academicJournals

Vol. 7(44), pp. 5097-5100, 7 November, 2013
DOI: 10.5897/AJMR2013.5850
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Antibacterial activity of leaves extracts of *Jatropha curcas* and *Euphorbia heterophylla*

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Accepted 8 October, 2013

The antibacterial activity of aqueous and ethanolic extracts of two Nigerian medicinal plants, *Euphorbia heterophylla* and *Jatropha curcas* was investigated using disc agar diffusion and broth dilution assays against four clinical isolates of bacteria consisting of two gram positive (*Staphylococcus aureus and Enterococcus faecalis)* and two gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa)*. The antibacterial activity was measured by the diameter zone of inhibition and minimum inhibitory concentration (MIC). The extracts exhibited broad spectrum antibacterial activities against the microorganisms. The diameter of zone of inhibition ranged from 10-15 mm at 2000 µg of crude extract per disc. The MIC values were between 125 -1000 µg/ml. The results of this study indicate that these plants contain compounds with antibacterial activity which validates their use for treatment of various microbial infections in traditional medicine. The findings in this study provide the basis for further study on the plants with the aim of isolating and identifying the active substances. The plants could also be standardized to develop cheap, culturally acceptable herbal medicines.

Key words: Antimicrobial activity, medicinal plants, *Jatropha curcas*, *Euphorbia heterophylla*, minimum inhibitory concentration.

INTRODUCTION

Medicinal plants serve as the major source of medicines for treatment of various ailments in the primary health care for majority of the rural populace in Nigeria as in other parts of Africa. According to the World Health Organization (WHO, 2002), over 80% of the world's population especially in the developing world relies on medicinal plants as sources of medicines for their primary healthcare. Traditional system of medicine which depends mainly on medicinal plants is rich in ethnomedical knowledge of the uses of medicinal plants in the treatment of infectious conditions (Iwu, 1993). These medicinal plants employed in traditional medicine represent potential sources of cheap and effective standardized herbal medicines (phytomedicine) and leads in the discovery of novel molecules for the development of new chemotherapeutic agents (Farnsworth and Morris, 1976). Several infectious diseases including malaria, diarrhea, dysentery, gonorrhea and fungal infections have been successfully managed in traditional medical practice employing medicinal plants (Sofowora, 1993).

Jatropha curcas Linn and *Euphorbia heterophylla* Linn are two plants in the family Euphorbiaceae that have been identified as plants widely used in traditional medicine in various parts of Africa (Iwu, 1993, Burkill, 2008).

J. curcas variously known as physic nut, purging nut or pig nut (Uche and Aprioku, 2008; Igbinosa et al., 2009) and "*Lapalapa*" in Yoruba Language (Burkill, 1994) is used in folklore remedies for treatment of various ailments such as skin infections, gonorrhea, jaundice and fever (Akinpelu et al., 2008). In Akwa Ibom State, the oil from

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the crushed seeds is used for treatment of skin diseases and as a laxative (Ajibesin et al., 2008). *E. heterophylla* with the common name “spurge weed” grows in semi-humid places especially in cassava, cowpea and soya beans plantations (Falodun et al., 2006). *E. heterophylla* has been used in the traditional medicine for the treatment of constipation, bronchitis and asthma. The plant has also been reported to be used as purgative (Erdem et al., 1999, Ajibesin et al., 2008).

The desire to scientifically validate the medicinal properties of these plants has resulted in the investigation of their various biological activities. The antibacterial activity of the methanolic extract of the leaves of *J. curcas* was investigated against 13 bacterial species including *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The extract showed appreciable inhibitory activity against these organisms (Akinpelu et al., 2009).

Although *E*. *heterophylla* is reputed to be widely used in folklore medicine, reports on the previous pharmacological studies are few. Falodun et al. (2003) reported the antibacterial activity of the petroleum ether, butanolic and ethanolic extracts of the leaves against strains of typed culture organisms. Only the butanolic extract showed antibacterial activity.

The aim of this study was to further evaluate the antibacterial activity of the aqueous and ethanolic extracts of the leaves of *J. curcas* and *E. heterophylla* against four clinical isolates of common bacterial pathogens namely, *S. aureus, S. faecalis, E. coli and P. aeruginosa.*

Collection of plant materials

Fresh leaves of *J. curcas* and *E. heterophylla* were collected during the rainy season in the month of August from Ibinaukwu Igbere, Bende L.G.A. of Abia State, Nigeria. Dr. Osuagwu of the Department of Plant Sciences and Biotechnology, Michael Okpara University of Agriculture, Umudike, taxonomically authenticated the plants and voucher samples were deposited in the Department herbarium. The leaves were air dried at ambient temperature for several days until well dried. The dried leaves were reduced to fine powder using laboratory mortar and the powder stored in an air-tight container until needed.

Extraction of plant materials

20 g amount of the powdered leaves was weighed and percolated in 200 ml of 96% ethanol contained in 500 ml conical flask. The flask was agitated manually several times over a period of 24 h. The extract was filtered using Whatman No. 1 filter paper and the filtrate collected in a clean beaker was concentrated to dryness by evaporation over a steam bath at 80°C. The aqueous extract was similarly prepared using 20 g of the powdered leaves material in 200 ml of distilled water.

Test organisms

The four test bacteria used in the study were *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa*. The bacteria were clinical isolates obtained from the Microbiology Laboratory of the Federal Medical Centre (FMC), Umuahia, Abia State. The microorganisms were maintained at 4°C on Nutrient Agar slant in the Department of Microbiology, Michael Okpara University of Agriculture, Umudike and fresh subcultures were made before use.

Preparation of extract impregnated paper discs

The extract impregnated paper discs were prepared as described by Ekundayo and Ezeogu (2006). Whatman No. 1 filter paper was cut into discs of 6 mm diameter using an office perforator. The discs were placed in glass Petri dish and sterilized in hot air oven at 160°C for 1 hour. Each disc was impregnated with 20 µl portion of stock solution of the extract (100 mg/ml) to give a concentration of 2000 µg of crude extract per disc. The discs were dried in an incubator at 35-37°C for 2 h. Discs of Ampicillin and Tetracycline used as control antibiotic discs were similarly prepared.

Antimicrobial activity by zone of inhibition

The antimicrobial activity of the extracts was assessed using the disc diffusion and broth dilution methods. A cell suspension of each test bacterial strain was prepared by transferring 4-5 isolated colonies on Nutrient agar plate into sterile normal saline in a bijou bottle. The turbidity was adjusted to McFarland turbidity standard tube No. 0.5 by adding sterile normal saline. The surface of Mueller Hinton Agar (Fluka BioChemika, Fluka Chemie GmbH,Buchs) plate was inoculated by swabbing the surface with a sterile swab stick dipped into the bottle containing the standardized cell suspension. The prepared disc was aseptically transferred unto the inoculated culture plate using a pair of flame sterilized forceps. The plates were incubated aerobically at 37°C for 18-24 h. The diameter zone of inhibition was measured using a transparent plastic ruler. The tests were carried out in duplicate.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of each of the extracts was determined using the tube serial dilution method. Extracts (100 mg/ml) were dissolved in water or ethanol and diluted with Nutrient broth in two fold serial dilutions in test tubes to obtain the following concentrations: 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.82, 3.91, 1.96 µg/ml. An overnight broth culture of the test organism was adjusted to McFarland turbidity standard No. 0.5 (10^6 CFUs/ml) and 50 µl (0.05 ml) of the cell suspension added to each of the tubes. The tubes were incubated aerobically at 37°C for 18 h. The MIC was defined as the lowest extract concentration that inhibited the growth of the test organism as indicated by absence of visible turbidity in the tube compared with the control tubes.

RESULTS

The antimicrobial activities of the leaves extracts of *J. curcas* and *E. heterophylla* measured by diameter of the zone of inhibition against the test organisms are shown in Table 1. The aqueous extract of *J. curcas* inhibited *S. aureus* and *E. coli* with diameters of zone of inhibition of 10 and 14 mm, respectively. The ethanol extract of the plant had no activity against all the test organisms. Similarly, the aqueous extract did not show activity against *E. faecalis* and *P. aeruginosa.* The aqueous extract of the leaves of *E. heterophylla* had antimicrobial activity

Table 1. Diameter of zone of inhibition (mm) of *J. curcas* and *E. heterophylla* extracts against clinical isolates of four bacterial pathogens.

Plant specie	Extract type	Bacterial isolate			
		S. aureus	***E. faecalis***	***P. aeruginosa***	***E. coli***
J. curcas	Ethanol (2000 µg)	No inhibition	No inhibition	No inhibition	No inhibition
	Aqueous (2000 µg)	10	No inhibition	No inhibition	14
E. heterophylla	Ethano l (2000 µg)	12	No inhibition	No inhibition	12
	Aqueous (2000 µg)	14	13	11	15
Antibiotics	Ampicilin (10 µg)	20	12	15	No inhibition
	Tetracycline (30 µg)	34	17	20	No inhibition

Table 2. Minimum inhibitory concentrations (MIC, µg/l) of *J. curcas* and *E. heterophylla* extracts against clinical isolates of four bacterial pathogens.

Plant extract		Bacterial isolates			
		S. aureus	***E. faecalis***	***P. aeruginosa***	***E. coli***
J. curcas Aqueous		1000	>1000*	>1000*	500
E. heterophylla	Aqueous	125	250	250	125
	Ethanol	250	>1000*	>1000*	250

*Extracts showed no inhibitory activity in disc agar diffusion assay

against all the test organisms with mean diameter of zone of inhibition ranging from 11 to 15 mm. The activity was highest against *E. coli* and least against *P. aeruginosa.* The ethanol extract was active only against *S. aureus* and *E. coli* but not against *E. faecalis* and *P. aeruginosa.* Table 2 shows the MIC values of the two plant extracts against the test organisms. The MIC values ranged from 125 to 1000 µg/ml of crude extract. The aqueous extract of *E. heterophylla* exhibited the highest activity against *S. aureus* and *E. coli* with MIC of 125 µg/ml, respectively.

DISCUSSION

The aqueous extract of *E. heterophylla* leaves produced diameters of zone of inhibition of 14 and 15 mm against *S. aureus* and *E. coli*, respectively, 13 mm against *E. faecalis* and 11 mm against *P. aeroginosa*. Thus, the aqueous extract of the leaves showed a broad spectrum of antimicrobial activity against the test organisms. Falodun et al. (2003), had previously investigated antibacterial activity of the petroleum ether, butanolic and ethanolic extracts of the leaves of *E. heterophylla* against *E. coli*, *Klebsiella pneumonia*, *S. aureus*, *P. aeruginosa* and *Bacillus subtilis*. The butanolic extract showed a broad spectrum of antibacterial activity against the test organisms at concentration of 100, 150 and 200 mg/ml. The petroleum ether and ethanolic extracts did not show any antibacterial activity against any of the test organisms. Similar to the results of Falodun et al. (2003), the ethanolic extract of the leaves at 2000 µg/20 µl/disc (equivalent to 100 mg/ml) did not show antibacterial activity against the test organisms in our study. In traditional medical practice, it is the aqueous extract or decoction of the leaves that is used to prepare food such as yam porridge or taken directly to “wash out the bowels” or as a purgative (Oksuz et al., 1994, Falodun et al., 2003). According to Falodun and Agbakwuru (2004), the leaves of *E. heterophylla* contain quercetin, saponins, tannins and flavonoids. Antimicrobial activity of medicinal plants has been linked with the presence of these chemical substances in medicinal plants (Cowan, 1999; Cushnie and Lamb, 2005; Ebana et al., 2005). The antibacterial activity of *E. heterophylla* may be due to the presence of these chemical substances in the leaves (Falodun et al., 2006).

The aqueous and ethanolic extracts of *J. curcas* tested in this study showed moderate antibacterial activity against *S. aureus* and *E. coli* with diameter of zone of inhibition of 10 and 14 mm, respectively. The results of our study are similar to those of Akinpelu et al. (2009) who reported strong inhibitory activity against *S. aureus* and *E. coli* in the methanolic extract of the leaves of *J. curcas*. Aiyelaagbe et al. (2007) reported the antimicrobial activities of some secondary metabolites from the root extract of the plant against some microorganisms associated with sexually transmitted diseases. Igbinosa et al. (2009) also reported the antimicrobial activity of the stem bark extracts against 12 bacterial species consisting both gram positive and gram negative organisms while Igbinosa et al. (2009) found little or no antibacterial activity in the aqueous extract of the stem bark of *J. curcas*; in our study, both aqueous and ethanolic extracts of the leaves showed moderate antibacterial activity against both *S. aureus* and *E. coli*. While the differences in results

may be due to the varying concentrations of active substances in the stem bark and leaves, it may also be due to the different concentrations used. The concentration in our study was 2000 µg/20 µl/disc (100 mg/ml) but the highest concentration used in their study was 10 mg/ml. However, the actual concentration of the extract used in agar well diffusion method employed by Igbinosa et al. (2009) may be difficult to determine as the stated concentration is nominal and does not represent the actual amount of substance present in the volume of extract dispensed into agar well. The work of Kisangau et al. (2007) showed the possibility of discrepancy in the results of antibacterial activity tested by agar well diffusion method and the paper disc diffusion method. Kisangau et al. (2007) found no activity in the water extract of *J. curcas* when agar well was used but strong inhibitory activity of the extract was seen in the disc diffusion method. The effect of these two methods of antibacterial activity screening of plant extracts may require further study.

It is interesting to note that the extracts of the leaves of *J. curcas* and *E. heterophylla* had activity against the *E. coli* isolate which was resistant to Ampicillin and Tetracycline. A major goal of the antimicrobial screening of medicinal plants is to find substances with novel mechanism of action against drug resistant strains. The aqueous extract of *E. heterophylla* was also active against *P. aeruginosa*, a gram negative bacterium commonly resistant to many antibiotics.

The results obtained in this study contribute to the scientific validation for the use of these medicinal plants in traditional medicine and serve as a guide for selection of plants with antimicrobial activity for further phytochemical work on isolation and identification of the active compounds. Furthermore, these results show the potential of some of these medicinal plants for development of standardized culturally acceptable herbal medicines for local use as broad spectrum antimicrobial agents.

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