JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/44094

RESEARCH ARTICLE

Standardization of *Urena lobata* Linn: Unveiling Pharmacognostical Features, Preliminary Phytochemical Analysis and HPTLC Fingerprinting

Ajay Shelke[1*], Archana Navale[1] and Komal Patel[2]

[1]Department of Pharmacology, Parul Institute of Pharmacy, Parul University, Vadodara – 391760, Gujarat, India; ajaybshelke457@gmail.com

[2]Department of Pharmacognosy, Parul Institute of Pharmacy, Parul University, Vadodara – 391760, Gujarat, India

Abstract

Background: *Urena lobata*, a member of the Malvaceae family, is valued in *Ayurvedic* medicine, exploring its pharmacognostic features and conducting HPTLC analysis could reveal its medicinal potential for modern healthcare applications. **Objectives:** Explore the pharmacognostic, physicochemical, and HPTLC profiles of *Urena lobata* Linn. **Materials and Methods:** The following methods were used for pharmacognostic characterization: fluorescence analysis, macroscopy, microscopy, powder microscopy, organoleptic investigation, primary phytochemical screening, and HPTLC profile. **Results:** *Urena lobata*, an erect herbaceous or semi-woody shrub, exhibits distinctive botanical and chemical characteristics. Its leaves are simple, lobed, and alternate, with a cordate base and hairy stems and branches. The stem structure includes a single epidermis with stellate trichomes, collenchymatous cells, and a well-defined vascular system comprising phloem, xylem, and medullary rays. Root cross-sections reveal cork, phellogen, cortex, and well-developed phloem and xylem. Leaf anatomy features a single epidermal layer, chlorenchyma, and a collateral vascular bundle with elongated palisade and parenchymatous cells. Chemical analysis using methanol and water extracts identified alkaloids, flavonoids, phenolic compounds, and tannins, confirmed by High-Performance Thin-Layer Chromatography (HPTLC) profiling. These studies aim to standardize the plant's identification and quality through quantitative, physicochemical, and fluorescence analyses. The comprehensive approach enhances understanding of *Urena lobata's* potential uses in medicine and industry, ensuring consistency and efficacy in its applications. **Conclusion:** An essential investigative tool for the identification, authentication, and establishment of value characteristics of the class is provided by the current report on the pharmacognostic characterization and HPTLC analysis of *U. lobata.* The data from this study could serve as a benchmark for research projects in the future.

Keywords: HPTLC Analysis, Microscopy, Macroscopy, Physicochemical, Quantitative Estimation, *Urena lobata*

1. Introduction

Since ancient times, people have utilized plants as medicine to preserve their health[1] and as a primary natural source of therapeutics[2]. Since the last two decades, people have turned towards natural based remedies due to an increasing in awareness about the unwanted side effect of long-term use of conventional medication. The use of plant-based preparation as a nutraceutical, preventive approach, or as treatment increasing worldwide and hence, there is an urge for scientific validation of plant or their preparations for their pharmacological potential and safety. Incorrect identification, adulteration and lack of standardization are major challenges encountered in field of herbal drug. World Health Organization (WHO) stated evaluation parameters like study of microscopy, physicochemical evaluation, and biological evaluations to generate standard quality control profiles of plants[3]. Also, over the period different tools and techniques like analytical methods and chromatography have been developed to support creating a reliable plant profile[4].

*Author for correspondence

Article Received on: 19.05.2024 Revised on: 09.07.2024 Accepted on: 16.07.2024

Urena lobata Linn belonging to the family malvaceaeis native to India and tropical Asia. This plant may be found growing beside highways and in wastelands throughout India. It is also known as araminafiber, caesarweed, and congojute. *U. lobata* is traditionally used as a diuretic, febrifuge, and anti-rheumatic. All plant components have therapeutic value and are frequently used in folk and traditional remedies to treat a variety of illnesses, including gonorrhea, leucorrhoea, hematemesis, trauma, bleeding, colds, fevers, pain, and numbness brought on by rheumatism, wounds, toothaches, and inflammation. The plant has a wide range of pharmacological properties, such as antioxidant[5], antimicrobial[6], antidiarrheal[7], immunomodulatory[8], anxiolytic, antidepressant and anti-inflammatory[9], antidiabetic[10], anti-bacterial[11,12], antifertility/spermatogenesis[13], cytotoxic and anti-proliferative[14,15], anti-hyperglycemic and anti-nociceptive[16,17], wound healing[18] and anti-arthritic activity[19].

Despite its many therapeutic benefits, there is little published study on the pharmacognostical characterization and HPTLC examination of *U. lobata* leaves. Thus, the purpose of this study is to set a quality standard for the plant's leaves. The study examines the macroscopic and microscopic properties of *U. lobata* leaves, as well as quantitative metrics including physical parameters, phytochemical screening, and chromatographic profiles. This study will be an important first step in assessing the crude drug quality for further development.

2. Materials and Methods

2.1 Collection and Authentication of Plant Material

The plant material was authenticated by Dr. M. S. Khyade and Dr. S. D. Jadhav, professors at the Department of Botany, S. N. Arts, D. J. Malpani Commerce, and B. N. Sarda Science College, Sangamner, Maharashtra. A voucher specimen (SC-139) was deposited in the department for reference. Following collection, the crude drug was subjected to shade drying, followed by coarse powdering and sieving through a 100-mesh sieve. The powdered material was then stored in airtight containers for subsequent use.

2.2 Chemicals

All chemicals employed were of the highest purity and analytical grade. Methanol, N-hexane, chloroform, G. acetic acid, ethyl acetate, Folin ciocalteu's phenol reagent, $AlCl_3$, and other chemicals were purchased from sigma aldrich ltd, India. Gallic acid and quercetin were purchased from yucca Enterprises, Mumbai, India.

2.3 Macroscopic Characteristics of *Urena lobata*

The fresh leaves, stem and root of plant were evaluated for organoleptic parameters like; shape, color, margin, texture, apex, petiole, phyllotaxy etc as per standard methods[20].

2.4 Microscopic Characteristics of *Urena lobata*

2.4.1 Study of Transverse Section

Under a photomicrograph, transverse slices of the leaf, stem, and root were examined. After the application of staining reagents ($(C_6H_3(OH)_3$ with conc. HCl) followed conventional procedures. Numerous distinguishing features were seen both with and without staining. Olympus light microscope BX23 was used to observe the detailed microscopy. Microphotographs were taken using a digital camera Olympus DP70.

2.4.2 Powdered Drug Microscopy

Under a microscope, finely powdered leaves, stems, and roots that had been shade-dried were examined. A tiny quantity of the powdered parts of plants were placed on slides using intense hcl and phloroglucinol and then looked at under a microscope. Numerous cell components have been observed, including stomatal cells, calcium oxalate crystals, cork cells, sieve tube cells, lignified fibers, and cortical cells. Digital photos were captured with an olympus DP70 camera[21].

2.5 Physiochemical Analysis

The ash values, extractive values and loss on drying were performed according to the official methods prescribed in Indian pharmacopeia 2014[22,23].

2.6 Chemical Test

The leaf, stem and root of *U. lobata* were subjected to extraction with water and methanol to explore the phytochemical profile by phytochemical test[24].

2.7 Estimation of Tannin Content

Total tannins in the leaves, stem and root of the methanolic extract were resolute using the folin-ciocalteu method. A 0.1 ml sample of 1 ppm concentration was mixed with 7.5 ml dis. H_2O, 0.5ml folin-ciocalteu's reagent, and 1ml 35% Na_2CO_3 solution in a 10 ml flask made up to volume with dis. H_2O. After 45 minutes at room temperature, absorbance at 700 nm was measured using a spectrophotometer against a water blank. Tannin content, stated in mgs of tannic acid equals per gram of dried sample, was determined in triplicate[25,26].

2.8 Estimation of Total Phenolic Content

The total phenolic content in the leaves, stems, and roots of the methanolic extracts was determined using folin-ciocalteu's assay. A 1 mL sample of 1 ppm concentration was mixed with the reagent, followed by 10 mL of 7% Na_2CO_3 and 13ml of water. After 90 minutes in the dark at 23°C, absorbance was measured at 760 nm[27,28]. Phenolic content was calculated using a gallic acid calibration curve and expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of dried sample. The analysis was done in triplicate[29,30].

2.9 Estimation of Flavonoid Content

Quercetin served as the standard for determining total flavonoid content. Solutions ranging from 25 to 150 µg/mL of quercetin were ready in water. Respectively solution had 4 mL of water, followed by 0.3 mL of $NaNO_2$ (5%) after 5 minutes. Then, 0.3 ml of $AlCl_3$ (10%) was add, and 2 mL of NaOH (1M) at the 6th minute. The total volume was adjusted to 10 ml with dis. H_2O. Absorbance was taken at 510 nm against a blank without $AlCl_3$ using a spectrophotometer[31,32].

2.10 Fluorescence Analysis

One of the most important methods for determining the different components of natural goods is fluorescence analysis. Certain components of this mixture glow only when exposed to UV radiation; in the presence of daylight, they do not fluoresce. This phenomenon may result from the presence of particular fluorescent materials or from fluorescent materials that have been treated with particular chemicals to generate fluorescent derivatives. The analysis was carried out by treating powder leaves with various organic and inorganic solvents and observed under short ultraviolet wavelength and long ultraviolet[33].

2.11 Fractionation of Leaf Powder

Using a Soxhlet apparatus, the powdered leaf was extracted sequentially using n-hexane, chloroform, ethyl acetate, and methanol. To obtain a dried fraction, each fraction remained filtered while still hot and then evaporated using a rotating vacuum evaporator. The dried fractions were further subjected to phytochemical screening to explore possible phytochemicals in respective fractions and the remaining dried fractions were kept at 2-8 °C in the refrigerator for additional study[24].

2.12 Expansion of Chromatographical Profile by HPTLC of Several Fractions of *Urena lobata*

2.12.1 Mobile Phase Optimization

Through rigorous experimentation with various mobile phases, the composition of toluene: Ethyl Acetate: Glacial Acetic Acid in a ratio of 9:1:0.1 achieved remarkable separations. After the mobile phase optimization, this optimized mobile phase has been employed to obtain HPTLC chromatograms.

2.12.2 HPTLC Analysis

Using a CAMAG-HPTLC system with a linomat 5 sample applier, HPTLC chromatograms were obtained for various fractions of *U. lobata*. The mobile phase consisted of Toluene: Ethyl Acetate: G. Acetic Acid (09: 01:0.1). Chromatographic separation was performed on silica gel-g precoated TLC aluminum plates. Each fraction (10 µL) was applied as 10 mm bands using an autosampler. The development occurred in a CAMAG twin through chamber with a pre-saturated mobile phase for 45 minutes. After drying, the plate was scanned at 366 nm to record the R_f values for each spot.

3. Results

3.1 Macroscopic Evaluation of the Leaf of *Urena lobata*

Hairy stems and branches adorn the upright, herbaceous or semi-woody shrub *U. lobata.* The leaves

are broadly oval or wide, cordate at the base, simple, lobed, petiolate, alternating, and measure around 10-15 cm in dimension (Figure 1).

Figure 1. Macroscopic evaluation of leaf of *Urena lobata*.

3.2 Microscopic Characteristic of *Urena lobata*

Microscopic studies were carried out by preparing thin sections of leaf, stem and root. The thin sections were washed with water, stained (phloroglucinol + HCl), mounted with glycerin, and observed under a simple microscope (10X, 45X). Biological microscope was used to take photomicrographs.

3.2.1 Transverse Sections of Leaf

The transverse section of the *U. lobata* leaf showed distinct dorsoventral characteristics, as depicted in Figure 2. The midrib showed a convex shape on the ventral side and a hemispherical shape on the dorsal side. The leaf is covered by one film of epidermis with a tinny cuticle. Positioned between the upper and lower epidermis are

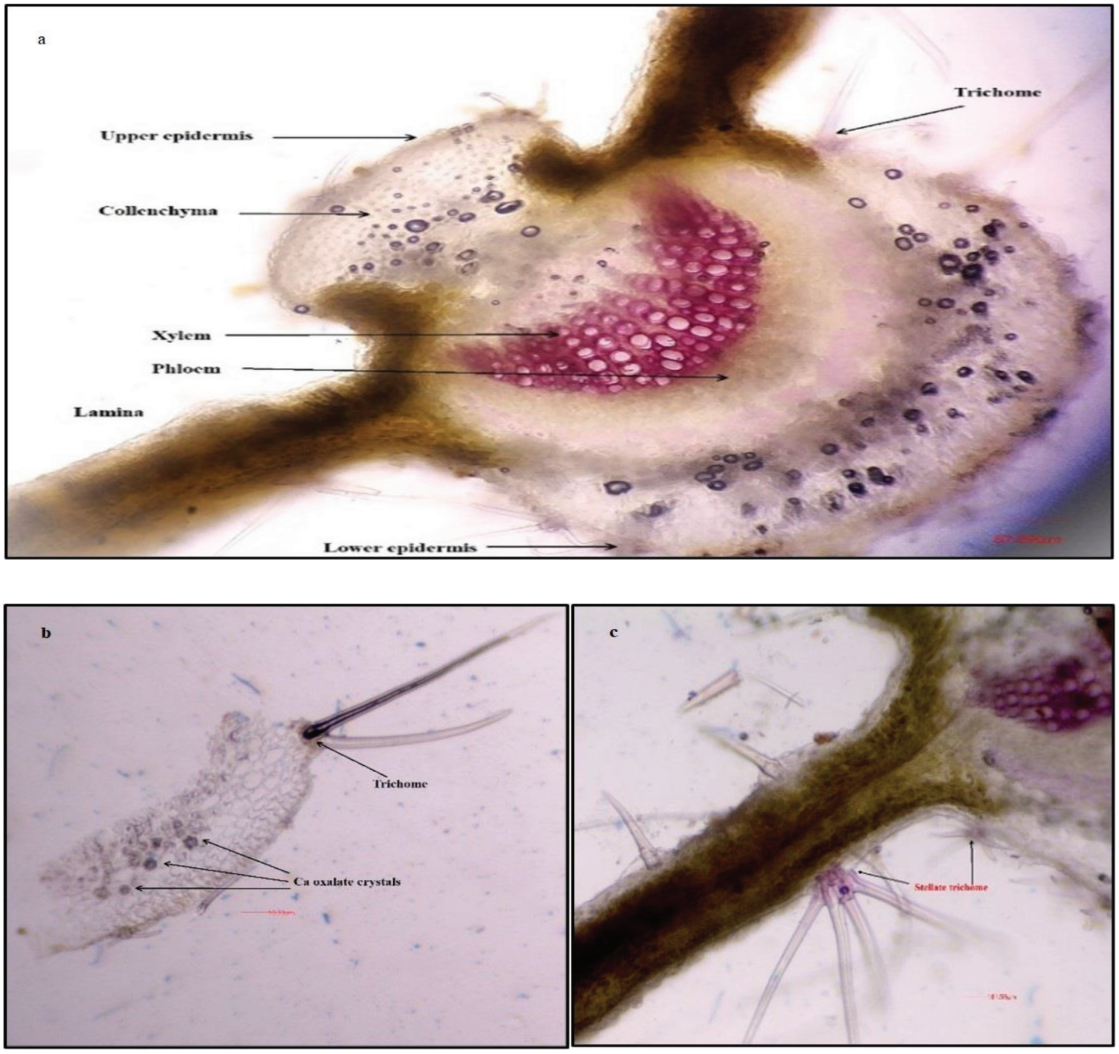

Figure 2. Leaf microscopy of *Urena lobata*.

3-4 layers of tightly arranged chollenchymatous tissue. A single collateral vascular bundle is located at the center of the midrib, containing both xylem and phloem. Furthermore, parenchymatous cells beneath the vascular bundle exhibited the presence of calcium oxalate crystals, as shown in Figure 2(b). The mesophyll tissue contained a compactly arranged single layer of elongated palisade cells filled with chlorophyll on the dorsal side, followed by loosely arranged spongy parenchymatous cells. Simple, long unicellular trichomes, as well as 2-7 armed stellate trichomes, were observed on the dorsal and ventral surfaces of the leaf, as shown in Figure 2(c). Trichomes were more numerous on the ventral side compared to the dorsal side of the leaf.

3.2.2 Transverse Sections of Stem

The transverse section of the *U. lobata* stems as Figure 3 revealed a sole film of the epidermis enclosed with a shell. Remarkable stellate trichomes were observed on the epidermal layer. After the epidermis, a few coatings of collenchymatous cells were present, followed by a well-defined cortex portion. A well-defined vascular system was noted, with the phloem positioned peripherally and the xylem towards the center, with the cambium located between the phloem and xylem. Biseriate to triseriate medullary rays radiated from the center toward the periphery. A well-defined pith was present in the center portion.

3.2.3 Transverse Sections of Root

The transverse section of the root, as illustrated in Figure 4, revealed that the outer periderm comprises cork, a few layers of phellogen, and phelloderm, followed by a cortex consisting of slightly flattened parenchymatous cells. The phloem was well-developed and included parenchyma, sieve tubes, companion cells, and lignified fibers. Well-defined xylem identified as protoxylem and metaxylem observed medullary rays were present as biserrate or triserrate.

3.2.4 Powdered Drug Microscopy

The powder microscopical characters are shown in Figures 5, 6 and 7.

3.3 Physicochemical Evaluation

Table 1 provides information on the ash values, foreign organic matter, loss of drying, fluorescence analysis of the crude material, and extractive values (water-soluble and alcohol-soluble) of leaf powder.

3.4 Phytochemical Screening of Different Extracts

The results of the phytochemical screening of the different extracts are provided in Table 2.

3.5 Total Tannin, Phenolic and Flavonoid Content

The quantity of total tannins, phenolics, and flavanoids in the leaf, stem, and root samples were calculated. The overall phenolic content was determined using the folin-ciocalteu method, with gallic acid as the standard. Using the calibration curve's regression equation ($Y = 0.0277x + 0.1382$, $R^2 = 0.9972$), the phenol content of the different extracts

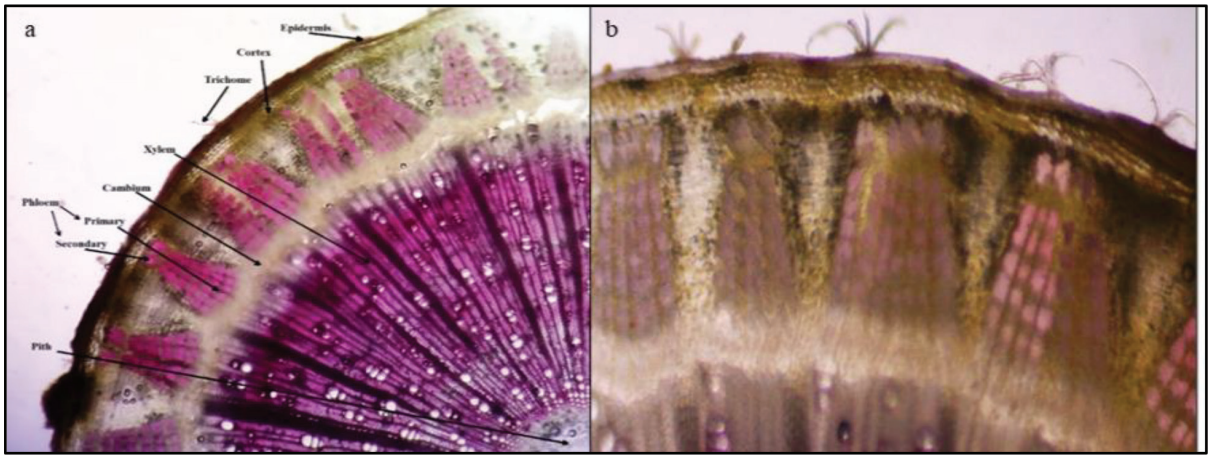

Figure 3. Stem microscopy of *Urena lobata*.

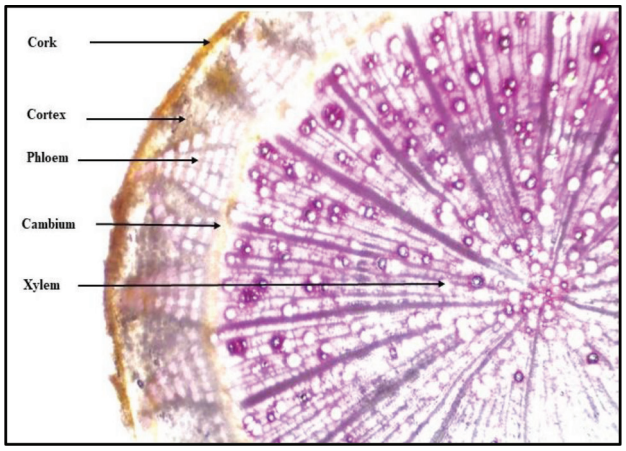

Figure 4. Root microscopy of *Urena lobata*.

Table 1. Pharmacognostic evaluation of leaves of *U. lobata*

Sr. No.	Physiochemical Parameter	Result (% w/w)
1	**Ash Value** I. Total Ash	8.07
	II. 'Acidin soluble ash'	2.25
	III. 'Water-soluble ash'	2.23
2	Foreign organic matter	0.43
3	Moisture content	4.72
4	**Extractive value** I. 'Water-soluble extractive value'	10.89
	II. 'Alcohol-soluble extractive value'	6.15

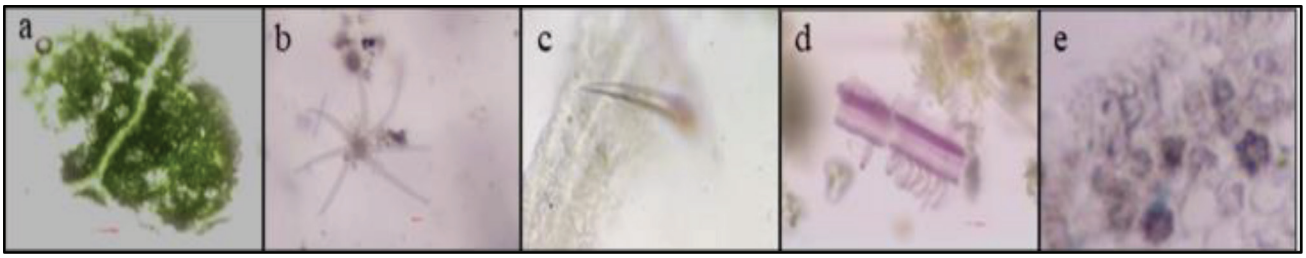

Figure 5. Powder microscopy of leaf as figure, the epidermal cells with paracytic stomata, and chlorophyll tissues (a), stellate trichomes (b), unicellular trichome (c), xylem vessel (d) and crystals of calcium oxalate (e).

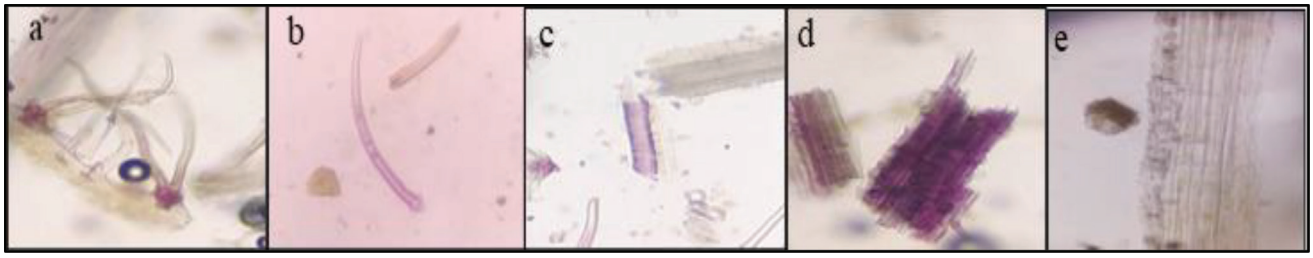

Figure 6. Powder microscopy of stem as figure, the epidermal cells with stellate trichomes (a), unicellular trichome (b), spiral xylem vessel (c), medullary ray (d) and crystals of calcium oxalate in fiber (e).

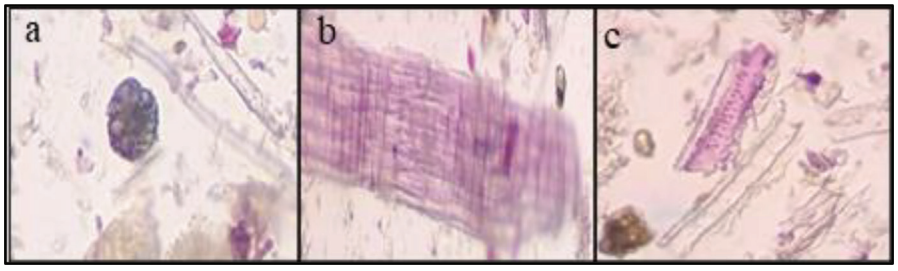

Figure 7. Powder microscopy of the root as a figure, crystals of calcium oxalate (a), medullary ray (b), lignified pitted xylem vessel (c).

Table 2. Phytochemical screening of *U. lobata* extract

Chemical Constituents	Chemical Test	Extract of Leaves		Extract of Stem		Extract of Roots	
		70% Methanol extract	Aqueous extract	70% Methanol extract	Aqueous extract	70% Methanol extract	Aqueous extract
Flavonoids	Shinoda test.	+	-	+	+	-	+
	Lead Acetate test.	+	+	+	-	-	+
Glycoside	Killer Killani test.	-	+	+	+	+	+
Carbohydrate	Molish test.	+	+	+	+	+	+
	Fehling's test.	+	+	+	-	+	+
Triterpene	Vanillin-Sulphuric Acid test.	+	-	+	+	+	+
Amino Acids	Ninhydrin test.	-	-	-	-	+	-
Sterols	Liebermann's test.	+	+	+	+	+	+
	Salkowskis test.	+	-	+	+	+	+
Phenol	$FeCl_3$ test.	+	+	-	+	-	+
Alkaloid	Mayers test.	+	+	-	-	-	+
	Dragendorffs test.	+	+	-	-	-	+
Saponin	Foam test.	+	+	-	+	+	+
Tannin	$FeCl_3$ test.	+	+	+	+	+	-
	Dil. nitric acid test.	+	+	+	+	-	-
Protein	Million's test.	-	-	-	+	+	-
	Biuret test.	-	-	-	+	-	-

'+' indicates present and '-' indicates absent

was determined and reported as milligrams of Gallic Acid Equivalents (GAE) per gramme of dry sample. The phenolic content of the leaf extract was found to be the greatest at 29.14 ± 3.26 mg GAE/g, but the phenolic contents of the root and stem extracts were much lower at 7.21 ± 0.84 mg GAE/g and 4.02 ± 0.14 mg GAE/g, respectively. Using the calibration curve regression equation ($Y = 0.0165x + 0.0431$, $R^2 = 0.9955$), the total flavonoid content was determined and represented as mg/g of quercetin equivalents per gramme of dry material. Significantly high amount of flavonoid content present in the methanolic extract of the leaf was about 21.88 ± 1.14 mg QE/g while root and leaf showed about 8.90 ± 0.90 mg QE/g and 4.21 ± 0.82 mg QE/g of flavonoid, respectively.

The total amount of tannin was calculated from the standard equation ($y = 0.0047x + 0.016$ $R^2 = 0.9982$) obtained from the calibration curve of the tannic acid standard and expressed as mg of tannic acid equivalents per gram of sample in dry weight (mg/g).

Table 3. TFC, TTC and TPC content of *U. lobata*

Plant Part	TFC (mg of Quercetin E /g of extract)	TTC (mg of tannic acid E/g of extract)	TPC (mg of gallic acid E/g of extract)
Leaf	21.88 ± 1.14	25.96 ± 3.14	29.14 ± 3.26
Root	8.90 ± 0.90	19.30 ± 1.89	7.21 ± 0.84
Stem	4.21 ± 0.82	8.28 ± 0.74	4.02 ± 0.14

The obtained findings were parallel with the number of flavonoids and phenols were highest in the leaf at about 25.96 ± 3.14 mg of TAE/g of extract and 8.90 ± 0.90 TAE/g in root and 4.21 ± 0.82 TAE/g in stem. The obtained findings are depicted in Table 3.

3.6 Fluorescence Analysis

After being treated with several chemical reagents, the fluorescence properties of a powdered material were examined under ultraviolet light at two separate

Table 4. Fluorescence analysis of leaf powder of *U. lobata*

Sr. No.	Chemical Treatment	Observed Fluorescence	
		254nm	366nm
1	Leaf Powder + NaOH in Methanol (1N)	Light Green	Light Greenish
2	Leaf Powder + NaOH in water (1N)	Dark Brown	Light Greenish
3	Leaf Powder + 50% HCl	Yellowish Green	Slightly Green
4	Leaf Powder + 50% H_2SO_4	Dark Green	Light Green
5	Leaf Powder + 50% HNO_3	Dark Yellow	Darkyellowish
6	Leaf Powder + Chloroform	Dark Green	Light Green
7	Leaf Powder + Picric acid	Yellow	Light Green
8	Leaf Powder + 5% $Fecl_3$	Dark Brown	Yellowish Green
9	Leaf Powder + 5% Iodine	Dark Brown	Faint Green
10	Leaf Powder + Petroleum ether	Yellow	Faint Green
11	Leaf Powder + Methanol	Black	Dark Green

wavelengths, 254 nm and 366 nm, the fluorescence were seen in Table 4.

3.7 Phytochemical Screening of Different Fractions

The results of phytochemical screening of fractions are mentioned in Table 5.

3.8 Chromatographic Studies

3.8.1 Mobile Phase Optimization over TLC

Through experimentation with various mobile phases in chromatography, an effective resolution was achieved with the composition of toluene: ethyl acetate: glacial acetic acid in a ratio of 9:1:0.1. After the mobile phase optimization this optimized mobile phase was used to develop HPTLC chromatograms shown in Figure 8.

The result of HPTLC fingerprint profiles of various fraction of *U. lobata* leaf was performed using the solvent system toluene: ethyl acetate: glacial acetic acid (09:01:0.1) and R_f values are shown in Table 6. Figure 8 shows the developed plate at day light, UV 254 and 366 nm, while Figure 9 highlights the chromatogram.

Table 5. Phytochemical screening of *U. lobata* fractions

Chemical Constituents	Chemical Test	Fractions of Leaves			
		n-hexane fractionof UL	Chloroform fractionof UL	Ethyl acetate fractionof UL	Methanol fractionof UL
Flavonoid	Shinoda test.	+	+	+	-
	Lead Acetate test.	+	+	+	+
Glycoside	Killer Killani test.	-	-	-	-
Carbohydrate	Molish test.	-	-	+	-
	Fehling test.	+	+	-	-
Triterpene	Vanillin-Sulphuric Acid test	+	+	+	+
Amino Acid	Ninhydrin test.	-	-	+	-
Sterol	Liebermann- Burchard's test.	+	-	+	+
	Salkowski's test.	+	+	+	+
Phenol	$FeCl_3$ test.	+	+	+	+
Alkaloid	Mayer test.	+	-	+	+
	Dragendorff test.	+	+	-	+
Saponin	Foam test.	+	+	+	+
Tannin	$FeCl_3$ test.	+	+	-	+
	Dil. nitric acid test.	-	-	+	+
Protein	Million's test.	-	+	+	-
	Biuret test.	-	+	-	-

'+' indicates present and '-' indicates absent.

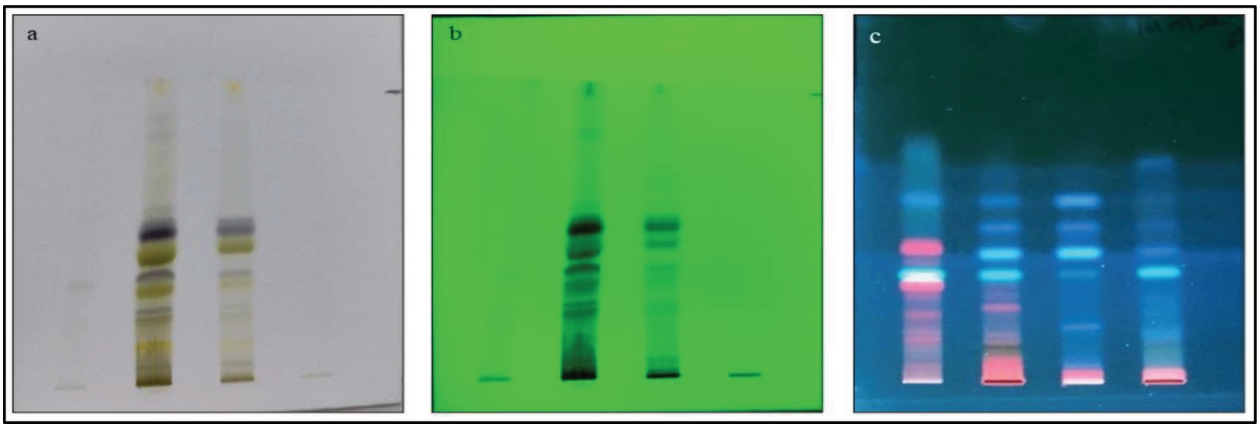

Figure 8. Visualization HPTLC fingerprint of four different fractions of *U. lobata* leaves at daylight (a), 254nm (b), 366nm (c).

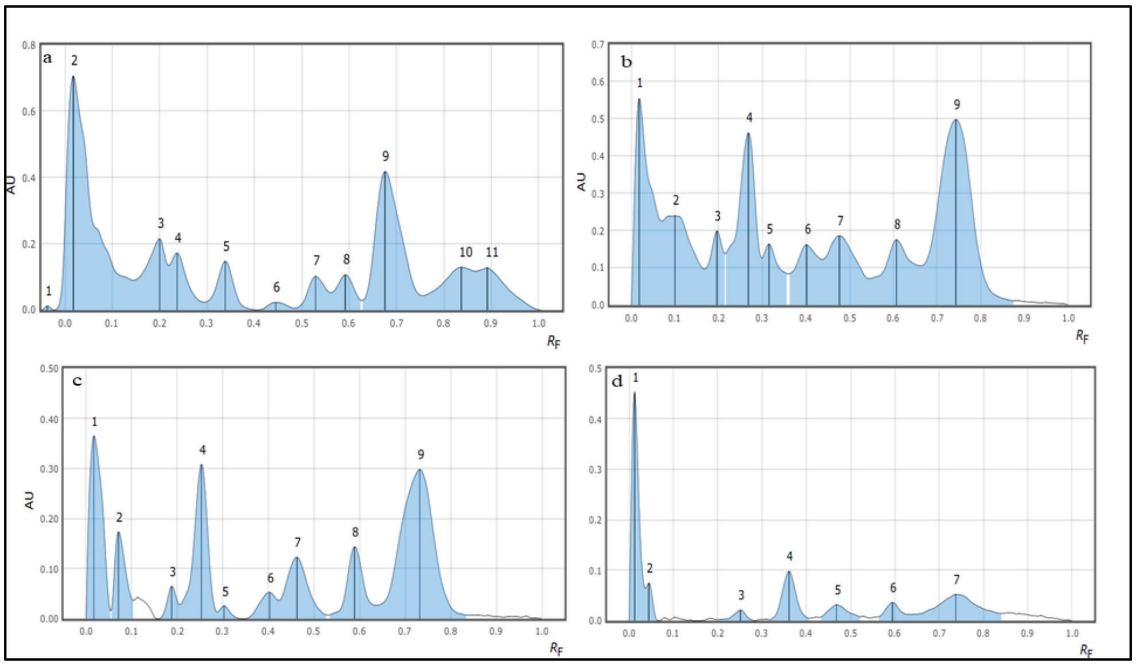

Figure 9. Chromatogram of four different fractions of *U. lobata* leaves at 366 nm.

Table 6. RF value of different spots of *U. lobata* fraction

Peak No.	N- Hexane Fractionof UL	Chloroform Fractionof UL	Ethyl Acetate Fractionof UL	Methanol Fractionof UL
1	0.20	0.20	0.25	0.25
2	0.24	0.27	0.30	0.36
3	0.34	0.32	0.40	0.47
4	0.45	0.40	0.46	0.59
5	0.53	0.48	0.59	0.74
6	0.59	0.61	0.73	-
7	0.68	0.74	-	-
8	0.84	-	-	-
9	0.89	-	-	-

4. Discussion

In recent years, there has been a repossession of interest in the beneficial potential of herbal medicines as people are turning toward natural and alternative healthcare options. To provide reliable herbal products it is essential to standardize the raw material. An organoleptic analysis is a result of visual inspection and gives a primary idea about the identification of drug[34,35]. The pharmacognostic examination of certain physical parameters plays a pivotal role in establishing standards for crude drugs, as these parameters often exhibit constancy within a plant species. *U. lobata* under macro-microscopic studies revealed that leaves are erect herbaceous or semi-woody shrub with hairy stem and branches. Leaves were simple loabed, petiolate, alternate, wide or oval, 10-15 cm long, cordate at base. The *U. lobata* leaf is characterized by a convex midrib and hemispherical shape. It has a single epidermal layer, 3-4 layers of chlorenchyma, and a collateral vascular bundle. The mesophyll contains elongated palisade cells and parenchymatous cells, with various trichomes on both surfaces. Stem has a single epidermis covered with cuticle, stellate trichomes, collenchymatous cells, a cortex, a well-defined vascular system, phloem and xylem, cambium, biseriate to triseriate medullary rays, and a well-defined pith in the center. The root's transverse section shows cork, phellogen, and phelloderm outer periderm, cortex, and well-developed phloem, with well-defined xylem and medullary rays. Analysis of the quantitative parameters, physicochemical parameters and fluorescence properties produced useful information for standardizing the plant. Phytochemical screening is employed to ascertain the presence of plant secondary metabolites[35,36]. Through qualitative analysis, the leaf demonstrates the existence of tannins, flavonoids, steroids, triterpenoids, and alkaloids. Likewise, the stem powder exhibits tannins, steroids, triterpenoids, and flavonoids. Additionally, the root powder displays similar constituents to the leaf and stem. All three parts of the plant have shown a remarkable presence of tannins and flavonoids the plant was further subjected to estimation of total tannin, phenol, and flavonoid content. Total phenolic compounds in the leaf, root, and stem of *U. lobata* were about 29.14±3.26, 7.21±0.84, and 4.02±0.14 respectively, while the total amount of flavonoids was about 21.88±1.14, 8.90±0.90, 4.21±0.82 in leaf, root, and stem respectively. The total amount of tannins in the leaf was 25.96±3.14, the root 19.30±1.89, and the stem 8.28±0.74 was estimated. These phyto constituents are potent antioxidants and can protect against oxidative stress-induced disorders. The presence of polyphenols, tannins and flavonoids in the extract is thought to enhance its capability to hunt free radicals by donating hydrogen. Analysis has revealed higher concentrations of total phenolic, total tannin and total flavonoids in the leaf sample compared to other plant parts and hence leaf was further studied for physicochemical analysis and HPTLC fingerprinting to develop its quality control profile. Physico-chemical parameters serve as critical indicators for discerning adulteration and ensuring the integrity of crude drugs[37]. Key parameters, including moisture content, ash value, and extractive value, were analyzed for *U. lobata* leaf. Moisture detection in crude drugs is imperative to mitigate microbial proliferation and degradation risks[38,39]. The moisture content of the leaf was about 4.72%. Ash value determination elucidates the presence of organic impurities such as carbonates, oxalates, and silicates[40]. The leaf was subjected to the determination of total ash, acid-insoluble ash, and water-soluble where the amount was 8.07, 2.25, 2.23, respectively. Extractive value assessment facilitates the quantification of soluble phytoconstituents in specific solvents, thereby offering insights into their composition and bioactivity[41,42]. The water-soluble extractives were 10.89, while soluble alcohol-soluble extractives were about 6.15 in leaf. HPTLC fingerprinting is a valuable tool for ensuring the quality control of herbal products and for detecting adulterants[43]. HPTLC profile of n-hexane, chloroform, ethyl acetate, and methanolic fraction leaf were generated using solvent system toluene: ethyl acetate: glacial acetic acid in ratio of 09:01:0.1. The hexane fraction of the leaf exhibited nine spots at R_f values of 0.20, 0.24, 0.34, 0.45, 0.53, 0.59, 0.68, 0.84, 0.89 in ascending sequence. The chloroform fraction has exhibited seven pots at Rf 0.20, 0.27, 0.32, 0.40, 0.48, 0.61, 0.74 in increasing order six spots at R_f 0.25, 0.30, 0.40, 0.46, 0.59, and 0.73 were found in the ethyl acetate fraction. While five spots were observed on methanolic fraction at R_f 0.25, 0.36, 0.47, 0.59, 0.74 at 254 nm.

5. Conclusion

Urena lobata, commonly known as caesarweed, is widely distributed in India. It is traditionally utilized for its anti-inflammatory and diuretic properties, and in treating conditions such as bronchitis, gastritis, cough, nephritis, diarrhea, and fever. Various extracts from its leaves and roots have exhibited antibacterial, anti-inflammatory, and potential benefits in rheumatism. The present study showed anatomical details of the leaf, stem, and root. The study also showed the presence of flavonoids, phenolics, tannins, alkaloids, carbohydrates, steroids, and terpenoid compounds upon phytochemical screening. The leaves are particularly abundant in tannins, phenols, and flavonoids. Physicochemical analysis of the leaf yielded satisfactory results across all parameters examined. High-Performance Thin Layer Chromatography (HPTLC) fingerprinting profiles were developed for various fractions of the leaf, which is crucial for the standardization and authentication of herbal drugs. These findings provide essential tools for establishing quality standards and identifying *U. lobata*.

8. Reference

1. Upadhya V, Hegde HV, Bhat S, Hurkadale PJ, Kholkute SD, Hegde GR. Ethno medicinal plants used to treat bone fracture from North-Central Western Ghats of India. J Ethnopharmacol. 2012; 142:557-62. https://doi.org/10.1016/j.jep.2012.05.051 PMid:22668503
2. Kingston DG. Modern natural products drug discovery and its relevance to biodiversity conservation. J Nat Prod. 2011; 74:496-511. https://doi.org/10.1021/np100550t PMid:21138324 PMCid:PMC3061248
3. Patil VV, Patil RV. *Ficus bengalensis* Linn. An overview. Int J Pharm Bio Sci. 2010; 1(2):1-11. https://doi.org/10.4103/0975-1483.51879
4. Rakholiya K, Chanda S. Pharmacognostic, physicochemical and phytochemical investigation of *Mangifera indica* L. var. Kesar leaf. Asian Pac J Trop Biomed. 2012; 2:S680-4. https://doi.org/10.1016/S2221-1691(12)60295-0
5. Omonkhua AA, Onoagbe IO. Effects of *Irvingia grandifolia*, *Urena lobata* and *Carica papaya* on the oxidative status of normal rabbits. Internet J Nutr Wellness. 2008; 6(2):2.
6. Adewale AO, David AA, Abiodun OO. Studies on antimicrobial, antioxidant and phytochemical analysis of *Urena lobata* leave extract. J Phys Nat Sci. 2007; 1(2):1220.
7. Yadav AK, Tangpu V. Antidiarrheal activity of *Lithocarpus dealbata* and *Urena lobata* extracts: Therapeutic implications. Pharm Biol. 2007; 45(3):2239. https://doi.org/10.1080/13880200701213153
8. Rinku M, Prasanth V, Parthasarathy G. Immunomodulatory activity of the methanolic extract of *Urena lobata* Linn. Internet J Pharmacol. 2008; 7(1):14. https://doi.org/10.5580/ba4
9. Babu SS, Madhuri DB, Reddy DS. Anxiolytic antidepressant and anti-inflammatory activity of ethanolic extract of *Urena lobata* leaf. Int J Pharm Res Health Sci. 2016; 4:1284-90. https://doi.org/10.21276/ijprhs.2016.04.07
10. Onoagbe IO, Negbenebor EO, Ogbeide VO, Dawha IH, Attah V, Lau HU, *et al*. A study of the antidiabetic effects of *Urena lobata* and *Sphenostylis stenocarpa* in streptozotocin induced diabetic rats. Eur J Sci Res. 2010; 43(1):614.
11. Mathappan R, Prasanth VV, Jolly CI, Somanath M. Comparative study on the antibacterial activity of the methanolic extract of *Urena lobata* root and a standard marketed herbal formulation. J Pharm Res. 2010; 3(5):953.
12. Lissy KP, Simon TK, Lathab MS. Antioxidant potential of *Sida retusa, Urena lobata* and *Triumfettar homboidea*. Anc Sci Life. 2006; 25(34):105.
13. Dhanapal R, Ratna JV, Gupta M, Sarathchandran I. Preliminary study on antifertility activity of *Enicostem maaxillare* leaves and *Urena lobata* root used in Indian traditional folk medicine. Asian Pac J Trop Med. 2012; 5:61622. https://doi.org/10.1016/S1995-7645(12)60127-8 PMid:22840449
14. Ali S, Faruq KO, Rahman AA, Hossain MA. Antioxidant and cytotoxic activities of methanol extract of *Urena lobata* (L.) Leaves. Pharm Innov J. 2013; 2(2):914.
15. Pieme CA, Ngogang J, Costache M. *In vitro* antiproliferative and antioxidant activities of methanol extracts of *Urena lobata* and *Viscum album* against breast cancer cell lines. Toxicol Environ Chem. 2012; 94(5):113. https://doi.org/10.1080/02772248.2012.674135
16. Islam H, Hasanur Rahman KM, Rahman S, Rahmatullah M. Preliminary antihyperglycemic, antinociceptive activity, phytochemical analysis and toxicity studies on leaves of *Urena lobata* L. J Chem Pharm Res. 2015; 7(4):55963.
17. Oladele GM, Abatan MO. Haematological and sperm count changes following exposure to *Hyptissuaveolens, Cleome viscosa* and *Urena lobata* in rats. Nig Vet J. 2010; 31(2):1706. https://doi.org/10.4314/nvj.v31i2.68956
18. Mathappan R, Umachigi SP, Prasanth VV. Wound healing activity of the methanolic extract of *Urena lobata* Linn. Int J Pharm Chem Sci. 2013; 2(2):793800.
19. Rajagopal PL, *et al*, Anti-arthritic activity of the leaves or *Urena lobata*. Int J Res Rev. 2019; 6(1):86-9.
20. Amponsah IK, Mensah AY, Otoo A, Mensah MLK, Jonathan J. Pharmacognotic standardisation of *Hilleria latifolia* (Lam.) H. Watt. (Phytolaccaceae). Asian Pac J Trop Biomed. 2014; 4:9410-946. https://doi.org/10.12980/APJTB.4.2014APJTB-2014-0416

21. Khaabadi SS, Deore SL, Baviskar BA. Experimental Phytopharmacognosy. A Comprehensive Guide. First edition, Nirali prakashan, Pune. 2011; 4(13).
22. Akbar S, Hanif U, Ali J, Ishtiaq S. Pharmacognostic studies of stem, roots and leaves of *Malva parviflora* L. Asian Pac J Trop Biomed 2014; 4(5):410-5. https://doi.org/10.12980/APJTB.4.2014C1107 PMid:25182728 PMCid:PMC3985058
23. Najafi S, Deokule SS. Pharmacognostic study of *Tylophora dalzelli* Hook. F. J Med Plant Res. 2010; 4(5):403-6.
24. Menpara D, Chanda S. Phytochemical and pharmacognostic evaluation of leaves of *Pongamia pinnata* L. (Fabaceae). Phcog Commun. 2014; 4(2):3-7. https://doi.org/10.5530/pc.2014.2.2
25. Govindappa M, Naga Sravya S, Poojashri MN, Sadananda TS, Chandrappa CP. Antimicrobial, antioxidant and *in virto* anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc. Pharmacognosy Journal. 2011; 3(3):43-51. https://doi.org/10.5530/pj.2011.25.15
26. SaeedN, Khan M R, Shabbir M. Antioxdant activity, total Phenolic and Total Flavanoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complement Altern Med. 2012; 12:221. https://doi.org/10.1186/1472-6882-12-221 PMid:23153304 PMCid:PMC3524761
27. Tambe V D, Bhambar R S. Estimation of total phenol, tannin, alkaloid and flavanoid in *Hibiscus tiliaceus* linn. wood extracts. JPP. 2014; 2(4):41-7.
28. Sultana M, Verma P, Raina R, Prawez S, Dar M A. Quantitative analysis of total phenolic, flavanoids and tannin contents in acetone and n- hexane extracts of ageratum conyzoides. Int J Chemtech Res. 2012; 4(3):996-9.
29. Alhakmani F, Kumar S, Khan S A. Estimation of total phenolic content, *in-vitro* antioxidant and anti-inflammatory activity of *Moringa oleifera*. Asian Pac Trop Biomed. 2013; 3(8):623-7. https://doi.org/10.1016/S2221-1691(13)60126-4 PMid:23905019
30. Aliyu A B. Free radical scavenging ANF Total antioxidant capacity of root extracts of *Anchomanes difformis* Engl (Araceae). Acta Pol Pharm. 2013; 70(1):115-21.
31. Riaz T. Phytochemical screening, free radical scavenging, antioxidant activity and phenolic content of *Dodonaea viscose* Jacq J Serb Chem Soc. 202; 77(4):423-35. https://doi.org/10.2298/JSC110621183R
32. Babu K, Shankar SG, Rai S. Comparative pharmacognostic studies on the barks of four *ficus* species. Turk J Bot. 2010; 34(3): 215-24. https://doi.org/10.3906/bot-0907-115
33. Sethi PD. High performance thin layer chromatography. Student 1st ed., vol. X. New Delhi: CBS Publishers and Distributers; 1996.
34. Organization WHO. Quality control methods for medicinal plant materials: World Health Organization; 1998.
35. Neyanila SK, Prakash Y, Gopal V. Preliminary phytochemical and pharmacognostical standardization of aerial parts of *Merremia tridentata* (linn) Hallier. f. Convolvulaceae. Int J Pharm Res. 2013; 3(2):99-105.
36. Doctor T, Manuel J. phytochemical screening of selected indigenous medicinal plants of Tublay, Benguet Province, Cordillera administrative region, Philippines. Int J Sci Res Publ. 2014; 4(4):1.
37. Chanda S. Importance of pharmacognostic study of medicinal plants: An overview. J Pharmacogn Phytochem. 2014; 2(5):69-73.
38. Okhale SE, Amanabo MO, Jegede IA, Egharevba HO, Muazzam IW, Kunle OF. Phytochemical and pharmacognostic investigation of antidiabetic *Scoparia dulcis* Linn. Scrophulariaceae whole plant grown in Nigeria. Researcher. 2010; 2(6):7-16.
39. Musa K, Katsayal A, Ahmed A, Mohammed Z, Danmalam U. Pharmacognostic investigation of the leaves of *Gisekia pharnacioides*. Afr J Biotechnol. 2006; 5(10):1-2.
40. Tatiya A, Surana S, Bhavsar S, Patil D, Patil Y. Pharmacognostic and preliminary phytochemical investigation of *Eulophia lind* tubers (Orchidaceae). Asian Paci J Trop Dis. 2012; 2:S50-5. https://doi.org/10.1016/S2222-1808(12)60123-6
41. Shinde K, Shinde V, Sharma K, Mahadik KR. Phytochemical and Pharmacological Investigation on *Vitex negundo* Linn. Planta Medica. 2010; 76(05):P77. https://doi.org/10.1055/s-0030-1251839
42. Senguttuvan J, Subramaniam P. HPTLC Fingerprints of various secondary metabolites in the traditional medicinal herb *Hypochaeris radicata* L. J Bot. 2016; 1:1-11. https://doi.org/10.1155/2016/5429625
43. Navale AM, Patel KS, Paranjape AN. Phytochemical screening and HPTLC fingerprinting of *Anogeissus acuminata* extracts. Int. J Res Ayurveda Pharm. 2016; 7(2):207-9. https://doi.org/10.7897/2277-4343.07288