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Phytochemical Constituents and Antibacterial Activities of *Jatropha curcas* and *Jatropha gossypiifolia* Leaves

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Abstract

Medicinal plants play key role in wellbeing of people across several countries, most especially in unindustrialized countries; many of these plants are believed to have antimicrobial properties. The phytochemical screening and antibacterial potentials of *Jatropha curcas* and *J. gossypiifolia* leaves extracts were investigated in this study. Phytochemical constituents of the plants were extracted using methanol and ethanol as solvent. The bioactive chemicals were determined qualitatively. The antagonistic property of the crude extracts (200 mg/mL) was evaluated by agar well diffusion technique. The minimum inhibitory concentration and minimum bactericidal concentration were evaluated by broth dilution and streak methods, respectively. The occurrence of alkaloids, flavonoids, phenols, saponins and tannins was revealed in the two plants. Glycoside was also present in *J. gossypiifolia* extracts but not in *J. curcas*. The extracts showed different degrees of activity against strain of *Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae* but no visible activity against *Salmonella typhi* and *Pseudomona aeruginosa.* Diameters of clear zones around the wells ranged from 14.0±1.0 – 22.0±1.0 mm. Ethanolic extracts were higher in antibacterial activity (22.0±1.0 mm). The minimum inhibitory concentration values of the extracts ranged from 100 – 200 mg/mL. *Jatropha curcas* extracts minimum bactericidal concentration values were 100 mg/mL and >200 mg/mL while that of *J. gossypiifolia* were 200 mg/mL and >200 mg/mL. The extracts compete favourably with ciprofloxacin; therefore, the plants could be source of drug(s) of important value.

Keywords: Agar well diffusion, ethanolic extract, *Jatropha*, methanolic extract, phytochemical

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1. Introduction

Plants play important roles in the life of mankind; plants and plant products serve as foods with nutritional and health benefits. They have also been recognized for their therapeutic values. Several plants (tree, shrubs, herbs, leafy vegetables and twigs) serve as foods or are used for medicinal purposes (Nwaogu, Alisi, Ibegbulem, & Igwe, 2007). Most inhabitants in Africa duel in the rural areas and depend on plants for their health care to deal with infectious and non-infectious diseases. More so, great percentage of world population (80%) depends on medicinal plants to solve health related issues (Upadhyay, Saini, & Srivastava, 2011). Various parts of medicinal plants such as leafy part, root, shoot, bark, fruits and seeds are recognized for their medicinal values. Medicinal plants are rich in bioactive compounds; alkaloids, saponins, tannins, phenolic compounds, flavonoids, terpenoids, steroids etc., the medicinal value of the plants depends on these phytochemicals. The bioactive compounds have the ability to produce definite physiological activity on the body (Dahiya, & Purkayastha, 2012). The extracts of these plants are

believed to possess antimicrobial properties. Several authors have reported the bioactive components and antimicrobial potentials of medicinal plant extracts (Ajaiyeoba, 2000; Daoud *et al*., 2015; Dhale, & Birari, 2010, Faparusi *et al*., 2012), and many plants are yet to be studied or studied adequately. Ornamental and hedge plants such as *Jatropha* spp. are also used in folk medicine for health care delivery. *Jatropha* is widely distributed across Africa and Americas (Felix-Silva, Giordani, da Silva-Jr, Zucolotto, & Fernandes, -Pednosa, 2014.). *Jatropha* is used in folklore medicine as remedy for various ailments, and they are of various species including *J. elliptica, J. curcas, J. gossypiifolia* and so on.

Jatropha gossypifolia Linn belongs to the family Euphorbiaceae. Its common name is bellyache bush or black physic-nut (Felix-Silva *et al*., 2014), it is known by the Yoruba of Southern Nigeria as "*Lapalapa*" (Agbogidi, 2013) and it is popularly known as *Jangali yerend*" in India (Dhale, & Birari, 2010). *Jatropha gossypiifolia* is a valuable shrub widely distributed and largely used across Africa and Americas (Felix-Silva *et al*., 2014; Mariz, Borges, Melo-Diniz, & Medeiros, 2010), and thrives as a hedge plant in different parts of Nigeria. Different parts of the plant play important roles in folklore medicine (Felix-Silva *et al*., 2014). It is used for the treatment of diverse disease conditions, predominantly in developing countries (Sabandar, Ahmat, Jaafar, & Sahidin, 2013). It has antimicrobial, antidiarrheal, analgesic properties among others (Felix-Silva *et al*., 2014). *Jatropha gossypiifolia* has dark green or purple-red dark leaves. The young stem of the plant with an acrid juice is used as toothbrush and for cleaning tongue (treatment of thrush).

Jatropha curcas is a woody shrub of the family Euphorbiaceae. It thrives in arid and semiarid tropical parts of the world. It is a small tree of about 6 m high. The upper surface of its leaves is dark green while the reverse side is pale green. Each fruit of *J. curcas* has 2-3 oblong seeds. The plant has many common names such as Barbados nut, black vomit nut, Curcas bean, and so on (Sumit *et al*., 2013). It is used in traditional medicine for different purposes including skin infection, diarrhea, gonorrhea and many others (Dada, Ekundayo, & Makanjuola, 2014). Its seeds are used for the production of biodiesel (Keneni, & Marchetti, 2017). The plant is also used to secure boundaries, as a hedge plant. Several studies have been carried on *Jatropha* species by various authors. This study compared the bioactive constituents and antibacterial properties of *J. curcas* and *J. gossypiifolia*.

2. Materials and Methods

Sample Collection and Preparation

Jatropha curcas and *J. gossypiifolia* plants fresh leaves were collected at Ikosi area, in Ilaro, Ogun state, Nigeria using shears. The plants were identified by a botanist in the Department of Science Laboratory Technology, The Federal Polytechnic, Ilaro. The leaves were rinsed with 2-3 changes clear water and subsequently with sterile distilled water. The leaf samples were air-dried (27±2ºC) and pulverized with the aid of warring blender. The resultant fine powder was store in airtight universal bottle for subsequent use.

Test Organisms

The organisms: *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi* and *Pseudomonas aeruginosa* were gotten at University College Hospital, Ibadan, Oyo State. The organisms were sub-cultured and preliminarily identified for their confirmation; they were maintained on agar slants at 4ºC before use.

Preparation of Crude Extracts

The crude extracts were obtained using maceration technique (Azwanida, 2015). The plant powdered (250 g) was weighed into a clean five-litre round bottom flask and 1000 mL methanol or ethanol (95%) was dispensed into it. The flask was cotton plugged and covered with aluminum foil and the leaves powder was macerated at room

temperature (27±2ºC) for 72 h. The mixture was agitated manually every 24 h for proper solvent-powder mixing. The mixture was filtered into a clean baker using sterile double folded muslin-cloth (cheese cloth). The filtrate obtained was subsequently re-filtered with the aid of a clean Whatman no 1 paper. Rotary evaporator was used to concentrate the resultant extract and the thick extract obtained was evaporated to dryness in the water-bath (100ºC). The dried extract was stored in airtight container at 4ºC until required.

Phytochemicals Analysis

Methanolic and ethanolic extracts of *J. curcas* and *J. gossypiifolia* leaves were screened for their phytochemical constituents (flavonoids, saponins, tannins, alkaloids, phenols and glycosides) as described by Ajaiyeoba (2000) and Dhale and Birari (2010).

Preparation of Inoculum

The test organisms were propagated in Muller Hinton broth at 37ºC for 18 - 24 h prior to antibacterial analysis. Inoculum concentration of each bacterium was adjusted to 1.0 x 10^8 cells/mL using 0.5 McFarland standards (Bhalodia, & Shukla, 2011). The turbidity that corresponds to 10^5 cells/mL was achieved with the addition of sterile normal saline.

Crude Extracts Antibacterial Activity

Agar well technique described by Daoud *et al*. (2015) was slightly amended for the antibacterial action of the plants extracts. Fresh suspension of test organism (1 mL) was introduced to the centre of a sterile Petri dish. About 20 mL cool molten Muller Hinton agar (45ºC) was dispensed into the Petri-dish containing the inoculum and then swirled gently for even distribution of the cells. The seeded plate was allowed to solidify, and wells were cut using a 6 mm diameter, sterile cork borer under aseptic condition. The same process was adopted for all the test organisms (bacteria). 100 µL suspended extract (200 mg/mL) was introduced into each well. Negative control (extraction solvent well) and positive control (ciprofloxacin well, 10 µg/mL) were also set up. The plates were maintained at 4ºC of 1 h in order for the extracts to diffuse into the agar, before incubated at 37ºC for 24 h (Trigui, Hsouna, Tounsi, & Jaoua, 2013). The diameter of halo area around the well was used as antibacterial activity. The diameters of clear zones round the wells were measured with the aid of millilitre calibrated transparent ruler. The assay was carried out in triplicates.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration Evaluation

Broth dilution (macrodilution) method as described by El-Mahmood (2010) was adopted for the minimum inhibitory concentration (MIC) using Muller Hinton broth. The reconstituted extracts concentration (200 mg/mL) that showed antibacterial activity was further diluted to obtain the following concentrations: 200, 100, 50, and 25 mg/mL by a two-fold serial dilution. In order to obtain the various concentrations, Muller Hinton broth (1 mL) was dispensed into four (4) test-tubes, they were sterilized and labeled accordingly. One millilitre of stock solution (reconstituted extract) was transferred to the first and the second test-tubes with the aid of sterile syringe; 1 ml was also transferred from the second test-tube to the third one and from the third test-tube to the fourth test-tube. 1 ml was removed from the last (forth) test-tubes, for all the test-tubes to have uniform volume of difference concentration. Each test-tube was inoculated with 10 µL of fresh test bacterium suspension (10^8 cell/mL) with aid of micropipette. Incubation of the test-tubes was at 37ºC for 24 h and growth was observed thereafter, lowest concentration of the extract at which no visible growth of microorganism after incubation was considered as MIC. Turbidity was used as indices of growth.

Minimum Bactericidal Concentration (MBC) for each extract was determined from the MIC tubes that showed no visible bacterial growth. A loopful from each tube was streaked on Muller Hinton agar plate. Incubation of the plates

was done at 37°C for 24 h. The lowest concentration of each extract that yielded no growth (colony) was documented as the MBC (Mann, Mishra, Kehri, Sharma & Pandey, 2008; Faparusi, Bello-Akinosho, Oyede, Adewole, Bankole, & Ali, 2012).

Statistical Analysis

Mean of the replicates and standard deviations (SD) of the results of zone of inhibition were determined using Statistical Package for the Social Sciences (SPSS) version 16.

3. Results and Discussion

Results

The bioactive constituents of the crude extracts of *J. curcas* and *J. gossypiifolia* leaves are as shown in Table 1. The occurrence of alkaloids, saponins, tannins, flavonoids, and phenols were recorded in all the extracts. The results also revealed glycosides in the extract of *J. gossypifolia* leaves.

The potency of *J. curcas* and *J. gossypiifolia* leaves extracts was determined against five bacterial strains: *E. coli, S. aureus, S. typhi, K. pneumoniae* and *P. aeruginosa*. As stated in Table 2, the extracts of the two plants showed activity against the test strains in varying degrees. *Jatropha curcas* extracts had antagonistic action against *K. pneumonia*e, *E. coli*, and *S. aureus*. *Jatropha gossypiifolia* extracts showed narrow spectrum of activity against *E. coli*, and *K. pneumonia*e (Gram-negative bacteria). *J. curcas* leaves methanolic extracts diameters of clear zones ranged from 15.0±2.0 mm to 18.7±1.5 mm while the ethanolic extract of same plant had inhibition that ranged from 19.3±1.5 mm to 22.0±1.0 mm. The highest antagonistic activity (22.0±1.0 mm) was recorded by *J. curcas* against *K. pneumoniae*. The least zone of inhibition (14.0±1.0 mm) was recorded by methanolic extract of *J. gossypiifolia* against *E. coli* and the highest inhibition zone (20.3±1.5 mm) was against *K. pneumonia*e. The extracts were active against *S. typhi* and *P. aeruginosa*.

The results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are presented in Table 3. The extracts showed varying MIC and MBC against the test organisms. Ethanolic extract of *J. curcas* inhibited *S. aureus, K. pneumoniae* and *E. coli* at minimum concentration of 100, 200 and 100 mg/mL, respectively. Methanolic extract of *J. gossypifolia* had MIC against *S. aureus, K. pneumoniae* and *E. coli* at 100 mg/mL. More so, Ethanolic extracts of *J. gossypiifolia* recorded MIC against *K. pneumoniae* and *E. coli* at 100 mg/mL while the methanolic extract of the plant (*J. gossypiifolia*) inhibited *K. pneumoniae* and *E. coli* at minimum concentration of 200, and100 mg/mL, respectively. The minimum bactericidal concentrations of *J. curcas* ethanolic leaves extract against *S. aureus, K. pneumoniae* and *E. coli* were 100, > 200 and 200 mg/mL, respectively. Methanolic extract of *J. curcas* had minimum bactericidal concentrations of 100, 200 and 200 mg/mL against *S. aureus, K. pneumoniae* and *E. coli*, respectively. The extracts of *J. gossypiifolia* exhibited MBC at >200 and 200 mg/mL against *K. pneumoniae* and *E. coli* respectively. *Jatropha curcas* extracts had the least MBC (100 mg/mL) against *E. coli*.

Table 1: Phytochemical constituents of *J. curcas* and *J. gossypiifolia* leaves

Phytochemical	*J. curcas* Extract	*J. gossypiifolia* Extract

	Ethanolic	Methanolic	Ethanolic	Methanolic
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Phenols	+	+	+	+
Glycosides	–	–	+	+

+: positive –: negative

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Table 2: Antibacterial activities of crude extracts of *J. curcas* and *J. gossypiifolia* leaves

Test strains	Diameter of Inhibition Zones in mm[x]				Ciprofloxacin (10 µg/ml)
	J. curcas		*J. gossypiifolia*		
	Methanolic	Ethanolic	Methanolic	Ethanolic	
S. aureus	18.3±1.5	20.3±1.5	N	N	53.3±0.6
E. coli	15.0±2.0	19.3±1.5	14.0±1.0	17.0±1.0	40.7±0.6
S. typhi	N	N	N	N	40.0±1.0
K. pneumoniae	18.7±1.5	22.0±1.0	20.3±1.5	20.3±1.5	51.7±0.6
P. aeruginosa	N	N	N	N	43.3±0.6

[x]**:** mean of replicates (n=3) ± standard deviation (SD) N: no zone of inhibition

Table 3: Minimum inhibitory concentration and minimum bactericidal concentration of *J. curcas* and *J. gossypiifolia* leaves extracts

Test strains	Plant	Solvent	Concentrations of extract (mg/mL)				MIC (mg/mL)	MBC (mg/mL)
			25	50	100	200		
S. aureus	*J. curcas*	Ethanol	+	+	–	–	100	100
K. pnuemoniae			+	+	+	–	200	>200
E. coli			+	+	–	–	100	200
S. aureus		Methanol	+	+	–	–	100	100
K. pnuemoniae			+	+	–	–	100	200
E. coli			+	+	–	–	100	200
K. pnuemoniae	*J. gossypiifolia*	Ethanol	+	+	–	–	100	>200
E. coli			+	+	–	–	100	200
K. pnuemoniae		Methanol	+	+	+	–	200	>200
E. coli			+	+	–	–	100	200

+: turbid –: not turbid

Discussion

The different solvent extracts of *J. curcas* and *J. gossypiifolia* leaves extracts revealed the presence of bioactive constituents; alkaloids, flavonoids, saponins, tannins, phenols, and glycosides. The presence of these secondary metabolites (phytochemicals) might be responsible for their use in traditional medicine and antimicrobial activity of plants extracts. Several authors have reported the presence of phytochemical in plants used for traditional health care delivery (Ajaiyeoba, 2000; Dhale & Birari, 2010; Faparusi *et al*., 2012). Presence of more phytochemicals was recorded in *J. gossypiifolia*. Glycosides were not detected in *J. curcas* leaves. The presence of a good number of bioactive components in the extracts could be due to solubility, high extraction capability and different polarity in the extraction solvent (Dhale, Birari, & Dhulgande, 2010; Daoud *et al*., 2015). Genetics factor and geographical location might be due to little variation in their phytochemical constituents, but the two species revealed the presence of good number of bioactive constituents.

The findings in this study with respect to the presence of flavonoids is at variance with the reports of Dhale and Birari (2010) and Vijayta, Renu, Sikha, and Nishi (2015), that showed the absence flavonoids and alkaloids in the methanolic extracts of *J. gossypiifolia.* There was no variation in the extraction ability of the solvent used contrary to precious findings by other authors (Faparusi *et al*., 2012; Daoud *et al*., 2015). The bioactive compounds in medicinal plants are believed to possess antimicrobial activities.

The antibacterial property of the plants extracts was measured on the basis of diameters of inhibition zones against the test organisms. Both plants displayed relative action against greater number of the bacterial strains at concentration of 200 mg/ml, but *J. curcas* was more potent on the basis of number of bacteria inhibited. Antimicrobial potential of plants is usually ascribed to presence of phytochemicals (Faparusi *et al*., 2012; Daoud *et al*., 2015). Flavonoids and tannins are known for antibacterial activity (Loizzo *et al*., 2004; Mattana, Satorres, Sosa, Fusco, & Alcaraz, 2010). Various studies on the antimicrobial activity medicinal plants are many in literature (Faparusi *et al*., 2012, Trigui *et al*., 2013; Daoud *et al*., 2015). The antagonistic action of *J. curcas* and *J. gossypiifolia* against the test bacterial strains revealed ethanolic extracts of the two plants had higher potency, based on diameters of inhibition zones. This might be due to the ability of ethanol to extract high polar and non-polar components from the plant materials. It also has very low toxicity, volatile, completely miscible in water and easily remove in plant materials at low temperature (Roy, 2014). Both methanolic and ethanolic extracts of *J. gossypiifolia* possessed antagonistic activity against *E. coli*, in contrary to the findings of Ruchi and Renu (2010) that reported methanolic extract of *J. gossypiifolia* had no activity against *E. coli*. More so, the two plants exhibit no activity against *P. aeruginosa* and *S. typhi*. This could be due to low concentration of the bioactive compounds in the extract to inhibit the bacteria (Itelima, Nwokedi, Ogbonna, & Hyam, 2016). The bacteria (*P. aeruginosa* and *S. typhi*) might also been carrying resistant gene which could be chromosomal and plasmid mediated. The standard antibiotic, ciprofloxacin, showed display better activity against all the test strains than the extracts. This could be to the fact that the standard antibiotic is a refined and purified product (El-mahmood, & Amey, 2007).

After preliminary screening of antibacterial activity of the extracts, those that exhibited antagonistic activity against the test strains were exposed to minimum inhibitory concentration and minimum bactericidal concentration analyses. There was variation in the MIC and MBC of the extracts against the test strains. The most effective extract(s) showed MIC and MBC at 100 mg/mL. This could be due to the concentration of bioactive compounds in the extracts. Similar concentrations had earlier been reported by Faparusi *et al*. (2013). The MBC (100 mg/mL) recorded by *J. curcas* extracts could be due to the concentration of potent chemicals in the extracts.

4. Conclusion

Jatropha curcas and *J. gossypiifolia* leaves demonstrated presence of a good number of bioactive chemicals; alkaloids, saponins, tannins, flavonoids, and phenols, but the latter is richer in phytochemicals. The plants also showed antagonistic action against *S. aureus*, *E. coli* and *K. pneumoniae* in varying degrees; the ethanolic extracts were more potent. These extracts compete favourably well with standard antibiotic (ciprofloxacin); therefore, the plant could be sources of drug (s) of important value.

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