Current Research in Pharmaceutical Sciences

Available online at www.crpsonline.com

ISSN: 2250 – 2688

CODEN: CRPSBZ (USA)

Received: 30/03/2017
Revised: 21/04/2017
Accepted: 18/05/2017
Published: 08/07/2017

PA Shelar, VG Gharge, AV Yadav
Gourishankar Institute of Pharmaceutical Education & Research, Limb, Satara, India-415015

Correspondence

Pournima A Shelar
Gourishankar Institute of Pharmaceutical Education & Research, Limb, Satara, India-415015

Email: pshelar82@gmail.com

DOI: 10.24092/CRPS.2017.070202

Website: www.crpsonline.com

Quick Response Code:

Pharmacognostic Evaluation, Phytochemical Screening and Antimicrobial Study of Leaves Extracts of *Urena lobata* Linn.

PA Shelar, VG Gharge, AV Yadav

ABSTRACT

Urena lobata is a plant (Malvaceae family) found abundantly in tropical and sub-tropical regions. Traditionally plant is used in febrifuge, rheumatism, wound and as antiseptic. To evaluate the scientific basis for the use of the plant, the antimicrobial activities of extracts of the leaves were evaluated against some common Gram negative and Gram positive bacteria. Pulverized *Urena lobata* leaves were investigated for physical constant (LOD, ash value, fluorescence analysis). The fresh leaf of *Urena lobata* was studied for microscopical characterization which shows upper and lower epidermis, palisade cells, spongy parenchyma in lamina while collenchyma and vascular bundles were observed in midrib. The antimicrobial activity of the ethanolic and aqueous extracts were evaluated by determination of the diameter of zone of inhibition against both Gram negative and Gram positive bacteria using agar well diffusion and minimum inhibitory concentration (MIC). Phytochemical studies revealed the presence of alkaloids, glycosides, steroids, flavonoids, tannins and both the extracts were active against Gram positive and Gram negative bacteria, of which Aqueous extract shows significant results.

Key words: *Urena Lobata* Linn, Physical constants, Microscopy, Antimicrobial activity.

1. INTRODUCTION

Urena lobata Linn (Malvaceae), otherwise called Caesar weed, is a shrub that grows between 0.6- 3 m tall and up to 7 cm in basal diameter.[1] The plant found abundantly in tropical and sub-tropical regions of the land. The plant blooms with pink coloured flowers.[2] Various extracts of leaves and roots are used in herbal medicine to treat such diverse ailments as colic, malaria, gonorrhea, fever, wounds, toothache and rheumatism.[3] Antihyperglycemic effect of *Urena lob*ata Leaf extact by inhibition of DPP-IV on diabetic rats.[4] Aerial parts of *Urena lobata* is reported to contain Mangiferin and Quercetin and roots having imperatonin and furanocoumarin.[5] *Urena lobata* is one of the medicinal plants used to treat diabetes in Nigeria. Its hypoglycaemic and antidiabetic activities have been demonstrated.[6] Plants are the only economic source of a number of well established and important drugs; in addition they are the sources of some chemical intermediates needed for the production of a number of drugs. The popularity of natural drugs all over the world in recent years is an indication of significant contribution of Pharmacognosy in modern medicine.[7]

The nature has provided abundant plant wealth for all the living creatures, which possess medicinal virtues. The essential values of some plants have long been published, but a large number of them have remained unexplored till date. Therefore, there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties. Medicinal plants are of great importance to the health of individuals and communities. Hence an attempt has been made to investigate antimicrobial activity of *Urena lobata Linn.*

2. MATERIALS AND METHODS

2.1 Collection of Plant material

The plant material *Urena lobata Linn (Malvaceae*) were collected from the Satara district, Maharashtra and authenticated by Dept. of Botany, Y.C.I.S, Satara, Maharashtra, India specimen voucher was deposited in the college hebarium for future reference. Fresh drug obtained were shade dried and coarsely powdered and passed through sieve 100 mesh sizes and stored in air - tight containers for further use.

2.2 Preparation of Extract [8]

The pulverized dried *Urena lobata* leaves were extracted with ethanol using soxhlet apparatus. The powder of *Urena lobata* leaves were also macerated with chloroform water. Ethanol and aqueous extracts were filtered & evaporated to dryness.

2.3 Macroscopic characteristic[9, 10]

The macroscopy of fresh leaves were studied according to standard methods.

2.4 Microscopic characteristics[11]

For microscopy hand section of leaf was taken, stained & mounted following usual micro-techniques.

2.5 Physical evaluation[12, 13, 14]

The ash values, extractive values and loss on drying were performed according to the officinal methods prescribed in Indian Pharmacopeia.

2.6 Phytochemical screening

The dried leaves were extracted with ethanol and water. The behavior of powder with various chemical reagent and preliminary chemical tests for ethanolic and aqueous extracts were also carried out according to the standard procedures described by Kokate[15] and Horborne.[16]

2.7 Antimicrobial study[17,18,19,20,21]

2.7.1 Collection of microbes

Bacterial strains such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* were used for the study. The collected microbes were maintained in Nutrient agar broth and cultured in Nutrient Agar media. (Hi Media (P) Ltd Mumbai).

2.7.2 Preparation of the medium

Nutrient agar medium was prepared by dissolving 2.8 g of nutrient agar in 100 ml of distilled water. The solution was sterilized in an autoclave at 121°C for 15 min. It was cooled and poured into sterile Petri dishes to solidify. The agar depth of the medium was measured (10mm).

2.7.3 Determination of antimicrobial activity

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) plates were swabbed (sterile cotton plug) with 8 hour old-broth culture of respective bacteria. Three wells (10mm diameter) were made in each of the plates using sterile cork borer. About 0.3 ml of different concentration of plant solvent extract were added using sterilized dropping pipettes in to the wells and allowed to diffuse at room temperature for two hours. The plates were incubated at 37^0C for 18-24 hr for bacterial pathogen. Respective proper controls of solvent plant extracts were also maintained. Diameter of the inhibition zones and the values were recorded.

2.8 Chromatographic studies[22, 23, 24]

Thin Layer Chromatography studies were carried out for extracts to confirm the presence of different phytoconstituents in these extracts. TLC is a mode of liquid chromatography, in which the extract is applied as a small spot or band at the origin of thin sorbent layer supported on a glass/plastic/metal plate. The mobile phase migrates through the stationary phase by capillary action. The separation of solutes takes place due to their differential absorption/ partition coefficient with respect to both mobile and stationary phases. Each separated component has same migration time but different migration distance. The mobile phase consists of a single solvent or a mixture of solvents. Although, a number of sorbent like silica gel, cellulose, polyamide, alumina, chemically modified silica gel etc. are used, silica gel (type 60) is most commonly used sorbent. Handmade plates are prepared by using pouring technique. The retardation factor (R_f) is calculated using following formula,

$$R_f = \frac{\text{Distance traveled by sample from base line}}{\text{Distance traveled by solvent from base line}}$$

2.8.1 Thin layer chromatography[25, 26, 27]

The extracts were subjected to thin layer chromatography for the presence of phytoconstituents. In this technique, the Silica

gel-GF254 (for TLC) was used as an adsorbent and plates were prepared by pouring technique, then air dried for an over-night and activated for one hour at 110°C and used.

Thin layer chromatography of Ethanolic extract-
Stationary phase: Silica gel GF-254
Mobile Phase: Toluene: ethyl acetate: Formic Acid (8.5:1:0.5).
Detection: UV-366
Solvent front:5.2
Spot detection: 2.3

Thin layer chromatography of Aqueous extract-
Stationary phase: Silica gel GF-254
Mobile Phase: Toluene: ethyl acetate: Formic Acid (8.5:1:0.5).
Detection: UV-366
Solvent front: 5.3
Spot detection: 2.6

2.8.2 Preparative thin layer chromatography

A thick layer of silica gel GF-254 was coated on the square shaped plate and activated at 110^0C for one hour. The spot of extracted sample was applied on the plate.

2.8.3 Characterization of isolated compound

From the separated bands, the substance of interest was scrapped from the plate and it dissolved in methanol. The mixture was filtered and the filtrate was evaporated to dryness. The isolated compound was then subjected for further studies. (1mg/ml concentrations of the extracts were used).

2.9 IR of isolated compound[28, 29, 30]

IR spectrum was recorded in IR- spectrometer in 400-4000 frequency in cm^{-1} for isolated moiety. IR spectrum of compound was carried in KBR pellet. The important absorption can be correlated (Fig 6, 7).

3. RESULTS & DISCUSSION

3.1 Macroscopic characteristic leaves of *Urena lobata Linn.*

Urena lobata Linn, is an erect herbaceous or semi woody, tomentose under shrub. The stems and branches are covered with hairy structures. The plant possesses pink colored flowers. The leaves are simple, petiolate, alternate, stipulate usually broader or ovate, about 10-15 cm long and cordate at the bare angled or 5-7 lobed. For macroscopy refer Fig. 1.

3.2 Microscopic characteristics leaves of *Urena Lobata Linn.*

The Transverse Section of leaf is dorsiventral consists of Midrib and Lamina.

3.2.1 Midrib

It consist of single layered epidermis, on either side, upper epidermis composed of single layer closely arranged elongated cells externally covered with striated cuticle. Leaf surface contains simple, multicellular covering trichomes and anomocytic type of stomata. Below the upper epidermis 3-4 layers of well developed more or less isodiametric collenchymatous tissue were observed.

Midrib contains centrally located vascular bundle which is collateral surrounded by some parenchymatous cells filled with dark content. Xylem is well developed and the phloem consists of strands of sieve tubes and small celled parenchyma.

Lower epidermis consisted of single layer elongated cells with cuticle and just above the lower epidermis 2-3 layers of parenchymatous cells followed by the layers of collenchymatous cells were present. Calcium-oxalate crystals were found in spongy parenchyma. Lower epidermis contains more number of covering trichomes as compared to upper epidermis.

3.2.2 Lamina

Dorsi-ventral structure with single layered upper and lower epidermis with a layer of elongated closely arranged cells externally covered with cuticle. Epidermal cells show anomocytic stomata; below upper epidermis single layered palisade cells followed by 5-7 layers of mesophyll parenchyma which are rounded in shape and are devoid of intracellular spaces.

Microscopic evaluation is reproduced in Fig. 2.

3.3 Physical evaluation

The Loss on Drying, Ash Values likes (Total Ash, Acid insoluble ash, and Water soluble ash), Ethanol soluble extractive and Water soluble extractive of leaf powder are given in Table No.-1.

3.4 Phytochemical screening

Results of phytochemical screening are mentioned in Table No. 2.

3.5 Antimicrobial study

Results of antimicrobial study of ethanolic and aqueous extracts are mentioned in Fig 3, 4 and Table No. 3, 4.

Table No. 1. Physical evaluation of leaves of *Urena Lobata Linn.*

Sr. No.	**Physical Constants**	**Result**
1.	Ash Value (% w/w) • Total Ash • Acid Insoluble Ash • Water Soluble Ash	 12.3 3.75 3.47
2.	Loss on Drying (% w/w)	81.3
3.	Extractive Values (% w/w) ➢ Ethanol soluble extractive. ➢ Aqueous soluble extractive	 0.249 0.320

Table No. 2. Phytochemical investigation of Ethanolic and Aqueous extracts of *Urena Lobata Linn.*

Sr No.	Name of the test	Leaves	
		Ethanolic extract	Aqueous extract
1.	Test for sterols	+	+
2.	Test for Triterpenoids	+	-
3.	Test for glycosides	+	+
4.	Test for carbohydrates	+	+
5.	Test for alkaloids	+	+
6.	Test for flavonoids	+	-
7.	Test for tannins	+	+
8.	Tests for proteins	-	-
9.	Test for amino acids	-	+
10.	Test for fats	+	-
11	Test for Volatile oils	-	-

Table No. 3. Antimicrobial activity of Ethanolic extract of *Urena Lobata Linn.*

Organism	Diameter of inhibition zone in cm		
	Ethanolic extract		Streptomycin 100(μ/ml)
	200(μ/ml)	300(μ/ml)	
Staphylococcus aureus	2.3	4	3
Escherichia coli	3	3.2	4
Salmonella typhi	2	3	3.5
Pseudomonas aeruginosa	2.5	2.6	3.2

Table No. 4. Antimicrobial activity of Aqueous extract of *Urena Lobata Linn.*

Organism	Diameter of inhibition zone in cm		
	Aqueous Extract		Streptomycin 100(μ/ml)
	200(μ/ml)	300(μ/ml)	
Staphylococcus aureus	2.2	2.8	3
Escherichia coli	1.9	3.1	3.5
Salmonella typhi	2	3.3	3.4
Pseudomonas aeruginosa	2.5	3	3.5

Table No. 5. TLC Profile of steroids

Extract	Observation		R_f values
	No. of spots	Colour of spots	
Ethanolic	1	Yellow	0.44
Aqueous	1	Yellow	0.49

Table No. 6. TLC Profile of steroids

Peak Observed	Assignment	Absorption Expected (cm^{-1})
719.45	Alkanes	600-1500
1035.77	Alcohol, Ether, Esters	1000-1300
1228.66	Amines	1180-1360
1373.32	Nitro compound	1330-1540
1635.34	Alkenes	1620-1680
1722.47	Aldehydes, Ketones	1680-1760
2924.09	Hydrogen bonded Acid	2500-3000
3061.09	Aromatic Ring	3000-3100
3224.98	Amines	3300-3500

Table No. 7. TLC Profile of steroids

Peak Observed	Assignment	Absorption Expected (cm^{-1})
792.84	Alkanes	600-1500
1188.15	Alcohol, Ether, Esters	1000-1300
1255.66	-F	1400-1000
1531.63	-C=C-	1400-1600
2918.30	Hydrogen bonded Acid	2500-3000
3140.11	-CH	3150-3050
3219.19	-OH	3400-3200

Fig 1. Whole Plant of *Urena lobata Linn.*

Upper Chollenchyma
Phloem
Upper Epidermis
Xylem
Lower Epidermis
Lower Chollenchyma
Upper Epidermis
Palisade
Spongy Parenchyma
Lower Epidermis

Stomata

Covering Trichomes

Fig 2. Microscopy of leaf of *Urena Lobata Linn.*

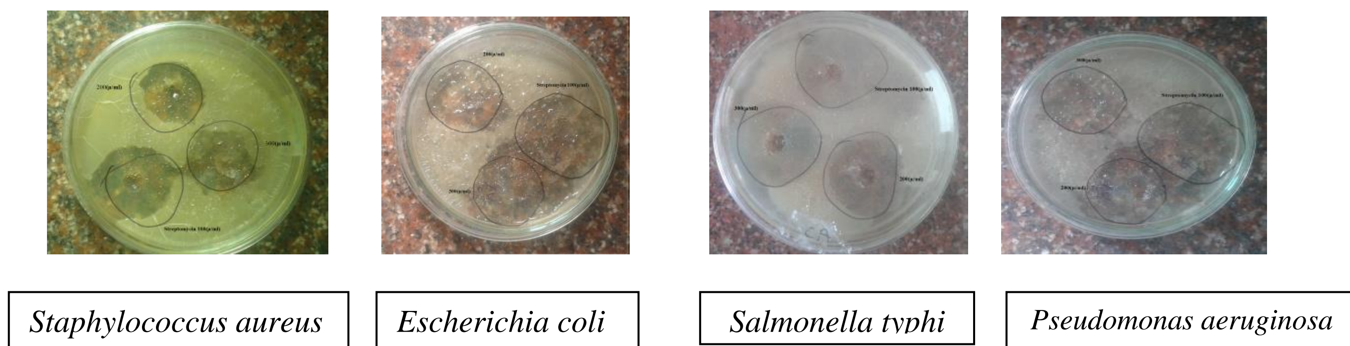

Fig 3. Antimicrobial activity of Ethanolic extract

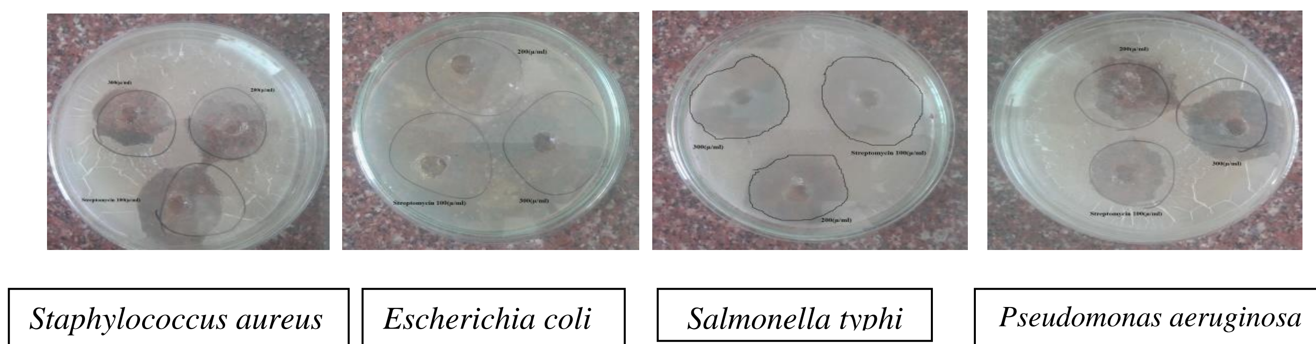

Fig 4. Antimicrobial activity of Aqueous extract

Ethanolic extract Aqueous extract

Fig 5. Thin layer chromatography of Ethanolic and Aqueous extracts

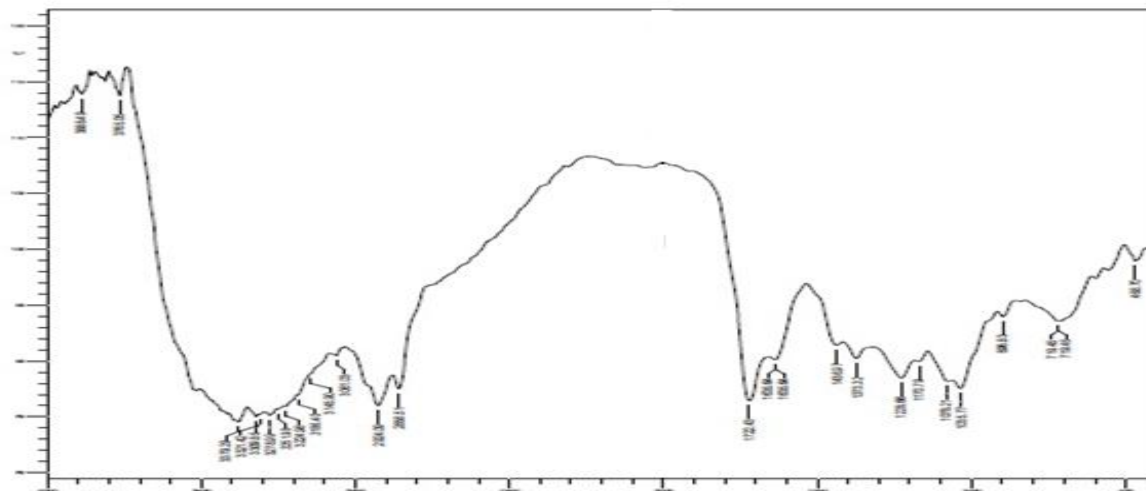

Fig 6. IR Spectra of Ethanolic extract of *Urena Lobata Linn*

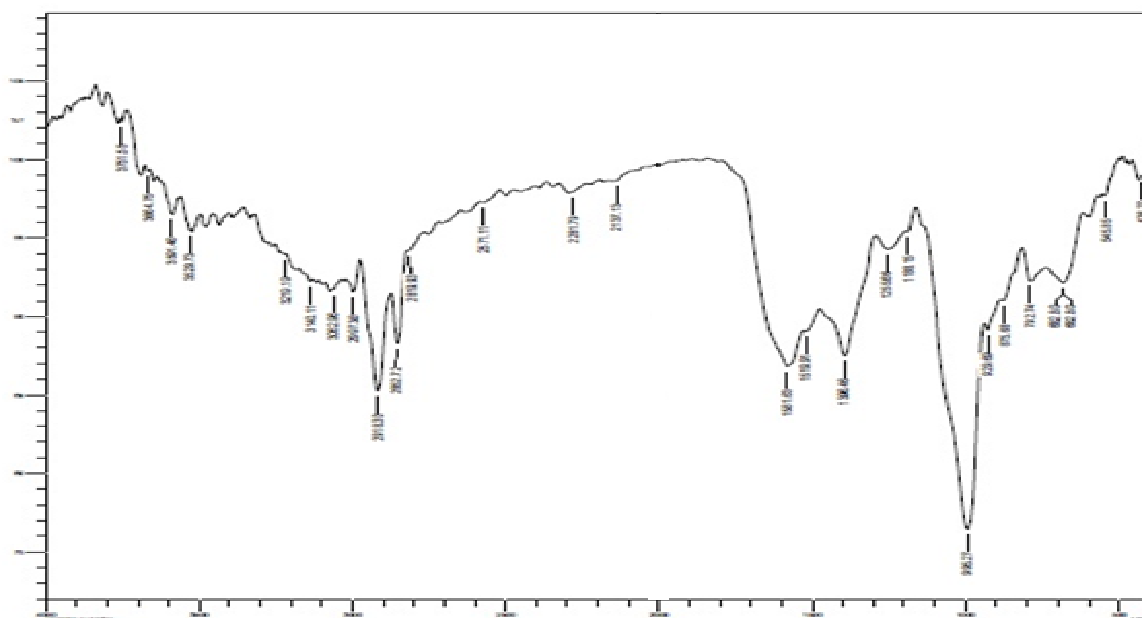

Fig 7. IR Spectra of Aqueous extract of *Urena Lobata Linn.*

3.6 Chromatographic study

Details of TLC are mentioned in Table No. 5 and Fig 5.

From the present study, it has been discussed that

1. The plant material was collected from Satara district, Maharashtra region and authenticated. The plant material was subjected for Pharmacognostic investigation which includes determination of physical constants such as ash value, extractive values determination and Loss on Drying.
2. Macroscopic and microscopic characteristics of the leaf were studied. The microscopic study shows that it contains midrib and lamina portion. The lamina shows upper and lower epidermis, spongy parenchyma, palisade

cell layer while midrib portion shows upper and lower epidermis, collenchyma, vascular bundles, etc., Powder characteristics shows presence of anomocytic stomata and covering trichomes.

3. The leaves were subjected to extraction by using ethanol and water and these extracts were subjected to phytochemical investigation.
4. Phytochemical investigation of extracts of *Urena Lobata Linn,* shows that Aqueous extract contains sterols, glycosides, carbohydrates, alkaloids. While Ethanolic extract shows presence of sterols, flavonoids, glycosides, carbohydrate, alkaloids and tannins.
5. For antimicrobial activity, 200(μ/ml) and 300(μ/ml) concentrations of ethanolic and aqueous extracts were used. Streptomycin was used as standard. The ethanolic as well as aqueous extracts possess antimicrobial activity. Where the study shows that aqueous extract shows potent antimicrobial activity as compared to ethanolic extract.
6. Chromatographic study of the extracts was carried out. Where Thin layer chromatography were carried out by using mobile phase Toluene: Ethyl acetate: Formic Acid (8.5:1:0.5) which shows R_f value 0.44 and 0.49 for steroids for Ethanolic and Aqueous extracts respectively.
7. For these isolated compounds of Ethanolic and Aqueous extracts, infrared spectroscopy was carried out which shows that Aqueous extract of *Urena Lobata Linn,* contains hydroxyl group, fluorine, alkenes, ethers, esters, etc. While Ethanolic extract shows alkenes, amines, nitro-group, esters, aldehydes, ketones, etc.

4. CONCLUSION

Urena Lobata Linn is widely found in India during rainy season. As there is less information available on pharmacognostical work on leaves hence the morphological study, microscopical studies, physico-chemical parameters and phytochemical study will guide in the proper identification of the plant species as well as help in authentication of the purity of the plant. All these parameters also help to build up a suitable plant profile.

REFERENCES

1. Adeloye O, Akinpelu A, Ogundaini O, Obafemi A. "Studies on antimicrobial, antioxidant and phytochemical analysis of *Urena lobata* Leave extract." Journal of physical and natural sciences, 2007; 1(2): 1-9.
2. Dixa S, Vimal S. *"Urena lobata* Flowers: A Green Route to Volumetric Analysis." Green and Sustainable Chemistry, 2015; 5, 1-5.
3. Marietta C, Jeanne N. "*In vitro* antiproliferative and anti-oxidant activities of methanol extracts of *Urena lobata* and *Viscum album* against breast cancer cell lines." Toxicological and environmental chemistry, 2012; 94(5), 987- 999.
4. Yudi P, Dgoko WS. "Antihyper glycemic effect of *Urena lob*ata Leaf extact by inhibition of DPP-IV on diabetic rats." International Journal of Pharmacognosy and Phytochemical Research, 2015; 7(5):1073-1079.
5. Keshab G, "A furocoumarin, Imperatorin isolated from *Urena lobata* L. (Malvaceae)," *Molbank* 2004, 1-2.
6. Akhere A. Omonkhua and Iyere O. Onoagbe, "Evaluation of the long-term effects of *Urena lobata* root extracts on blood glucose and hepatic function of normal rabbits," Journal of Toxicology and Environmental Health Sciences, 2011; 3(8) : 204-213.
7. Thirumalaikumaran R, Chamundeeswari D, Seethalakshmi S, Gopa V. "Pharmacognostical, phytochemical and Anti oxidant studies of the aerial parts of *Urena lobata L."* Basic Research Journal of Medicine and Clinical Sciences. 2013; 2(2):32-36.
8. Chatwal GR, Anand SK. Instrumental methods of chemical analysis, 7th ed., Himalaya Publishing House. 1992; 588-598.
9. Mukherjee PK, Quality control of herbal drugs, Bussiness Horizon's, Pharmaceutical publisher, New Delhi, 2002, 138-141.
10. Evans WC, Trease and Evans, Pharmacognosy, W.B. Saunders, Edinburgh London, New York Philadelphia, 15th Edition, Pg. No.519-520, 545-547.
11. Johansen DA. Plant microtechniques, McGraw-hill Book Company, New York & London, 1st Edn 1940, 182-203.
12. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, Controller of Publication, 4th edition, New Delhi, 1996. 4(II), A53-A54.
13. Pradeep KS, Mohammad A and Dinesh KY. "Physicochemical and Phytochemical evaluation of different black tea brands." Journal of Applied Pharmaceutical Science, 2011; 01 (03):121-124.
14. Rinku M, Sanjay PU. "Antioxidant Activity of the Methanolic and Aqueous Extracts of *Urena lobata (Lin*n.) By DPPH Method RRJPP," Research & Reviews: Journal of Pharmacognosy and Phytochemistry, 2013; 1(1): 6-9.

15. Kokate, CK. Practical Pharmacognosy, 1st Ed, Vallabh Prakashan, New Delhi 1986, 1, 15-30.
16. Harborne, JB. Methods of extraction and isolation. In: Phytochemical methods, 3rd Chapman and Hall, London 1998, 60-66.
17. Sayyad S, Dasari B, Shaik L. "A Pharmacological Review Of *Urena Lobata* Plant," Asian Journal Pharm Clin Res,2016; 9(2): 20-22.
18. Perez C, Paul M, Bazerque P. "Antibiotic assay by Agar well diffusion method," Acta Boil Med Ex, 1990, 15, 113-115.
19. Ali MS, Faruq KA, Md. Rahman MAA. "Aslam Hossain, Antioxidant and Cytotoxic Activities of Methanol Extract of *Urena lobata (L)* Leaves," hepharmajournal, 2013; l (2): 9-14.
20. Son R and Cheah YK. "Preliminary Screening Of Endophytic Fungi Frommedicinal Plants In Malaysia For Antimicrobial And Antitumor Activity," Malaysian Journal of Medical Sciences, 2002; 9(2):23-33.
21. Mshelia IY, Dalori BM. "Effect of the Aqueous Root Extract of *Urena lobata (Linn)* on the Liver of Albino Rat." Res. J. Appl. Sci., Engine. Technol., 2013; 5(1): 01-06.
22. William Kemp. Organic Spectroscopy, 3rd ed. 1991; 58-269.
23. Chatwal GR, Anand SK. Spectroscopy (Atomic ad Molecular), Himalaya Publishing House, 5th ed. 2001; 2.29-2.234
24. Sharma BK. Instrumental method of chemical analysis, 22nd ed., Goel Publishing House, Merrut. 2002; 180-224
25. Ergon Stahl. Thin Layer Chromatography, A Laboratory Hand Book, 2nd ed., New York 1990; 52-59.
26. Khamkar SP, Shelar PA.,Kakade TB, Ubale AT. "Physicochemical And Phytochemical Investigation Of Herbs Of Kas Pathar," International Journal of Universal Pharmacy and Bio Sciences,2014; 3(6): 155-176.
27. Khamkar SP, Raskar,S. "Preliminary Phytochemical Investigation and Microbial Activity." International Journal of Current Pharmaceutical Sciences, 2015; 1 (3):216-226.
28. Shelar PA, Jawlikar BV. "Comparative study of isolated moiety from Senna leaves, Pods and Powder." Current Pharma Research, 2013; 3(4):1019-1022.
29. Chatwal G, R, Anand SK. Instrumental methods of chemical analysis, 7th edition, Himalaya Publishing House. 1992; 588-598.
30. Chatwal GR, Anand SK. Spectroscopy (Atomic ad Molecular), Himalaya Publishing House, 5th edition, 2001; 2.29-2.234.