African Journal of Biotechnology Vol. 8 (8), pp. 1660-1664, 20 April, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

The bioactive potentials of two medicinal plants commonly used as folklore remedies among some tribes in West Africa

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Accepted 12 January, 2009

***Jatropha curcas* and *Newboulda laevis* are medicinal plants used for the treatment of ailments such as diarrhoea, dysentery, sexually transmitted diseases, jaundice and several other diseases caused by micro-organisms. The antibacterial activities of the leaves of the plants were assessed against a panel of selected bacterial pathogens. Methanolic leaf extracts of *J. curcas* and *N. laevis* exhibited antibacterial activity against eight of the thirteen tested bacterial isolates at a final concentration of 20 mg/ml. The zones of inhibition exhibited by *J. curcas* ranged between 12 and 17 mm while that of *N. laevis* varied between 10 and 23 mm. The minimum inhibitory concentration of *J. curcas* extract ranged between 0.625 and 10.00 mg/ml, while that of *N. laevis* extract varied between 0.313 and 10 mg/ml. The standard antibiotic – streptomycin had MIC values of between 0.0313 and 0.0625 mg/ml. Phytochemical compounds present in the extract of *J. curcas* include alkaloids, saponins, steroids and tannins, while those present in *N. laevis* extract includes alkaloids, flavonoids and tannins.**

Key words: *Jatropha curcas, Newbouldia laevis,* antibacterial, phytochemicals.

INTRODUCTION

African plants have long been the source of important products with nutritional and therapeutical properties. Plant cells fundamentally are chemical factories and many possess a rich supply of therapeutically useful constituents. There is an urgent need for the development of alternative antimicrobial substances of natural origin for the treatment of infection in the light of growing cases of microbial resistance to the available synthetic antibiotics. Thus, plants around us can be investigated for the purpose of identifying those that may be potent against infectious organisms and hence useful in treating ailments caused by micro-organisms. Africa is reputed for the extraordinary richness of its flora totalling several tens of thousands of species. Many of these plants contain large varieties of chemical substances referred to as secondary metabolites such as flavonoids, tannins among others and these have significant biological effects on humans. *Jatropha curcas* belongs to the family Euphorbiaceae and commonly found in Africa, Asia and Latin America. It is a shrub and is known as “Lapalapa” in Yoruba Language (Burkill, 1994). This plant is used in folklore remedies for the treatment of various ailments such as skin infections, gonorrhoea, jaundice and fever (Dalziel, 1937; Chopra et al., 1956). Aiyelaagbe et al. (1998), Sanni et al. (1988) reported antibacterial, antitumor and antiinsect activities of this plant. The sap from *J. curcas* is employed for treating sores, cleaning teeth and for toothache (Burkill, 1994; Langdon, 1977). The root methanolic extract of the plant was shown to exhibit anti-diarrhoeal activity in mice (Mujumdar et al., 2001). In addition, Mujumdar and Misar (2003) revealed that the root extract from *J. curcas* showed antiinflamatory activity on local inflammatory induced in albino rat. Naengchommong et al. (1986) reported the isolation of two lathyranes from *J. curcas* while Olapeju et al. (2007) isolated diterpenoids from the same plant and also reported its antibacterial activity on some bacterial isolates.*Newbouldia laevis* an angiosperm belongs to the

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family Bignoniaceae. The plant is commonly found in Tropical Africa (Kaey, 1989). *N. laevis* is widely used as folklore remedies for the treatment of diseases such as diarrhoea, dysentery, malaria, dental caries and sexually transmitted diseases (Eyong et al*.,* 2005), it is also used as worm expellant. Kuete et al. (2007) reported the antimicrobial activity of root bark methanolic extract of *N. laevis* on some bacterial isolates, while the leaf extract also exhibited antimicrobial activity (Usman and Osuji, 2007). Gafner et al. (1998) reported the isolation of naphthaquinone a phytochemical compound from the root extract of *N. laevis*. This compound also showed antifungal and antibacterial activities. The methanolic stem bark extract of this plant also exhibited anti-inflammatory activity on oedema induced in rat (Olajide et al., 1997). Eyong et al. (2006) studied the chemical constituents of the root extract of *N. laevis* in which newbouldiaquinone was isolated. The compound exhibited antimicrobial and antimalaria activities.*J. curcas* and *N. laevis* are used as folklore remedies in the treatment of different ailments among many tribes in West Africa. This study therefore focused on the comparison of the antimicrobial activities of these two plants to support their usefulness in folklore remedies to combat the effect of pathogens. Research is continuing on the medicinal plants around us in an endeavour to find new compounds of therapeutical interest. Our investigation is thus one of such researches carried out by ethnobotanists.

MATERIALS and METHODS

Plant materials

Fresh leaves of *J. curcas* and *N. laevis* were collected in the month of May, 2007 in Abeokuta, Nigeria and were identified by Dr. H. C. Illoh of Botany Department, Obafemi Awolowo University, Ile Ife, Nigeria. Voucher samples were prepared and deposited in the Herbarium of Botany Department, Obafemi Awolowo University, Ile Ife, Nigeria for future reference. The leaves were later dried to a constant weight in hot-air oven at 40^{o}C, powdered and stored in an air-tight container for further use.

Preparation of the extract

Exactly 280 g each of the powdered leaves were separately extracted in cold using mixture of methanol and sterile distilled water in ratio 3:2 for 4 days. The supernatant collected was concentrated to dryness *in vacuo* using rotary evaporator. The yield collected for *J. curcas* was 35% (w/w) while that of *N. laevis* was 38% (w/w).

Preparation of micro-organism for the experiment

The following typed cultures and locally isolated organisms obtained from culture collection of Dr. D. A. Akinpelu of Microbiology Department, Obafemi Awolowo University, Ile Ife, Nigeria were used for the experiment. These bacteria isolates include Gram-positive *Bacillus subtilis* (NCIB 3610), *Staphylococcus aureus* (NCIB 8588), *Streptococcus faecalis* (NCIB 775), *Bacillus stearothermophilus* (NCIB 8222), *Bacillus polymyxa* (LIO), *Bacillus anthracis* (LIO), *Staphylococcus epidermidis* (LIO), *Clostridium sporogenes* (NCIB 532), and *Corynebacterium pyogenes* (LIO). The Gram-negative include *Escherichia coli* (NCIB 86), *Pseudomonas aeruginosa* (NCIB 950), *Pseudomonas fluorescens* (NCIB 3756), *Klebsiella pneumoniae* (NCIB 418). For the experiment the bacterial isolates were first sub-cultured in nutrient broth (Oxoid, Ltd.) and incubated at 37^{o}C for 18-h.

Phytochemical screening test for the extract

A small portion of the dry extract was subjected to the phytochemical test using Trease and Evans (1983); Harbourne (1983) methods to test for alkaloids, tannins, flavonoids, steroids and saponins.

Test for alkaloids

Exactly 0.5 g of the plant extract was dissolved in 5 ml of1% HCl on steam bath. A millilitre of the filtrate was treated with drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloids.

Test for tannins

About 1 g of the extract was dissolved in 20 ml of distilled water and filtered. Two to three drops of 10% of $FeCl_3$ was added to 2 ml of the filtrate. The production of a blackish-blue or blackish-green colouration was indicative of tannins. To another 2 ml of the filtrate was added 1 ml of bromine water. A precipitate was taken as positive for tannins.

Test for flavonoids

A 0.2 g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange colouration was indicative of the flavonoids.

Test for saponins

Freshly prepared 7% blood agar medium was used and wells were made in it. The extract in methanol was applied with distilled water and methanol used as negative control while commercial saponin (BDH) solution was used as positive control. The plates were incubated at 35^{o}C for 6-h. Complete haemolysis of the blood around the extract was indicative of saponins.

Test for steroids

About 0.5 g of the extract was dissolved in 3 ml of $CHCl_3$ and filtered. To the filtrate was added concentrated H_2SO_4 to form a lower layer. A reddish brown colour was taken as positive for steroid ring.

Sensitivity testing of the two plant extracts on bacterial isolates

The sensitivity testing of the plants extracts were determined using agar-well diffusion method as described by Irobi et al. (1994),

Russell and Furr (1977) with little modifications. The bacterial isolates were first grown in nutrient broth for 18 h before use. The isolates were later subcultured on to Mueller-Hinton agar (Oxoid, Ltd.). Wells were then bored into the agar medium using a sterile 6 mm cork borer. The wells were then filled up with 100 µl of the solution of the extract and care was taken not to allow the solution to spill to the surface of the medium. The plates were allowed to stand on the laboratory bench for between 1-2 h to allow proper inflow of the solution into the medium before incubating the plates in an incubator at 37°C for 24 h. The plates were later observed for the zones of inhibition. The effects of the extract on bacterial isolates were compared with those of standard antibiotics, streptomycin and tetracycline at a concentration of 1 mg/ml each.

Minimum inhibitory concentrations (MICs) of the extracts on the bacterial isolates

The MIC of the extract was determined using method of Akinpelu and Kolawole (2004). Two-fold dilutions of the plant extract was prepared and 2 ml of different concentration of the solution was added to 18 ml of pre-sterilized molten nutrient agar at temperature of 40°C to give final concentrations ranging between 0.040 and 10 mg/ml. The medium was then poured into sterile Petri dishes and allowed to set. The surface of the medium was allowed to dry before streaking with 18 h old isolates. The plates were later incubated in an incubator at 37°C for up to 72 h after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that will prevent the bacterial growth. Minimum bactericidal concentrations (MBC) of the plants extracts on bacterial isolates. The MBC of the extract was determined in accordance with the method of Olorundare et al. (1992) with modification. Samples were taken from plates with no visible growth in the MIC assay and subcultured on to freshly prepared nutrient agar medium and later incubated at 37°C for 48 h. The MBC was taken as the lowest concentration of the extract that did not allow any bacterial growth on the surface of the agar plates.

RESULTS AND DISCUSSION

The investigations carried out on the two plants, viz*., J. curcas* and *N. laevis* showed that extracts from the plants possess antibacterial activities both at a final concentration of 20 mg/ml. The zones of inhibition exhibited by *J. curcas* ranged between 12 and 17 mm, while that of *N. laevis* ranged between 10 and 23 mm. On the other hand zones of inhibition exhibited by streptomycin are between 15 and 30 mm (Table 1). The minimum inhibitory concentration exhibited by *J. curcas* extract against the tested bacterial isolates ranged between 5.00 and 10.00 mg/ml while those of *N. laevis* extract varied between 1.563 and 10.00 mg/ml (Table 2). From all indications, *N. laevis* exhibited stronger activity against the tested bacterial isolates than *J. curcas*. The standard streptomycin showed MIC values ranging between 0.0313 and 0.500 mg/ml. The results indicated that streptomycin has stronger activity when compared with those of the two plants extracts which is expected considering that it is a pure compound. *J. curcas* leaf extract was active against such pathogens as *E. coli*, *Staphylococcus aureus* and in particular *Pseudomonas aeruginosa* known to be resistant to most synthetic antibiotics. Pathogens which include *Clostridium sporogenes, E. coli*, *Klebsiella pneumoniae* and *Streptococcus faecalis* were susceptible to the *N. laevis* extract. These results further corroborate the usefulness of these two medicinal plants in folklore remedies among many tribes in West Africa. The phytochemical analysis conducted on *J. curcas* extract revealed the presence of tannins, alkaloids, steroids and saponins while *N. laevis* leaf extract contains flavonoids, tannins and alkaloids (Table 3). These phytochemical compounds are known to support bioactive activities in medicinal plants and thus supported the antimicrobial activities of these plant extracts used in this study. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer (Motar et al., 1985; Li et al., 2003). Thus*, J. curcas* and *N. laevis* containing this compound may serve as sources of bioactive compound in the treatment of cancer if such extracts from these plants are improved upon by chemists. *N. laevis* leaf extract contains flavonoids while this was absent in *J. curcas* extract. Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A_2 (Hausteen, 1983), and this property may explain the mechanisms of antibacterial action of *N. laevis* against the test bacteria. Flavonoids serve as health promoting compound as a results of its anion radicals (Ferguson, 2001). These observations support the usefulness of *N. laevis* in folklore remedies in the treatment of infections caused by bacteria. Alkaloid was among the phytochemical compounds present in the extracts of the two plants studied. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their toxicity against cells of foreign organisms. Extensive studies have been carried out on their potential use in the elimination and reduction of human cancer cell lines (Nobori et al., 1994). Thus, *J. curcas* and *N. laevis* can therefore serve as source of drug that can be used in the treatment of cancerous cells in future. *J. curcas* extract revealed the presence of saponins which are absent in *N. laevis* extract. Saponins are known to produce inhibitory effect on inflammation (Just et al., 1998) and are key ingredient in traditional Chinese medicine and thus responsible for most of the observed biological effects (Liu and Henkel, 2002). These facts justified the use of *J. curcas* in traditional medicine. Lastly, steroids were observed only in *J. curcas* extract and not in *N. laevis* leaf extract and this phytochemical compound are of importance and interest due to their relationship with such compounds as sex hormone (Okwu, 2001). The presence of these phenolic compounds in *J. curcas* and *N. laevis* contributed to their antimicrobial properties and thus the usefulness of these plants in herbal medicament. Phenols have been found to be useful in the preparation of some antimicrobial compounds such as dettol and cresol. *J. curcas* and *N. laevis* are used routinely among many tribes in West Africa for the treatment of various diseases

Table 1. Sensitivity patterns of zones of inhibition exhibited by *J. curcas* and *N. laevis* extract on the test isolates.

Bacterial Isolates	Zones of inhibition (mm)*			
	J. curcas **(20 mg/ml)**	***N. laevis*** **(20 mg/ml)**	**STR (1 mg/ml)**	**TET (1 mg/ml)**
Bacillus anthracis (LIO)	0	11	18	25
Bacillus polymyxa (LIO)	12	0	15	20
Bacillus stearothermophilus (NCIB 3222)	0	0	23	22
Bacillus subtilis(NCIB 3610)	13	23	20	22
Clostridium sporogenes (NCIB 532)	0	20	25	20
Corynebacterium pyogenes (LIO)	12	10	20	20
Escherichia coli (NCIB 86)	15	12	0	18
Klebsiella pneumoniae (NCIB 418)	0	18	0	12
Pseudomonas aeruginosa (NCIB 950)	14	0	21	0
Pseudomonas fluorescens (NCIB 3756)	12	0	30	0
Staphylococcus aureus (NCIB 8588)	15	0	21	0
Staphylococcus epidermidis (LIO)	17	10	21	10
Streptococcus faecalis (NCIB 775)	0	23	23	28

*Values are means of replicates; NCIB, National Collection of Industrial Bacteria; LIO, Locally Isolated Organism; STR, Streptomycin; TET, Tetracycline.

Table 2. The minimum inhibitory concentrations of leaves extract of *J. curcas, N. laevis* and streptomycin.

Microorganism	Minimum inhibitory concentration (mg/ml)		
	J. curcas	***N. laevis***	**Streptomycin**
Bacillus anthracis (LIO)	ND	10.00	0.313
Bacillus polymyxa (LIO)	5.00	ND	0.0625
Bacillus subtilis (NCIB 3610)	5.00	0.625	0.0625
Clostridium sporogenes (NCIB 532)	ND	0.313	0.0625
Corynebacterium pyogenes (LIO)	10.00	10.00	0.0313
Escherichia coli (NCIB 86)	5.00	5.00	ND
Klebsiella pneumoniae (NCIB 418)	ND	1.563	ND
Pseudomonas aeruginosa (NCIB 950)	5.00	ND	0.0625
Pseudomonas fluorescens (NCIB 3756)	10.00	ND	0.0625
Staphylococcus aureus (NCIB 8588)	5.00	ND	0.0625
Staphylococcus epidermidis (LIO)	0.625	10.00	0.5
Streptococcus faecalis (NCIB 775)	ND	10.00	0.0625

ND, Not done; NCIB, National Collection of Industrial Bacteria; LIO, Locally Isolated Organism.

Table 3. Phytochemical compounds present in the extracts of the leaves of *J. curcas* and *N. laevis*.

Phytochemical compounds	***J. curcas***	***N. laevis***
Alkaloids	Positive	Positive
Flavonoids	Negative	Positive
Saponins	Positive	Negative
Steroids	Positive	Negative
Tannins	Positive	Positive

ranging from dysentery, diarrhoea, chest pain, stomach disorder and gastrointestinal infections. These plants are used to prepare decoction which are consumed by the patients with no documented report of toxicity so far. Hence, these plants stand as potential candidates as sources of ingredients for drug formulations for the treatment of bacterial infections.

ACKNOWLEDGEMENTS

We wish to thank Govan Mbeki Research and Develop-

mental Centre (GMRDC) of the University of Fort Hare, Alice, South Africa for the postdoctoral fellowship granted the first author. Dr. HC Illoh of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, is appreciated for identifying the plant used in this study.

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