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Antimicrobial effect of *Jatropha tanjorensis* leaves on multi antibiotic resistant bacterial isolated from poultry droppings

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Abstract

The widespread use of antibiotics as prophylactics and therapeutic agents has increased the risk of emergence and spread of resistant bacteria leading to prolonged and expensive treatment and greater risk of death in humans. The aim of this study was to determine the antimicrobial effect of the methanol extract of *Jatropha tanjorensis* leaves on multi antibiotic resistant bacteria isolated from poultry droppings. Multi antibiotic resistant bacterial isolates from poultry dropping already identified from previous research were used. The plant extract was obtained by soxhlet extraction process, susceptibility test was caried out using well in agar diffusion method. The percentage yield of the plant extract using soxhlet extraction process with methanol was 22.84%. The qualitative phytochemical analysis revealed the presence of bioactive compounds such as tannins, flavonoids, cardenolide, steroids, terpenoids, anthraquinone, alkaloids, fixed oil and carbohydrate. The extract showed competitive zones of inhibition on *Escherichia fergusonii, Stenotrophomonas maltophilia, Enterobacter clocae, Lysinibacillus sphaericus* and *Salmonella typhimerium*. *Enterobacter clocae* had the highest zone of inhibition of 17mm at a concentration of 100mg/ml, 10mm at 80mg/ml and 8mm at 50 mg/ml. *Stenotrophomonas maltophilia* had the lowest zone of inhibition of 10mm at 100mg/ml, 8mm at 80mg/l and 7mm at 50mg/ml. These zones of inhibitions show the antimicrobial potency of *Jatropha tajorensis* on antibiotic resistant bacteria. Compared with synthetic antibiotics or inorganic chemicals, this plant-derived extract is natural, less toxic, typically residue free with high rate of metabolism. The result of this study gives credence to claims of the medicinal and nutritional properties of *Jatropha tanjorensis.*

Keywords: Antimicrobial; *Jatropha tanjorensis*; Antibiotic; Multi antibiotic resistant bacterial

1. Introduction

Antibiotics are without doubt miracle medications, they provide the main basis for the therapy of microbial (bacterial and fungal) infections. For decades, they have fought many infectious diseases and saved millions of lives. However, the recent failure of antibiotics due to the rapid spread of new infections and the dramatic emergence of multidrug resistant pathogens has prompted health organizations and pharmaceutical industries around the world to begin screening plants for the development of new pharmaceuticals to address both old and new health problems [1]. The use of antibiotic as prophylaxis and as growth promoters in animal production has also contributed to the burden of antibiotic resistant bacterial [2].The rapid rise of microbial resistance to traditional antibiotics has caused major concern in the treatment of infectious diseases. Many studies have recently been conducted in order to uncover possible answers to these issues. Phytochemicals have been shown to exhibit antibacterial activity against sensitive and resistant infections through a variety of ways [3].The plant kingdom offers a wide range of natural phytobiotics and medicinal plants. Medicinal plants, according to the World Health Organization (WHO), are the best source of a wide range of medications. Many plants have been employed for their antibacterial properties, which are related to phytochemicals produced in the plant's secondary metabolism. Secondary metabolites present in plants include tannins, alkaloids, phenolic

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chemicals, and flavonoids, which have been shown to have antibacterial activities in vitro. Various medicinal plants have been mentioned in a number of phytotherapy books for treating infectious diseases such as urinary tract infections, gastrointestinal disorders, respiratory disease, and skin infections [4]. A number of studies have revealed the antioxidant activities of phytochemical constituents of medicinal plants (e.g. polyphenols, carotenoids, phenolics and vitamins C and E). These phytochemicals, which function as antioxidants, protect cell membranes and cellular oxidative processes from damage that can lead to disease. Natural polyphenols from plant vegetables, for example, have been proven to have anti-oxidant properties by eliminating free radicals, chelating metal catalysts, and activating antioxidant enzymes. Plant-based antioxidants have gotten a lot of attention recently, and they're even favoured over synthetic antioxidants because of their possible health advantages, availability, cost, and, in many cases, lower toxicity [5]. *Jatropha tanjorensis* commonly referred to as Catholic vegetable, Iyana-Ipaja (Yoruba), Ugu-Oyibo (Igbo), hospital too far (Pidgin English) is a vegetable plant widely grown in southern and mid-western states of Nigeria and used primarily for fencing while its secondary uses are as source of edible leafy vegetable and medicine. This vegetable plant is believed to be an excellent blood building herb, a super antioxidant, beneficial in the treatment of diabetes mellitus (due to its hypoglycemic action), malaria and hypertension in some parts of Nigeria—though there is a dearth in scientific validation of these claims [6]. Different parts of Jatropha plants are used in many ways and in different countries. Nutritionally, the leaves of *J. tanjorensis* are locally consumed as vegetable. The leaves also serve medicinal purposes as they are used for the treatment of fevers, cabuncles, eczema, itches, sores on the tongues of babies, stomach ache and venereal diseases. In the southern parts of Nigeria, the leaves of *J. tanjorensis* are used for the treatment of diabetes mellitus. It is also popular as a natural remedy against malarial infection and hypertension in some parts of Nigeria. It grows on all types of soils and barren land. Jatropha is a versatile plant owing to its excellent regeneration capability and long, productive life. According to Omobuwajo et al*.,* [7], the leaves contain the antioxidant vitamins (vitamins C and E).

The aim of this study is to investigate the antimicrobial potential of the methanol extract of *J. tanjorensis* leaves on multiantibiotic resistant bacteria isolates from poultry droppings.

2. Material and methods

The leaves of *Jatropha tanjorensis* was collected from Rumualogu Road, Choba, Port Harcourt, Rivers State, Nigeria and identified in the Herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt. The plant leaves were then air dried and pulverized at the Pharmacognosy Laboratory of the Department of Pharmacy, University of Port Harcourt.

2.1. Extraction and Concentration

A quantity (287 g) of the dried and pulverized leaves of *Jatropha tanjorensis* was extracted by using soxhlet extraction process with 98% methanol for 48 hours. The methanol in the extract was recovered leaving behind the raw pure extract of *Jatropha tanjorensis* by using a rotary evaporator at 40ºC. The pure raw extract was then dried to paste form by using water bath and a desicator. The desired concentration of 100mg/ml, 80mg/ml and 50mg/ml were then prepared.

2.2. Qualitative Phytochemical Analyses

The qualitative phytochemical analyses of the leaves of *Jatropha tanjorensis* were carried out using the methods of Daniyan et al., [8].

2.3. Test for alkaloids

0.2 g quantity of the extract was mixed with 10ml 2% HCl, heated for 5 minutes then filtered. To 1ml filtrate was added 1 ml of Wagner‘s reagent. A creamy white precipitate indicated the presence of alkaloids.

2.4. Test for reducing sugar

0.2 g quantity of the extract was dissolved in 10 ml of distilled water and boiled for 10 minutes before filtering. To 1 ml of the filtrate, 200µl of Fehling‘s Solution B was added and then heated for 5 minutes. A brick red precipitate indicated the presence of reducing sugar.

2.5. Test for steroids

To 0.2 g of methanol extract was added 2 ml of acetic anhydride. To the solution was subsequently added 2 ml of concentrated H2SO4 carefully. A colour change from violet to green or bluish green indicated the presence of steroids.

2.6. Test for Flavonoids

Sodium hydroxide test: 5g of the sample was weighed and detanned completely with acetone. The mixture was warmed on water bath to evaporate the acetone. The residue was then extracted with water on a water bath. The mixture was filtered and the filtrate was used for the test. 5ml of 10% sodium hydroxide was added to an equal volume of the detanned water extract. A yellow solution indicates the presence of Flavonoids.

2.7. Test for Terpenoid

0.2g extract was mixed with 2ml Chloroform and 3ml of concentrated sulphuric acid was added carefully to form a layer. A reddish brown coloration of the interface formed indicates the presence of terpenoids.

2.8. Test for Tannins

2g of the sample was boiled in 50 ml distilled water for 30 minutes on a hot plate. The mixture was then filtered and a portion of the filtrate was diluted with sterile water in a ratio of 1:4 and 3 drops of 10% ferric chloride solution was added. A blue or green color indicates the presence of tannins.

2.9. Test for Anthraquinones

0.5g of the plant extract was shaken with 10 ml of aqueous sulphuric acid and then filtered while hot, the filtrate was then shaken with 5 ml of benzene, the benzene layer separates and half its own volume of 10% ammonia solution was added. A violet or red coloration in the ammonical (lower) phase indicates the presence of combined Anthraquinones [9].

2.10. Test for Phenol

25 ml of extract was added to 2 ml of ferric chloride solution, formation of deep bluish green solution indicates the presence of phenols.

2.11. Test for Glycosides

25 ml of 1% Sulphuric acid was added to 5ml of the extract in a test tube and boiled for 15minutes, cool and neutralize with 10% sodium hydroxide, and then 5ml of Fehling's solution **A** and **B** was added. A brick red precipitate of reducing sugars indicates the presence of Glycosides.

2.12. Carbohydrates

A quantity of extract (0.2 g) was macerated with 5 ml of water and heated for 10 minutes. Thereafter, it was centrifuged at 4000 rpm for 5 minutes. The supernatant (0.5 ml) was added 0.5 ml of phenol reagent, mixed and subsequently added 2 ml of concentrated H2SO4. It was then shaken carefully and allowed to stand for 20 minutes in a cold water bath. The absorbance of the resulting mixture was read spectrophotometrically at 490 nm.

2.13. Test organisms and maintenance

The microorganisms (*Escherichia fergusonii, Stenotrophomonas maltophilia, Enterobacter clocae, Lysinibacillus sphaericus* and *Salmonella typhimerium*) employed in this study were some clinical Gram negative bacteria maintained on Nutrient agar slants and stored in the refrigerator at 4^0C.

2.14. Bacterial Sub Culture

Nutrient agar media was prepared and the bacteria isolates were aseptically introduced unto the surface of the agar using a platinum wire loop. The plates were incubated at 37^0C for 18-24 hours to check for the organism's viability and formation of distinct colonies. This process was repeated in duplicate for three times in order to get pure distinct colonies.

2.15. Well in Agar Diffusion Technique (Sensitivity)

A standard concentration of each of the extracts (100mg/ml, 80mg/ml and 50mg/ml) was prepared for the preliminary antimicrobial testing for invitro well in agar diffusion technique. A loopful of each bacterial isolate was transferred aseptically from the stock slants onto a nutrient broth and incubated at 35^0C for 18-24 hours. A little quantity of the nutrient broth was dispensed into a sterile test tube and was diluted with sterile water until it became clear as the McFarland standard solution. Using a sterile swab stick, the microorganism in each test tube was transferred unto Mueller Hinton Agar (MHA) plates. The broth culture was spread on the surface of the dried Mueller Hinton agar (MHA) plates for homogeneity. Using a sterilized 5mm cork borer, three distinct holes were bored aseptically on the surface of the agar and labeled as 100mg/ml, 80mg/ml and 50mg/ml. 0.1ml of each concentration of the extract was carefully dispensed into the wells. Equal volumes of Tween eighty and crude aqueous extract of *J. tanjorensis* served as controls.

All plates were kept at low temperature for 5-10minutes to allow for diffusion of extracts into the agar ahead of growth of the test organisms after which the plates were incubated for 18-24 hours at 35^0C. The plates were then examined for presence or absence of zone of inhibition and measured.

3. Results

The phytochemical screening of the plant extract showed the presence of phytochemicals such as flavonoids, alkaloids, tannins, terpenoids, carbohydrates, anthraquinones, steroids, fixed oils and cardenolide as shown in Table 1 below:

Table 1 Phytochemical constituents of the methanol extract of *Jatropha tanjorensis* leaves

Biochemical Availability			**Result**
1	Alkaloid	Drangedorff's Test	+ve
		Mayer's Test	+ve
		Hager's Test	-ve
2	Flavonoids	Shinoda Test	+ve
		Leadacctate Test	ND
		A1C13 Test	+ve
3	Tannins	FeC13 Test	+ve
		Phlobatannins	+ve
		Gelatin Test	ND
		Albumin Test	ND
4	Anthraquinone	Free Anthraquinone	-ve
		Combined Anthraquinone	+ve
5	Triterpenoid/Steroid	Leibermann-Buchard Test	+ve
		Salwoski Test	+ve
6	Fixed oils		+ve
7	Carbohydrate	Molisch Test	+ve
		Fehling's Test	+ve
8	Cyanogenic glycosides		-ve
9	Saponins	Frothing Test	-ve
		Haemolysis Test	ND
		Emulsion Test	-ve
10.	Cardenolide	Keller Killuni Test	+ve
		Kedde Test	-ve

Key: -ve = Negative, +ve = Positive, ND = Not determined

The antimicrobial effect of the methanol extract of *Jatropha tanjorensis leaves* on *Escherichia fergusonii, Stenotrophomonas maltophilia*, *Enterobacter clocae*, *Lysinibacillus sphaericus* and *Salmonella typhimerium* was investigated using well in agar diffusion technique. The respective zones of inhibition obtained are shown in table 2:

Table 2 Well in agar zones of inhibition exerted by the methanol extract of *Jatropha tanjorensis* leaves on bacteria isolates from poultry droppings

	Various Concentration of Methanol Extract/Zones of Inhibition		
Organism	**100mg/ml (mm)**	**80mg/ml (mm)**	**50mg/ml (mm)**
Escherichia fergusonii	11	9	6.5
Stenotrophomonas maltophilia	10	8	7
Enterobacter clocae	17	10	8
Lysinibacillus sphaericus	11	10	7
Salmonella typhimerium	12	10	7

4. Conclusion

The leaves of *Jatropha tanjorensis* possess antimicrobial properties. The antimicrobial potency of the methanol extract of *J. tanjorensis* is leaves is attributed to the innate presence of bioactive compounds which includes tannins, flavonoids, alkaloids, anthraquinone, terpenoid, fixed oil, carbohydrate and cardenolide as succinctly reported by Tarawneh *et al.*, [5]. The methanol extract of *J. tanjorensis* showed different levels of potency against all the tested multi drug resistant bacteria isolates from poultry droppings. The extract showed a greater antimicrobial potency against *Enterobacter clocae* with a zone of inhibition of 17mm at a concentration of 100mg/ml, 10mm at 80mg/ml and 8mm at 50 mg/ml. It was also potent against *Escherichia fergusonii, Stenotrophomonas maltophilia, Lysinibacillus sphaericus* and *Salmonella typhimerium* and may help in the cure of diseases caused by these organisms. *Escherichia fergusonii* for instance causes urinary tract infection and other infectious disorders of open wounds. Thus, the methanol extract of *J. tanjorensis* leaf may be used as agent for the treatment of these diseases. *Salmonella typhimerium* can also be controlled by the methanol extract of *J. tanjorensis*. The percentage yield of 22.84% shows that methanol is a good solvent for extraction of important secondary metabolites from plants. This is a higher value than that reported by Madubuike *et al*.,[10] who extracted with 80% methanol and 20% distilled water and obtained a percentage yield of 8.45%. This difference could be as a result of the slight difference in the solvent used for extraction [8]. Apart from their roles in human health, phytochemicals also play important roles in plant survival. They give plants their colour, flavour, aroma and are parts of a plant's natural defence system protecting them from environmental hazards such as stress, UV exposure and pathogenic attacks. The presence of these phytochemicals as shown in this study could be responsible for the lush green colour of the plant and its ability to resist insect pests. The presence of alkaloids in the extract might be responsible for the plant's use in the treatment of malarial infection and hypertension as reported by Daniyan et al., [8]. Alkaloids have many pharmacological properties including antihypertensive, antimalarial and antiarrhythmic effects. The use of the leaves of *Jatropha Tanjorensis* to treat veneral diseases could be due to the presence of tannins as obtained in the leaf extract in this study. Tannins have been reported to possess antimicrobial activities as they inhibit the growth of many viruses, bacteria, fungi and yeast [6]. Though from reviewed literatures, there is no account for the presence of cardenolide and fixed oils as detected in this study, this variation could be attributed to differences in extraction solvents and techniques and also the environment where the plant was obtained. Cardenolide constitute a class of cardiac glycosides used for the treatment of heart failure and abnormal heart rhythms. This buttresses the claim that consumption of *Jatropha Tanjorensis* leaves as vegetable beefs blood volume**.** [11]. The presence of Fixed oils— a non-volatile compound which is chemically a glyceride of fatty acids, self purgative and nutritive is a further appraisal of the bioactive constituents of *Jatropha tanjorensis* as a suitable alternative for the production of natural antimicrobials. The result of this study has further given credence to the various claims of the medicinal and nutritional properties of this plant which can be exploited in the production of pharmaceutically important antimicrobial drug alternatives.

Compliance with ethical standards

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Disclosure of conflict of interest

Ohemu Godwin Pius and Fajoyomi Bridget Uredo-ojo declare that there is no conflict of interest.

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