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

Abstract

The crude extracts of *Jatrophatajorensis* 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 *Jatrophatajorensis* leaf inhibited the growth of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*with inhibition zone of 6.0±0.04mm, 5.5±0.70mm and 7.5±0.70mm respectively. This study reveals that the ethanol extracts of *Jatrophatajorensis* have antimicrobial effect on three test pathogens, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Keywords: *Jatrophatajorensis*, ethanol extracts, antimicrobial, pathogens

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].

The use of plants in medicine is highly dependent on experimental screening to ascertain active components, safety, and efficacy of the plant products [4]. Medicinal values of plants depend on these inherent substances that produce a definite physiological action on the human body. Among plants valued for their medicinal properties is *Jatrophatanjorensis* (chaya leaf). The name Jatropha is derived from the Greek words jatrós (doctor) and trophé (food) which implies medicinal use [9]. *Jatrophatanjorensis* 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 [10]. The Igbo people of South Eastern Nigeria call it 'Ugu-Oyibo'. The active chemical compounds of medicinal plants, especially terpenoids, are thought to have potential as an antibacterial, antidiabetic potentials, antihypertensive properties, antioxidative and anticancer effects [11].

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 [12]. This study aim to ascertain the antibacterial effect of *J. tajorensis*extractsagainst three clinical isolates, *Escherichia coli*, *Staphylococcus aureus*and *Pseudomonas aeruginosa.*

Materials and Methods

Sample collection

Fresh healthy leaves of *J.tajorensis*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. 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.

Preparation of the Leaf Extracts

The powdered material was extracted successively with ethanol and petroleum ether solvents in the increasing order of their polarity.To obtain ethanolic and petroleum ether of *J.tajorensis* powdered material of the leaves weighing 60.07g was introduced into extraction chamber of sohxlet extractor and extraction done for 48hours with temperature maintained at 70^{o}c with ethanol solvent and 45^{o}c for petroleum ether solvent. For aqueous extracts of *J.tajorensis*, after the petroleum ether extraction the leaves were left to dry before soaking in distilled water for 24hours and filtered, then soaked again for another 24hours and filtered for a second time. The extracts produced were concentrated to dryness on water bath and then weighed. These were according to methods of Daniyan and Muhammad [13].

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Phytochemical Screening

Phytochemical screening was carried out in Pharmacognocy laboratory Madonna University, Elele campus. The phytochemical analysisperformed were test for alkaloids (Wagner's reagent test andMeyer's reagent test), test for flavonoids (lead acetate test and sodium hydroxide test), test for reducing sugartest 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. [14].

Collection and Preparation of Bacterial Test Isolates

Clinical bacterial isolates were obtained from samples collected from patients attending Madonna University Teaching Hospital, Elele. The microorganisms suspected to be *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* 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.

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 [15]. The isolate were further identified on the bases of their 16S rRNA sequences as described by Briggs et al. [16].

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 10^8CFU/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. 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.tajorensis* leaf extracts (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml). Using a separate sterile Pasteur's pipette 0.1ml of each concentration of *J.tajorensis* 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 [15].

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) [16].

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.

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.

Table 1: Phytochemicals composition of *Jatrophatajorensis*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

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%.

Table 2: mest Microorganisms

Bacterial Sample ID	Accession Number	Similarity (%)
N1	*Escherichia coli* (CP093368)	100
N2	*Staphylococcus aureus*(KFO83978)	100
N3	*Pseudomonas aeruginosa*(MB65745)	100

Susceptibility of test organisms to extracts

Table 3 shows test organisms were susceptible to only ethanolic extract of *J.tajorensis*. *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.tajorensis* against *E. coli*, *S.aureus* and *P.aeruginosa* were 25 mg/ml, 50 mg/ml and 25 mg/ml respectively.

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Table 3: Susceptibility of test organisms to *Jatrophatajorensis* leaf extracts

Organism	Ethanol	Water	Pet. ether	Positive Control
Escherichia coli	5.5±0.70	0.00±0.00	0.00±0.00	9.50±0.70
Staphylococusaureus	6.0±0.40	0.00±0.00	0.00±0.00	19.50±0.70
Pseudomonas aeruginosa	7.50±0.70	0.00±0.00	0.00±0.00	14.50±0.70

Table 4: Antibacterial activity of various concentrations of ethanol extract of *Jatrophatajorensis* against test organisms

Organism	Concentrations (mg/ml)					Positive control
	100	50	25	12.5	6.25	
Staphylococcusaureus	4.0± 0.00	1.00±0.4	1.0±0.0	0.00±0.00	0.00±0.00	19.5±0.70
Escherichia 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
Pseudomonas 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

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.tajorensis* leaf extracts were flavonoids, tannin, alkaloids, glycosides, terpenoids, phenols, steroids and saponin. This is in agreement with the study by Elinge et al. [17] that reported the presence of flavonoids, tannin, alkaloids, glycosides, terpenoids, phenols, steroids and saponin in ethanolic and aqueous extracts of *J.tajorensis*. 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 [14].

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 report is in agreement with previous report of Oboh and Masodje et al. [18] that *S. aureus* and *E. coli* were susceptible to ethanol extract of *J.tajorensis*. *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 *Jatrophatajorensis* 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 [19].

The MICs of ethanolic extract of *J.tajorensis* against *E coli, S. aureus* and *P. aeruginosa* were 25 mg/ml, 50 mg/ml and 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.

Conclusion

This study provide further evidence that plants are reservoirs of potentially useful chemical compounds which serve as drugs, and provides newer leads and clues for modern drug design by synthesis. This study has demonstrated that the ethanolic extract of *Jatrophatajorensis* is active against the three bacterial pathogen studied. These extracts could be of value in the management of disease conditions caused by *Escherichia coli*, *Staphylococusaureus* and *Pseudomonas aeruginosa.*

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