
67 bath at 100°C.

68 **2.4. Phytochemical Analysis of the Plant Extracts**

69 The extracts were subjected to qualitative phytochemical tests for tannins, saponins, flavonoids,
70 alkaloids, oxalates and glycosides in accordance with Trease and Evans. [20]

71 **2.5. Antimicrobial Activity**

72 **2.5.1. Preparation of Culture Media**

73 *2.5.1.1. Nutrient agar preparation:* The medium was prepared by suspending 2.8g of the
74 agar powder into a 100ml of distilled water, in a conical flask and shaken very well to dissolve. It
75 was then autoclaved at 121°C for 15mins and allowed to cool at 47°C before pouring into plates
76 aseptically in the required amount. The medium was allowed to solidify. [21]

77 *2.5.1.2. Macconkey agar preparation:* This medium was prepared by weighing 4.8g of
78 agar powder into a conical flask; 100ml of distilled water was poured into the flask. The flask
79 was corked, shaken and allowed to soak for about 10minutes. The medium was autoclaved at
80 121°C for 15minutes. After autoclaving, it was allowed to cool to 47°C before pouring into the
81 plates aseptically in the required amount and allowed to solidify. [21]

82 **2.5.2. Incubation and Inoculation**

83 The agar well diffusion method was used in the assessment of the antimicrobial activity of the
84 plant. This method was achieved by using a sterile cork borer to make six wells on each Nutrient
85 and MacConkey agar plates. Then 100µl of the plant extracts at 1mgml⁻¹ were dropped into wells
86 and allowed to diffuse for 5 minutes. Tetracycline, at 1mgml⁻¹, was used as control. Plates were
87 then kept to stand in the incubator for 24hours at 37°C. [22] Zones of inhibition were measured
88 and recorded. The experiment was done in triplicate and the results are expressed as mean ±
89 standard deviation.

90 **3. RESULT AND DISCUSSION**

91 **Table 1:** Phytochemical screening of methanolic, ethanolic and aqueous extract of *Jatropha*
92 *curcas* Leaves

Phytochemicals	Methanolic extract	Ethanolic extract	Aqueous extract
Saponin	-	-	+++
Tannins	-	++	+
Flavonoids	+++	+	-
Cyanogenic glycosides	+++	++	++
Alkaloids	-	+	+++
Oxalate	++	+++	-

93 +++Highly present, ++Moderately present, +Present, -Absent

94
95 **Table 2:** Antibacterial Activity of *Jatropha Curcas* Leaves Extracts Using Each Solvent (Water,
96 Ethanol And Methanol) at Different Concentrations.

Organisms	Zone of Inhibition (mm)						
	W ₁	W ₂	E ₁	E ₂	M ₁	M ₂	Control
<i>Escherichia coli</i>	8.0±2.0	7.7±0.8	8.1±1.2	9.1±1.4	10.1±2.2	8.8±1.1	8.0±0.54.
<i>Staphylococcus aureus</i>	8.7±1.71	8.3±1.1	7.5±0.9	7.7±1.0	7.0±1.5	8.1±1.4	7.4±1.3
<i>Proteus spp</i>	6.3±0.76	8.7 ± 1.8	6.2±0.2	5.9±0.3	7.7±1.1	6.9±1.2	6.1±0.81
<i>Klebsiella pneumonia</i>	7.1±0.29	7.2±1.3	6.9±1.1	7.4±1.2	10.6±2.5	9.7±1.3	7.7±1.2
<i>Pseudomonas aeruginosa</i>	8.1±0.91	8.1±0.56	7.3±0.71	6.8±0.2	7.6±1.1	7.8±2.1	6.9±2.2

97 Means±Standard Deviation

98 W₁ = Water extract (refrigerated) 4°C

99 W₂ = Water extract (evaporated) 100°C

100 E₁ = Ethanolic extract (refrigerated) 4°C

101 E₂ = Ethanolic extract (evaporated) 100°C

102 M₁ = Methanolic extract (refrigerated) 4°C

103 M₂ = Methanolic extract (evaporated) 100°C

104 Control = Tetracycline

105
106
107 The phytochemical screening of *Jatropha curcas* leaf, presented in Table 1, revealed the
108 presence of tannins, saponins, flavonoids, alkaloids, oxalates and cyanogenic glycosides. These
109 phytochemicals are biologically active and can be responsible for the antimicrobial activity of
110 the plant. The mechanisms through which these secondary metabolites exert their antimicrobial
111 activities differ. Tannins have been reported to form irreversible complexes with proline rich
112 protein, [23] thereby inhibiting cell protein synthesis. [24] Intestinal disorders such as diarrhea
113 and dysentery have been treated with herbs that have tannins as their main components. [25]

114 Another secondary metabolite observed in the leave extract of *J. curcas* was alkaloid. Alkaloids
115 have analgesic effects [26] and have been clinically used. [27] Alkaloids have been acclaimed

for their antimicrobial activities, especially against gram negative bacteria. [28] Other secondary metabolites present in *J. curcas* whose antimicrobial activities have been documented are flavonoids and saponins. [29]

Though traditionally used in the fermentation of cassava, *J. curcas* is inedible. This is due to the presence of oxalate and cyanogenic glycosides in it as presented in Table 1. Oxalate is an antinutrient and an acute toxin. [30] Cyanogenic glycosides are not just distasteful but also toxic, as they release hydrogen cyanide which generates free radicals and causes oxidative stress. [31]

As presented in Table 2, all the extracts demonstrated significant inhibition against the test microorganisms. Ethanolic extract however demonstrated the greatest inhibition. This is consistent with the high level of phytochemicals in the ethanolic extract. The inhibition was most effective against *E. coli*. This is consistent with the inhibition by stem bark extracts of *Jatropha curcas*. [32] The use of *J. curcas* as a medication for diarrhea may however not be feasible because of the presence of oxalate and cyanogenic glycosides in it. The extracts are also active against *Staphylococcus aureus* and *Klebsiella pneumonia*. This agrees with the report of Ekundayo and co workers [33] on the antimicrobial activities of the nut extracts of *J. curcas*. *Staphylococcus aureus* is the causative organism for skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning. [34] *Klebsiella pneumonia* infections are seen mostly in people with a weakened immune system. *Klebsiella* infections are often nosocomial. The most common condition caused by *Klebsiella* outside the hospital is pneumonia. [35] *Klebsiella* is also implicated in urinary tract, lower biliary tract, and surgical wound sites infections. [36] The inhibition of *S. aureus* and *K. pneumonia* by the leave extract substantiates the trade-medicinal use of *J. curcas* in wound healing, scabies and other skin infections. [10]

Other microorganisms inhibited include *Proteus spp* and *Pseudomonas aeruginosa*. Similar inhibitions by the nuts and stem bark of *J. curcas* have been reported. [22,33] *Pseudomonas aeruginosa* is a ubiquitous multi drug resistant pathogen that is associated with serious illnesses - especially nosocomial infections such as ventilator-associated pneumonia and various sepsis syndromes as well as hot-tub rash. [37] *Proteus spp* is implicated in urinary, septic and wound infections, often nosocomial. [38] However, it is opportunistic and only affects immunosuppressed individuals. [35] The inhibition of *Proteus spp* by *Jatropha curcas*, as shown in Table 2, substantiates the reported wound healing ability of *Jatropha curcas*. [39,40] There were no significant differences between the inhibitions by the refrigerated and evaporated extracts. This demonstrates that the preservation methods have no significant effect on the antimicrobial properties of the extracts.

4. CONCLUSION

Phytochemical screening of *Jatropha curcas* leaf revealed the presence of several biological active compounds including tannins, saponins, flavonoids, alkaloids, oxalates and cyanogenic glycosides; which can be responsible for the observed antimicrobial action of the leave. The leaf demonstrated considerable inhibition zone against the test microorganisms but can only be used for topical treatment because of its inedibility.

REFERENCES

1. Li X and Krumholz LR. Regulation of asenate resistance in *Desulfovibrio desulfuricans* G20 by an *arsRBCC* operon and an *arsC* Gene. *J. Bacteriol.* 2007; **189**(10): 3705-11.
2. Jain SK . Medicinal plants1996; National Book Trust. India, pp. 3.
3. Mothanaa RA and Lindequist U. . Antimicrobial activity of some medicinal plants. *Journal of Ethnopharmacology.* 2005; **96**(1): 177-181.
4. WHO (World Health Organization) . Traditional Medicine 2003; Fact sheet No 134.
5. Mark MS . Phytochemicals in Nutrition and Health. 2002; CRC Press. p. 123. ISBN 9781587160837.
6. Al-Sayed E, Martiskainen O, Seif el-Din SH, Sabra AA, Hammam AO, El-Lakkany NM, and Abdel-Daim MM. . Hepatoprotective and Antioxidant Effect of Bauhinia hookeri Extract against Carbon Tetrachloride-Induced Hepatotoxicity in Mice and Characterization of Its Bioactive Compounds by HPLC-PDA-ESI-MS/MS. *BioMed Research International.* 2014; Article ID 245171. doi:10.1155/2014/245171.
7. Eldahshan OA and Abdel-Daim MM. Phytochemical study, cytotoxic, analgesic, antipyretic and anti-inflammatory activities of Strychnos nux-vomica. *Cytotechnology.* 2015; **67**(5):831-44. doi: 10.1007/s10616-014-9723-2.
8. Ivanova DD, Geroval T, Chervenkov and Yankova T. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *J.Ethnopharmacol.* 2005; **96**:145-150.
9. Helal MS, Youssef FM, Moursi MK, Khalil WF, Abdel-Daim MM . Effectiveness of prebiotic as an alternative to the antimicrobial growth promoter on growth performance, blood constituents, intestinal healthiness and immunity of broilers. *AJVS.* 2015; **45**(1): 13-25. doi:10.5455/ajvs.179869
10. Thomas T, Sah NK and Sharma P B . Therapeutic Biology of *Jatropha curcas*: A Mini Review. *Curr Pharm Biotechnol.* 2008; **9**(4):315-24.

11. Dalziel JM . The Useful Plants of West-Tropical Africa. 1955; Crown Agents for Oversea Governments and Administration, London, P.147
12. Duke JA . CRC Handbook of Medicinal Herbs. 1988; CRC Press, Boca Raton, Florida, pp. 253–254.
13. Duke JA Biologically active compounds in important species. *In*: Charalambous, E. ed. .1994 . Spices, herbs and edible fungi. Elsevier Science Oxford. Pp 225-250.
14. Edeoga HO, Okwu DE, Mbaebre BO. Phytochemical constituents of some Nigerian plants. *African Journal of Biotechnology* 2005; 44 7 :685-688.
15. Agbogidi OM, Ekeke EA . *Jatropha curcas*: Linn an important but neglected plant species in Nigeria. *J Biol Chem Res.* 2011; **281** : 52-62.
16. Duke JA, Bogenschutz-Godwin MJ, DuCellier J, Duke PAK . Handbook of medicinal herbs. 2002; 2nd Edition, Boca Raton, CRC Press.
17. Rajore S, Batra A. *Jatropha curcas*: a plant of immense potential value. *Journal of Ecology, Taxonomy and Botany* 2003; 27:36-41.
18. Agbogidi OM, Eruotor PG. Morphological Changes due to spent engine oil contamination and its heavy metal components of *Jatropha curcas*. *In*: Baby, S. and Sandhu, P.S. eds. Proceedings of the International Conference on Bioscience, Biotechnology and Health Sciences ICBBHs' 2012 organized by Planetary Science Centre Research December 14 and 15, 2012 in Singapore. Pp. 88-93.
19. Esimone CO, Nworu CS and Jackson CL. Cutaneous wound healing activity of an herbal ointment containing the leaf extract of *Jatropha curcas* L. (*Euphorbiaceae*). *International Journal of Applied Research in Natural Products.* 2009; **1**(4), pp. 1-4.
20. Trease GE, Evans WC. Textbook of Pharmacognosy. 1989; 12th Edn. Balliere, Tinadl London.
21. Cheesbrough M. Medical Laboratory Manual. Tropical Health Technology. 2003; Low Priced Edition. Doddington, Cambridge shire, England. Pp.132-143.
22. Igbiosa OO, Igbiosa EO and Aiyegoro OA. Antimicrobial activity and Phytochemical screening of stem bark extracts from *Jatropha curcas*. *African Journal of Pharmacology.* 2009; **3** (2): 58-62.
23. Shimada T. Salivary proteins as a defense against dietary tannins. *J. Chem. Ecol.* 2006; **32** (6): 1149-1163.

24. Parekh J, Chanda S (). *In vitro* antibacterial activity of crude methanol extract of Wood for dia fruticosa Kurz flower (Lythaceae). *Braz. J. Microbiol.* 2007; 38: 2.
25. Dharmananda S. Gallnuts and the uses of Tannins in Chinese Medicine. In: Proceedings of Institute for Traditional Medicine, 2003; Portland, Oregon.
26. Sutradhar RK, Rahman AM, Ahmad M, Bachar SC, Saha A, Roy TG. Anti-inflammatory and analgesic alkaloid from *Sida cordifolia* linn. *Pak J Pharm Sci.* 2007; **20**(3):185-8.
27. Kam PCA, Liew S. Traditional Chinese herbal medicine and anaesthesia. *Anaesthesia* 2002; 57(11): 1083-1089.
28. Cushnie TPT, Cushnie B, Lamb AJ. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and anti-virulence activities. *International Journal of Antimicrobial Agents*: 2014; **44**(5): 124-129
29. Hodek P, Trefil P, Stiborova M. Flavonoids - Potent and versatile biologically active compounds interacting with cytochrome P450. *Chemico-Biol. Intern.*, 2002; **139**(1): 1-21.
30. Munro A, Bassir O. Oxalates in Nigerian Vegetables. *West African Journal of Biological and Applied Chem.* 1996; **12**:14-18.
31. Gleadow RM, Woodrow IE. Mini-Review: Constraints on Effectiveness of Cyanogenic Glycosides in Herbivore Defense. *Journal of Chemical Ecology.* 2002; **28**(7): 1301-1313.
32. Igbinosa OO, Igbinosa EO and Aiyegoro OA. Antimicrobial activity and Phytochemical screening of stem bark extracts from *Jatropha curcas*. *African Journal of Pharmacology.* 2009; **3**(2): 58-62.
33. Ekundayo FO, Adeboye CA and Ekundayo EA. Antimicrobial activities and phytochemical screening of pignut (*Jatropha curcas* Linn.) on some pathogenic bacteria. *J. Med. Plant. Res.* 2011; **5**(7): 1261-1264.
34. Curran JP, Al-Salihi FL; Al-Salihi (). "Neonatal staphylococcal scalded skin syndrome: massive outbreak due to an unusual phage type". *Pediatrics* 1980; **66**(2): 285-90. PMID 6447271.
35. Brooks GF, Butel JS, Morse SA., Carroll, KC, and Mietzner TA. Medical Microbiology, 23rd Ed. New York: McGraw-Hill Companies, 2004.

- 244 36. Ryan KJ and Ray CG. Sherris Medical Microbiology (4th ed.). McGraw Hill. ISBN
245 0-8385-8529-9, 2004.
- 246 37. Balcht A and Smith R. Pseudomonas aeruginosa: Infections and Treatment. Informa
247 Health Care. pp. 83–84. ISBN 0-8247-9210-6, 1994.
- 248 38. Guentzel MN. Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter, and Proteus.
249 In: Barron's Medical Microbiology (Barron S *et al.*, eds.) (4th ed.). Univ of Texas
250 Medical Branch. ISBN 0-9631172-1-1. (via NCBI Bookshelf), 1996.
- 251 39. Shetty S, Udupa SL, Udupa AL and Vollala VR. Wound healing activities of Bark
252 Extract of Jatropha curcas Linn in albino rats. *Saudi Med J*. 2006; **27**(10):1473-6.
- 253 40. Esimone CO, Nworu CS and Jackson CL.. Cutaneous wound healing activity of a
254 herbal ointment containing the leaf extract of Jatropha curcas L. (Euphorbiaceae).
255 *IJARNP*. 2008; **1**(4): 1-4.