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Assessment of Antibacterial Properties of *Jatropha tanjorensis*

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Authors' contributions

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

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ABSTRACT

Aim: This study aimed at evaluating the Phytochemical and antibacterial properties of *Jatropha tanjorensis* against selected test bacteria.
Methods: The antibacterial properties were tested using the agar-well diffusion technique. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts were also determined. Phytochemical analysis of the hot and cold extracts revealed presence of flavonoids, glycosides, phenols, saponins, anthraquinones and tannis.
Results: Results obtained revealed that the cold extracts were more effective than the hot extracts. Cold extracts of *Jatropha tanjorensis* leaf has antibacterial activity against *E. coli* and *S. aureus*. Hot extracts of *Jatropha tanjorensis* stem has very little antibacterial activity against *S. marcescens* at

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low concentrations with no MBC. Cold extract of *Jatropha tanjorensis* leaf was bactericidal for *S. aureus* at 250 mg/ml. *Salmonella* sp., *E. coli*, *Pseudomonas* sp., *S. pyogenes*, *Serratia marcescens* and *Serratia marcescens* were resistant to HE of *Jatropha tanjorensis* leaf and stem respectively.
Conclusion: This study shows that there are phytochemicals present in *Jatropha tanjorensis*. The plant shows antibacterial properties against some bacteria, namely: *Serratia marcescens*, *Staphylococcus aureus, Streptococcus pyogenes* and *Escherichia coli*, indicating that they can be used as antimicrobials.

Keywords: traditional medicine; antibacterial; phytochemical analysis; glycosides; cold extract.

1. INTRODUCTION

"Plants extracts have been used in folk medicinal practices for the treatment of different types of ailments since antiquity. In the last century, herbalism became popularized throughout the world. Despite the great advances achieved in modern medicine, plants still make an invaluable contribution to health care. This is attributed to the recognition of the value of traditional medicinal systems" [1]. "Traditional medicine is the sum total of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve, or treat physical and mental illnesses. This practice that has been adopted by other populations (other than its indigenous culture) is often called complementary or alternative medicine. African traditional medicine is the oldest, and perhaps the most assorted, of all therapeutic systems. Africa is considered to be the cradle of mankind with a rich biological and cultural diversity marked by regional differences in healing practices" [2]. "African traditional medicine in its varied forms is holistic involving both the body and the mind. The traditional healer typically diagnoses and treats the psychological basis of an illness before prescribing medicines, particularly medicinal plants to treat the symptoms" [3] "The concept of traditional medicine has recently become so highly and formerly distinguished that a Presidential Initiative Committee on the Development, Promotion, and Commercialisation of Nigerian Herbal Medicinal Products was inaugurated on 30th May 2006" [4].

"A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. This description makes it possible to distinguish between medicinal plants whose therapeutic properties and constituents have been established scientifically, and plants that are regarded as medicinal but which have not yet been subjected to a thorough scientific study. A number of plants have been used in traditional medicine for many years. Some do seem to work although there may not be sufficient scientific data (double-blind trials, for example) to confirm their efficacy. Such plants should qualify as medicinal plants" [4].

"Medicinal plants have been the basis of treatment of various diseases in African traditional medicine as well as other forms of treatment from diverse cultures of the world. About 80% of the world's population still depends solely on traditional or herbal medicine for treatment of diseases, mostly in Africa and other developing nations. Most of the potent medicinal plants have relatively no toxic or adverse effects when used by humans, while some are very toxic to both humans and animals with the potential of damaging certain organs in the body" [5]. "Plants typically contain mixtures of different phytochemicals, also known as secondary metabolites that may act individually, additively, or in synergy to improve health. Indeed, medicinal plants, unlike pharmacological drugs, commonly have several chemicals working together catalytically and synergistically to produce a combined effect that surpasses the total activity of the individual constituents. The combined actions of these substances tend to increase the activity of the main medicinal constituent by speeding up or slowing down its assimilation in the body. Secondary metabolites from plant's origins might increase the stability of the active compound(s) or phytochemicals, minimize the rate of undesired adverse side effects, and have an additive, potentiating, or antagonistic effect" [4] "A single plant may, for example, contain bitter substances that stimulate digestion and possess anti-inflammatory compounds that reduce swellings and pain, phenolic compounds that can act as an antioxidant and venotonics, antibacterial and antifungal tannins that act as natural antibiotics, diuretic substances that enhance the elimination

of waste products and toxins, and alkaloids that enhance mood and give a sense of well-being" [5].

New species of plants with medicinal properties are constantly being brought to light. Such plants have limited data and research that confirm their potential. One of such plants is *Jatropha tanjorensis*. The genus Jatropha that belongs to tribe Joannesieae in the Euphorbiaceae family contains approximately 170 known species. The name Jatropha is derived from the Greek word ''jatros'' meaning 'doctor' and ''trophe'' meaning 'food', which indicates its medicinal uses [6]. "Jatropha *tanjorensis* Ellis & Saroja is a common weed of field crops. The leaf is a commonly consumed vegetable in many parts of Southern Nigeria. It is commonly called 'hospital too far', catholic vegetable, 'Iyana-Ipaja' or 'lapalapa'. It is also popular as a natural remedy against diabetes in this region. Though it has been in existence for many years, research to support its medicinal properties is not much" [7].

2. METHODOLOGY

2.1 Collection of Samples and Extraction (Cold Extract) (Ce)

Jatropha tanjorensis fresh leaves and stem were collected from Gilead-Balm farm in Kpaduma village, Guzape, Abuja. The samples were properly cleaned with clear water, then dried outside in the sun before being individually homogenized. *Jatropha tanjorensis* homogenized leaves weighing 30g and stem weighing 10g were each combined with 100ml of methanol and left to stand for 48 hours. After standing for 48 hours, each preparation was given a shake every 30 minutes for 6 hours. With No. 1 Wattman filter paper, each preparation was filtered. To obtain the dried extract, they were each put into a separate beaker and submerged in water for 48 hours at 37°C.

2.2 (Hot Extract) Alcohol Soxhlet Extraction Method (He)

Jatropha tanjorensis stem and homogenized leaves were placed separately in thimbles. A flask with a flat bottom was filled with 100ml of methanol and attached to a soxhlet extractor. A condenser attached to the soxhlet extractor heated this setup. The extracts went through an inflow and efflux reaction as it heated. Up to four cycles of this method were required to fully remove the contents of the leaves. The methanol extracts, along with the stem mixture and each leaf, were all collected in the same manner.

Tests for the extracts' sterility: Testing was done on the extracts to look for contaminant growth. Each extract was inoculated aseptically into a loopful of Nutrient Agar, which was then incubated at 37°C for 24 hours. We looked for any evidence of visible development on the plates. The absence of growth on the plates demonstrated the sterility of the extracts.

2.3 Dilution of the Extracts

Six test tubes were set up to make the dilutions. 2ml of peptone water was added to the *Jatropha tanjorensis* leaf extract in tube 1 to get a dilution of 450mg/ml. 1ml of peptone water was added into tubes 2 to 6. 1ml from tube 1 was pipette and transferred to tube 2. This same process was repeated to tube 6. 1ml from tube 6 was pipette and discarded. This same set of procedure was repeated for all other extracts of *Jatropha tanjorensis* to get their various dilutions. Doubling dilutions of the extracts were made for *Jatropha tanjorensis* leaf CEs in 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.63 mg/ml and 7.81 mg/ml; **f**or *Jatropha tanjorensis* leaf and stem HEs in 500mg/ml, 250mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml and 15.63 mg/ml.

2.4 Collection of Bacterial Isolates

Clinical bacterial isolates were obtained from the microbiology laboratory of Veritas University, Bwari. These organisms include; *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Serratia marcescens, Salmonella* and *Pseudomonas* species. Each of these organisms was subcultured on a sterile plate of Chocolate agar, Nutrient agar, Blood agar, MacConkey agar and Salmonella-Shigella agar according to the type of the organism.

2.5 Inoculum Size Standardization

All bacterial isolates to be used for were prepared using McFarland standard. This was done by inoculating the organism in a preparation of 0.05ml of 1.175% Barium Chloride and 9.95ml of 1% Sulphuric acid.

2.6 Evaluation of Antibacterial Activity using Agar Well Diffusion Method

A Mueller Hinton Agar plate was prepared according to the manufacturer's direction and poured in 20m1 amount into petri dish. A loopful of the test organism from the tube containing 102cfu/ml concentration of each of the test isolates were spread on the surface of the 20m Mueller Hinton Agar plate with a sterile glass

spreader. These were allowed for 30 minutes to pre-diffuse and a sterile Number 4 cork borer was used to bore hole of 8mm diameter on each of the agar plates containing the isolates. A volume of 0.02ml (20u1) of each of the different extract was used to fill the agar wells made in the Mueller Hinton agar plates and Ciprofloxacin was used as control. The plates were allowed to stand for 1 hour to allow the drug to pre-diffuse into the agar and were incubated at 37°C for 24 hours. After incubation, the zones of inhibition were recorded as resistant or susceptible using the criteria for viable antimicrobial activity for plant extracts. When D1≥ 8mm it is taken to be susceptible, while D1≤ 8mm is taken as resistance. Minimum inhibitory concentration of the extracts was determined only on extracts exhibiting apparent zones of Inhibition against test organism greater than D1≥ 8mm.

2.7 Determination of Minimum Inhibitory Concentration (Mic In Mg/Ml) Using The Tube Dilution Method

The MIC was determined for the methanol leaf and stem extracts.

2.8 Dilution of the Extracts

The original concentration of the *Jatropha tanjorensis* leaf extract was 500mg/ml, 1 in 2 dilution of the extract was made to get an extract concentration of 250mg/ml. Six test tubes were set up in a row for each of the extracts. 1ml sterile Nutrient broth was pipette into the tubes. 1 ml of *Jatropha tanjorensis* leaf extract with concentration of 250mg/ml was pipette into all 6 tubes. A doubling dilution was prepared from tube 1 up to tube 6 in 1mI amount to get the following concentrations 250mg/ml, 125mg/mi, 62.5mgIml, 31 .25mg/mI,15.63mg/mi and 7.8mg/ml concentrations respectively. A loopful of isolate I was pipette into tubes 1 to 6. The same procedure was repeated for the remaining isolates. The tubes were incubated at 37°C for 18-24hrs. It was observed for turbidity or cloudiness which indicates a positive result. The lowest concentration showing no turbidity is the MIC of the extract. These procedures were repeated for stem extract.

2.9 Determination of the Minimum Bactericidal Concentration (Mbc)

Minimum bactericidal Concentration is the lowest concentration of the antimicrobial required to produce a sterile culture. The MBC was determined by subculturing 0.01ml of the highest concentration of the extract which showed visible growth and all the tubes showing no visible signs of growth in the MIC tube dilution test, to fresh extract-free media, like Chocolate agar and Nutrient agar. The plates were then incubated at 37oC for 18-24hrs.

2.10 Screening for Qualitative Phyto-chemical Properties

2.10.1 Test for saponins

10ml of distilled water was added to 2m1 each of the extracts in a test tube. The mixture was shaking vigorously. It was examined for persistent frothing even after heating which indicates the presence of saponins .

2.10.2 Test for anthroquinone

To about 2m1 each of the extracts, 5m1 of 10% ammonia was added and mixed by shaking vigorously. It was observed for a color change which is an indication of a positive test (the color change is from its original color to another color). No color change is an indication of a negative test [1].

2.10.3 Test for phenol

10ml of each of the extracts was mixed with 8m1 of distilled water in a test tube. 6m1 of ferric chloride solution was added to the mixture. A color change to light brown was checked for, which is an indication of a positive test.

2.10.4 Test for tannins

To 1ml each of the extracts 1% ferric chloride was added. It was checked for a color change which indicates positive test

2.10.5 Test for phylobatanning

To 2m1 each of the extracts, 1% aqueous hydrochloric acid was added and boiled. It was observed for the presence of white precipitate which indicates positive test.

2.10.6 Test for alkaloids

Each extract of 0.5 g was dissolved in 3 drops of Dragendoffs reagent. An orange precipitate indicates the presence of Alkaloid.

2.10.7 Test for flavonoids

Each extract of 0.2 g was dissolved in 2 ml of sodium hydroxide solution. The occurrence of a yellow solution which disappears on addition of HCl acid indicates the presence of flavonoids [1].

2.10.8 Test for glycosides

Legal's test: To a small portion of the extracts, sodium nitropruside in pyridine and sodium hydroxide was added and observed.

3. RESULTS

Table 1 shows the phytochemical constituents of *Jatropha tanjorensis* (Hospital too far). Hot extracts of the leaf contain flavonoids, phenols and glycosides with absence of alkaloids, saponins, anthraquinones, phylobataning and tannins. Cold extracts of the leaf contain saponins, anthrequinones, phenols, tannins and glycosides with absence of flavonoids, phylobataning and alkaloids. Hot extracts of the stem contain flavonoids, phenols, alkanoids and glycosides with absence of saponins, anthraquinones, phylobataning and tannins.

Table 2 shows antibacterial activity of cold extract of *Jatropha tanjorensis* (Hospital too far) leaf against *Escherichia coli* and *Staphylococcus aureus*. *Escherichia coli* was sensitive at concentrations 250 mg/ml and 125 mg/ml with 18mm and 16 mm zones of inhibition respectively. *Staphylococcus aureus* was sensitive at concentrations 250 mg/ml, 125 mg/ml and 62.6 mg/ml with 28 mm, 20 mm and 18 mm zones of inhibition respectively.

Table 3 shows antibacterial activity of hot extract of *Jatropha tanjorensis* (Hospital too far) stem against *Serratia marcescens*. *Serratia marcescens* shows sensitivity at concentrations 62.5 mg/ml and 31.25 mg/ml with 15 mm and 12 mm zones of inhibition.

Table 4 shows Minimum Inhibitory Concentration (MIC) for cold extract of *Jatropha tanjorensis* (Hospital too far) leaf for *Staphylococcus aureus* and *Escherichia coli*. *Staphylococcus aureus* is inhibited at concentrations 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25mg/ml, making 31.25 mg/ml the MIC. *Escherichia coli* is inhibited at concentrations 250mg/ml and 125 mg/ml, making 125 mg/ml the MIC.

Table 1. Phytochemical composition of *Jatropha tanjorensis*

Phytochemical components	HE (leaf)	CE (leaf)	HE (stem)
Flavonoids	+	-	+
Saponins	-	+	-
Anthraquinone	-	+	-
Phenol	+	+	+
Phylobataning	-	-	-
Alkaloids	-	-	+
Tannins	-	+	-
Glycosides	+	+	+

CE= Cold Extract
HE= Hot Extract
+ = positive
- = negative

Table 2. Antibacterial activities of cold extracts of *Jatropha tanjorensis* leaf

Isolates	Mean zone	Diameter of	Inhibition	(mm)		
E. coli	18	16	0	0	0	0
S. aureus	28	20	18	0	0	0
Extract conc. in mg/ml	250	125	62.5	31.25	15.63	7.81

Table 3. Antibacterial activities of hot extract of *Jatropha tanjorensis* stem

Isolate	Mean zone	Diameter of	Inhibition	(mm)		
S. marcescens	0	0	0	15	12	0
Extract conc. in mg/ml	500	250	125	62.5	31.25	15.63

Table 4. Minimum inhibitory concentrations (MIC) for cold extracts of *Jatropha tanjorensis* leaf

	Extract conc.	in mg/ml				
Isolates	250	125	62.5	31.25	15.63	7.81
S. aureus	++	+	+	+	-	-
E. coli	+	+	-	-	-	-

Table 5. Minimum inhibitory concentrations (MIC) for Hot Extract of *Jatropha tanjorensis* stem

	Extract conc. in mg/ml					
Isolates	500	250	125	62.5	31.25	15.63
S. marcescens	-	-	-	-	-	-

Table 6. Minimum bactericidal concentration (MBC) for cold extracts of *Jatropha tanjorensis* leaf against *Staphylococcus aureus*

	Extract conc. in mg/ml					
Isolates	250	125	62.5	31.25	15.63	7.81
S. aureus	+	-	-	-	-	-

Table 4 shows Minimum Inhibitory Concentrations (MIC) for hot extract of *Ipomoea batatas L*. (OFSP) bark for *Serratia marcescens*. *Serratia marcescens* is inhibited at concentration 500 mg/ml, making 500 mg/ml the MIC.

Table 5 shows Minimum Inhibitory Concentration (MIC) for hot extract of *Jatropha tanjorensis* (Hospital too far) stem for *Serratia marcescens*. *Serratia marcescens* shows no inhibition.

Table 6 shows Minimum Bactericidal Concentration (MBC) for cold extract of *Jatropha tanjorensis* (Hospital too far) leaf for *Staphylococcus aureus*. *Staphylococcus aureus* is killed at concentration 250 mg/ml, making 250 mg/ml the MBC.

4. DISCUSSION

Results obtained in this present study reveal that cold extracts of *Jatropha tanjorensis* contain saponins, phenols, glycosides, anthroquinone and tannins. The hot extracts of *Jatropha tanjorensis* contain flavonoids, phenols and glycosides. Results here are similar to those reported by [8,9] and [6], which found tannins, saponins, flavonoids and alkaloids. This study however does not report the presence of alkaloids.

Results obtained in this present study reveal that cold extracts of *Jatropha tanjorensis* leaf have antimicrobial activity against *E. coli* and *S. aureus* with the lowest activity at 16 mm for *E. coli* and the highest at 28mm for *S. aureus*. This result is in line with what was reported by [8] where the extract had the highest antibacterial activity of 13mm and 15mm and lowest antibacterial activity of 8mm and 12 mm for *Staphylococcus aureus* and *Escherichia coli* respectively. Unlike that report however, *Pseudomonas* sp showed no zone of inhibition for the cold extract in this work [8] reported highest antibacterial activity of 14mm and lowest antibacterial activity of 7mm for *Pseudomonas* sp. Cold extract of *Jatropha tanjorensis* leaf in this study was bactericidal for *S. aureus* at concentration 250 mg/ml. Hot extracts of *Jatropha tanjorensis* stem have very little antimicrobial activity against *S. marcescens* at lower concentrations of 62.5 mg/ml and 31.25 mg/ml with no MBC. No previous studies on the stem of *Jatropha tanjorernsis* and on antibacterial properties against *S. marcescens* particularly have been seen.

5. CONCLUSION

This study shows that there are phytochemicals present in *Jatropha tanjorensis*. The plant shows antibacterial properties against some bacteria, namely: *Serratia marcescens*, *Staphylococcus aureus, Streptococcus pyogenes* and *Escherichia coli*, indicating that they can be used as antimicrobials.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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