Phytochemical Analysis and Antibacterial Efficacy of *Jatropha Tanjorensis*

Abstract

The use of plants in traditional medicine has been in existence almost as long as man. The practice started in Africa a long time ago before the discovery of even chemotherapeutics. With time, the practice spread to Asian countries and other parts of the world. Over the past century it has gotten more attention from places like Europe and the US and has been employed more extensively in curing various ailments. In light of the manifestation of the power of traditional herbs, this study aimed at evaluating the Phytochemical and antibacterial properties of *Jatropha tanjorensis* against selected test bacteria. 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 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 low concentrations with no MBC. Cold extract of *Jatropha tanjorensis* leaf was bactericidal for *S. aureus* at 250mg/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.
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.

Key words: traditional medicine, antibacterial, Phytochemical analysis,glycosides, cold extract

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 (Unegbu *et al., 2020)*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 (Mahomoodally, 2013) 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(Gurib and Mahomoodally, 2013) 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 (Sofowora *et al., 2013).*

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 (Sofowora et al., 2013).

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 .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. 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(Nun Koo and Mahomoodally, 2012)

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 (Sabandar *et al., 2013).* 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 (Idu *et al., 2014).*

Materials And Methods

Sample Collection And Extraction (Cold Extract (Ce)

Fresh leaves and stem of *Jatropha tanjorensis* were harvested from Gilead-Balm farm in Kpaduma village, Guzape, Abuja. The samples were washed thoroughly with clean water, dried under the sun and homogenized separately with an electric blender. 30g of homogenize leaves and 10g of stem of *Jatropha tanjorensis* were mixed separately with 100ml of methanol and allowed to stand for 48hours. Each preparation was shaken every 30 minutes for 6 hours and

allowed to stand for 48 hours. Each preparation was filtered with No 1 wattman filter paper. They were placed separately in a beaker and put into a water bath to be evaporated to dryness at37°C for 48 hours to get the dried extract.

Alcohol Soxhlet Extraction Method (Hot Extract (He)

The homogenized leaves and stem of *Jatropha tanjorensis* respectively were put separately into a thimble. 100mI of methanol was poured into a round bottom flask and connected to the soxhlet extractor. This set up was heated with a condenser fixed to the soxhlet extractor. As it heated, the extracts underwent influx and efflux reaction. This procedure was repeated up to 4 times until the content of the leaves had been completely extracted. The methanol extracts, each of the leaves , and stem mixture were collected likewise.

Sterility Tests for The Extracts

The extracts were tested for growth of contaminants. A loopful of each extract was inoculated aseptically unto Nutrient Agar and incubated at 37oC for 24hrs. The plates were observed for any sign of visible growth. No growth on the plates indicated that the extracts were sterile.

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 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.63mg/ml and 7.81mg/ml; **f**or *Jatropha tanjorensis* leaf and stem HEs in 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml and 15.63mg/ml.

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.

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 .

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.

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.

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.

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.

Screening For Qualitative Phytochemical Properties

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 .

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 (Unegbu et al., 2020)

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

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

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

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 .

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 (Unegbu *et al*., 2020).

Test For Glycosides

Legal's test

To a small portion of the extracts, sodium nitropruside in pyridine and sodium hydroxide was added and observed .

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 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 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 250mg/ml and 125mg/ml with 18mm and 16mm zones of inhibition respectively. *Staphylococcus aureus* was sensitive at concentrations 250mg/ml, 125mg/ml and 62.6mg/ml with 28mm, 20mm and 18mm zones of inhibition respectively.

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

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 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 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml, making 31.25mg/ml the MIC. *Escherichia coli* is inhibited at concentrations 250mg/ml and 125mg/ml, making 125mg/ml the MIC.

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 4. shows Minimum Inhibitory Concentrations (MIC) for hot extract of *Ipomoea batatas L.* (OFSP) bark for *Serratia marcescens*. *Serratia marcescens* is inhibited at concentration 500mg/ml, making 500mg/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 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 shows Minimum Bactericidal Concentration (MBC) for cold extract of *Jatropha tanjorensis* (Hospital too far) leaf for *Staphylococcus aureus*. *Staphylococcus aureus* is killed at concentration 250mg/ml, making 250mg/ml the MBC.

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	+	-	-	-	-	-

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 (Arun *et al.*, 2012), (Nwachukwu, 2018) and (Sabandar *et al* ., 2013), 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 16mm for *E. coli* and the highest at 28mm for *S. aureus*. This result is in line with what was reported by (Arun *et al* ., 2012) where the extract had the highest antibacterial activity of 13mm and 15mm and lowest antibacterial activity of 8mm and 12mm 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 250mg/ml. Hot extracts of *Jatropha tanjorensis* stem have very little antimicrobial activity against *S. marcescens* at lower concentrations of 62.5mg/ml and 31.25mg/ml with no MBC. No previous studies on the stem of *Jatropha tanjorernsis* and on antibacterial properties against *S. marcescens* particularly have been seen.

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.

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