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Research Article

Bioefficacy of *Lipoblepharis urticifolia* (Blume) Orchard: Pharmacognostic Insights, Essential Oil Analysis, Evaluation of Antioxidant and Antidiabetic Properties

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ABSTRACT

The study comprehensively characterized *Lipoblepharis urticifolia* (syn. *Wedelia urticifolia*) leaves, focusing on elucidating the phytochemical composition of their essential oil, assessing the antioxidant and antidiabetic activities of its methanolic leaf extract. The study also involved a detailed pharmacognostic analysis of macroscopic, microscopic, fluorescence, and physicochemical properties. The essential oil was found to contain 17 individual chemical components through a gas chromatography-mass spectrometry (GC-MS) study, predominantly octadecanoic acid ethenyl ester (69.28%), followed by β-caryophyllene (7.54%) and β-elemene (4.05%). The methanolic extract of *L. urticifolia* leaves demonstrated dose-dependent total antioxidant capacity with increasing absorbance observed in the phosphomolybdate assay. Furthermore, it exhibited significant nitric oxide scavenging activity, showing an IC_{50} of 29.10 µg/mL comparable to the standard Vitamin C (IC_{50}=18.25 µg/mL). Pharmacognostic analysis characterized *L. urticifolia* leaves as bitter, ovate, and serrated with bristly hairs; quantitative microscopy revealed a stomatal index of 33.1%, trichome index of 23.3%, and vein islet/termination numbers of 1 per mm^2. Powder microscopy confirmed anomocytic stomata and uniseriate non-glandular trichomes, complemented by varied UV fluorescence. Physicochemical analysis revealed low moisture, high acid-soluble ash, superior methanolic extract yield and abundant K, Ca, Na, detectable Fe and P. This research offers crucial data for authenticating, identifying, and assessing the therapeutic potential of *L. urticifolia*.

Introduction

Lipoblepharis urticifolia, formerly known as *Wedelia urticifolia* (L.) DC., has been traditionally employed in the treatment of various health conditions, especially inflammation, skin diseases, and gastrointestinal disorders. [1] It has been widely used in various indigenous medicinal systems, highlighting its ethnopharmacological importance. Recent advancements in molecular phylogenetics and detailed morphological assessments have led to a taxonomic revision of this species. As a result *W. urticifolia* has been reclassified under the genus *Lipoblepharis* and the currently accepted scientific name for this species is *L. urticifolia* (Blume) Orchard.[2] This reclassification has important implications for botanical research, conservation and pharmacognostic standardization, ensuring accurate species identification and preventing misapplication of pharmacological data. *L. urticifolia* is a fragrant, perennial herb of medium height. It features elliptic leaves and bright yellow flowers arranged in terminal capitulum inflorescence. [3] Primarily found in tropical and subtropical Asia, this Asteraceae member has not been extensively studied. The Irula tribal communities in the Bolampatty valley of the Nilgiri Biosphere Reserve have extensive traditional

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knowledge of medicinal plants, including using the entire *W. urticifolia* plant to treat jaundice.[4] This traditional remedy is supported by scientific findings, as *W. urticifolia* and similar species contain coumestan derivatives like wedelolactone and demethylwedelolactone, which are recognized for their strong anti-hepatotoxic properties.[5] Essential oils and extracts from *W. urticifolia* and other *Wedelia* species have demonstrated significant antimicrobial activity. In addition to being effective against yeast and molds, they demonstrate activity against several bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. [6] This antimicrobial effect is primarily due to key components such as α-pinene and limonene. [7]

Phytochemical profiling of *W. urticifolia* extracts demonstrates a high concentration of various secondary metabolites, including flavonoids, phenolic compounds, terpenoids, alkaloids, and saponins.[8] These classes of compounds are well-recognized for their diverse biological activities, notably their potent antioxidant and antidiabetic properties.[9] Certain plant compounds, such as flavonoids and phenolics, are recognized for their capacity to directly neutralize free radicals, bind with metal ions, and modulate the systems of antioxidant enzymes.[10] Plant-based compounds are reported to exert antidiabetic effects by regulating key metabolic pathways. For example, they can block carbohydrate-digesting enzymes like α-amylase and α-glucosidase, stimulate insulin secretion from β-cells, or improve how well peripheral tissues respond to insulin. Additionally, some compounds can lower the rate of gluconeogenesis. [11] Given the established role of oxidative stress in diabetes pathogenesis, the traditional uses of *L. urticifolia* and its identified phytochemical constituents with known antioxidant and antidiabetic properties, a scientific investigation into these activities is highly warranted. While some studies have explored general phytochemical profiles, detailed scientific validation of its specific antidiabetic and antioxidant mechanisms remains largely underexplored.

Despite the limited existing data on its physicochemical and pharmacognostic characteristics, and the scarcity of detailed GC-MS analyses of its essential oil, the research aims to fill these gaps. The present study will systematically identify the chemical makeup of *L. urticifolia* essential oil using GC-MS analysis, investigate the antioxidant and antidiabetic potency of its methanolic leaf extract *in-vitro* and thoroughly define its botanical identity and quality through pharmacognostic studies. This integrated approach is expected to both scientifically affirm the traditional uses of *L. urticifolia* and build a strong scientific basis for its therapeutic potential. The extensive data collected will be invaluable as a reference for future in-depth pharmacological research, potentially aiding in the creation of new plant-derived medicines.

Materials And Methods

Plant Material

Samples of *L. urticifolia* leaves, collected from Vazhathope, Idukki district, Kerala, were taxonomically authenticated by Dr. V.B. Sreekumar, a Principal Scientist at the Kerala Forest Research Institute (KFRI). A voucher specimen (accession no. 17701) is preserved in the KFRI herbarium. For chemical analysis, the collected leaves were shade-dried, coarsely powdered, and sieved through a 100-mesh sieve before being stored in an airtight container. Separately, fresh leaves were used for micromorphological and anatomical investigations. [12]

Isolation of Essential Oil and GC-MS Analysis

Hydrodistillation was the method used to extract essential oil from 1 kg of fresh *L. urticifolia* leaves in a Clevenger-type apparatus. [13] The plant material was subjected to hydrodistillation by boiling it in 500 mL of distilled water for a duration of 3 hours. The resulting essential oil was then isolated, and any residual moisture was removed by treatment with anhydrous sodium sulfate (Na_2SO_4). The purified oil was subsequently stored in amber screw-cap vials to protect it from photodegradation. The final oil yield was expressed as a percentage of the total mass of the dried plant matter.

The GC-MS analysis was performed using a Shimadzu Nexis GC-2030 system, which included an AOC-30/20i autosampler. For the separation of compounds, a SH-I-5 Sil MS capillary column with dimensions of 30 m x 0.25 mm ID and a 0.25 µm film thickness was employed. Helium served as the carrier gas at a constant flow rate of 1 mL/min, and a 1:100 split injection ratio was utilized.

The oven temperature was programmed to ramp from 60 to 280°C, while the ion source was held at 230°C. The mass spectrometer operated in electron ionization (EI) mode at 70 eV, scanning mass-to-charge ratios (m/z) from 41 to 450 amu with a scan interval of 0.5 seconds. Compounds were identified by cross-referencing their retention times with a standard n-alkane series (C_6–C_{22}) and matching their mass spectra and retention indices with the NIST 20 spectral library.

Antioxidant Activity

Nitric oxide radical scavenging assay

A spectrophotometric method was used to measure the nitric oxide scavenging activity of the methanolic leaf extract. The assessment was performed using various extract concentrations, from 10 to 200 µg/mL. The procedure began by mixing 100 µL of each concentration with an equal volume of a 10 mM sodium nitroprusside solution, which was prepared in saline phosphate buffer. Following this, 1-mL of Griess reagent was added to the reaction mixtures, which were then incubated for 3 hours. The Griess reagent was formulated as a 1:1 mixture of

1% sulphanilamide in 2% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride in water. The absorbance of the solutions was subsequently measured at 540 nm against their respective blank solutions to determine the scavenging effect. [14]

Total antioxidant capacity

The total antioxidant capacity was determined using the phosphomolybdate method.[15] In this procedure, 0.3 mL of each test sample was combined with 3 mL of a reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The resulting reaction mixtures were incubated at 95°C for 90 minutes. Following incubation, the absorbance of the solutions was measured at 695 nm using UV-vis spectrophotometer 117 (Systronics) with a blank solution serving as the reference. The total antioxidant activity was then reported as milligrams of ascorbic acid equivalents per gram of sample.

Antidiabetic activity

The quantitative α-amylase inhibition assay was performed spectrophotometrically using a modified 3,5-dinitrosalicylic acid (DNSA) method. [16] The α-amylase inhibition assay was initiated by mixing 100 µL of the *L. urticifolia* methanolic extract (1-mg/mL) with 100 µL of α-amylase solution (1-mg/mL) and 100 µL of 0.1 M phosphate buffer (pH 7.0). After a 10-minute pre-incubation at room temperature, the enzymatic reaction was started by adding 100 µL of a 0.1% starch solution and incubated for an additional 30 minutes.

To stop the reaction, 1-mL of DNSA reagent was added and the samples were heated for 5 minutes. After cooling and diluting to 10 mL with water, absorbance was read at 540 nm. The experiment included an enzyme-only control, sample blanks to correct for background noise, and a positive control with 100 µg/mL of acarbose.

The percentage inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

where A_{control} is the absorbance of the enzyme control and A_{sample} is the corrected absorbance of the test sample. The IC_{50} value was determined from the dose-response curve.

Pharmacognostic Characterization

Organoleptic and morpho anatomical characterization

The organoleptic and morphological features of the leaves were systematically documented. This included their shape, size, margin, apex, color, odor, and venation pattern. For detailed anatomical characterization comprehensive observations of stomatal and trichome morphology, alongside quantification of vein islet and vein termination numbers were conducted. These analyses were done utilizing both paradermal sections and a leaf clearing technique employing a 5% sodium hydroxide solution. Epidermal layers were isolated by partial maceration using Jeffrey's maceration fluid. [17]

Powder microscopy

Powdered leaf samples were cleared using 75% chloral hydrate and mounted on slides following standard microscopic preparation methods. [18]

Fluorescence analysis

Fluorescence analysis was conducted on the powdered leaf material. This involved exposing small quantities of the dried powder to a range of chemical reagents. The treated samples were analyzed with visible light and ultraviolet light, specifically at 254 nm (shortwave) and 366 nm (longwave). We then recorded any changes in colour to evaluate their fluorescence characteristics. [19]

Physicochemical Analysis

Physicochemical analysis of the powdered leaf was conducted to determine parameters, including extractive values, moisture content (loss on drying), total ash, water-soluble ash, and water-insoluble ash.

Ash values

The ash content of the leaf material was quantified in accordance with standard pharmacopoeial methods to evaluate the total mineral content and purity of the sample. [20]

Total ash

To determine the total ash content, a 2 g sample of the powdered plant material was precisely weighed into a crucible. The material was distributed evenly before being slowly heated in a muffle furnace to a temperature ranging from 500–600°C. The heating continued until a uniform grey ash, free of carbon, was obtained. The resulting ash was then cooled in a desiccator, and the total ash content (w/w) of the air-dried material was calculated using a specific formula. [21]

$$\text{Percentage of total ash} = \frac{\text{weight of ash sample (g)}}{\text{weight of sample (g)}} \times 100$$

Determination of acid-insoluble ash content

About 25 mL solution of 2N HCl was added to the total ash in a crucible, which was then covered with a watch glass and boiled for 5 minutes. After rinsing the watch glass with 5 mL of hot water and returning the rinse to the crucible, the acid-insoluble residue was collected on ashless filter paper. This paper, along with the ash, was transferred to a pre-weighed crucible for drying in an oven, followed by cooling in a desiccator and final weighing. [22] The acid-insoluble ash content (w/w) of the air-dried material was calculated using the following formula

$$\text{Percentage of acid insoluble ash} = \frac{\text{weight of ash (g)}}{\text{weight of sample (g)}} \times 100$$

Determination of water-soluble ash content

The ash was boiled for 5 minutes with 25 mL of distilled water. The insoluble residue was then collected in a crucible or on an ashless filter paper, washed with hot water, ignited, and weighed. The residue was allowed to cool in a desiccator and weighed. The weight of the residue in milligrams was subtracted from the total ash weight and the water-soluble ash content was calculated per gram of air-dried material. [23]

Loss on drying (Moisture Content)

For the estimation of moisture content in the raw drug, one gram of the pulverized leaf sample was accurately weighed and transferred into a clean, dry crucible prior to further analysis. This was then dried in an oven at 100°C for one hour. Following the drying period, the crucible and its contents were cooled in a desiccator before being reweighed. The resulting loss in weight was used to calculate the moisture content present in the crude drug. [24]

Extractive values

The extraction yield was measured according to a standardized protocol. [25] Ten grams of powdered plant material were successively extracted with 50 mL each of petroleum ether, ethyl acetate, and methanol, employing ultrasonication at 50 kHz for 30 minutes in repeated 2-minute cycles. The filtered extracts were subjected to evaporation using a rotary evaporator until they were completely dry. The extractive values were then calculated as a percentage using the standard formula.

Elemental Analysis

The concentrations of alkali metals (sodium and potassium) and calcium in the extracts were quantified from the acid-insoluble ash fraction using a flame emission spectrophotometer.[26] To calibrate the instrument, standard sodium chloride solutions were used to establish the peak readings for concentrated solutions. The extracts, after being diluted with water, were analysed for sodium using a specific sodium optical filter. Subsequently, the sodium filter was replaced with potassium and calcium filters, respectively, to measure the potassium and calcium content, and their readings were recorded. Iron and phosphorus contents were determined separately using spectrophotometric methods. The iron content was analysed by the phenanthroline method, while phosphorus was analysed using the phosphomolybdenum blue reaction method.[27,28]

Results And Discussion

Phytochemical Profile of Essential Oil

GC-MS analysis plays a crucial role in identifying bioactive compounds that could validate the traditional ethnobotanical uses of the plant. Hydrodistillation of *L.urticifolia* leaves yielded an essential oil of dark yellowish with a brown tint and moderately intense aroma. Essential oil yield was about 0.35 mL/kg. Gas chromatography–mass spectrometry profiling of the essential oil showed 17 distinct peaks. The major compounds identified were octadecanoic acid ethenyl ester (69.28%), Kaur-16-en-18-al, (4.alpha) (0.38%), Phytol (0.64%), alpha-cadinol (0.50%), germacrene D (3.43%), trans- alpha-bisabolene (0.32%), cadina-1(10),4-diene (2.10%), alpha-farnesene (1.40%), humulene (2.19%), alpha-gurjunene (0.85%), β-caryophyllene (7.54%), cyperene (1.38%), copaene (2.86%), beta-elemene (4.05%). The major constituents of the essential oil included sesquiterpenes, oxygenated sesquiterpenoids, diterpenoids, and octadecanoic acid [Fig. 1 and Table 1]. The compounds were identified by cross-referencing their retention times and mass spectra with the NIST 20 library and existing scientific data.

Antioxidant Activity

Nitric oxide radical scavenging assay

Excessive production of nitric oxide can contribute to oxidative damage and inflammatory processes.[65] The methanolic extract of the leaf from *L. urticifolia* demonstrated strong and dose-dependent nitric oxide scavenging activity, as revealed by the results in [Fig. 2]. The extract achieved an IC_{50} value of 29.10 µg/mL, demonstrating its effectiveness in neutralizing NO radicals. This activity is notably comparable to that of the standard antioxidant, vitamin C, which showed an IC_{50} of 18.25 µg/mL. The ability of *L. urticifolia* to scavenge nitric oxide aligns with findings in other *Wedelia* species. For example, essential oils and extracts of *W. chinensis* have been reported to effectively scavenge nitric oxide, contributing to their overall antioxidant profile.[66] The presence of various terpenes (e.g., β-Caryophyllene, β-elemene identified in the essential oil) and other phenolic constituents in *L. urticifolia* likely contributes to this observed NO scavenging potential, as these compounds are well-known for their free radical neutralizing properties.[67] The potent nitric oxide scavenging activity noted in the *L. urticifolia* extract may be attributed to the combined, synergistic effects of its various phytochemicals.

Total Antioxidant Capacity

The assessment of total antioxidant capacity through the phosphomolybdate assay is based on the sample's ability

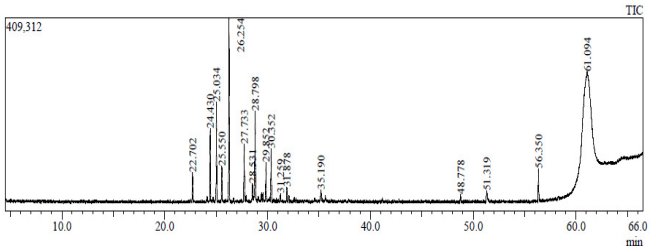


Fig. 1: GC-MS Chromatogram of Essential Oil from Leaves of *L. urticifolia*

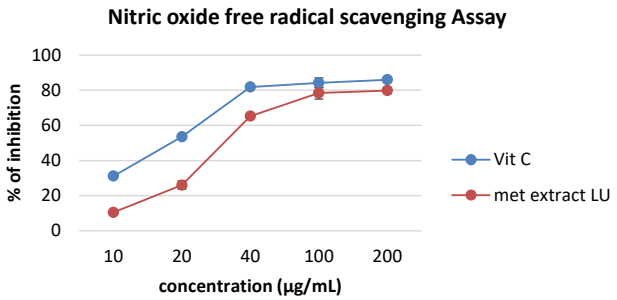


Fig. 2: Nitric Oxide Scavenging Activity of Methanolic leaf Extract of *L. urticifolia* (LU)

to reduce molybdenum (VI) into its lower oxidation state, molybdenum (V). This reaction results in a green-colored complex forming in an acidic solution.[68] The data in [Fig. 3] suggests a concentration-dependent increase in the overall antioxidant ability of the *L. urticifolia* methanolic extract. With increasing extract concentrations from 10 to 500 µg/mL, the absorbance at 695 nm showed a steady rise, indicating enhanced reducing power, whereas ascorbic acid, a strong reference antioxidant, demonstrated higher absorbance values at all tested concentrations.

The presence of numerous compounds capable of donating electrons and neutralizing free radicals likely accounts for the activity seen in *L. urticifolia*. This observation is in agreement with prior studies that have attributed the high total antioxidant capacity of various plant extracts to their rich content of phenolic compounds, flavonoids, and other phytochemicals. [69]

Antidiabetic activity (Alpha-Amylase Inhibition Assay)

Type 2 diabetes mellitus is characterized by hyperglycemia, particularly postprandial hyperglycemia, which contributes significantly to long-term complications.[70] A key therapeutic strategy involves inhibiting carbohydrate-hydrolyzing enzymes like α-amylase, thereby slowing down glucose absorption from the gastrointestinal tract and mitigating postprandial glucose spikes.[71] While synthetic α-amylase inhibitors like acarbose are available, they are often associated with gastrointestinal side effects, prompting a search for safer, natural alternatives from medicinal plants. [72]

Our investigation into the antidiabetic potential of the methanolic extract of *L. urticifolia* leaves revealed a dose-dependent α-amylase inhibitory activity [Fig. 4]. The control absorbance for the enzyme reaction was 0.799. Upon treatment with increasing concentrations of the *L. urticifolia* extract, a reduction in absorbance at 540 nm was observed, corresponding to an increase in the percentage of α-amylase inhibition. Specifically, at concentrations of 25, 50, 100, and 200 µL of the extract, the inhibition percentages were 22.27, 23.40, 23.77, and 27.65%, respectively. While a definitive IC_{50} value could not be calculated within the tested concentration range, the consistent dose-dependent inhibitory effect indicates that *L. urticifolia* possesses compounds capable of modulating α-amylase activity.

These findings are consistent with previous studies on other species within the *Wedelia* genus, which have demonstrated notable α-amylase inhibitory properties. Methanolic extracts of *W. trilobata* have been investigated for their α-amylase and α-glucosidase inhibitory activities, with diterpenoid glycosides identified as potential active constituents.[73] The α-amylase inhibition noted in *L. urticifolia* can likely be attributed to its abundant phytochemicals, such as flavonoids, phenolics, and other secondary metabolites, all of which are known to be effective enzyme inhibitors.[74] Further studies involving higher concentrations of the extract and isolation of active compounds would be beneficial to fully elucidate the antidiabetic potential and underlying mechanisms of *L. urticifolia*.

Pharmacognostic Evaluation

Macroscopic evaluation and Organoleptic characters

Pharmacognostic examination revealed that leaves of *L. urticifolia* are opposite, petiolated, ovate with sharply serrated margins, and covered in bristly hairs, giving

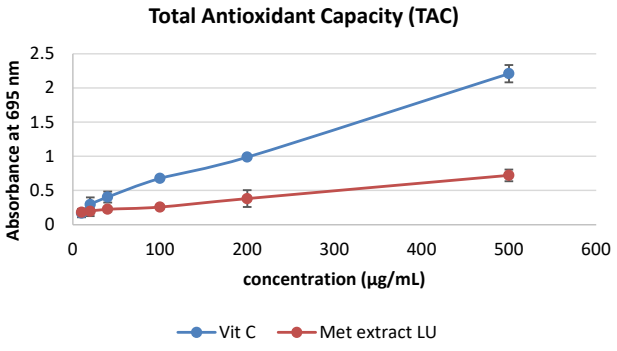


Fig. 3: Total Antioxidant Capacity of methanolic leaf extract of *L.urticifolia* (LU)

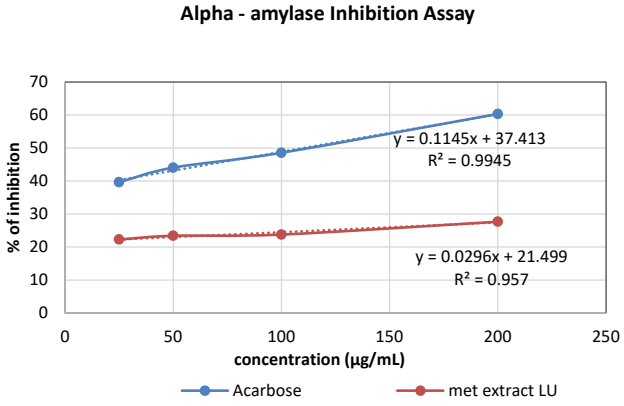
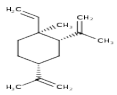
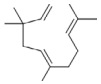
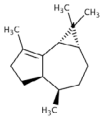
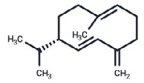
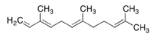
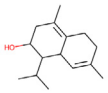
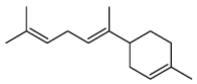
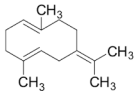
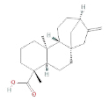
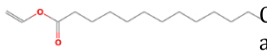


Fig. 4: Alpha Amylase inhibition Activity of methanolic leaf extract of *L .urticifolia* (LU)

Table 1: Major Compounds Identified in the Essential oil of Leaves of *L.urticifolia*

Peak No.	*Name of the compound*	*R.Time (min)*	*Area%*	*Molecular weight (g/mol)*	*Molecular Formula*	*Structure*	*Medicinal uses*
1	Delta-elemene	22.702	1.17	204.35	C15H24		The sesquiterpene delta-elemene demonstrates powerful anti-cancer properties by causing apoptosis and cell cycle arrest across a range of cancer types. [29] It also exhibits anti-inflammatory potential through its ability to modulate inflammatory cytokines such as TNF-α and IL-6. [30] Additionally, delta-elemene possesses antioxidant and antimicrobial properties. [31]
2	Copaene	24.430	2.86	204.35	C15H24		Copaene a tricyclic sesquiterpene, has demonstrated significant anti-inflammatory activity. It can help reduce inflammation by inhibiting the production and release of inflammatory mediators, such as prostaglandin E2. [32] Copaene is recognized as a natural antioxidant. Anticancer/ Antiproliferative Properties. [33] Certain enantiomers of copaene, particularly (+)-α-copaene, are known to be strong attractants for agricultural pests, such as the Mediterranean fruit fly (*Ceratitis capitata*). [34] Copaene has shown neuroprotective potential, particularly against oxidative stress-induced neurotoxicity. [35]
3	Beta- elemene	25.034	4.05	204.36	C15H24		It is a Sesquiterpene, possess strong anti-cancer properties [36], β-elemene exhibits notable anti-inflammatory effects,antioxidant properties [37], Shows antimicrobial activity against certain bacterial strains like Mycobacterium tuberculosis. [38]
4	Cyperene	25.550	1.38	204.35	C15H24		Cyperene is a tricyclic sesquiterpene hydrocarbon possess significant antioxidant activity and cytotoxic properties [39], possesses antimicrobial activity [40], exerts anti-inflammatory effects by modulating inflammatory mediators and enzymes like COX-2 and iNOS.[41]
5	β-Caryophyllene	26.254	7.54	204.36	C15H24		It is a natural bicyclic sesquiterpene compound, possess Anticancer, Antioxidant and Antimicrobial Properties. [42]
6	Humulene	27.733	2.19	204.351	C15H24		This naturally occurring monocyclic sesquiterpene demonstrates anti-inflammatory effects.[43] It has demonstrated anticancer and cytotoxic effects against several cancer cell lines. [44]
7	alpha-Gurjunene	28.531	0.85	204.35	C15H24		It is a sesquiterpene hydrocarbon, possessing Anti-inflammatory antioxidant Properties.[45]

8	Germacrene D	28.798	3.43	204.35	C15H24		It is a sesquiterpene hydrocarbon, possess Antimicrobial [46], Anti-inflammatory Effects [47], antioxidant capacity[48] and Anticancer Potential. [49]
9	alpha-farnesene	29.852	1.40	204.35	C15H24		It is a naturally occurring acyclic sesquiterpene, possess Antioxidant Activity [50], anti-inflammatory effect [51], Antimicrobial and Antifungal Activity. [52]
10	Cadina-1(10),4-diene	30.352	2.10	204.35	C15H24		It is a sesquiterpenoid compound, possessing Antioxidant activity [53], Cholinesterase inhibitory activity. [54]
11	trans-alpha-Bisabolene	31.259	0.32	204.35	C15H24		It is a sesquiterpene hydrocarbon shows antioxidant, antimicrobial and cytotoxic activities. [55]
12	Germacrene B	31.878	0.57	204.35	C15H24		It is a naturally occurring sesquiterpenoid, showing Antimicrobial, antioxidant, Insecticidal properties.[56]
13	alpha-Cadinol	35.190	0.50	222.37	$C_{15}H_{26}O$		It is a sesquiterpenoid alcohol, possess Antimicrobial Activity [57], Angiotensin-Converting Enzyme (ACE) Inhibitory Activity.[58]
14	Kaur-16-ene(8beta,13 beta)	48.778	0.38	272.46	$C_{20}H_{32}$		It is a diterpene compound, exhibits antimicrobial activity [59], anti-inflammatory. [60]
15	Phytol	51.319	0.64	296.5	$C_{20}H_{40}O$		It is a acyclic diterpene alcohol exhibits Anti-inflammatory, Antioxidant, antimicrobial, Anti-tumor/Anticancer properties. [61]
16	Kaur-16-en-18-al, (4 alpha)	56.350	1.36	286.45	$C_{20}H_{30}O$		Diterpenoid, Anticancer/Antitumor Activity.[62]
17	Octadecanoic acid, ethenyl ester	61.094	69.28	310.514	$C_{20}H_{38}O_2$		Octadecanoic acid (stearic acid) and its esters are reported to have antimicrobial[63], antioxidant activities. [64]

them a rough, nettle-like texture, bitter in taste, as shown in Table 2.

Anatomical Characterization

Microscopic examination of the leaf's transverse section showed a dorsiventral lamina, characterized by a well-defined midrib and lateral veins. A discrete epidermal layer covered the veins, and directly beneath it were two to three strata of tightly packed collenchyma cells. Each lateral vein features a single, top-shaped collateral vascular bundle at its core. This bundle contains 3 to 5 short, radial rows of xylem elements and an arc-shaped phloem region.

The leaf exhibits clear differentiation into palisade and spongy mesophyll. The palisade tissue comprises two layers of slender, rod-shaped cells. The leaf has stomata on both its upper and lower surfaces. The leaf lamina features uniseriate, 2 to 3-celled, pointed non-glandular trichomes on both its dorsal and ventral sides, as illustrated in Fig. 5. A single layer of epidermis covered by a thin cuticle was observed in a transverse section of the petiole. Epidermal layer features uniseriate, 1 to 2 celled, non-glandular trichomes with distinctive pointed tips [Fig. 6].

Stomatal Index , Trichome Index, Vein islet Number and Vein Termination Number

Leaf constants are fundamental quantitative metrics used in the microscopic evaluation and standardization of crude drugs. These include key parameters such as stomatal number, stomatal index, vein islet number, and veinlet termination number. These specific anatomical features provide critical data that contribute directly to the standardization of herbal medicines.[75] The quantitative microscopic analysis of *L. urticifolia* leaves revealed characteristic Vein islet number and vein termination number as 1-per mm^2 [Fig. 7]. Anomocytic stomata were found in both upper and lower epidermis [Fig. 8]. Stomatal index was calculated to be 33.1 ± 0.04, and trichome index was found to be 23.3 ± 0.69. Trichomes

Table 2: Macroscopic and Organoleptic evaluation of leaves of *L. urticifolia*

Features	*Observations*
Size	7-12 cm long
Shape	blade ovate
Apex	Acuminate
Margin	sharply serrated or toothed margins
Base	rounded
Venation	reticulate
Petiole	1-1.5 cm
Odour	Slightly aromatic
Taste	Slightly bitter
Colour	dark green
Texture	Hairy rough texture (leaves are typically covered with bristly hair)

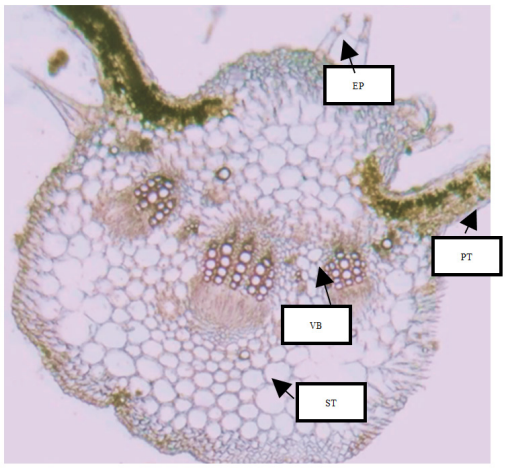
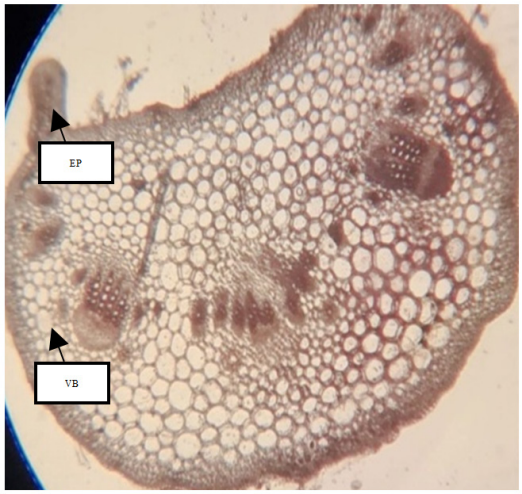


Fig. 5: T.S of leaf lamina



(TC- Trichome, EP – Epidermis, PT – Palisade Tissue, VB – Vascular Bundle, ST- Spongy Tissue)

Fig. 6: T.S of petiole

are present on both epidermis, which are of uniseriate, 2-3 celled, nonglandular type with bulbous base and pointed tip [Fig. 9]. The morphology, distribution, and density of nonglandular trichomes are highly conserved within species but often vary significantly between different species or genera of Asteraceae. This makes them valuable diagnostic characters for plant identification, especially when floral parts are unavailable or for distinguishing closely related taxa. [76]

Powder Microscopy

Powdered microscopic examination revealed the presence of anomocytic stomata and non-glandular, 2-3 celled uniseriate trichomes characterized by pointed tips.

Fluorescence Analysis

The leaves of *L. urticifolia* were treated with different reagents and analysed for their fluorescence properties. Different colour variations were observed under visible

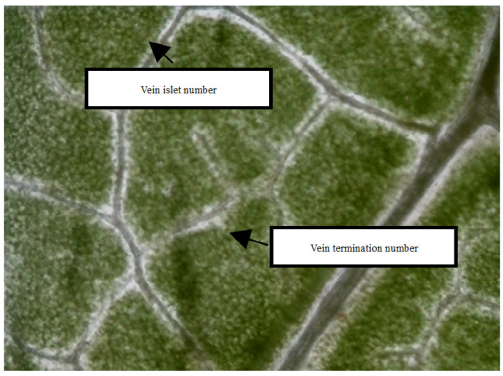


Fig. 7: Vein islet number and vein termination number

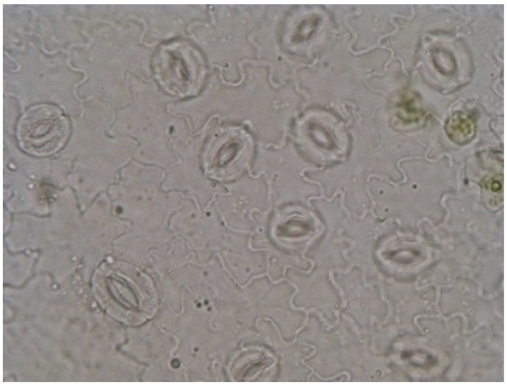


Fig. 8: Anomocytic stomata

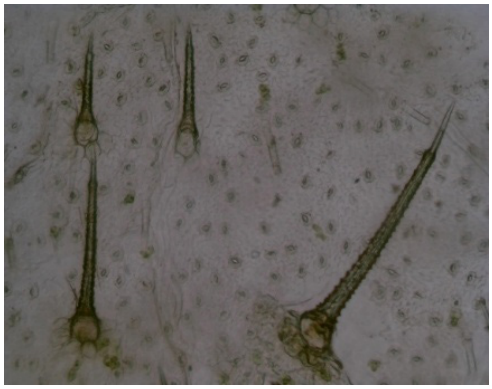


Fig. 9: leaf epidermal peeling showing trichomes

and ultraviolet light as depicted in Table 3. The consistency between the fluorescence pattern of the authenticated plant sample and established pharmacognostic data provides a reliable indicator of the drug's authenticity and purity. [77]

Physicochemical Analysis

The plant sample was subjected to a physicochemical analysis to determine its ash values and loss on drying. Successive extractive values using petroleum ether, ethyl acetate and methanol were also calculated. The amount of ash in a drug can provide insight into its mineral composition and any other inorganic impurities that are present.[78] The calculated values for total ash, acid-insoluble ash, and water-soluble ash were 15.11, 9.44, and 5.68%, respectively. Pharmacopoeias worldwide (e.g., European Pharmacopoeia, Indian Pharmacopoeia, USP, WHO guidelines) specify maximum permissible limits for moisture content (often between 10 to 14% for crude drugs). The powdered leaf sample was determined to have

Table 3: Fluorescence analysis of leaves of *L. urticifolia*

S. No	*Reagents*	*Visible light*	*UV 254nm*	*UV 366 nm*
1	Powder drug	Dark green	Light green	Dark green
2	Powder drug +distilled water	Dark green	Blackish green	Black
3	Powder drug +10% aq. Sodium hydroxide	Dark green	Blackish green	Black
4	Powder drug + ammonia	Dark green	Blackish green	Black
5	Powder drug + conc. Sulfuric acid	Brownish green	Blackish green	orange
6	Powder drug + sulfuric acid + water	Brownish green	Blackish green	orange
7	Powder drug + conc. Hydrochloric acid	Dark green	Dark green	Black
8	Powder drug + Hydrochloric acid + water	Reddish green	Dark green	Black
9	Powder drug + conc. Nitric acid	Red	Dark green	Black
10	Powder drug + nitric acid + water	Orange	Green	Black
11	Powder drug + iodine	Dark green	Dark green	Black
12	Powder drug + 5% ferric chloride	Yellowish green	Dark green	Black
13	Powder drug + picric acid	Yellowish green	Yellowish green	Black
14	Powder drug + picric acid +water	Yellowish green	Yellowish green	Black
15	Powder drug + glacial acetic acid	Dark green	Dark green	Red
16	Powder drug + petroleum ether	Yellowish green	Pale green	Brown
17	Powder drug + chloroform	Yellowish green	Pale green	Dark green
18	Powder drug +ethyl acetate	Pale green	Dark green	Black
19	Powder drug + methanol	Dark green	Black	Black
20	Powder drug + 5% potassium dichromate	Dark green	Black	Black
21	Powder drug + alcoholic potassium hydroxide	Dark green	Blackish green	Black

a moisture content of 5.37%. The solubility of the powdered plant material was assessed with three solvents chosen for their different polarities: methanol, ethyl acetate, and petroleum ether.[79] The highest extractive yield was observed with methanol (5.36 ± 0.28% w/w), followed by ethyl acetate (2.17 ± 0.13% w/w) and petroleum ether (1.21 ± 0.02% w/w), Table 4. Methanol, characterized by its high polarity and capacity for hydrogen bonding, is highly effective at dissolving a diverse array of phytochemicals. This includes numerous abundant polar compounds like glycosides, highly polar phenolics, and specific alkaloids, which often prove insoluble in less polar solvents.

This broad-spectrum solubility leads to a higher overall extractive value or yield. The higher extractive value of methanol compared to ethyl acetate and petroleum ether in pharmacognostic research is a well-established phenomenon attributed to methanol's higher polarity and its resultant ability to dissolve a broader and more abundant range of phytochemicals (particularly polar and moderately polar secondary metabolites) from complex plant matrices.[80]

Elemental Analysis

The medicinal properties of plants are frequently attributed to the essential minerals they contain, such as sodium, potassium, calcium, and magnesium.[81] Flame photometry helps to quantify these beneficial minerals, ensuring that herbal formulations meet specified nutritional or therapeutic profiles. The elemental analyses by flame photometer showed significant amounts of sodium (44.81 mg/100 g), calcium (281.71 mg/100 g) and potassium (390.49 mg/100 g) in *L. urticifolia* leaves. While the other elements like iron and phosphorus contents were found in only trace quantities, 21.43 mg/100g and 0.98 mg/100 g, respectively Table 5.

Table 4: Physicochemical analysis of *L. urticifolia* leaves

S. No.	*Parameters*	*Values % (w/w)*
1	Total ash value	15.11± 0.021
2	Acid insoluble ash	9.44 ± 0.015
3	Water soluble ash	5.68 ± 0.015
4	Loss on drying	5.37 ± 0.006
5	Extractive value	
	• Petroleum ether soluble extractive	1.21 ± 0.02
	• Ethyl acetate soluble extractive	2.17 ± 0.13
	• Methanol soluble extractive	5.36 ± 0.28

Values are mean ± standard deviation (SD)

Table 5: Elemental analysis of *L. urticifolia* leaves

Elements	*Concentration (mg/100g)*
Potassium	390.49 ± 0.11
Calcium	281.71± 0.02
Sodium	44.81± 0.11
Iron	21.43±0.05
Phosphorus	0.98± 0.02

Values are mean ± standard deviation (SD)

Conclusion

This study comprehensively characterized *L. urticifolia* (syn. *W. urticifolia*) leaves by elucidating the phytochemical profile of its essential oil via GC-MS analysis, evaluating the antioxidant and antidiabetic activities of its methanolic extract, and conducting detailed pharmacognostic studies. The essential oil's GC-MS chromatogram indicated the presence of numerous chemical compounds, which, based on literature, exhibit diverse bioactivities, including antioxidant, antimicrobial, anti-inflammatory, cytotoxic, analgesic, cholinesterase inhibitory, and insecticidal effects. The methanolic extract from *L. urticifolia* leaves exhibited a concentration-dependent increase in antioxidant properties. This was evidenced by both its total antioxidant capacity and a notable nitric oxide scavenging activity, with an IC_{50} value of 29.10 µg/mL. The inhibitory effect of the extract on α-amylase activity was concentration-dependent, suggesting its potential application as an antidiabetic agent. This research establishes foundational data for future investigations into *L. urticifolia* leaves. To fully understand the specific bioactivities of the key components in both the essential oil and the methanolic extract, a detailed investigation is warranted. Significantly, this study provides the first comprehensive pharmacognostic and physicochemical data for *L. urticifolia* leaves, offering essential standards for future quality and purity assessments of this plant.

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