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Research Article

EXPLORING THE PHARMACOGNOSTIC CHARACTERISTICS AND ANTIMICROBIAL POTENTIAL OF LEAVES OF *URENA LOBATA* LINN.

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ABSTRACT

Although herbal drugs are known to elicit their response quite slowly but they are now overtaking allopathic medicine owing to its high cost, side effects, drug resistance and development of tolerance. This article spots some light on an obscured herbal drug named *Urena lobata* Linn (Malvaceae) for its antimicrobial activity and also provides some pharmacognostic studies of the drug.

Keywords: *Urena lobata*, Malvaceae, Pharmacognostic studies, Antimicrobial activity.

INTRODUCTION

Herbal medicines have been a main source of primary healthcare all over the world[1].According to the World Health Organization, more than 70% of the world's population must use traditional medicine to satisfy their principal health needs[2].India is the largest producer of medicinal herbs and is called as botanical garden of the World. Herbal medicine is gaining popularity both in developing and developed countries because of their natural origin[3]. Current estimates suggest that, in many developing countries, about two-third of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs[4]. India has an officially recorded list of 45,000 plant species and a various estimation of 7500 species of medicinal importance[5]. Globally sales of herbal medicines are growing by about 10% annually. Over 25% of our common medicines contain at least some compounds obtained from plants. The use of traditional medicines increased mainly due to the failure of modern medicines to provide effective treatment for chronic diseases and emergence of multidrug resistant bacteria and parasites[6]. Herbal drugs referred as plant materials or herbalism, involves the use of whole plant or parts of plant, to treat injuries or illnesses. The wide acceptance of herbal medicines are due to low/minimum cost , potency and efficiency , enhanced tolerance , more protection , fewer side-effects, complete accessibility and recyclable nature[7,8,9]. Although newer synthetic antimicrobial agents are being developed nowadays, a variety of infective microorganisms have also been identified causing persistent and chronic infections in humans[10]. Infections caused by *Escherichia coli, Klebsiella spp., Salmonella spp., Pseudomonas spp.* and *Staphylococcus aureus* are the most common ones. The continuous use of antibiotics has led to the development of resistant microorganisms. Also antibiotics are also known to cause hypersensititivity. This problem can be tackled by exploring alternative antimicrobial agents which can overcome the drawbacks of antibiotics. Hence, some local medicinal plants are now been studied for possible antimicrobial activity [11].

Plant description

Table 1: Taxonomy of *Urena lobata* L.

Taxonomical classification[12]	Synonyms[13]	Common names[12,14]
Kingdom: Plantae **Subkingdom:** Tracheobionta **Super division:** Spermatophyta **Division:**Mangoliophyta **Class:**Mangoliopsida **Sub class:**Dilleniidae **Order:**Malvales **Family:** Malvaceae **Genus:** Urena **Species:** Lobata	***Urena lobata* Linn.** *Urena americana* L. f. *Urena grandiflora* DC. *Urena trilobata* Vell. *Urena lobata* L. *Urena diversifolia* Schumach.	**Sanskrit:** Vanadenda or Vanabenda **English:** Caesarweed, congo jute, hibiscus bur, aramina, pink chineseburr, bur mallow, grand cousin, cadillo, carrapicho do mata, malva, mahot cousin, cousin petit, cousinrouge, jut africain, coozemahot, dadangsi, and mautofu. **Malayalam:** Udiram, Uram, Uran, Vatto, Uren. **Tamil:** Ottatti, Ottuthuthi, Piliyamankena. **Hindi:** Lotloti, Unga, Lepetua, Kunjia, Kungooya, Bachata, Bacnit, Bachita, Brachta **Telugu:** Peddabanda, Nallabenda. **Bengali:** Panokhra. **Tamil:** Ottatti, Ottututti. **Kannada:**Otte.**Oriya:** Bilokapasiva. **Manipuri:** Sampakpi.**Marathi:** Vanbhendi.

Botany

The plant *Urena lobata* Linn of Malvaceae family is an erect herbaceous or semi-woody, a tomentose shrub growing 60-250 cm or more in height and has a basal diameter of 7cm. *Urena lobata* is annual in subtropic and perennial in the tropics. It grows in moist regions. *Urena* grows best in hot, humid climates, with direct sunlight and rich, well-drained soil. It is found wild in the tropical and temperate zones of North and South America and in Asia, Indonesia, the Philippines, and Africa. Cultivated crops, usually grown as annuals, are found mainly in the Congo Basin and Central Africa, with smaller plantings in Brazil, India, and Madagascar.The young stem and branches are covered with a bit of harsh scattering stellate hair and sessile or shortly stalked pinkish auxiliary flowers. Leaves are simple, alternate, petiolate, stipulate; blade-very variable, usually broader, long round or ovate, up to 10-15 cm long and cordate at the base angled or shallowly 5-7 lobed[12, 15, 16].

Chemical Constituents

The main constituents of *Urena lobata* Linn, includes flavonoids, flavonoids glycosides, β-sitosterol, stigmasterol, furocoumarin, imperatorin, mangiferin and quercetin. Also, it contains kaempferol, luteolin, hypolatin and gossypetin. Roots contain carbohydrate 33%, protein 1.9%, fat 1.8%, fiber 51.7%, moisture 6.6%, and ash 5%. Roots also, contain ephedrine, 4'-O-Me-apigenin and phenolic acids such as vanillic, *p*- coumaric, and melilotie acids. Mannose and xylose are present in mucilage. Raw leaves are reported to contain 81.8% moisture, 54 cal, 3.2 g of 57 protein, 0.1 g fat, 12.8 g carbohydrates, 1.8 g fiber, 2.1 g ash, 558 mg calcium and 67 mg of phosphorous per 100 g. The leaf contains only traces of alkaloids also contains flavonoid like 4'- O-Me-kaempferol and other constituents like kaempferol, rutin,, afzelin, astragalin, tiliroside, kaempferol-3-O-β-D-glycopyranoside-7-O-α-L-rhamnoside,kaempferol-7-O-α-L-rhamnoside,kaempferol-7-O-α-L-rhamnoside-4'-O -β-D-glycopyranoside, and crenuloside. Leaf also contains phenolic acids such as vanillic, syringic, cis and trans p coumaric and gallic acids.. The mucilage contains homopolysaccharide of glucose (glucan). Stem contains vanillic and *cis* and *trans p*- coumaric acids. The sugar monomer present in stem mucilage is reported as xylose[12,14-16].

Pharmacological actions

The root of *Urena lobata* is a popular diuretic in Assam. A decoction of its stem and root is used in brazil as a remedy in severe windy colic. A poultice prepared from the roots and leaves is used as an emollient. The flowers are administered as a pectoral and expectorant in dry and inveterate coughs. An infusion of the flowers is used as a gargle for aphthae and sore throat. The root is used in Assam as an abortifacient[12]. Traditionally the plant is being used in the treatment of febrifuge and rheumatism. It is useful for wounds, toothache, gonorrhea and as food for animals as well as humans[14,16,17]. It was also reported that, the plant parts exhibits antioxidant activity[18.19], cytotoxic activity[19],radical scavenging potential[20], antimicrobial[21], anti-motility, analgesic, anti-inflammatory, membrane sensitizing activity[22], immunomodulatory[23,24], hypoglycemic effect[25], anti-diarrheal[26], hypolipidemic[27] and anti-fertility/spermatogenesis effect[28].

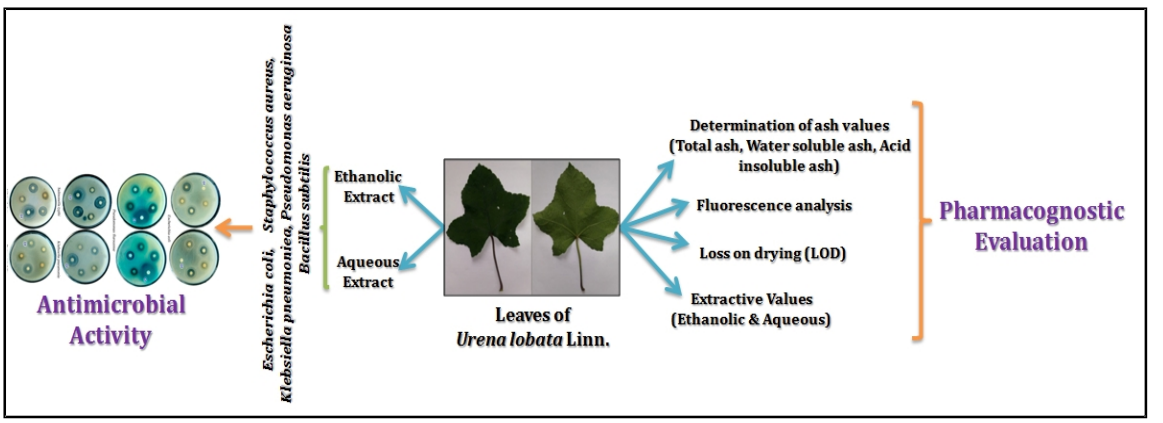


Figure 1: Graphical abstract

MATERIALS AND METHODS

All the experiments of these investigations were carried out at the laboratories of the Department of Pharmacognosy, Gourishankar Institute of Pharmaceutical Education and Research, Satara, Maharashtra, India. All the chemicals used in this study were of analytical grade.

Collection of Plant material

The plant material i.e. leaves of *Urena lobata* L. (Malvaceae) were collected from the Satara district, Maharashtra, during the month of July in the year 2014 and authenticated by Department of Botany, Y.C. Institute of Science, Satara, Maharashtra, India. The fresh crude drug obtained was shade dried, coarsely powdered, passed through 100 mesh sieve and stored in an air - tight containers for further use.

Figure 2: *Urena lobata* Linn.

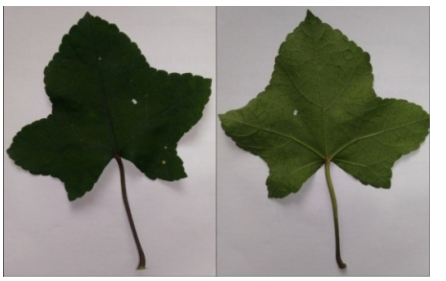


Figure 3: Leaf of *Urena lobata* Linn.

Figure 4: Fruit of *Urena lobata* Linn.

Figure 5: Flower of *Urena lobata* Linn.

PHARMACOGNOSTIC EVALUATIONS

Analytical parameters like ash values (total ash value, water soluble ash value, acid insoluble ash value), fluorescence analysis of crude drug, loss on drying and extractive values, were carried out as per the Indian Pharmacopoeia[29].

Determination of ash values

Ash values are helpful in determining the quality and purity of a crude drug, especially in the powdered form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere with an analytical determination. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates, silicates of sodium, potassium, calcium and magnesium. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high.

Total ash value: About 2 to 3 gms of powdered drug was weighed accurately in a tarred silica crucible and incinerated at a temperature not exceeding 450^{0}C for 4 hrs, until free from carbon. It was then cooled and weighed. The percentage of ash with reference to air-dried drug was calculated using the following formula,

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of crude drug taken}} X100$$

Water soluble ash value: The ash was boiled with 25 ml of water. It was then filtered and the insoluble matter was collected on an ashless filter paper, washed with hot water and ignited in a tarred crucible at a temperature not exceeding 450^{0}C for 4 hrs. It was cooled in desiccator, weighed and the weight of insoluble matter was subtracted from the total weight of ash. The difference in weight represented the weight of water soluble ash. The percentage of water soluble ash was calculated with reference to the air-dried drug using the following formula-

$$\%\text{Water soluble ash value} = \frac{\text{Weight of total ash} - \text{weight of water in soluble ash}}{\text{Weight of crude drug taken}} X100$$

Acid insoluble ash value: The ash was boiled for 5 min with 25 ml of 2 M HCl, filtered and the insoluble matter was collected on an ashless filter paper, washed with hot water and ignited in a tarred crucible at a temperature not exceeding 450^{0}C for 4 h. It was then cooled in a desiccator and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug using following formula,

$$\% \text{ Acid insoluble ash value} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of crude drug taken}} X100$$

Fluorescence analysis of the drug

Many crude drugs show fluorescence when the sample is exposed to ultraviolet radiation. Evaluation of crude drugs based on fluorescence in daylight is not much used, as it is usually unreliable due to the weakness of the fluorescence effect. Hence, fluorescent lamps are used which are fitted with suitable filters, which eliminate visible radiation from the lamp and transmit ultraviolet radiation of definite wavelength. Several crude drugs show characteristic fluorescence useful for their evaluation. The fluorescence characteristics of powdered drug were studied under U.V. light after treating with different chemical reagents. The fluorescence was observed at two different wavelengths i.e. at 254 nm and at 366 nm.

Loss on drying

Loss on drying is the loss of mass expressed as per cent w/w. The test for loss on drying determines both water and volatile matter in the crude drug. Moisture is an inevitable component of the crude drug, which must be eliminated as far as possible.
5 gm of powdered leaves were taken in a tarred porcelain dish. The powder was distributed evenly in the porcelain dish. The porcelain dish was kept open in the vacuum oven and the sample was dried at a temperature 110°C for 2 hrs until a constant weight was recorded. Then it was cooled in a desiccator to room temperature and weighed. % Loss on drying was calculated using the following formula.

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}}$$

Preparation of the extract

Preparation of the ethanolic extract- The coarsely powdered Leaves (25gms) of *Urena lobata* were accurately weighed and 150 ml of ethanol was added to a 250 ml round bottom flask which was fitted with a reflux condenser. The mixture was refluxed at 50 ℃ for about 48 hrs. Finally, the mixture was filtered and the extract was collected. The water was then evaporated under reduced pressure in an evaporator at 50 ℃, to obtain of powder extract.
Preparation of the aqueous extract- Aqueous extract of *Urena lobata* was developed as explained previously under the preparation of ethanolic extract. But in this case, 150 ml of water was added and the temperature was maintained at 100 ℃ for refluxing the mixture.

EVALUATION OF ANTIMICROBIAL ACTIVITY

Source of microorganisms

The test bacteria used were *E. coli* (ATCC 8739), *staphylococcus aureus* (ATCC 8739), *Klebsiella pneumoniea* (ATCC 20031), *Pseudomonas aeruginosa* (ATCC 9027),and *Bacillus subtilis* (ATCC 6633). They were obtained from Gourishankar Institute of Pharmaceutical Education and Research, Satara, Maharashtra, India. The bacteria were maintained on Nutrient agar (NA).

Antimicrobial assay

The sensitivity testing of the plant extracts were determined using agar-well diffusion method/ punch agar method[20,28]. Nutrient broth and nutrient agar were used for sub-culturing the bacterial isolates, while diagnostic sensitivity test agar was used for sensitivity testing. The bacterial isolates were first grown in nutrient broth for 24 hours before use. The inoculum suspensions were standardized and then tested against the effect of the extracts at a concentration of 20 mg/ml in diagnostic sensitivity test agar. Water-ethanol mixture was used as solvent for dissolution of various extracts and control. The plates were observed for zones of inhibition after 24 hours incubation at 37^0C. The effects were compared with Ciprofloxacin eye drops (standard antibiotic) having a strength of 0.3% w/v. The diameters of the zones of inhibition were measured to the nearest millimeter.

RESULTS AND DISCUSSION

Ash values

Table 2: Estimation of the Percentage of Ash values

Ash	Powdered
Total ash	11.67 %w/w
Water soluble ash	3.50 %w/w
Acid insoluble ash	4.24 %w/w

Fluorescence Analysis

The fluorescence characteristics of powdered drug were studied under U.V. light after treating with different chemical reagents. The fluorescence was observed at two different wavelengths i.e. at 254 nm and at 366 nm.

Table 3: Fluorescence analysis of leaves of *Urena lobata* L.

Reagents	Fluorescence Observed					
	Leaf		Leaf powder		Stem Powder	
	At 254	At 366	At 254	At 366	At 254	At 366
Powder + 1N NaOH in Methanol	Green	Green	Light Green	Light Green	Dark Brown	Dark Brown
Powder + 1N NaOH in Water	Green	Green	Dark Brown	Light Green	Dark Brown	Light Brown
Powder + 50% HCl	Blue	Yellow	Yellowish Green	Green	Dark Brown	Yellowish Green
Powder + 50% H_2SO_4	Light Green	Light Green	Dark Green	Light Green	Light Brown	Light Brown
Powder + 50% HNO_3	Dark Yellow	Greenish Yellow	Dark Yellow	Dark Yellow	Dark brown	Light Yellow
Powder + Petroleum Ether	Faint Yellowish Green	Faint Yellowish Green	Dark Yellow	Faint Green	Dark brown	Light brown
Powder + Chloroform	Faint Green	Faint Green	Dark Green	Faint Green	Faint Green	Faint Green
Powder + Picric Acid	Faint Green	Faint Green	Yellow	Dark Green	Dark brown	Faint Green
Powder +5% $Fecl_3$	Yellowish Green	Yellowish Green	Dark brown	Faint Green	Yellowish	Yellowish Green
Powder +5% Iodine	Green	Faint Green	Dark brown	Dark Green	Dark brown	Black Green
Powder +Methanol	Black	Dark Green	Black	Dark Green	Light brown	Dark Green
Powder + (HNO_3+NH_3)	Faint Green	Yellowish Green	Faint brown	Light Green	Dark brown	Yellowish

Loss on Drying

Table 4: Estimation of the LOD

LOD	84.8 %w/w

Extractive Values

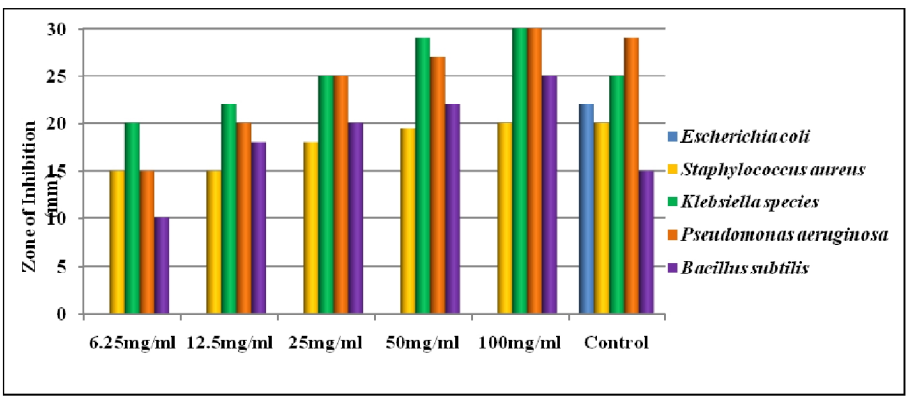
Table 5: Extractive values

Extract	Result
Ethanol soluble extractive.	0.200
Aqueous soluble extractive	0.249

Antimicrobial activity

Table 6: Antimicrobial activity of ethanolic extract of leaves of *U. lobata* L. (Malvaceae) on some selected bacteria using punch agar method (Zone of Inhibition in mm)

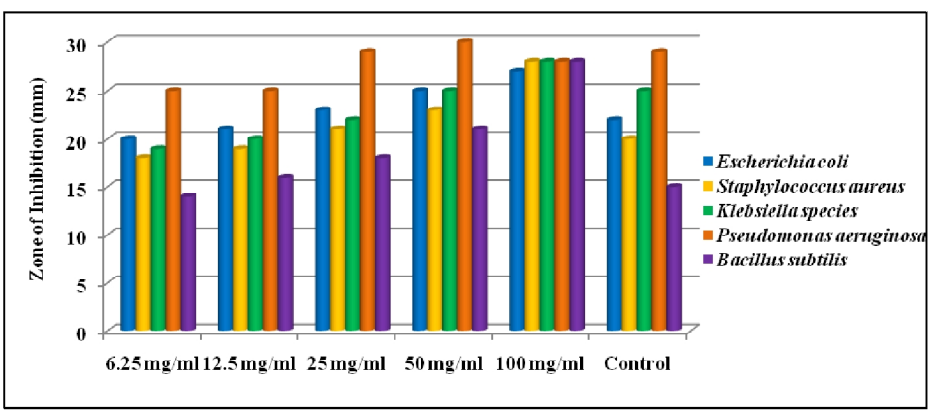
Microorganisms	Concentrations					
	6.25 mg/ml	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	Control
Escherichia coli	0	0	0	0	0	22
Staphylococcus aureus	15	15	18	19.5	20	20
Klebsiellapneumoniea	20	22	25	29	30	25
Pseudomonas aeruginosa	*15*	20	25	27	30	29
Bacillus subtilis	10	18	20	22	25	15



Graph 1: Antimicrobial activity of ethanolic extract of leaves of *Urena lobata* L.

Table 7: Antimicrobial activity of aqueous extract of leaves of *U. lobata* L. (Malvaceae) on some selected bacteria using punch agar method (Zone of Inhibition in mm)

Microorganisms	Concentrations					
	6.25 mg/ml	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	Control
Escherichia coli	20	21	23	25	27	22
Staphylococcus aureus	18	19	21	23	28	20
Klebsiellapneumoniea	19	20	22	25	28	25
Pseudomonas aeruginosa	*25*	25	29	30	28	29
Bacillus subtilis	14	16	18	21	28	15



Graph 2: Antimicrobial activity of aqueous extract of leaves of *Urena lobata* L.

Antibiotics are very effective against bacterial infections but pose a threat of developing allergies or resistant bacterial species. Herbal medicines are therefore comparatively safer for treating bacterial infections. Here an attempt was made to explore the antimicrobial activity of a less renowned herbal drug *Urena lobata.* Linn. According to the pharmacognostic studies performed; The values of total ash, water soluble ash and acid insoluble ash were found to be 11.67 %w/w, 3.50%w/w and 4.24%w/w respectively. All types of ash values of the crude drug were found to be in agreement with the earlier studies[21,30]. The crude drug also exhibits versatile fluorescence characteristics with different colors in different solvent. This can be used in its evaluation, to check its quality, purity or presence of some chemical constituents. The loss on drying was found to be 84.8 %w/w. Loss on drying value was also in agreement with the previously done studies and is indicative of the moisture and volatile matter content of the crude drug. The ethanolic and aqueous soluble extractive values are 0.200 and 0.249 respectively. Ethanolic and aqueous extractive values are indicative of the presence of ethanol and water soluble chemical constituents of the crude drug which was also found to be equivalent with the previously reported studies[21]. The ethanolic extract of *U. lobata* showed good antimicrobial activity against all the microorganisms used in the study except E. coli and the highest activity was shown against *Klebsiella pneumoniea* at all the concentrations of the extract used, followed by *Pseudomonas aeruginosa.* It was also found that the antimicrobial activity of ethanolic extract increased with increasing concentration of the extract for all the microorganisms studied except *E.coli*. The aqueous extract was also found to be effective against all the microorganisms tested. It was most effective against *Pseudomonas aeruginosa* at all the concentrations when compared with its effect on other microorganisms. But its highest activity was seen at a concentration of 50 mg/ml rather than 100mg/ml. For *Pseudomonas aeruginosa* the aqueous extract of the drug did not show a linear increase in activity with concentration. From the above results, the use of the *Urena lobata* L. plant by traditional practitioners to treat bacterial diseases is, therefore, justified.

CONCLUSION

Based on the results of the present study, it can be concluded that ethanolic and aqueous extracts of leaves of *Urena lobata* L. shows potential antimicrobial activity. Literature review of *Urena lobata* L. reveals moderate antimicrobial activity against some microorganisms used in the test. Moreover, it is possible to extract antimicrobial agents from different parts of Urena *lobala* L. Literature review of *Urena lobata* L. indicated that phytochemical and pharmacological investigations have been done on this plant previously. However, all the investigations were of preliminary type and more sophisticated technology should be adopted. More precise methods should be adopted for the complete investigation of phytochemicals which have different pharmacological activities.

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