Journal of Medicinal Plants Research Vol. 6(12), pp. 2256-2260, 30 March, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR10.233
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Chemical composition and antimicrobial activities of *Urena lobata* L. (*Malvaceae*)

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Accepted 21 June, 2011

The chemical composition and antimicrobial activity of the leaves of *Urena lobata* L. were investigated. The proximate analysis showed that the leaves contained moisture (7.21%), crude fibre (6.31%), carbohydrate (47.53%), crude protein (19.79%), fat (10.21%) and ash (8.95%). The mineral analysis in mg/100 g indicated that the leaves contained calcium (42.09), copper (0.31), iron (9.35), magnesium (35.38), manganese (0.81), phosphorus (24.91), potassium (35.38), sodium (29.48) and zinc (51.55). The phytochemicals detected in the leaves of *U. lobata* L. were alkaloids, cardiac glycoside, tannins, terpenoid and saponin; while flaonoid, phlobatanin and steroid were not detected. Antibacterial activities of the leaf extract showed that *Escherichia coli* was sensitive to the methanolic extract of the plant and had a zone of inhibition that varied from 1 - 4 mm with concentration that varied from 6.25 - 50 mg/ml. *Staphylococcus aureus* had zone of inhibition that varied from 1.0 - 3.0 mm, *Enterococcus* spp. had zone of inhibition that varied form 1.0 - 2.0 mm with concentration that varied between 25 - 50 mg/ml respectively. *Klebsiella* spp. had a zone of inhibition of 2.0 mm at 50 mg/ml concentration, while *Pseudomonas aeruginosa* was resistant to the extract at all the concentrations tested. The effect of methanolic extract of *U. lobata* L on radial mycelial growth of the test fungi revealed that *Botryodiplodia theobromae* had a percentage inhibition which varied from 20 - 50% with concentration of 6.25 - 50 mg/ml, however, *B. theobromae* was not sensitive at 3.13 mg/ml. However, *Rhizopus* spp. had a percentage inhibition that varied from 20 - 50% with concentration that varied from 12.5 - 50 mg/ml.

Key words: Chemical composition, phytochemical analysis, antimicrobial activities, *Urena lobata* L.

INTRODUCTION

Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, 2008; Gill, 1992). In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported to be safe and without any adverse side effect especially when compared with synthetic drugs and because of low income of the majority of the populace.

Thus, a search for new drugs with better and cheaper substitute of plant origin is a natural choice. The importance of higher plants cut across all aspects of life and economy of man. Compounds from plant materials have been reported to possess *in vitro* activities against pathogenic microorganisms (Bringman et al., 1999) and reduce or cure infections of microbial origin (Ekwuete, 1992; Kim et al., 2002). Medicinal plants have been formulated into powders, concoctions, decortions, soap and ointment. Various parts of plants are used which include the leaves, stem, roots, stem bark and fruits (Gbile and Adeshina, 1999).

Urena lobata L. is a member of the Malvaceae. The mallow family are found all over the world with a primary concentration in the tropics. There are about 110 genera and over 2,300 species divided into five tribes. The plant has pink flowers like a miniature hollyhocks. It grows to about 2 m high stellate trichomes (star-shaped plant hairs) which gives the leaves a grayish colour and raspy feel (Dalziel, 1937). The origin of the plant is not well known

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and it has been traced to South America, Florida and Australia but taxonomist believed it evolved in Africa. It is clutivated in Brazil and Congo for its fibres which are tough, flexible and used for making sack and twine (Wunderlin, 1998). The Nigerian local names are: akeri, ilasa-agbunrin, ilasa-omode (Yoruba), rama-rama (Hausa),Oronhon (Benin), ridiri (Efik) and Ebe-izili (Edo). The leaves and the whole plant are used as an emollient and expectorant. In Edo North, the herbalists use the juice of the leaves for treatment of dysentery. The plant is reputed for its antimicrobial properties (Gill, 1992). Phytochemical study of the aerial parts of the plant reported the presence of mangeferin and quercetin while imperation has been isolated from the roots (Keshab, 1976).

The aim of this work was to investigate the chemical composition, phytochemical properties and antimicrobial activities of the methanolic extract of the leaves of *U. lobata* L.

MATERIALS AND METHODS

Collection of plant materials

The fresh leaves of *U. lobata* L. (Malvaceae) were collected from a local farm in Ado-Ekiti, Ekiti-State, Nigeria. Identification and authetication were carried out in the herbarium section of the Department of Plant Sciences, University of Ado-Ekiti, Ekiti-State, Nigeria.

Processing of plant materials

The leaves of the plants were air-dried at room temperature for 30 days. This was grinded into fine powder using mortar and pestle. The powdered leaves materials were stored in a cool dry container until use.

Determination of antimicrobial activity

Source of microorganisms

The test bacteria used were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella* spp. and *Enterococcus* spp. while the fungi were *Botryodiplodia theobromae* and *Rhizopus* spp. They were obtained from the Department of Microbiology, University of Ado-Ekiti. The bacteria were maintained on Nutrient Agar (NA) and the fungi on Potato Dextrose Agar (PDA) and stored at 4°C until ready for use.

Standardizaion of inocula

The test bacteria were grown (in separate tubes) at 37°C in Mueller-Hilton (Oxoid)broth McFarland standard) at optical activity of 625 nm with Mueller- Hilton (Oxoid) broth and stored at 4°C to arrest further bacteria growth/multiplication (Bauer et al., 1966).

Antibacterial testing

This was determined using agar diffusion method (Gnanamanickam and Smith, 1980; Odeyemi and Fagbohun, 2005). About 0.2 ml of standardized 24 h old culture of each of the test organisms (containing 10^5 cfu/ml) was aseptically transferred to seed the agar plate. A sterile cork borer (8 mm diameter) was used to cut six wells on the agar plate. The wells in each plate were filled with various concentrations of the extracts 50, 25, 12.5, 6.25 and 3.13 mg/ml while sixth hole in centre was filled with the extracting solvent (methanol) as control. The tests were set up in duplicates. The dishes were incubated at 28°C for 18 – 24 h and zones of inhibition were measured in millimeter.

Antifungal testing

Radial mycelial growth assay technique of Smith (1978) and Odeyemi and Fagbohun (2005) were used whereby sterile plant extracts of the following concentration 50, 25, 12.5, 6.25 and 3.13 mg/ml were introduced aseptically into sterile petri-dishes. About 18 ml of sterilized PDA was added to each of the dishes containing the various concentration of the plant extracts. The plates were swirled carefully to ensure proper mixing and allowed to set. Mycelial discs (6 mm diameter) taken from the advancing edges of 3 – 5 days old culture of each of the test fungi on PDA were placed centrally on the cooled seeded agar plates, incubated at 28°C. The radial mycelial growth was measured every 24 h for 5 days. All the plates were in duplicate and the test carried out twice. Control plates were treated as described above using the extracting solvent (methanol) only.

Phytochemical analysis of *U. lobata* L.

Quantitative phytochemical screenings to determine the presence of alkaloids, tannins, saponins steroids, phlobatannin, terpenoids, flavonoid and cardiac glycosides using standard methods as described by Trease and Evans (1985), Harbone (1984) and Sofowora (2008) were carried out.

Proximate analysis

The proximate analyses of the sample for moisture, ash, fibre and fat were done by the method of AOAC (2005). The nitrogen was determined by micro-Kjeldahl method as described by Pearson (1976) and the percentage nitrogen was converted to crude protein by multiplying with 6.25. Carbohydrate was determined by difference. All determinations were performed in duplicates.

Mineral analysis

The mineral was analyzed by using a flame photometer (Model 405 Corning, UK), using NaCl and KCl to prepare the standards. Phosphorus was determined colometrically using Spectronic 20 (Gallenkap, UK) as described by Pearson (1976) with KH_2PO_4 as standard.

All other metals were determined by atomic absorption spectrophotometer (Pekin-Elmar Model 403, Norwalk CT, USA). All determinations were done in duplicates. All chemicals used were analytical grade (BDH, London). Earlier, the detection limit of the metals was determined according to Techtron (1975). The optimum analytical range was 0.1 - 0.5 absorbance unit with a coeffient of variation of 0.87 - 2.20%. All the proximate values were reported as percentage while the minerals were reported as milligram/100 grams.

RESULTS AND DISCUSSION

The results of proximate analysis of *U. lobata* L. leaves are

Table 1. Results of proximate analysis of *U. lobata* L. (Malvaceae) (%).

Test	Percentage of dry sample
Ash content	8.95
Carbohydrate	47.53
Crude protein	19.79
Fat	10.21
Crude fibre	6.31
Moisture content	7.21

Table 2. Results of mineral analysis of *U. lobata* L. (Malvaceae) (mg/100 g).

Test	Results (mg/100 g)
Calcium	42.09
Copper	0.31
Iron	9.35
Magnessium	35.38
Manganese	0.81
Phosphorus	24.91
Sodium	29.48
Zinc	51.55

shown in Table 1. The plant contained higher amount of carbohydrate 47.53%. This was similar to the findings of Abolaji et al. (2007) who reported that *Blighia sapida* contained 44.09% carbohydrate but is higher than that of *Senna obtusfolia* 23.70% and *Amaranthus incurvatus* 39.05% (Faruq et al., 2002). It is however, lower than the value for *Corchorus tridens* 75.0% and sweet potato leaves 82.8% (Asibey-Berko and Taiye., 1999). Carbohydrates are essential for the maintenance of life in both plants and animals and also provide raw materials for many industries (Ebun-Oluwa et al., 2007). The plant is a good source of carbohydrate when consumed because it meets the recommended dietary allowance (RDA) values (FND, 2002).

The plant contained crude protein value of 19.79% which is higher than the value reported for *Momordica balsania* L. 11.29% and *Lesianthera africanas* 13.1% (Isong and Idiong, 1997), but lower than those value reported for *Piper guineeses* 29.78% and *Talinum. triangulare* 31.00% (Etuk et al., 1998; Akindahunsi and Salawu, 2005). However, it compared favourably with the value reported for *Gnetum africana* 17.50% and *Leptadenia hastata* by Ekop (2007). The plant is considered a good source of protein because it provides more than 12% of caloric value from protein (Pearson, 1976).The leaves contained 10.21% of crude fat which is a moderate amount when compared to those of *T. triangulare* 5.9%, *Baseila alba* 8.71% and *Amaranthus hybridus* 4.80% (Akindahunsi and Salawu, 2005). Dietary fat increases the palatability of food by absorbing and retaining flavours (Antia et al., 2006). A diet providing 1 - 2% of its caloric of energy as fat is said to be sufficient for human being as excess fat consumption is implicated in certain cardiovascular disorders (Antia et al., 2006).The moisture content value for the leaves of *U. lobata* L. was 7.21% which is relatively low, therefore it would hinder the growth of spoilage microorganisms and enhance the shelf life.

The ash content of *U. lobata* L. leaves was 8.95% which is lower than the value reported for the leaves of *T. triangulare* 20.05% (Ifon and Bassir, 1980; Ladan et al., 1996), *Ipomea batatas* 11.10%, *Vernonia colorate* 15.86% and *Moringa oleiifera* 15.09% (Lockett et al., 2000; Antia et al., 2006). It is however higher than that of some Nigerian leafy vegetables such as *Ocimum gratissium* 8.0% (Akindahunsi and Salawu, 2005).

The crude fibre of *U. lobata* was 6.31%. This value compares favourably with those reported for *I. batatas* 7.20%, *T. triangulare* 6.20% and *P. guineeses* 6.40% (Akindahunsi and Salawu, 2005).

The mineral composition of *U. lobata* L. leaves in mg/100 g were shown in Table 2. It contained sodium 29.45 mg/100 g and potassium 35.38 mg/100 g. The ratio of sodium to potassium is less than 1(0.8); therefore consumption of the plants would reduce high blood pressure disease because Na:K is less than one as recommended by FND (2002).

The value of calcium and phosphorus in the leaves of *U. lobata* L. were 42.09 mg/100 g and 24.91 mg/100 g respectively. Calcium and phosphorus are associated with each other for growth and maintenance of bones, teeth and muscles (Okaka et al., 2006). The phosphorus content of the leaves 24.91 mg/100 g compared favourably with that of *I. batatas* 37.28 mg/100 g (Antia et al., 2006). For good calcium and phosphorus intestinal absorption, calcium and phosphorus ratio should be close to 1 or unity (Gull-Guerrero et al., 1998) hence, calcium and phosphorus would be well absorbed in the intestine because the ratio or Ca:P in leaves was (0.6) close to unity.

Magnessium content of the leaves of *U. lobata* L. was found to be 35.38% mg/100 g. This value is high when compared to that of *Xylopia aethiopica* 24 mg/100 g (Abolaji et al., 2007). Magnessium is a component of chlorophyll and it is an important mineral element in connection with ischemic heart disease and calcium metabolism in bones (Ishida et al., 2000).

The values of copper and manganese in the leaves of *U. lobata* L. were 0.31 mg/100 g and 0.81 mg/100 g respectively. This suggests that the leaves of *U. lobata* L. does not contribute or rather cannot be used as a substitute for blood forming leafy vegetables because it fell far below RDA values (Bogert et al., 1973).

Iron content of the leaves of *U. lobata* L. was 9.35 mg/100 g. This value compared favourably with the value reported for *I. batatas* 16.00 mg/100 g by Antia et al.

Table 3. Results of phytochemical analysis of *U. lobata* L. (Malvaceae).

Test	Results
Alkaloid	+ve
Cardiaglycoside	+ve
Flavonoid	-ve
Phlobatanin	-ve
Tannins	+ve
Terpenoid	+ve
Saponin	+ve
Steroid	-ve

+ve = Presence of constituents; -ve = Absence of constituents.

Table 4. Antibacterial activity of methanolic extract of *U. lobata* L. (Malvaceae) on some selected bacterial using punch agar method (Zone of Inhibition in mm).

	Concentrations					
	50 mg/ml	**25 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**	**3.13 mg/ml**	**Control**
Escherichia coli	4.0	4.0	2.0	1.0	0.0	0.0
Staphylococcus aureus	3.0	1.0	0.0	0.0	0.0	0.0
Klebsiella species	2.0	0.0	0.0	0.0	0.0	0.0
Enterococcus species	2.0	1.0	0.0	0.0	0.0	0.0
Pseudomonas aeruginosa	0.0	0.0	0.0	0.0	0.0	0.0

(2006) but low when compared to the vaules of other green leafy vegetables (Ibrahim et al., 2001). Iron is an essential element for haemoglobin formation, normal functioning of central nervous system and oxidation of carbohydrate, protein and fats (Adeyeye and Otokili, 1999). The leaves are a good source of iron because it meets the RDA value of 11.2 mg (Bogert et al., 1973).

The zinc value for the leaves of *U. lobata* L. was 51.55 mg/100 g. This is the most abundant mineral found in the leaves in this work. Zinc is involved in normal function of immune system and is a component of over 50 enzymes in the body (Okaka et al., 2006). The leaves are a good source of zinc because it is far above 6.23 mg recommended by RDA (Borgert et al., 1973).

The result of phytochemical analysis of leaves of *U. lobata* L. are shown in Table 3. The plant contained alkaloid, saponin, tannins, terpenoid and cardiac glycoside while flavonoid, steroid and phlobatanin were absent. Alkaloids has been found to have microbiocidal effect and the major anti-diarrheal effect is probably due to their effects on small intestine and antihypertensive effect (Trease and Evans, 1978) Some alkaloids are useful against HIV infection as well as intestinal infection associated with AIDS (McDevitt et al., 1976). Saponin was detected in the leaves of *U. lobata* L. This compound has been reported to have antihyper-cholesterol, hypotensive and cardiac depressant properties (Trease and Evans, 1985; Ghoshal, 1996). The plant also showed the presence of tannins. The presence of tannin in *U. lobata* L. suggests the ability of the plant to play a major role as anti diarrheaic and anti-haemorrhagic agent (Asquith and Butler, 1986). The leaves of *U. lobata* L. showed a positive result for cardiac glycoside, which have been used for two centuries as stimulants in treatment of cardiac failure and diseases (Trease and Evans, 1985; Olayinka et al., 1992). This perhaps justifies its use by local herbalists for treatment and management of hypertension (Cowan, 1999). The plant also showed presence of terpenoids. The anti-bacterial and anti-protozoan properties of this compound have been reported by Ghoshal et al. (1996); and Mendoza et al. (1997).The result of antibacterial activities of methnolic extract of leaves of *U. lobata* L. are shown on Table 4. *E. coli* was sensitive to the methanolic extract with zones of inhibition that varied from 1.00 to 4.00 mm at concentration that varied from 6.25 – 50 mg/ml while *S. aureus* had zone of inhibition that varied from 1.0 - 3.0 mm. *Enterococcus* sp. had zone of inhibition that varied from 1.0 - 2.0 mm at concentration that varied between 25 - 50 mg/ml. However, *P. aeruginosa* was not sensitive to the extract at all the concentrations tested. The antifungal activities of the methanolic extract of *U. lobata* L. on the radial mycelia growth of the test fungi are shown shown in Table 5. *B. theobromae* had a percentage of inhibition that varied from 20 - 50% at concentration of 6.25 - 50 mg/ml while *Rhizopus* sp. had a percentage of inhibition that varied from 20 - 50% at concentration of

Table 5. Effects of the methanolic extract of *U. lobata* L. (Malvaceae) on the radial mycelial growth on the test fungi (mm).

Concentration of extract (mg/ml)	*Botrydiplodia theobromae*			*Rhizopus* species		
	Control	Test	% inhibition	Control	Test	% inhibition
50	34	17.0	50	39	19.0	50
25	34	21.0	40	39	26.0	30
12.5	34	24.0	30	39	32.0	20
6.25	34	29.0	20	39	22	40
3.13	34	34.0	0	39	23.0	40

12.5 - 50.0 mg/ml. From the above results *U. lobata* L. may serve as a constituent of human diet supplying the body with minerals, protein and energy. The use of the plant by traditional practitioners to treat bacterial and fungal diseases is therefore justified.

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