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 document-ted (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 populations (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 compounds found in *L. hastata* were: triterpenes, fatty acids, amino acids, poly-oxypregnane, lutein, β-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 separately 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°C. The dried extracts obtained were reconstituted in different solvents and used for the determination of antimicrobial activities.

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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

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

Values are means of three replicates

Key: $-^a = < 8.0$ mm

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°C. PDA was prepared and autoclaved before the addition of the extracts. Extracts were mixed with the molten agar (at 45°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°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 guite 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

^{1.} Staphylococcus aureus 2. Salmonella paratyphi 3. Escherichia coli,

^{4.} Bacillus metagerium 5. Pseudomonas aeruginosa.

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

Extracts	Con. (mg/ml)	Growth inhibition (%)		
LAHACIS	Con. (mg/m)	A. niger	F. oxysporum	
Acetone	5	0.00 ^c	0.00 ^d	
	10	0.00^{c}	0.00 ^d	
	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 ^e	0.00 ^e	
	10	12.00 ^d	30.00 ^d	
	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.

REFERENCES

- Adebooye OC, Opabode JT (2004). Status of conservation of the indigenous leaf vegetables and fruits of Africa. Afr. J. Biotech. 3: 700–705.
- Afolayan AJ (2003). Extracts from the shoots of *Arctotis arctotoides* inhibit the growth of bacteria and fungi. Pharm. Biol. 41: 22-25.
- Afolayan AJ, Meyer JJM (1997). The antibacterial activity of 3, 5, 7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens*. J. Ethnopharmacol. 57: 177-181.
- Aliero BL, Umar MA, Suberu HA, Abubakar A (2001). A hand Book of common plant in northern Western Nigeria pp. 78.
- Aliero AA, Grierson DS, Afolayan AJ (2006). Antifungal activity of Solanum pseudocapsicum. Res. J. Bot. 1(3): 129-133.
- Aquino R, Peluso G, DE Tommassi N, DE Simone F, Pizza C (1996). New polyoxypregnane ester derivates from *Leptadenia hastata*. J. Nat. Prod. 59(6): 555-564.
- Ayodele AE (2005). The medicinally important leafy vegetables of south western Nigeria. Ethnobotanical leaflets. Web journal http://www.siu.edu/~ebl/leaflets/ayodele.htm).
- Cheesbrough M (2000). District Laboratory Practice in Tropical Countries, low price edition. The press syndicate of the University of Cambridge, Trumpington Street Cambridge part two. pp. 157-206.
- Collee JG, Grulo JP, Fraser AG, Marmion BP (1989). In: Mackie and McCartney. Practical medical microbiology; 13th edition: Churchill and living stone. New York pp. 76.
- Cowan MM (1999). Plant products as antimicrobial agents. Clin.

- Microbiol. Rev. 12: 564.
- Darmon N, Darmon M, Maillot M, Drewnowski AA (2005). Nutrient Density Standard for Vegetables and Fruits: Nutrients per Calorie and Nutrients per Unit Cost. J. Am. Diet Assoc. 105: 1881 – 1887.
- Etkin NL (1996). Medicinal cuisines: Diet and ethnopharmacology. Int. J. Pharmacognosy 34: 313-326.
- Etkin NL, Ross PJ (1982). Food as medicine and medicine as food: An adaptive framework for the interpretation of plant utilization among the Hausa of northern Nigeria. Soc. Sci. and Med. 16: 1559-1573.
- Erasto P, Grierson DS, Afolayan AJ (2006). Bioactive lactones from the leaves of *Vernonia amydalina*. J. Ethnopharmacol. 106: 117-120.
- Freiberger CE, Vandergagt DJ, Pastuszyn A, Glew RS, Mounkarla G, Millson M, Glew RH (1998). Nutrient content of edible leaves of seven wild plants from Niger. Plant. Foods. Hum. Nutr. 53(1): 57-69.
- Gulcin I, Oktay M, Kirecci E, Kufrevioglu IO (2003) Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. Food Chem. 83: 371-382.
- Hutchinson J, Dalziel JM (1937). Leptadenia, the useful plants of tropical Africa. London (1): 387–388.
- Jimoh FO, Adedapo AA, Aliero AA, Afolayan AJ (2008). Polyphenol Contents and Biological Activities of *Rumex ecklonianus*. Pharm. Biol. 46(5): 333-340.
- Kishi K (1974). Disease and pest control in enclosed environments in Japan. Outlook Agric. 8: 100-104.
- Krebs-Smith SM, Kantor LS (2001). Choose a variety of fruits and vegetables daily: understanding the complexities. J. Nutr. 131, 487S–501S supplement.
- Lee Y, Cesario T, Wang Y, Shanbrom E, Thrupp L (2003). Antibacterial activity of vegetables and juices. Nutrition 19: 994–996.
- Liu S, Manson JE, Lee IM, Cole SR, Hennekens CH, Willett WC, Buring JE (2000). Fruit and vegetable intake and risk of cardio vascular disease: The women's health study. Am. J. Clin. Nutr. 72: 922-928.
- Mathekga ADM, Meyer JJM (1998). Antibacterial activity of South African *Helichrysum* species. South Afr. J. Bot. 64(5): 239-295.
- Nascimento GF, Locatelli J, Freitas PC, Silva GL (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz. J. Microbiol. 31: 247.
- Neuwinger HD (1996). African ethno botany: Poisons and drugs. Chapman & Hall 280-296.
- Nikiema JB, Vanhaelen-Fastre R, Vanhaelen M, Fontaine J, Gracf CDE, Heenen M (2001). Effects of anti-inflammatory triterpenes isolated from *Leptadenia hastata* latex on keratinocyte proliferation. Phytother. Res. 15(2): 131-134.
- Ogle BM, Tuyet HT, Duyet HN, Xuan D, Nguyen N (2003). Food, feed or medicine: the multiple functions of edible wild plants in Vietnam. Econ. Bot. 57: 103–117.
- Olivier-Bover BEP (1986). Medicinal plants in tropical West Africa. 1P Ed. P. 375.

- Portillo A, Vila R, Freixa B, Adzet T, Canigueral S (2001). Antifungal activity of Paraguayan plants used in traditional medicine. J Ethnopharmacol. 76: 347-354.
- Rayner M (1998). Vegetables and fruits are good for us so why don't we eat more? Br. J. Nutr. 80: 119-120.
- Salie F, Eagles PFK, Leng HMJ (1996). Preliminary antimicrobial screening of four South African Asteraceae species. Ethnopharmacol. 76: 347-354.
- Sena LP, Vanderjagt DJ, Rivera C, Tsin AT, Muhamada I, Millson M, Pastuszyn A, Glew RH (1998). Analysis of nutritional component of eight famine foods of the republic of Niger. Plant Foods Hum. Nutr. 52(1): 17- 30.
- Steel RGD, Torrie JH (1996). Principles and Procedures of Statistics. Mc Graw-Hill, New York, USA.
- Szeto YT, Tomlinson B, Benzie IFF (2002). Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implications for dietary planning and food preservation. Br. J. Nutr. 87: 55-59.
- Tamboura HH, Bayala B, Lompo M, Guissou IP, Sawadogo L (2005). Ecological distribution, morphological characteristics and acute toxicity of aqueous extracts of Holarrhena floribunda (G. Don) Durand & Schinz, Leptadenia hastata (Pers.) Decne and Cassia sieberiana (d c) used by veterinary healers in Burkina Faso. Afr. J. Trad. Cam. 2 (1): 13 - 24.

- Van't Veer P. Jansen MC, Klerk M, Kok FJ (2000). Fruits and vegetables in the prevention of cancer and cardiovascular disease. Public Health Nutr. 3(1): 103.
- Walingo KM (2005). Role of Vitamin C (Ascorbic acid) on human health—A review. Afr. J. Agric. Food, Nutr. Dev. 5: 1-13.
- Wedge DE, Galindo JCG, Macias FA (2000). Fungicidal activity of natural and synthetic sesquitepenes lactone analogs. Phytochemistry 53: 747:757.