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Phytochemical properties and effect of aqueous extract of *Jatropha caucus* root bark on some bacterial isolates

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Phytochemical properties and effect of aqueous extract of *Jatropha caucus* root bark on some bacteria isolates was investigated. The root bark extract revealed the presence of carbohydrates, alkaloids, flavonoids, saponins, cardiac glycosides and terpenes/steroids. The extract applied in graded concentrations of 200, 400 and 600 mg/ml inhibited the multiplication of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Corynebacterium pyogenes*, *Candida albicans, Pseudomonas aeroginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Escherichia coli* and *Proteus mirabilis.* Conclusively, the root bark extract of this plant has exhibited good antibacterial activity especially on organisms such as *B. subtilis*, *C. albicans*, *S. pyogenes*, *Proteus* species, *E. coli* and *S. typhi* that are incriminated in causing diseases such as anthrax, reproductive tract infection, skin infection and various gastrointestinal disorders.

Key words: *Jatropha curcas*, phytochemical properties, *in vitro* antibacterial activity, aqueous root bark extract.

INTRODUCTION

Jatropha curcas (Linnaeus) belongs to the family Euphorbiaceae and is closely related to other important cultivated plants like rubber and castor plants. The plant is believed to be a native of South America and Africa but later spread to other continents of the world by the Portuguese settlers (Gubitz et al., 1999).

Various parts of this plant have been documented to have medicinal uses for human and veterinary purposes. The plant has been used in the treatment of infectious and non infectious ailments such as gonorrhea, dropsy, gout, paralysis, scabies, eczema, dermatitis and rheumatoid arthritis (Srinivasan et al., 2001).

Many parts of this plant such as leaves, stem bark and latex have been reported to exhibit antibacterial activity (Oyi et al., 2007; Donlaporn and Suntornsuk, 2010). There are more than 35,000 plant species with various phytochemicals in them being used on various human cultures around the world for medicinal purpose. About 80% of individuals from developed and under developed countries use traditional medicine, which has compound derived from medicinal plants in various form of therapies (Galal et al., 1991).

Phytochemicals are biologically active compounds found in plants such as vegetables and grains in low,

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moderate and high amounts; these compounds are not established nutrients, but significantly protect the development of lots of degenerative diseases in animals and humans (Dreosti, 1998; Abo et al., 1991).

Researchers are increasingly turning their attention to natural plant products such as flavonoids, saponins, tannins and others to look for new products to develop better drugs against cancer, as well as mycotic, viral and microbial infections (Hoffmann et al., 1993; Srinivasan et al.,2001).Bacteria have the genetic ability to transmit and acquire resistance to drugs (Cohen, 1992). In the last three decades, numbers of new antibiotics have been produced, but clinical efficacy of these existing antibiotics is being threatened by the emergence of multi drug-resistant pathogens (Bandow et al., 2003). According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs (Santos et al., 1995).

The aim of this study was to evaluate the phyto-chemical properties and *in vitro* antibacterial activity of the aqueous extract of *J. caucus* root bark on some pathogenic bacterial organisms of medical importance.

MATERIALS AND METHODS

Collection of plant and identification

Fresh roots bark of *J. caucus was* collected from Borgu in Shani Local Government Area of Borno State. The root bark was taken to the Department of Biological Sciences, University of Maiduguri, where it was identified and authenticated by a taxonomist. Voucher specimen was deposited at the University Herbarium for reference. The root bark were kept under a well ventilated shade for several days and allowed to air-dry. The dried root bark were then crushed and pulverized into fine powder and kept in an amber bottle at 4°C.

Preparation of extract

Aqueous root bark extract of the plant was prepared according to the methods of Mittal et al. (1981) and Fernando et al. (1989). 200 g of the powdered root bark was mixed with 1 L of distilled water in a 5 L beaker. The mixture was steamed at 65°C for 1 h, allowed to cool and mixed vigorously. It was filtered using sterile Whatman No.1 filter paper. The aqueous extract filtered was then concentrated by evaporation to dryness at 60°C in a water bath and finally stored at 4°C for use.

Microbial cultures

Pure cultures of some Gram positive and Gram negative bacteria such as *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Corynebacterium pyogenes*, *Candida albicans, Pseudomonas aeroginosa, Klebsiella pneumoniae, Salmonella typhi, Escherichia coli* and *Proteus mirabilis* were obtained from the Department of Veterinary Medicine Laboratory, University of Maiduguri and were used. The isolates were separately cultured on a nutrient plate for 24 h. Twenty milliliters of the culture media was poured into sterile medium sized Petri–dish and allowed to solidify. A colony of each test organism was subcultured in 10 ml nutrient broth and incubated at 37°C for 8 h. One milliliter of the subcultured organisms were inoculated on the agar plates.

Extract concentration and standard drug preparation

Stock solution of the aqueous extract is prepared by dissolving 200, 400 and 600 mg of the extract in 1 ml of distilled water giving the concentration of 200, 400, and 600 mg/ml of the extract, respectively. Amoxicilli as a standard drug (Control) was used at the concentration of 250 mg/ml.

Antibacterial sensitivity testing

Disc diffusion method as described by the National Committee of Clinical Laboratory standards (1993) was used to determine the antimicrobial activity of the aqueous root bark extract. Discs of sterilized Whatman No.1 filter paper (6 mm) in diameter made using a paper puncher were soaked in beakers containing different concentrations of 200, 400 and 600 mg/ml of the extract and dried at 50°C. Each paper disc used for the antibacterial sensitivity test contains 200, 400 and 600 mg of the extract, respectively. Overnight cultures of each bacteria isolate were diluted using sterile normal saline to give an inoculum size of 10^6 CFU/ml. The inoculum was spread on the surface of dried nutrient agar plates with cotton wool swabs, which have been dipped in the diluted suspension of the organisms. The plates are then inoculated at 37°C for 30 min before the discs were applied aseptically. The treated plates were incubated at 37°C for 48 h. Discs of sterilized Whatman No.1 filter papers (6 mm) in diameter made using a paper puncher were soaked in a beaker containing 250 mg/ml of amoxicillin as a positive control. The paper discs used as positive control therefore contain 250 mg of amoxicillin. Plates without the extract or antibiotics were setup as negative control. The zone of inhibition above 6 mm diameter of each isolate was used as a measure of susceptibility of the organisms to the extract and this will be compared to the zone of inhibition of the standard antibiotic (Amoxicillin).

RESULTS

Preliminary phytochemical analysis of the crude extract of *J. curcas* root bark as shown in Table 1 revealed the presence of alkaloids, tannins, flavonoids, saponins, carbohydrates, terpenes, cardiac glycosides and steroids, whereas no anthraquinones were detected in the extract. The antibacterial activity of the aqueous extract of *J. curcas* root bark clearly showed that all the organisms were sensitive to the extract. The extract used in graded concentrations (200, 400 and 600 mg/ml) exhibited graded effect on the microorganisms. At higher concentration of 600 mg/ml, the organisms showed variation in their zones of inhibition as follows, *B. subtilis* (20 mm), *C. albicans* (20 mm), *E. coli* (17 mm), *P. mirabilis* (17 mm), *P. aeroginosa* (15 mm), *S. pyogenes* (15 mm), *S. typhi* (14 mm) with *S. aureus* (12 mm) and *K. pneumoniae* (11 mm) exhibiting less sensitivity. *Corynebacterium pyogenes* is the only microorganism that has exhibited resistance to the extract. Similar trend was shown by the microorganisms to 200 and 400 mg/ml of the extract used. Amoxicillin (control drug) inhibited the growth of all the microorganisms including *C. pyogenes*

Table 1. Phytochemistry of aqueous extract of *J. curcas* root bark.

Phytochemical constituents	Types of test	Inference
Carbohydrates	Molisch's	+
	Barfoed's	-
	Free reducing sugar	+
	Combined reducing sugar	-
	Ketones	+
	Pentoses	-
Tannins	Ferric chloride	+
	Lead ethanoate	+
Anthraquinones	Bournstrager	-
	Free/Combined anthraquinones	-
Saponins	Frothing	+
Glycosides	General test	+
Terpenes and steroids	Lieberman- Buchard's	+
	Salkowski's	+
Flavonoids	Lead acetate	+
	Sodium hydroxide	+
	Ferric chloride	+
	Pew	+
Alkaloids	Dragendorff's	+
	Mayer's	+

-: Not detected; +: Present.

that was resistant to the aqueous extract of *J. caucus* root bark (Table 2). The minimum inhibitory concentration for *S.aureus*, *S. pyogenes* and *S. typhi* is 25 and 50 mg/ml for *Escherichia* (Table 3). The sensitive laboratory bacterial isolates has a minimum bactericidal concentration of 50 mg/ml (Table 4).

DISCUSSION

Plant essential oils and extracts have been used for many thousands of years in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate these plants scientifically, which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens (Ramo-Tejada, 2002).

The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Sieradski et al., 1999).

The aqueous extract of *J. caucus* root bark has very important phytochemicals such as alkaloids, tannins, flavonoids, saponins, carbohydrates, terpenes, cardiac glycosides and steroids. Flavonoids are strong antioxidants also found to be effective antimicrobial substance *in vitro* and *in vivo* against wide range of microorganism by inhibiting their membrane bound proteins (Ramo-Tejada, 2002; Cowan, 1999). Tannins have also been reported to posse's antibacterial activity by suppressing certain key enzyme activities involved in metabolic processes in bacteria microorganisms (Narayana et al., 2001; Birk and Petri, 1980).

Plant parts from *Acacia albida, Anchomanes difformis, Boscia senegalensis, Bridelia ferruginea, Ficus ingens, Indigofera pulchra, Moringa oleifera, Mormodica basalmina, Pavetta crassipes, Phyllanthus amarus* and *Vernonia blumeoides* used for antibacterial studies have shown various levels of antibacterial activity (Aliyu et al., 2008).

Parts of *J. caucus* (Stem and Stem bark) used in *in vitro* antibacterial study (Bhaskarwar et al., 2008; Igbinosa et al., 2009) have shown activity, but the root

Table 2. Antibacterial activity of aqueous root bark extract of *J. curcas* on some bacterial organisms.

Extract / antibiotic	Amounts of extract and amoxicillin (mg)	Zone of inhibition diameter(mm)									
		P. aeroginosa	*S. aureus*	*K. pneumoniae*	*S. pyogenes*	*S. typhi*	*B. subtilis*	*E. coli*	*C. pyogenes*	*Proteus* spp.	*C. albicans*
Aqueous extract of *J. caucus*	600	15	12	11	15	14	20	17	R	17	20
	400	10	8	8	12	13	15	14	R	12	14
	200	9	6	6	7	12	13	8	R	11	13
Amoxicillin	250	12	20	15	35	35	30	25	25	30	30

Table 3. Determination of minimum inhibitory concentration (MIC) of *J. curcus* aqueous crude root bark extract.

Organism	Concentration of *Hyphaene thebaica* aqueous pericarp extract (mg/ml)				
	200	100	50	25	12.5
S. aureus	-	-	-	-	+
S. pyogenes	-	-	-	-	+
S. typhi	-	-	-	-	+
E. coli	-	-	-	+	+

+: Growth observed; -: Growth inhibited.

Table 4. Determination of minimum bactericidal concentration (MBC) of *J. curcas* aqueous crude root bark extract.

Organism	Concentration of *Hyphaene thebaica* aqueous pericarp extract (mg/ml)				
	200	100	50	25	12.5
S. aureus	-	-	-	+	+
S. pyogenes	-	-	-	+	+
S. typhi	-	-	-	+	+
E. coli	-	-	-	+	+

+: Growth observed; -: Growth inhibited.

bark extract had better activity. The result of antibacterial activity of the aqueous extract of *J. caucus* root bark exhibited promising activity that could assist lots of people in the sub-Saharan Africa since orthodox drugs are not easily accessible due to financial problem experienced by in the developing countries especially in Africans. All the Gram positive and negative organisms used in this study exhibited sensitivity to this extract except *C. pyogenes*. *S. typhi*, *S. aureus* and *K pneumoniae* that causes serious problems such as typhoid fever, dermal infections and pneumonia in sub-Saharan Africa due to poor primary health care could be effectively controlled by the aqueous extract of *J. caucus* root bark developed in form of suspension, poultices or creams.

The sensitivity shown by *C. albicans to* this

product also indicate the possibility of the extract being used in the management of reproductive tract infection (Candidiasis) that mostly affects female folk.

Conclusion

This research conclusively proved that theaqueous extract of *J. caucus* root bark has antibacterial property principally due to the presence of flavonoids and tannins as active principle, hence its folkloric application in the management of various bacterial disease condition by herbalists and traditionalist in Askira/Uba, Shani and Maiduguri metropolitan in Borno State, Nigeria.

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