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Phytochemical content and Anti-microbial Activity of Polar solvents leaf extracts of *Jatropha gossypifolia* L.

Kolapo Solomon Olawale[1]*and Tochukwu Kevin Ugwueze[2]

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[1]Department of General Studies, Federal College of Agriculture, Akure, Nigeria.
[2]Department of Medical Microbiology and Parasitology, University of Lagos, Nigeria.

ABSTRACT

In this study some polar solvents leaf extracts of *Jatropha gossypifolia* were screened for phytochemicals and tested for their antimicrobial activity using the agar well diffusion method against five (5) pathogenic organisms of clinical origin; namely, *Escherichia coli, Candida albicans, Klebsiella pneumoniae, Salmonella typhi* and *Staphylococcus aureus*. Phytochemical screening of the water, 95% ethanolic and 95% methanolic extracts of the leaves showed that the extracts contained Alkaloids, Tannins, Saponin, Glycosides, Flavonoids and Phenols. Antimicrobial activity studies indicated that aqueous extract inhibited only *Salmonella typhi* at a concentration of 200mg/ml while ethanolic and methanolic extract inhibited all isolates except *Klebsiella pneumoniae*. The Minimum Inhibitory Concentration (MIC) of the leaf extracts ranged between 25-100mg/ml while the Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) ranged from 75-150mg/ml. Antibiotic susceptibility test using standard antibiotics indicated multidrug resistance by the test organisms with only Gentamycin, Erythromycin and Ofloxacin eliciting inhibitory activity against the isolates. Hence, leaves of *Jatropha gossypifolia* contain basic pharmacologic compounds that could be exploited for the treatment of infections and a possible source of antimicrobial agents.

Keywords: *Jatropha gossypifolia*, inhibition, antimicrobial, phytochemicals, polar solvents.
Corresponding author Email: Olawalekolapo3649@gmail.com

INTRODUCTION

The earth is full of diverse kinds of plant with potent therapeutic prospects (Anibijuwon and Udeze, 2009). In Nigeria, medicinal plants are widely used by all sections of people both directly as folk medicines in different indigenous systems of medicine and indirectly in the pharmaceutical preparations (Srinivasan et al., 2001). *Jatropha gossypifolia* Linn belongs to the family *Euphorbiaceae* and has several uses in traditional medicine (Agra et al., 2008; Sabandar et al., 2013). It is a tropical species and occurs on all major continents. It has its origin from South America and is native to Brazil. Cultivated in tropical countries globally, the most frequently used parts being the leaves, root, seed, and latex (Rios and Pastore, 2011) which have been used for the treatment of various diseases of humans and animals. Most preparations of this plant that are of significant use are in the form of extraction through boiling (decoction), infusion and maceration and are applied either topically or orally (Di Stasi and Hiruma-Lima, 2002; de Albuquerque et al., 2007). Reports have indicated anti-ophidian, antipyretic, anti-inflammatory, anti-hypertensive, wound healing, anti-diabetic and antimicrobial activities (de Albuquerque et al., 2007; Hemraj and Anil, 2012) for this plant. The plant is also used for other purposes including pesticides, insecticides, biodiesel production, beautification as well as religious rituals (Falodun et al., 2012).

Based on the foregoing pharmacological activities reported for *J. gossypifolia*, this study was aimed at assessing the antibacterial and antifungal properties of the water, methanol and ethanolic extracts of its leaf on carefully selected Gram-negative organisms: *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*, Gram-positive organisms: *Staphylococcus aureus* and *Candida albicans,* a fungus. The selection was based on the notion that the morphological and physiological

characteristics of different parts of the plant can have possible effects on the relative susceptibility or resistance to the different antimicrobial agent.

MATERIALS AND METHODS

Collection of plant materials

Leaves of *J. gossypifolia* were collected and wrapped in clean, sterile polythene bags at Oke-odo area, Tanke road, Ilorin, while identification and authentication of the plant was done at the herbarium, Department of Plant Biology, Faculty of Life Science, University of Ilorin, Ilorin, Kwara State, Nigeria. The leaves were washed clean with distilled water to remove dust and other foreign particles and they were left in open air for two weeks under shade to dry after which the dried leaves were grounded into fine powder and stored in a clean, sterile container.

Collection of Test organisms

The microorganisms used for this study were clinical isolates (*Escherichia coli, Candida albicans, Klebsiella pneumoniae, Salmonella typhi and Staphylococcus aureus*) obtained from the Medical Microbiology Department of the University of Ilorin Teaching Hospital (U.I.T.H) and Wesley Guild Specialist Hospital, Ilesha. All the microorganisms used were maintained on agar slants (double strength) after collection and were then kept as stock cultures refrigerated at 4°C.
Preparation and standardization of test organisms were done by introducing each organism before use into sterile peptone water in Sterile Mac-Cartney bottles. About 3 loop-full was taken from a 24 hours old culture plate in each case. The turbidity was adjusted to 0.5 McFarland standards and then used for the test.

Preparation of Plant Extracts

Hundred grams (100 g) of the finely grounded leaves of *J. gossypifolia* was placed in conical flasks and 500 ml of the respective solvents (hot water, 95% ethanol and 95% methanol) were added. The preparation was then corked, mixed together properly and left on the shaker at 100 revolutions per minute (r.p.m) for 72 hours after which the preparation was then filtered through fine layers of muslin cloth. The filtrate obtained was then centrifuged at 2500 rpm for 5 minutes after which it was decanted. The supernatant was sterilized by using the membrane filtration unit with type HC filters. The filtrate obtained was concentrated to dry weights by evaporating the solvents used for extraction on a rotary evaporator. The extracts were stored in sterile bottles and refrigerated at 4^{o}C until use. A stock concentration of 200 mg/ml was obtained by dissolving 200 mg of extract in 1ml of solvent (distilled water). Dilutions were made to obtain different concentrations (i.e. 150, 100, 75, 50, 25 and 5 mg/ml).

Phytochemical screening of Extracts

Test for Alkaloids

Each extract (0.5g) was stirred with 5mL of 1% HCl on a Hot water bath. The solution obtained was then filtered and one 1mL of the filtrate was treated with a few drops of Mayer's reagent. The turbidity of the extract filtrate on addition of Mayer's reagent was taken as evidence of the presence of alkaloids in the extracts (Prashant et al., 2011).

Test for Saponins

Each extract (0.5g) was mixed with water in test tube. Foaming which persisted on warming was taken as an evidence for the presence of saponins (Prashant et al., 2011).

Test for Tannins

About 5 g of each plant extract was mixed stirred with 10 ml of distilled water. It was filtered and ferric chloride reagent was added to the filtrate. A blue-green, green or blue-black precipitate indicated the presence of tannin (Prashant et al., 2011).

Test for Flavonoids

Aluminium chloride colorimetric method was used to determine the flavonoid content in the leaf extracts. 1 ml of the plant extract was mixed with 3 ml of methanol, 0.2 ml of 10% Aluminium chloride, 0.2 ml of 1M potassium acetate, and 5.6 ml of distilled water. The entire mixture was allowed to stand at room temperature for 30 minutes, while the absorbance was measured at 420 nm, the total flavonoid content in each extract was expressed in terms of standardized quercetin equivalent (mg/g of the extracts) (Prashant et al., 2011)

Test for Phenols

Plant extracts 0.5 ml (from stock) each was added to 10 ml of deionized distilled water and 2.5 mL of 0.2N Folin-Ciocalteu's phenol reagent. The mixture was allowed to stand at room temperature for 5 minutes and then 2 mL of 2% sodium carbonate was added. The absorbance of the solution was measured at 780 nm after 10 minutes (Kolawole et al., 2014).

Test for Glycosides

0.5 ml of each Plant extract was dissolved in 2 mL of chloroform. Sulphuric acid (H_2SO_4) was carefully added to form a lower layer. A reddish brown colour at the

Table 1: Phytochemical Constituents of *J. gossypifolia* leaf extracts.

CONSTITUENTS	AQUEOUS EXTRACT	ETHANOLIC EXTRACT	METHANOLIC EXTRACT
ALKALOID	_	+	+
FLAVONOID	+	+	-
SAPONIN	+	+	+
TANNIN	_	+	+
GLYCOSIDES	_	+	+
PHENOLS	_	+	_

Key: + = Present**;-** = Absent.

interface indicated the presence of a steroidal ring, that is, a glycone portion of the cardiac glycosides (Kolawole et al., 2014).

Antimicrobial assay of the Leaf extracts using Agar Well diffusion method

Overnight culture of the test organisms was transferred into sterile peptone water aseptically and the turbidity was adjusted to 0.5 Mac-Farland standard (10^8 cells/ml) after which it was swabbed on sterile solidified Mueller Hinton Agar (Bacteria) and Potato Dextrose Agar (Fungi) in Petri dishes using sterile cotton swab sticks. A sterile cork borer of size 6 mm in diameter was used to make wells on the plates. About 0.5 ml of the respective leaf extracts were then put into each appropriately labeled well using sterile micropipettes. Control experiments were also carried out where the holes were filled with sterile distilled water. The inoculated plates were left on the table for 1 hour for the extracts to diffuse into the agar. The plates were then incubated at 37° C for 24 hours (Bacteria isolates) and at room temperature for 48 hours (Fungus). After the incubation period, the zone of inhibition was measured. The diameter of the zone of inhibition around each well was measured to the nearest millimeter along two axis of 90° to each other and the mean of the reading was calculated (Fawole and Oso, 2004; Anibijuwon and Udeze, 2009).

Determination of Minimum Inhibitory Concentration (MIC)

The organisms that were susceptible to *J. gossypifolia* extracts were inoculated unto sterile peptone water and incubated overnight at 37º C. Then, 9ml of sterile peptone water was added into sterile test tubes and 1 ml of the different concentrations of the extracts were added, followed by the addition of about 0.3 ml of culture of the test organism. A control was set up which contains only peptone water and the extract. The inoculated and control tubes were incubated at 37º C and 27^0C for 24 hours and 48 hours for Bacteria and Fungi respectively, after which they were observed for turbidity. The lowest concentration that shows no turbidity was taken as the MIC (Adetitun et al., 2013).

Determination of the Minimum Bactericidal or Fungicidal Concentration (MBC/MFC)

Sample from the tubes used in the MIC assays which did not show signs of turbidity after incubation were streaked out on solidified Nutrient Agar (Bacteria) and Potato Dextrose Agar (Fungi) plates respectively using a sterile cotton swab and incubated at 37° C and 27^0C respectively. The lowest concentration of the extract which showed no growth on plates after 24 and 48 hours of incubation indicated bactericidal or fungicidal effect and was taken as MBC (Adetitun et al., 2013).

Antimicrobial Susceptibility Test

The antibiotic susceptibility testing by a standardized disc method was employed. The antibiotics used for this study had been prepared into kit containing multiple discs, each with small discs impregnated with different types of antibiotics. The discs used were for Gram positive and Gram negative organisms respectively. The plate diffusion technique was used for the antibiotic sensitivity test. Overnight broth cultures of the organisms were swabbed on sterile Mueller Hinton agar plates using sterile swab sticks. The plates were allowed to solidify. The multiple antibiotic discs were then placed on the agar surface and pressed using sterile forceps to ensure complete contact with agar. All the plates were incubated at 37° C for 24 hours. The zones of inhibition generated by the antibiotics were measured to the nearest millimeters (mm) and interpreted as sensitive (+) and resistant (-) (Anibijuwon and Udeze, 2009).

RESULTS AND DISCUSSION

The results of phytochemical screening of *J. gossypifolia* leaf extracts (Table 1) showed that the ethanolic extract contained all the six constituents assessed i.e. alkaloids, flavonoids, saponin, tannin, glycosides and phenols, while the methanolic extract contained alkaloids, saponin, tannin and glycosides and the aqueous extract contained flavonoids and saponins only.

This may be due to the better solubility of the active components of the plant in organic solvents; hence they

Table 2: Initial sensitivity test of the extracts of *J. gossypifolia* on the isolates using the stock concentration of 200 mg/ml.

S/N	Test Organisms	Diameter of Zone of Inhibition (mm)		
		Aqueous Extract	**95% Ethanolic Extract**	**95% Methanolic Extract**
1	***Candida albicans***	_	**16.5**	**14.0**
2	***Escherichiacoli***	_	**14.0**	**11.0**
3	***Klebsiellapneumonia***	_	_	_
4	***Salmonella typhi***	**12.5**	**19.5**	**15.5**
5	***Staphylococcus aureus***	_	**20.5**	**15.0**

Key:(**-**) = No Inhibition.

Table 3: The MIC and MBC/MFC of the ethanolic extract of the leaf of *J. gossypifolia*.

	Various concentrations of extract (mg/mL)							
Test organisms	**5**	**25**	**50**	**75**	**100**	**150**	**MIC**	**MBC/MFC**
Candida albicans	**+**	_	_	_	_	_	**25mg/ml**	**75mg/ml**
Escherichia coli	**++**	**++**	**+**	_	_	_	**100mg/ml**	_
Salmonella typhi	**+**	**+**	_	_	_	_	**50mg/ml**	**150mg/ml**
Staphylococcus aureus	**++**	**++**	**+**	_	_	_	**75mg/ml**	_

Key: Turbid +, Very Turbid ++, Not Turbid -

could interact more efficiently with the bioactive compounds of the plant leaf and dissolve them (de Boer et al., 2005). It has been frequently reported that the presence of bioactive compounds in plants conferred resistance to organisms including bacteria, fungi, viruses and parasites and this explains why many plant extracts exhibits both inhibitory and cidal effects (Srinivasan et al., 2001), and these contribute to the various medicinal uses of these plant (Zhang et al., 2009; Sharwar et al., 2010).

The sensitivity test (Table 2) was carried out using the stock concentration of 200 mg/ml of each extract (Ogundare, 2007). This showed a weak activity of the aqueous extract which however inhibited *S. typhi*. Methanolic extract followed in activity after which was the ethanolic extract. However, all extracts showed no inhibitory activity on *K. pneumoniae* indicating its high degree of resistance and possible indications of being a multidrug-resistant organism.

The fact that the extracts were active against both Gram-negative and Gram-positive bacteria tested may indicate a broad spectrum of activity. This observation is of importance as it offers prospects of the use of the plant in the development of active therapeutic substance against organisms with multidrug resistance (Anibijuwon and Udeze, 2009). Saponins which was observe in all three extracts, as well as flavonoids, tanins and glycosides present in at least two of the extracts, are suggestive of their good antioxidant property. Flavonoids, glycosides are reported to be antioxidants and used as anti-inflammatories (Iwu et al., 1999), also, as effective constituents of several pharmaceuticals (Parekh and Chanda, 2007).

Results of MIC obtained showed that *C. albicans* was highly susceptible to the ethanolic extract. The low MIC value observed for *C. albicans* in Table 3, is a good indication of high efficacy against this fungus. This outcome is remarkable considering that a vast majority of infections such as inflammation of the mouth, intestinal tract and urinogenital tract as well as a lot of nosocomial infections and diseases of immune-compromised individuals caused by this fungus which is on the increase can be combated (Oduola et al., 2005; Olowokudejo, 2014). The MFC obtained for the fungus (75 mg/ml) also showed that the extract was potent enough as to be able to kill it hence having prospects of being used in the treatment of various illnesses caused by the fungus. *S. typhi* had a low MIC (25 mg/ml) for the ethanolic extract demonstrating susceptibility to the extract at a low concentration while *E. coli* had a high MIC. High MIC may be an indication of low efficacy or that the organism has the potential for developing resistance to the bioactive compounds (Anibijuwon and Udeze, 2009). The MIC obtained for *S. aureus* is indicative of the fact that the organism was susceptible to the ethanolic extract at a moderately low concentration (75 mg/ml). No MBC was recorded for *S. aureus* and *E.coli* while *S. typhi* had a MBC of 150mg/ml.

From the results obtained for the methanolic extract (Table 4), it was observed that *C. albicans* had a MIC of 50mg/ml and a MFC of 100 mg/ml. This also shows a high level of susceptibility of the fungus to the methanolic extract. *Escherichia coli* demonstrated no MIC thus indicating that *E. coli* had a very low level of susceptibility to the extract. *S. aureus* was also very susceptible with a MIC of 50mg/ml but without a MBC. *S. typhi* was very

Table 4: The MIC and MBC/MFC of the Methanolic extract of the leaf of *J. gossypifolia*.

	Various concentrations of extract (mg/mL)							
Test Organisms	**5**	**25**	**50**	**75**	**100**	**150**	**MIC**	**MBC/MFC**
Candida albicans	**++**	**+**	**-**	**-**	**-**	**-**	**50mg/ml**	**100mg/ml**
Escherichia Coli	**++**	**++**	**++**	**+**	**+**	**+**	**-**	**-**
Salmonella typhi	**+**	**+**	**-**	**-**	**+**	**-**	**50mg/ml**	**150mg/ml**
Staphylococcus aureus	**+**	**+**	**-**	**-**	**+**	**-**	**50mg/ml**	**-**

Key: Turbid +, Very Turbid ++, Not Turbid -

Table 5: Antimicrobial Activity of Standard Gram negative antibiotics on the test organisms.

	DIAMETER OF ZONES OF INHIBITION (mm)		
STANDARD ANTIMICROBIALAGENT	***Escherichia Coli***	***Klebsiella pneumoniae***	***Salmonella Typhi***
Ceptazidine (CAZ) (30µg)	**- (R)**	**- (R)**	**- (R)**
Cefumoxine (CFX) (30µg)	**- (R)**	**- (R)**	**- (R)**
Gentamycin (GEN) (10µg)	**14.0**	**- (R)**	**23.0**
Ceftriazone (CTR) (30µg)	**- (R)**	**- (R)**	**- (R)**
Erythromycin (ERY) (5µg)	**23.5**	**- (R)**	**26.5**
Cloxacilin (CXC) (5µg)	**- (R)**	**- (R)**	**- (R)**
Ofloxacin (OFL) (5µg)	**17.5**	**20.5**	**29.5**
Augmentin (AUG) (30 µg)	**- (R)**	**- (R)**	**- (R)**

Table 6: Antimicrobial Activity of Standard Gram positive antibiotics on the test organisms.

	DIAMETER OF ZONES OF INHIBITION (mm)
STANDARD ANTIMICROBIAL AGENT	***Staphylococcus aureus***
Amoxycilin (AMX) (25µg)	**- (R)**
Cotrimoxazole (COT) (25 µg)	**- (R)**
Notrofurantoin (NIT) (300 µg)	**- (R)**
Gentamycin (GEN) (10 µg)	**- (R)**
Nalidixic acid (NAL) (30 µg)	**- (R)**
Ofloxacin (OFL) (30 µg)	**33.5**
Augmentin (AUG) (30 µg)	**- (R)**
Tetracycline (TET) (30 µg)	**- (R)**

KEY : SUSCEPTIBLE :DIAMETER OF ZONE; RESISTANT: R

susceptible with a MIC of 50mg/ml and a MBC of 150 mg/ml. The results obtained shows that *S. typhi* was the most susceptible isolate while *E. coli* was the least susceptible.

In the antimicrobial sensitivity testing using standard antibiotics, the Gram-negative test organisms used showed high level of resistance to most of the antimicrobial agents (Table 5). Only three of the antimicrobial agents i.e. Gentamycin, Erythromycin and Ofloxacin were found to inhibit the growth of the organism. Of all the gram-negative isolates, *Klebsiella pneumoniae* exhibited the highest level of resistance against all the antimicrobial agents tested. This is similar to the results obtained from this study which showed a high level of resistance of *K. pneumoniae* to all the extracts. However, it was susceptible to only Ofloxacin, a broad spectrum antibiotic (Table 6). *S. typhi* and *E.coli* exhibited susceptibility to some of the antibiotics considered, namely Gentamycin, Erythromycin and Ofloxacin while Gram-positive bacteria *S. aureus* were found to be resistant to all the antimicrobials except Ofloxacin.

CONCLUSION

From this study, the extracts were found to contain various important phytochemicals which were particularly active against *S. aureus* and some other organisms studied, hence demonstrating the potency

against the organism, likewise, and isolates were susceptible to broad-spectrum antibiotics. However, studies on the pharmacokinetic properties and possible toxic effects of the extracts are of the essential before they can be adjudged to have a promising future of being used as antimicrobials in tackling the menace of multi-drug resistance of organisms owing to their broad spectrum activity.

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