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Full Length Research Paper

Validating the medicinal potential of *Leptadenia hastata*

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***Leptadenia hastata* (Pers.) Decne is a wild plant used as vegetable by many African populations and medicine due to its nutritive and therapeutic properties for the treatment of wounds and stomach upset in children. Acetone, methanol and aqueous extracts from its leaves were investigated against five selected bacterial species and two fungal species. Aqueous extract markedly inhibited the growth of *Salmonella paratyphi* and *Escherichia coli* at 30 mg/ml and *Pseudomonas aeruginosa* at 60 mg/ml. The activity exhibited by the methanol extract was generally low and acetone extract did not show any activity against the tested organisms. The result of antimycotic assay showed that methanol extract suppressed the growth of *Fusarium oxysporum* and *Aspergillus niger* at 80 mg/ml with inhibition percentages ranging from 58.89 to 73.30%. The activity of acetone extract was low with 40 and 50% inhibition on the growth of *A. niger* and *F. oxysporum* respectively. The result obtained in this study has provided a scientific support for the claimed ethnomedical uses of aqueous extracts of *L. hastata* in the treatment of bacterial diseases and suggest the potential of methanol extract as a source of antifungal agent.**

Keywords: *Leptadenia hastata,* antibacterial, antifungal, wild vegetable, ethnomedicine.

INTRODUCTION

The multiple roles of wild traditional vegetables as both food and medicinal sources have been widely documented (Lee et al., 2003; Ogle et al., 2003; Adebooye and Opabode, 2004; Ayodele, 2005). Wild vegetables have been reported to contain comparatively high amounts of Vitamins A and C and other antioxidant micronutrients (Szeto et al., 2002; Jimoh et al., 2008), promote good health by assisting in preventing cancer and high blood pressure, stimulating the immune system, improving drug metabolism, and tissue regeneration (Rayner, 1998; Krebs-Smith and Kantor, 2001; Walingo, 2005) . It has become obvious that food and medicine are closely related (Etkin, 1996).

Leptadenia hastata (Pers.) Decne belongs to the family Asclepiadaceae, used as food by many African popula-tions (Hutchinson and Dalziel, 1937) . It is commonly used as a vegetable and is considered as a famine food due to its high content of valuable nutrients in Niger (Freiberger et al., 1998; Sena et al., 1998). The major chemical com-pounds found in *L. hastata* were: triterpenes, fatty acids, amino acids, poly-oxypregnane, lutein, -carotene (Aquino et al., 1996; Nikièma et al., 2001). It is also used in herbal medicine against milk drying, sex-impotence, trypanosomosis, acute rhinopharyngitis and wounds (Neuwinger, 1996; Tamboura et al., 2005). The leaves are often chewed by shepherds against polydipsia and mouth dryness (Olivier-Bover, 1986). In some part of northern Nigeria, leaves extract is used for the treatment of stomach upset in children (Aliero et al., 2001). As far as our literature survey could ascertain, there is no information about the antimicrobial activity of *L*. *hastata*. The aim of this work was to evaluate the medicinal potential of *L*. *hastata* by bioassay screening against selected species of bacteria and fungi to validate its use in ethnomedicine.

MATERIALS AND METHODS

Plant material and extract preparation

The leaves of *L. hastata* were collected from a natural population on the campus of the Usmanu Danfodiyo University, Sokoto, Nigeria and air-dried at ambient temperature and pulverized into powder. A portion (100 g) each of the material was extracted sepa-rately in acetone, methanol and water for 18-24 h. Each extracts was filtered through Whatman No.1 filter paper and concentrated to dryness at 40^{o}C. The dried extracts obtained were reconstituted in different solvents and used for the determination of antimicrobial activities.

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Table 1. Antibacterial activity of *L. hastate*.

Extractant Conc. mg/ml		Diameter of inhibition zone (mm)				
		1	2	3	4	5
Aqueous	30	-	-	16.50	22.50	-[a]
	60	-	-	23.00	26.00	15.00
	90	-	-	26.00	29.00	17.00
Methanol	30	-	-	-	-	-
	60	-	-	15.50	18.00	-
	90	-	-	17.50	19.50	-
Acetone	30	-	-	-	-	-
	60	-	-	-	-	-
	90	-	-	-	-	-
Tetracycline	(0.33)	22.67	21.67	17.33	25.50	17.33

Values are means of three replicates
Key: -[a] = < 8.0mm
1. *Staphylococcus aureus* 2. *Salmonella paratyphi* 3. *Escherichia coli,*
4. *Bacillus metagerium 5. Pseudomonas aeruginosa.*

Antibacterial assay

Laboratory isolates of five bacteria species which included Gram positive and Gram negative strains were obtained from the Department of Microbiology of the Usmanu Danfodiyo University Teaching Hospital Sokoto, Nigeria. They were; *Bacillus metagarium*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella paratyphi* and *Pseudomonas aeruginosa*. Each organism was maintained on nutrient agar slants and recovered by sub-culturing in nutrient broth (Antec Diagnostic Products, UK) for 24 h.

Before use, each bacterial culture was standardized with fresh sterile nutrient broth. The organisms were suspended in prepared broth and incubated at 37°C for 24 h. They were then inoculated into prepared molten agar medium by surface plating method. Paper disc were impregnated with various concentrations of the extracts at 30, 60 and 90 mg/ml and 0.33 mg/ml tetracycline was used as a positive control. The paper discs prepared earlier were placed at 45° opposite to each other on the plate containing the bacterial culture, and plates were incubated at 37°C for 24 h. Clear zones of inhibition were observed after the incubation period (Cheesbrough, 2000). Diameters of the zones of growth inhibitions were measured for each concentration of the extract using a metre rule and diameter < 8.0 mm indicates low sensitivity, while > 8 mm indicates high sensitivity (Collee et al., 1989).

Antifungal assay

The cultures of *Aspergillus niger* and *Fusarium oxysporum* were obtained from Mycology Laboratory, Usmanu Danfodiyo University, Sokoto. The culture was maintained on Potato dextrose agar (PDA) and was recovered for testing by sub- culturing on fresh PDA for 3 days at 25^{O}C. PDA was prepared and autoclaved before the addition of the extracts. Extracts were mixed with the molten agar (at 45^{O}C) to final concentrations of 5, 10, 20, 40 and 80 mg/ml and poured into Petri dishes. Each plate was swirled carefully until the agar began to set and left for the solvent to evaporate. Blank plates containing PDA served as control. The prepared plates were inoculated with plugs obtained from the actively growing margin of the fungi plates and incubated at 25^{O}C for 5 days (Afolayan and Meyer, 1997; Aliero et al., 2006). The diameter of the fungal growth was measured and expressed as percentage growth inhibition of three replicates. Significant differences within the means of the treaments and the controls were calculated using the LSD statistical test at 5% probability (Steel and Torrie, 1996).

RESULTS AND DISCUSSION

Methanol and water extracts from the leaves of *L. hastata* showed antibacterial activities (Table 1). However, little or no activity was observed from the acetone extracts. The action of *L. hastata* on *E. coli, B. metagarium* and *P. aeruginosa* is instructive. Although, *E. coli* belongs to the normal flora of humans, an enterohemmoragic strain of *E. coli* has caused serious food poisoning and preservatives to eliminate its growth are needed (Gulcin et al., 2003). The susceptibility of *P. aeruginosa* to the extract of this plant may be an indicator to its potential as a drug that can be used against this organism. Infections caused by *Pseudomonas* species are often difficult to combat (Salie et al., 1996). The growth of *S. aureus* and *Salmonella paratyphi* was not inhibited by the extracts at the tested concentration. The activity of this plant extracts against the Gram negative bacteria is quite noteworthy as high resistance of this group of bacteria has been earlier reported (Afolayan, 2003). In this study, aqueous extract was more active than the methanol extracts. This result is however, noteworthy. Traditionally, plant extracts are prepared with water as infusions, decoction and poultices; therefore it would seem likely that the traditional healer is able to extract those compounds which are responsible for activity in the methanol and water extracts. According to Tamboura et al. (2005), *L. hastata* is considered safe to use due to its high LD quotient value of 0.78.

The results of antifungal assays of *L. hastata* are depicted in Table 2. The extracts did not show any activity on the growth of fungal species studied at 5 mg/ml. However, methanol extract suppressed the growth of *F. oxysporum* and *A. niger* at 80 mg/ml with inhibition percentages ranging from 58.89 to 73.30%. The activity of acetone extract on the growth of *A. niger* and *F. oxysporum* was low with 40 and 50% inhibition respectively. The activity exhibited by the methanol extract of this species is instructive, as the fungus is known to have high resistance to most fungicides (Wedge et al., 2000; Erasto et

Table 2. Antifungal activity of *L. hastata*.

Extracts	Con. (mg/ml)	Growth inhibition (%)	
		A. niger	*F. oxysporum*
Acetone	5	0.00[c]	0.00[c]
	10	0.00[c]	0.00[c]
	20	0.00[c]	5.56[c]
	40	31.00[b]	26.30[b]
	80	40.00[a]	50.00[a]
Methanol	5	0.00[c]	0.00[c]
	10	12.00[c]	30.00[c]
	20	37.56[c]	44.10[c]
	40	46.67[b]	58.89[b]
	80	58.89[a]	73.30[a]

Values are means of percentage growth inhibition of three replicates. Values within a column followed by the same superscript are not significantly different at P<0.05 according to LSD test.

al., 2006). In addition, *F. oxysporum* is a phytopathogen that causes vascular wilt and damping off in plants which could result in substantial stand reduction and yield loss (Gerlach, 1954; Kishi, 1974). The resistance of *A. niger* to dichloromethane, aqueous and methanolic extracts of 14 plants used for traditional medicine in Paraguay has been reported (Portillo et al., 2001). In this investigation, however, methanol extracts suppressed the growth of *A. niger* significantly. Water extract did not show any appreciable activity on the growth of the fungi at 5 mg/ml or lower, except on *F. oxysporum* which was weakly (36.56%) suppressed by the extract. The result obtained in this study has provided a scientific support for the claimed ethnomedical uses of aqueous extracts of *L. hastata* in the treatment of bacterial diseases and suggest its potential as antifungal agent that could be useful in the current search of antimycotic agent from plants.

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