International Journal of Pathogen Research

10(4): 12-17, 2022; Article no.IJPR.91066
ISSN: 2582-3876

Antibacterial Screening of *Jatropha tanjorensis* against *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*

N. E. Ewa-Udu [a*], F. C. Nwanebu [a], H. O. Stanley [b] and I. W. Okereke [c]

[a] Department of Microbiology, Madonna University, Elele, Rivers State, Nigeria.
[b] Department of Microbiology, University of Port Harcourt, Rivers State, Nigeria.
[c] Department of Oral Pathology and Oral Medicine, University of Nigeria, Enugu State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPR/2022/v10i430255

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/91066

Original Research Article

Received 09 June 2022
Accepted 19 August 2022
Published 23 August 2022

ABSTRACT

The crude extracts of *Jatropha tanjorensis* were investigated with the aim of determining the antibacterial activity, qualitative and quantitative properties, the best solvent used for extraction, the most active ingredients and the organism that is most susceptible to them. Ethanol, petroleum ether and water (warm) were used as solvents. Agar well diffusion method was used for the susceptibility of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* to the extracts, with streptomycin as positive control and sterile water as negative control. Ethanol extracts of the plant showed most activities, whereas petroleum ether and water (warm) extracts had no activity on the test organisms. The ethanol extracts of *Jatropha tanjorensis* leaf inhibited the growth of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* with inhibition zone of 6.0±0.04 mm, 5.5±0.70 mm and 7.5±0.70 mm respectively. This study reveals that the ethanol extracts of *Jatropha tanjorensis* have antimicrobial effect on three test pathogens, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Keywords: Jatropha tanjorensis; ethanol extracts; antimicrobial; pathogens.

**Corresponding author:*
Email: immanuelomega@gmail.com;

1. INTRODUCTION

"Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value" [1]. "Plants since origin have been recognized to contain natural products which serve as food as well as medicine in the event of human infections" [2]. "Green plants constitute a reservoir of potentially useful chemical compounds which serve as drugs, are provided newer leads and clues for modern drug design by synthesis" [3,4].

"The continuous and perpetual people's interest in medicinal plants has brought about today's modern and sophisticated fashion of their processing and usage" [5]. "In recent times, phytochemicals have received a lot of attention and are even preferred to synthetic ones especially due to their potential health benefits, availability, affordability and in many cases, reduced toxicity" [6]. "In addition, herbal medicines have received greater attention as an alternative to clinical therapy and the demand of these remedies has currently increased" [7,8].

"Among plants valued for their medicinal properties is *Jatropha tanjorensis* (chaya leaf). The name Jatropha is derived from the Greek words jatrós (doctor) and trophé (food) which implies medicinal use" [9]. "*Jatropha* is a member of the Euphorbiacea family. It is popularly referred to as 'Hospital Too Far', 'Catholic Vegetable', Iyana-Ipaja or 'Lapalapa' by the local folks in different parts of Nigeria. The Igbo people of South Eastern Nigeria call it 'Ugu-Oyibo'. *Jatropha tanjorensis* is a common weed of field crops in rain forest zones of West Africa. Its primary use is for fencing while its secondary uses are a source of edible leafy vegetables and medicine" [10]. "It is useful in herbal medicine, prepared locally in most parts of Southern Nigeria by collecting the leaves and squeezing out the juice" [11]. "Tanjorensis leaves are consumed in Nigeria as soups and as a tonic with the claim that it increases blood volume and it is also employed traditionally in the treatment of anaemia, diabetes and cardiovascular diseases" [10]. Chemical compounds present in *Jatropha tanjorensis* include phenolics, terpenoids and tannins which are thought to have potential against microorganism [12].

"Plant antimicrobials have numerous curative potentials and may provide relief from any of the side effects usually associated with synthetic antimicrobials. Many plants with antimicrobial properties are mainly produced during plant secondary metabolism" [13]. "They include steroids, phenolic compounds, gums, flavonoids, alkaloids, resins, fatty acids and tannins. Plants have the ability to synthesize aromatic substances such as phenolic compounds including phenolic acids, and flavonoids among others" [12,14].

"The search of alternative antimicrobial agents from natural plant origin have been on the rise due to increasing microbial drug resistance. The problem of drug resistance has prompted researchers to turn their attentions to folk medicines as alternative to conventional chemotherapeutic agents following several reports on the medicinal opportunities derived from higher plants" [15]. In Nigeria affordable medicine is out of the reach of many poor folks. Finding local plants with antimicrobial properties would be a boost to the treatment of infectious caused by susceptible microorganisms. This study aim to ascertain the antibacterial effect of *J. tanjorensis* extracts against three clinical isolates, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh healthy leaves of *J. tanjorensis* were locally collected from pharmacognocy garden in Madonna University, Elele campus and Elder Ewa-udu's compound, Afikpo town in Afikpo North local government area of Ebonyi State and properly authenticated by pharmacognocy department of Madonna University. The leaves were hand plucked aseptically and cleaned for debris using tap water and then rinsed in sterile water. "The leaves were oven-dried at 45^{o}C temperature for 15 minutes. The dried leaves were blended using a domestic blender; powdered samples were measured and stored in air-tight glass containers protected from sunlight for subsequent extraction and further bioassay These followed the method of Daniyan and Muhammad" [16].

2.2 Preparation of the Leaf Extracts

The powdered material was extracted successively with ethanol and petroleum ether solvents in the increasing order of their polarity. Extraction followed the method of Daniyan and Muhammad [16] with modification. Powdered material of *J. tanjorensis* leaves weighing 60.07g

was introduced into extraction chamber of sohxlet extractor (Buchi E-800) and extraction done for 48hours with temperature maintained at 45^{o}C for petroleum ether solvent, 70^{o}C with ethanol solvent and at room temperature for 24hours with distilled water. The extracts produced were concentrated to dryness on water bath and then weighed.

2.3 Phytochemical Screening

Phytochemical screening was carried out in Pharmacognocy laboratory Madonna University, Elele campus. The phytochemical analysis performed were test for alkaloids (Wagner's reagent test and Meyer's reagent test), test for flavonoids (lead acetate test and sodium hydroxide test), test for reducing sugar test for tannins**,** test for carbohydrates **(**molisch's test for glucose)**,** test for saponins, test for cardiac glycosides and test for terpenoids followed methods described by Shah et al. [17].

2.4 Collection and Preparation of Test Isolates

Clinical bacterial isolates (*E. coli, P. aeruginosa* and *S. aureus*) were obtained from patients attending Madonna University Teaching Hospital, Elele. Isolates were subjected to relevant cultures and biochemical tests and 16S rRNA sequencing to authenticate their identity. The stock cultures were transferred to nutrient agar slant and resuscitated at 37°C for 24hours.

2.5 Identification of Test Organisms

To further confirm the identity of all test isolates, Gram staining, motility test and biochemical test included catalase test, oxidase, urease test, Methyl Red and Voges-Proskauer test, indole test, citrate utilization test, haemolysis test sugar fermentation test and coagulase test as described by Cheesbrough [18]. The isolate were further identified on the bases of their 16S rRNA sequences as described by Briggs et al. [19].

2.6 Antimicrobial Susceptibility

Standardization of the test microorganisms was done from the slant culture of the identified microorganisms (*S. aureus, E. coli* and *P. aeruginosa*). A colony was suspended with a sterile wire loop into a sterile Bijou bottle containing sterile distilled water and the opacity was then matched with that of 0.5Mcfarland turbidity standard which corresponded to 10^{8}CFU/ml. Kirby-Bauer Agar diffusion method was used to carry out the susceptibility test experiment.

0.1ml of each of test microorganisms was added aseptically to the prepared Mueller Hinton Agar in the universal bottle and properly mixed by shaking. The mixture was then poured into correspondingly labelled Petri dish and allowed to solidify on the workbench. After the agar had solidified on the Petri dish, a sterile cork borer was used to remove 5 discs of agar from the agar layer in order to produce 5 wells in each agar plate. The Wells were labeled for the five (5) concentrations of *J. tanjorensis* leaf extracts (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml). "Using a separate sterile Pasteur's pipette 0.1ml of each concentration of *J. tanjorensis* leaf extracts was carefully added to each of the wells and allowed to stand on the workbench for 15 minutes for proper diffusion of the extracts. Antibiotic disk served and distilled water served as positive and negative control. All the plates were incubated at 37° C for 24 hours. The diameter of the resulting zones of inhibition was measured in millimeter (mm) through the base of the plates using a meter rule" [18].

2.7 Determination of the Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration was determined using tube dilution method. Sterile test tubes were arranged on a test tube rack. The initial concentration of the plant extract was diluted using a double fold dilution method by transferring 5 ml of the extract (stock solution) into 5 ml of sterile Mueller-Hinton broth to obtain 50 mg/ml concentration. The above procedure was repeated to obtain other concentrations (25 mg/ml, 12.5 mg/ml and 6.25 mg/ml). Each concentration was inoculated with 0.1 ml of bacterial cell suspension and incubated at 37±2°C for 24 hours. Growth was indicated by turbidity or cloudiness of the broth. The lowest concentration of the plant extracts that did not give any growth was taken as the minimum inhibitory concentration (MIC) [19].

2.8 Statistical Analysis

One-way analysis of variance (ANOVA) was used to compare the mean differences between the zones of inhibition of the extracts and controls. Significant difference between means were separated by Duncan multiple range test

(DMRT). All results were expressed as mean±SD, while all statistical decisions were taken at 95% level of significance.

3. RESULTS

Table 1 shows quantitative phytochemical composition of leaf extracts. Of all the phytochemicals tannin had the least concentration of 2.02 mg/100g and alkaloids had the highest concentration 72.11 mg/100g as detected in ethanol and water extracts respectively.

3.1 Test Microorganisms

Table 2 shows that the obtained 16s rRNA sequence from the test microorganisms are exact match with *E. coli*, *P. aeruginosa* and *S. aureus*, with percentage similarity of 100%.

3.2 Susceptibility of Test Organisms to Extracts

Table 3 shows test organisms were susceptible to only ethanolic extract of *J. tanjorensis*. *P. aeruginosa* was more susceptible to ethanolic extracts with 7.50±0.70 mm zone of inhibition while *E. coli* was the least susceptible with 5.5±0.70 mm diameter. The MICs of ethanolic extract of *J. tanjorensis* against *E. coli*, *S. aureus* and *P. aeruginosa* were 25 mg/ml, 50 mg/ml and 6.25 mg/ml respectively.

Table 1. Phytochemicals composition of *J. tanjorensis* leaf extracts

	Ethanol	Water	Ether
Flavonoid (mg/100g)	2.84	19.38	AB
Tannin (mg/100g)	2.02	AB	AB
Alkaloids (mg/100g)	53.28	72.11	AB
Glycos ides (mg/100g)	59.35	AB	AB
Saponin (mg/100g)	7.53	AB	AB
Triterpenes (mg/100g)	AB	AB	AB
Steroids (mg/100g)	AB	AB	AB
Terpenoids (mg/100g)	11.18	AB	31.68
Phenols (mg/100g)	22.18	AB	AB
Anthraquinone (mg/100g)	AB	AB	AB

Key: AB=Absent

Table 2. Test microorganisms

Bacterial sample ID	Accession number	Similarity (%)
N1	*E. coli* (CP093368)	100
N2	*S. aureus* (KFO83978)	100
N3	*P. aeruginosa* (MB65745)	100

Table 3. Susceptibility of test organisms to *J. tanjorensis* leaf extracts at 100 mg/ml

Organism	Pet. ether	Ethanol	Water	Positive control
E. coli	0.00±0.00	5.5±0.70	0.00±0.00	9.50±0.70
S. aureus	0.00±0.00	6.0±0.40	0.00±0.00	19.50±0.70
P. aeruginosa	0.00±0.00	7.50±0.70	0.00±0.00	14.50±0.70

Table 4. Antibacterial activity of various concentrations of ethanol extract of *J. tanjorensis* against test organisms

Organism	Concentrations (mg/ml)					Positive control
	100	50	25	12.5	6.25	
S. aureus	4.0± 0.00	1.00±0.4	1.0±0.0	0.00±0.00	0.00±0.00	19.5±0.70
E. coli	4.0 ±0.8	3.0±0.5	0.00±0.00	0.00±0.00	0.00±0.00	9.5±0.70
P. aeruginosa	3.0±0.0	2.5±0.7	1.5±0.7	0.00±0.00	0.00±0.00	14.5± 0.70

4. DISCUSSION

This research, evaluated the phytochemical components and antibacterial efficacy of *J. tanjorensis* leaf extracts against three clinical pathogens: *E. coli, S. aureus* and *P. aeruginosa.* Phytochemical detected as constituents of *J. tanjorensis* leaf extracts were flavonoids, tannin, alkaloids, glycosides, terpenoids, phenols, steroids and saponin. Elinge et al. [12] similarly reported the presence of flavonoids, tannin, alkaloids, glycosides, terpenoids, phenols, steroids and saponin in ethanolic and aqueous extracts of *J. tanjorensis*. Lack of detection of some phytochemicals does not suggest that the constituents are absent, they may be in a very small concentrations to detect or the test may not be so efficient to detect the constituents in some circumstance. The difference in the phytochemical properties constituents of extracts from the same plants could be due to the solvent of extraction.

Ethanolic extract of *J. tanjorensis* contained alkaloids (53.28mg/100g), phenols (22.18mg/100g), terpenoids (11.18mg/100g), saponins (7.53mg/100g), flavonoids (2.84mg/100g) and tannins (2.02mg/100g) when extracted with ethanol. There were higher yield of alkaloids (72.11mg/100g) and flavonoids (19.38mg/100g) when extracted with water. For petroleum ether, quantity of terpenoids (31.68mg/100g). The medicinal value of plants lies in the bioactive phytochemicals present in plants [3]. Secondary metabolites such as alkaloids terpenoids, phenols and tannins have antimicrobial properties [17].

The test organisms (*E. coli, S. aureus* and *P. aeruginosa*) were observed to be susceptible to the ethanolic extracts alone. This could be explained by the fact that ethanol was a better solvent for the bioactive phytochemicals than water and petroleum ether. This is in congruence with previous report by Oboh and Masodje et al. [20] that *S. aureus* and *E. coli* were susceptible to ethanol extract of *J. tanjorensis*. *Pseudomonas aeruginosa* was more susceptible to ethanolic extracts with 7.50±0.70 mm zone of inhibition while *E. coli* was the least susceptible with 5.5±0.70 mm diameter. The zones of inhibitions of the test organisms by the leaf extracts are significantly different ($p<0.05$) from the one obtained from the standard antibiotic (streptomycin 5 mg/ml) used as positive control. This shows that although, the leaves extracts have reasonable activities against the test organisms, their bactericidal effect is still limited. Although the antimicrobial activity of *Jatropha tanjorensis* leaf extract was lesser when compared to the standard antibiotic, it still possess the potential to be used in treatment of diseases caused by pathogenic bacteria [21].

The MICs of ethanolic extract of *J. tanjorensis* against *E coli*, *S. aureus* and *P. aeruginosa* were 25 mg/ml, 50 mg/ml and 6.25 mg/ml respectively. At lower concentrations (12.5 and 6.25 mg/ml), there was no activity observed across all the test isolates. Across the different concentrations, there were significant difference between the zones of inhibition compared to standard antibiotic (streptomycin) used as positive control.

5. CONCLUSION

This study provide evidence that *Jatropha tanjorensis* plants contains chemical compounds active against the three bacterial pathogen. *Jatropha tanjorensis* plants could be of value in the treatment of infections caused by *Escherichia coli*, *Staphylococus aureus* and *Pseudomonas aeruginosa.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nostro A, Germano MP, DÁngelo V, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letter. Applied. Microbiology. 2000;30:379-384.
2. Kumar AK, BinduPriya S, Sravani C, Amrutha Sai K, Poornodaya S, Reddy NR. Comparative evaluation of antibacterial efficacy of herbal extracts and mouth washes against subgingival plaque bacteria. An In vitro study. J. Dent. Her. 2014;1(1):1-3.
3. Coria-Tellez AV, Montalvo-Gónzalez E, Yahia EM, Obledo-Vázquez EN. *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. Arab Journal Chemical. 11:662-91.
4. Oyewole OI, Akingbala PF. Phytochemical analysis and hypolipidemic properties of jatropha leaf extract. European Journal of Medicinal Plants. 2011;1(4):180-185.

5. Kelly, EH, Anthony RJ, Dennis JB. Flavonoid antioxidant chemistry, metabolism and structure-activity relationship. Journal of Nutritional Biochemistry. 2002;13:572-548.
6. Tarawneh KA, Irshaid F, Jaran AS, Ezealarab M, Khlifat, KM. Evaluation of antibacterial and antioxidant activities of methanolic extract of some medicinal plants in Northern part of Jordan. Journal of Biological Sciences. 2010;10(4):325-332.
7. Qing Z.-X, Huang JL, Yang XY, Liu JH, Cao HL, Xiang F, Cheng P, Zeng JG. Anticancer and reversing multidrug resistance activities of natural isoquinoline alkaloids and their structure-activity relationship. Current. Medicine Chemotherapy. 2018;25:5088–5114.
8. Ross MST, Brain KR. An introduction to phytopharmacy. Pitman Medical Publishing Company Ltd. 1977;4-5.
9. Mousumi D, Bisen PS. *Jatropha curcas* L., a multipurpose stress resistant plant with a potential for ethnomedicine and renewable energy. Current Pharmaceutical Biotechnology. 2008;9 (4):288-306.
10. Iwalewa EO, Adewunmi CO, Omisore NO, Adebanji OA, Azike CK. Pro- and antioxidant effects and cytoprotective potentials of nine edible vegetables in Southwest Nigeria. Journal of Medicinal Foods. 2005;8(4):539-544.
11. Manthey J, Grohman K, Guhrie N. Biological properties of citrus flavonoids pertaining to cancer and inflammation. Current Medical Chemistry. 2013;8:135-153.
12. Elinge CM, Yanah YM, Habiba A, Obaro IO, Ogunleye AO, Yusuf H, Elinge RI. Phytochemical screening and antimicrobial activity of ethanolic leaves and stem bark extract of *Jatropha.* Direct Research Journal of Health and Pharmacology. 2020;8(1):7-13.
13. Kumar AK, BinduPriya S, Sravani C, Amrutha K, Poornodaya S Reddy NR. Comparative evaluation of antibacterial efficacy of herbal extracts and mouth washes against subgingival plaque bacteria. An In vitro study. J. Dent. Her. 2014;1(1):1-3.
14. Gavamukulya Y, Abou-Elella F, Wamunyokoli F, AEl-Shemy H. Phytochemical screening, anti-oxidant activity and in vitro anticancer potential of ethanolic and water leaves extracts of Annona muricata (Graviola). Asian Pacific Journal Tropical Medicine. 2014;7:55-63.
15. Gislene GF, Locatelli NJ, Paulo C, Giuliana LS. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazil journal on. Microbiology. 2000;31:247-256.
16. Daniyan SY, Ukubuiwe CC, Ukubuiwe AC, Oluwafemi OJ, Chukwudi PO. Antibacterial Activities of Leaf Extracts of *Jatropha Ellis* and *Saroja* (Euphorbiaceae) Medicinal Plant Research. 2018;8:(4). DOI:10.5376/mpr.2018.08.0004
17. Shah P, Modi HA, Shukla MD, Lahiri SM. Preliminary phytochemical analysis and antibacterial activity of *Ganoderma lucidum* collected from Dang District of Gujarat, India. Int. J. Curr. Microbiol. App. Sci. 2014;3(3):246-255.
18. Cheesbrough M. District laboratory practice in tropical countries. Cambridge University Press, UK; 2007.
19. Briggs WF, Stanley HO, Okpokwasili GC, Immanuel OM, Ugboma CJ. Isolation and molecular characterization of acid producing bacteria from selected oilfield environments within the Niger Delta. Journal of Advances in Microbiology. 2019;17(3):1-9.
20. Oboh FOJ, Masodje HI. Nutritional and antimicrobial properties of jatropha leaves. American – Eurasian Journal of ScientificResearch. 2009;4(1):7-10.
21. Debalke D, Birhan M, Kinubeh A, Yayeh M. Assessments of antibacterial effects of aqueous-ethanolic extracts of *Sida rhombifolia's* aerial part. Current Pharmaceutical Biotechnology. 2000; 9(4):288-306.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/91066