

Development of Pharmacognostic Profile and Preliminary Phytochemical Study of *Pluchea wallichiana* DC

Kinjal Bera, Madhavi Patel*

Department of Pharmacognosy, Parul Institute of Pharmacy, Parul University, Limda, Vadodara, Gujarat, INDIA.

ABSTRACT

Introduction: The genus *Pluchea* comprises of around 80 species native to tropical and warm temperate areas. It has been traditionally known as rasna and used to treat different ailments including inflammation and arthritis. One of the species of *Pluchea*, *Pluchea wallichiana* DC (Family: Asteraceae), commonly known as wallich camphor weeds. **Aim and Objectives:** The present study aims to develop pharmacognostic profile, set the qualitative and quantitative characteristics of phytochemicals present in leaf, stem and root parts of plant and determine antioxidant potential. **Materials and Methods:** The plant profile development includes authentication, macro- microscopical, physico-chemical, and chromatographic evaluation of root, stem and leaf of the plant. The presence of essential minerals in the plant was confirmed using ICP-MS. The DPPH technique was used to assess the antioxidant potential. **Results:** *P. wallichiana* is an erect branched shrub with camphoraceous, green, alternate, broadly obovate and sessile leaves and white compound corymb flowers. The stems are circular and green, and the tap root is branched, cylindrical and brownish in color. Microscopy of leaf, stem and root has demonstrated clear anatomical features. The phytochemical screening unveils the presence of steroids, terpenoids, flavonoids, tannins, and coumarins. The HPTLC fingerprinting profile was developed from the mentioned plant parts. The leaf expressed higher percentage of phenolics, flavonoids and steroids as compared to root and stem. ICP-MS analysis demonstrated presence of calcium, potassium, magnesium, zinc, and iron present as a mineral in leaves. The concentration of leaf methanol extract required to inhibit 50% of DPPH free radicals was 29.49 µg/mL. **Conclusion:** The developed pharmacognostic and phytochemical parameters may help to establish standards for identification, quality, and purity of *Pluchea wallichiana* DC.

Keywords: *Pluchea wallichiana*, Morphology, Microscopy, Flavonoids, HPTLC, Antioxidant activity, Mineral element.

Correspondence:

Dr. Madhavi Patel

Department of Pharmacognosy, Parul Institute of Pharmacy, Parul University, Limda, Vadodara-391760, Gujarat, INDIA.
Email: madhavii10@yahoo.co.in

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INTRODUCTION

WHO explains 'Traditional medicine' as the collection of all information and methods utilized in the diagnosis, prevention, and treatment of mental or behavioral imbalances, based solely on realistic experience and observations carried down to the next generation in verbal or written form.¹ The worldwide interest in the research and application of medicinal plants has been significantly rise in the last two decades as people become more aware of the toxicity and adverse effects of synthetic medications.² The considerable increase in utilization of herb over the world demands scientific validation of pharmacological potential and safety so research has been conducted to generate plant profile that represent the purity, safety, and potency of herbal remedies.² Hence, it is necessary to adopt scientific standardization

techniques for the development of plant profile of the traditional herbs. Standardization of natural products involves a collection of specific features that involve quantitative as well as qualitative reproducible parameters. Therefore, the essential task is to evaluate such parameters by different physico-chemical, chromatographic and spectrophotometric and biological methods that can help to detect and assess the different ingredients found in herbal drugs. However, the parameters are logically applied based on the nature of the herbal drug.³ *Pluchea wallichiana* DC is a species that belongs to the genus *Pluchea*, mainly distributed in America, Africa, Asia, India, Pakistan and Australia.⁴ The genus *Pluchea* has a variety of biological properties like anticancer, immunosuppressive, antioxidant anti-acetylcholinesterase, antimicrobial, hepatoprotective, anti-ulcer, anti-inflammatory and antinociceptive activities.⁵⁻¹² Considering the wide therapeutic potency and usefulness, the development of plant pharmacognostical profile may serve as the base for scientific evidence for future reference to assure the selected plant's quality and safety. Literature suggests, least research carried out for the *P. wallichiana*. Mehdi HK *et al.* have reported the



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presence of β -amyrin and β -amyrin acetate, (+) – syringaresinol, Pluviatilol, β -sitosterol, β -sitosterol 3-O- β -D-glucopyranoside, Apigenin 7-O- β -D-glucoside as a phytoconstituents using column chromatography.¹³ Sesquiterpenoids and flavonoids are the main constituents found in the species of this genus.¹³ The plant having remarkable antibacterial potential so active against the *Bacillus anthracis*, *Coryne bacterium*, *Pseudo diphthericum*, *Salmonella typhi* and *Shigella dysenteriae*.¹⁴ Traditionally, The plant is used to cure peptic ulcer, Ulcer burns, abdominal pain and bacterial diseases.¹⁴

Reagents and Chemicals

The standards for gallic acid and quercetin were procured from Yucca enterprise (Mumbai). Folin-Ciocalteu reagent, methanol, Aluminum chloride, sodium nitrite, sodium carbonate were obtained from Fisher Scientific (USA). The remaining chemicals and reagents used were of analytical grade.

MATERIALS AND METHODS

Collection, Identification and Plant Authentication

The mature and fresh plant was collected from the medicinal plants garden of Parul University in the month of November. It was identified on the basis of morphological and microscopical characters. Plant authentication was done by Dr. Padmanabhi Nagar, M. S. University, Botany Department, Vadodara, Gujarat, India.

Macroscopical Evaluation

The fresh plant samples were observed for the common surface characteristics like color, shape, odor, taste and size to evaluate organoleptic characteristics.¹⁴ The different plant parts were observed for their macroscopic features.

Microscopical Evaluation

Microscopical examination of the fresh plant parts (leaf, stem and root) was carried out using a binocular microscope. Cross sections of these plant parts were prepared, cleared with potassium hydroxide and stained with phloroglucinol and hydrochloric acid followed by examination under microscope. Quantitative microscopy of leaves was performed. Leaf samples were treated with potassium hydroxide and observed under microscope using camera lucida for the determination of leaf constants such as stomatal index, palisade ratio and vein islet and vein termination number.¹⁵

Physico-chemical Parameters

Physico-chemical parameters are significant to state the quality and purity of herbal drug like ash value, extractive value and loss on drying. These parameters were established for the powdered leaf, stem and root samples as per WHO guideline.¹⁴

Determination of Ash Values¹⁴

Total Ash

In a tared silica dish, 2 g of precisely weighed powdered crude drug was placed. The plant powder was burned in a muffle furnace at temperatures not higher than 450°C until it was carbon-free. Ash was allowed to cool. The recovered ash was weighed, and the percentage was calculated.

Acid Insoluble Ash

The total obtained ash was boiled with 25mL of dilute hydrochloric acid for the duration of 5 min. The ash-free filter paper was used to collect the insoluble materials. The insoluble residue was rinsed with hot distilled water before being ignited. The obtained ash was weighed, and the percentage was calculated.

Water Soluble Ash

The total ash was heated in 25mL of distilled water for 5 min. The insoluble materials were filtered and thoroughly washed with hot water using ash-free filter paper. The ash-free filter paper and insoluble residue were burnt in a muffled furnace at a temperature no higher than 45°C. The weight of the water insoluble ash was deducted from the total ash weight. The weight difference shows amount of water soluble ash based on which percentage of water soluble ash was calculated.

Determination of Extractive Values¹⁴

Water Soluble Extractive

In a well-closed conical flask, 5 g dried plant powder was soaked in 100 mL of distilled water for 24 hr, with regular shaking every 6 hr followed by placing at standing condition for 18 hr. After filtration, the volume was made up to 100 mL. In a tared flat bottomed shallow petri dish at 40°C, 25 mL of the filtrate was evaporated to dryness and weighed. The water soluble extractive was assessed using the air-dried powdered material as a standard.

Alcohol Soluble Extractive

The necessary strength of 100 mL of alcohol was used to macerate 5 g of air dried coarse powder of crude drug for 24 hr with regular shaking for 6 hr, and the mixture was permitted to stand for 18 hr. Filter quickly to avoid solvent loss. 25 mL filtrate was collected and evaporated in a petri dish at 40°C and weighed. The alcohol soluble extractive percentage was calculated with reference to dried plant powder.

Loss on drying

The procedure was used to determine the amount of moisture present in plant drug. The accurately weighed powder of drug was taken on a pre-weighed petri plate. The procured powder was then dried in an oven at 100°C, cooled in desiccators and

weighed. The process was continued until a constant weight was achieved. The percentage was calculated.

Phytochemical Screening

Leaf, stem and root parts of *Pluchea wallichiana* were dried under shade and powder was prepared. Alcoholic and aqueous extracts of leaf, stem and root were prepared and subjected to the preliminary phytochemical screening by performing chemical tests separately to track the presence of various phytoconstituents such as flavonoids, alkaloids, saponins, carbohydrates, sterols and terpenoids, coumarins, anthraquinone glycosides, tannins and phenolic constituents.¹⁶

Quantitative Evaluation of Phytochemicals

Sample preparation

Each plant portion (1 gm powder) was separately mixed with 100 mL of water and ethanol (10 mg/mL) and boiled for one hour on a water bath at 40°C. After filtration, the volume was adjusted and respective test solutions of leaf, root and stem were used for determination of the total tannins, phenolics and flavonoids.

Determination of Total Phenolics

Stock solution

Gallic acid (10 mg) reference standard was dissolved in 10 mL alcohol (100 µg/mL). Further, various concentrations such as 20, 40, 60, 80 and 100 µg/mL of gallic acid were prepared to obtain a standard calibration curve.

Procedure

Gallic acid (1 mL) solution was taken from each prepared concentration / 0.1 mL test solution was added in a 25 mL volumetric flask. In it, 9 mL of distilled water and 1.5 mL folinciocalteu reagent were added. The resulting solution was kept for 5 min. After 5 min, 4mL 1M Na₂CO₃ (20% w/w) was added and the final volume was adjusted with distilled water. After 30 min of stabilization, the absorbance at 765 nm was measured.^{17,18}

Determination of Total Tannin

Stock solution

The stock solution and different concentrations of gallic acid were prepared as per the method mentioned for the total phenolics estimation.

Procedure

The total tannin was estimated using the casein precipitation method.^{18,19} The extract (12 mL) was collected and treated with 2 g casein. The solution was agitated at ambient temperature for three hours before being filtered through whatman filter paper. 0.1 mL of filtrate was taken in a volumetric flask and evaluated for the presence of residual phenols using the folinciocalteu method.

The tannin quantity was calculated by subtracting the sample precipitated with casein from the total phenolics.^{19,20}

Determination of Total Flavonoids

Standard solution

Quercetin was utilized as a standard for the total flavonoids estimation. 10 mg quercetin was dissolved in 1 mL of alcohol (100 µg/mL). From this stock solution, different dilutions were prepared to obtain 20, 40, 60, 80, 100 µg/mL.

Procedure

The standard quercetin solution (1 mL) from each concentration or test solution was transferred to a 10 mL volumetric flask followed by addition of 4mL of distill water and 0.3 mL 5% NaNO₂. After 5 min, 0.3 mL 10% AlCl₃ was mixed and allowed to stabilize for 5 min. Then, 2 mL of 1M NaOH was added to neutralize and the volume was adjusted up to 10 mL with distilled water. Blank reading was taken by replacing the same quantity of AlCl₃ with water. Measurement of absorbance was done at 420 nm.²¹⁻²³

Steroid estimation

The powders of leaf, root and stem (5gm) were taken and mixed with 50 mL of 4N hydrochloric acid separately. The mixture was boiled for half an hour to hydrolyze the sample. The collected filtrate was transferred to a separating funnel containing an equivalent volume of ethyl acetate. It was mixed well and allowed to separate. The ethyl acetate layer was collected and heated at 50°C for 1 hr with 30 mL of concentrated amyl alcohol to separate the steroids. The turbid solution was filtered through pre-weighed whatman filter paper. After drying, the filter paper weight was measured.^{24,25}

The steroid concentration was estimated by using the formula mentioned below.

$$\% \text{ Steroids} = \frac{W2 - W1}{\text{Weight of powder taken}} \times 100$$

W1 = Filter paper weight, W2= Filter paper and Steroids weight.

Mineral Analysis

The accurately weighed 400 mg leaf powder was taken in PFA (Perfluoroalkoxy) Teflon vessel. The Nitric acid (5mL, Trace Metal Grade) was added along the sides of the vessel to remove the adhering matter. The sample was digested in a microwave Digester. After the completion of the digestion process, the vessel was kept in the fumigation chamber for 10 min. The vessel was opened slowly to release the pressure from the vessel. The vessel was kept undisturbed till the fumes were released completely. The solution was transferred to a 50 mL volumetric flask and the final volume was made up with distilled water, shaken well and filtered using 0.22-micron filter paper. This solution was used as

a test solution. The blank solution was prepared by omitting the test sample. Standard was prepared from 1000 ppm readymade multi-element stock solution and diluted accordingly to achieve 2, 5 and 10 ppb concentration.^{26,27} Analysis was carried out using the instrument ICP-MS Nexion 1000, Perkin Elmer.

Antioxidant activity

The leaf powder sample (2.5 g) was extracted in 50 mL of 50% methanol in a conical flask. The next day filtered with what man filter paper and volume was adjusted up to 100 mL with 50% methanol. This was stock solution 1. From this stock solution 1, 0.2 mL was collected and volume made up to 50 mL using 50% methanol to get stock solution 2 with a concentration of 100 µg respectively. From this stock solution 2, further 1, 2, 3, 4 and 5 mL were taken and volume made up to 10 mL with 50% methanol. The working solutions of 10 µg, 20 µg, 30 µg, 40 µg and 50 µg concentrations were prepared. The working solutions thus obtained were used for the antioxidant study. 3 mL of working solution was taken and mixed with 1 mL 0.1 mM solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical solution in methanol and the volume was made up to 10 mL. After 30 min of incubation at 27°C, absorbance was measured at 517 nm using a spectrophotometer.²⁸⁻³¹ The IC₅₀ value was derived using the Log dosage inhibition curve and compared to ascorbic acid as a control.

The percentage of inhibition was calculated using the equation described below.

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A₀ and A₁ are the absorbance values of the control and extract/standard, respectively.

HPTLC fingerprinting profile

Test solution preparation

The 15 mg of methanolic extract of root, stem and leaf part of plant was dissolved in 1 mL of methanol.

Procedure

Using a Hamilton syringe and LINOMAT 5 equipment (CAMAG, Muttensz, Switzerland) different concentrations of test solutions (5, 7, 9, and 12 µL) of the root, stem, and leaf (R₁, R₂, R₃, R₄, S₅, S₆, S₇, S₈, L₉, L₁₀, L₁₁, L₁₂) were spotted on a precoated silica gel 60 GF₂₅₄ plate as a 5 mm band length. After 20 min of saturation of the mobile phase (Toluene: Ethyl Acetate: Formic Acid - 6:4:0.3), the loaded plate was transferred into the TLC twin trough development chamber and allowed to develop in the corresponding mobile phase up to 70 mm. To eliminate solvents from the developed plate, the plate was air dried. After drying, the plate was photographed in natural day light, UV-visible, and fluorescent light. Densitometric scanning was performed at

254 and 366 nm. The R_f value, peak height and peak area, and densitogram were compared and recorded.³³ The developed plate was treated with anisaldehyde sulphuric acid spraying reagent and heated for five minutes at 100°C in a hot air oven and photographed at day light.

RESULTS

Authentication

The plant of *Pluchea wallichiana* DC. was identified and authenticated by Dr. Padmanabhi Nagar, Plant Taxonomist, M. S. University, Vadodara, Gujarat, India, where a specimens KMB-1 and KMB-2 have been deposited for future reference.

Macroscopic characteristics

Images of the plant and its parts are displayed in Figure 1. The plant is an erect branched shrub and 1-2 m tall. Camphoraceous leaves are 3-6 cm in length and 1.5-3 cm broad, green, alternate, broadly elliptic or obovate in shape, sessile, obtuse or acute apex, margins obscurely toothed or almost entire, somewhat leathery, velvet-hairy surface, venation is reticulate and prominent on both the surfaces. Flowers are white in color in the form of compound corymbs. Stems are 7-10 cm long, green branched, round with diameter 3 mm or more, striped, glandular velvet-hairy, young ones densely velvet-hairy. The tap root is branched cylindrical found along with lateral root; mature roots are dark brown in color while young one is pale yellowish brown in color with 10 cm or more length and 5 mm or more diameter.

Microscopic characteristics

The transverse section of *Pluchea wallichiana* fresh leaf displayed in Figure 2a revealed the presence of multicellular covering trichomes and glandular trichomes, isobilateral lamina, collenchyma, epidermis with anisocytic stomata, more than three vascular bundles in midrib surrounded by pitted parenchyma. Microscopical evaluation of the stem, as shown in Figure 2b, displayed the presence of an epidermis covered with cuticle, multicellular covering and glandular trichomes, collenchymatous endodermis, cortex with several layers of parenchyma, and lignified pericyclic fibers. Beneath the pericyclic fiber bundle, the vascular bundle of collateral open type and well developed pith along with pitted parenchyma were present in the center. The transverse section of the root as shown in Figure 2c, exhibited the presence of cork, cortex with secretory canals, lignified pericyclic fibers, vascular bundles and multi seriate medullary rays. Powder microscopical characteristics of leaf, stem and root are shown in Figure 3a, 3b and 3c respectively. Leaf powder evaluation indicates lamina portion, anisocytic stomata and multicellular covering trichomes. The stem powder microscopy described the presence of pericyclic fiber, calcium oxalate prisms, bordered pitted xylem vessel and pitted parenchyma cells. Starch grains, lignified pitted vessels, parenchymatous cells, cork cells, bordered pitted and



Figure 1: Macroscopic features of *Pluchea wallichiana* a: Stem, b: Leaves, c: Root

Table 1: Leaf constants.

Leaf Constants	Per Sq. mm
Vein Islet No.	6-8
Vein Termination No.	4-5
Stomatal No.	16 -18
Stomatal Index	20 -22

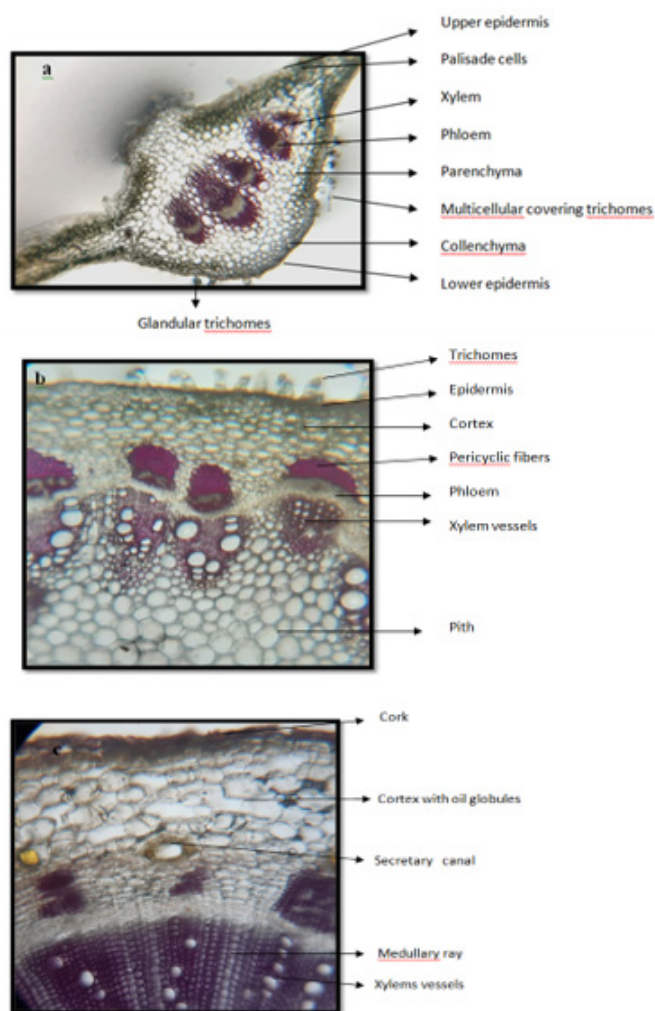


Figure 2: Transverse section of leaf (a), Stem (b) and Root (c) (Magnification, X10).

annular xylem vessels were present in powder microscopy of the root part of plant.

Quantitative microscopy of the leaf was performed 3 times for each parameter; the obtained values are displayed in Table 1 as leaf constants.

Physico-chemical Parameters

The different physico-chemical parameters of the leaf, stem and root are summarized in Table 2. The result suggests that the total ash (19.78 ± 0.48) and water soluble ash (06 ± 0.03) of the leaf were higher, while acid insoluble ash (5 ± 0.50) of the root was highest. Alcohol and water soluble extractive value of the leaf was reported to be more than root and stem parts of the plant. Loss on drying of the root was comparatively observed more in the root.

Phytochemical Screening

The preliminary phytochemical evaluation of water and alcoholic extracts of root, stem and leaf reveals the presence of flavonoids, sterols and triterpenoids, tannins, starch and coumarins, as shown in Table 3.

Quantitative evaluation of Phytochemicals

After performing preliminary phytochemical screening, the crude extracts of plant parts were screened for determination of total phenolic, total tannins, total flavonoid and total steroid content by looking at the presence of the said nature phytoconstituents. The amount of total phenolics and total tannins present in the leaf, stem and root sample were calculated from the standard equation ($y=0.04x + 0.032$, $R^2=0.997$) obtained from the calibration curve of gallic acid standard. Using the equation ($y=0.01x + 0.036$, $R^2=0.999$) derived from the calibration curve of the quercetin standard, the percentage of total flavonoids were calculated in the test samples of leaf, stem and root. The amount of phytochemicals present in these samples are shown in Table 4.

Table 2: Physical parameters of leaf, stem and root of *P. wallichiana*.

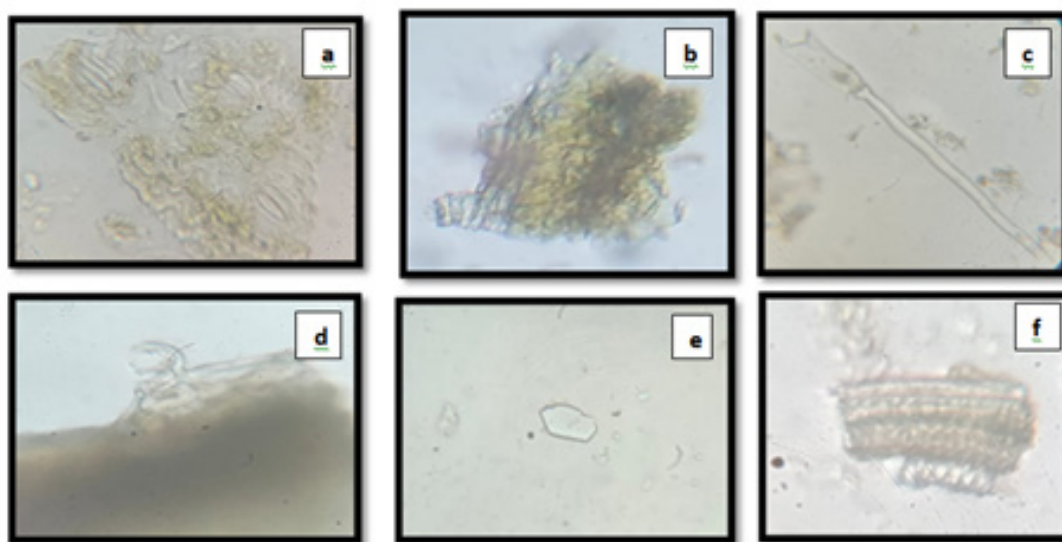
Parameters	Value (%W/W)		
	Leaf	Stem	Root
Total ash	19.78 ± 0.48	6.3 ± 0.29	6.1 ± 0.29
Acid insoluble ash	03 ± 0.00	1.5 ± 0.29	5 ± 0.50
Water soluble ash	06 ± 0.03	03 ± 0.29	2 ± 0.29
Water soluble extractive	16.33 ± 0.62	4 ± 0.58	11 ± 0.58
Alcohol Soluble extractive	7.73 ± 0.46	6.83 ± 0.29	4.33 ± 0.58
Loss on Drying (LOD)	7.28 ± 0.05	7.25 ± 0.06	8.525 ± 0.09

n = 3

Table 3: Phytochemical screening of plant parts.

Phyto Constituents	Leaf		Stem		Root	
	AE	EAE	AE	EAE	AE	EAE
Alkaloids	-	-	-	-	-	-
Flavonoids	-	+++	-	-	-	+
Saponins	-	-	-	-	-	-
Carbohydrates	-	-	-	-	-	-
Sterols and triterpenoids	-	+++	-	+	-	+
Tannins	++	+	+++	+	++	+
Coumarins	-	+	-	+	-	++
Anthraquinone glycosides	-	-	-	-	-	-
Starch	-	NA	++	NA	+++	NA

- Absent, +++HighlyPresent, ++ Moderate and + less present. Aqueous extract- AE, Alcoholic extract- EAE.

**Figure 3:** a: Powder microscopy of leaf (a) Stomata (x40), (b) Fragment of lamina (x40), (c) Multicellular trichomes d) Lamina along with glandular trichomes e) calcium oxalate crystals f) Annular xylem vessels.

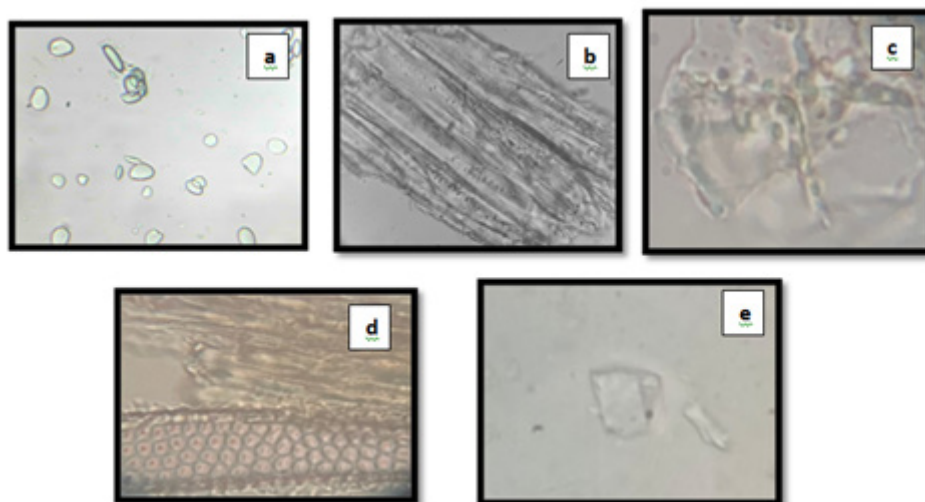


Figure 3: b: Powder microscopy of stem (a) Starch grains (x40), (b) pericyclic fibers (x40), (c) pitted Parenchymatous cells of pith (x40) (d) Bordered pitted xylem vessels with lignified pericyclic fibers (x40), (e) Calcium oxalate prism (x40).

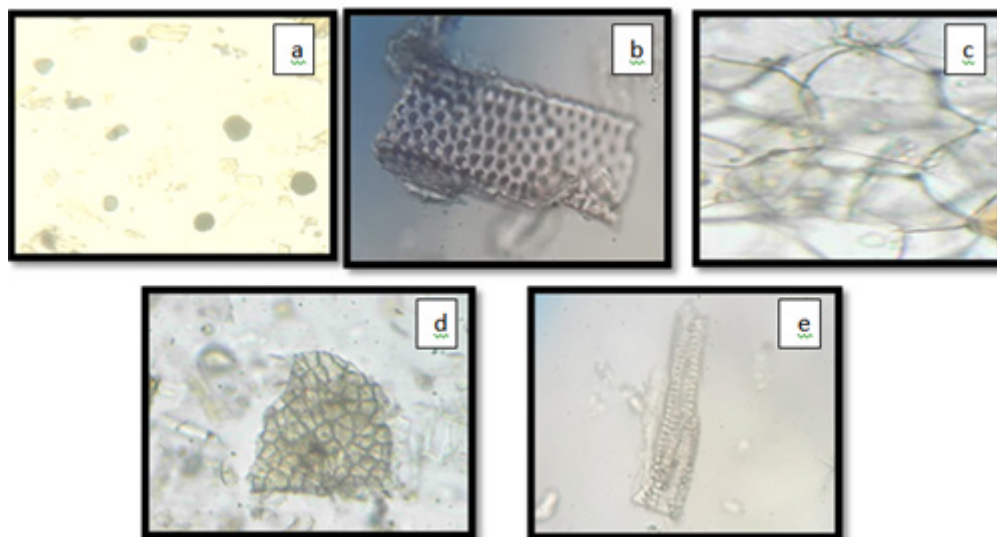


Figure 3: c: Powder microscopy of root. (a) Starch grains with iodine staining (x10), (b) Bordered pitted xylem vessels (x40), (c) Parenchymatous cells with oil globules (x40) (d) Cork cells (x110), (e) Annular xylem vessels (x40).

Elemental Analysis

The leaf sample was subjected to elemental analysis with the use of ICP-MS. The elements were identified, and quantified and the relative concentration of elements was predicted in the plant. The percentage of elements like Calcium (Ca), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Sodium (Na), Zinc (Zn) was found to be 0.271, 0.00078, 0.0771, 0.197, 0.205, 0.0196, 0.343 and 0.00395%, respectively.

Antioxidant activity

The leaf extract contains a high concentration of flavonoids and phenolics. As a result, the DPPH free radicals scavenging assay was performed on leaf part and compared to ascorbic acid as a standard. A quantity of 29.49 g/mL methanolic leaf extract and

15.42 g/mL ascorbic acid were required to inhibit 50% DPPH free radicals. This suggests that the methanolic extract of *P. wallichiana* leaf acts as free radical scavenger.

HPTLC Fingerprinting

The result reveals the simultaneous HPTLC fingerprint profiles of *P. wallichiana* root, stem and leaf methanolic extracts. The methanolic root extract exhibited seven spots with R_f values of 0.016, 0.05, 0.205, 0.439, 0.497, 0.837, and 0.963 in ascending sequence. The methanolic stem extract indicates 6 spots having R_f 0.019, 0.05, 0.118, 0.211, 0.869, 0.966 in increasing order. Five unknown compounds observed with their R_f values 0.019, 0.056, 0.131, 0.473, and 0.866 were found in leaf methanol extract. When separate bands on the plate were observed at 366

Table 4: Quantitative evaluation of phytochemicals in leaf, stem and root of *P. wallichiana*.

Plant Part	% Tannin		% Phenolics		% Flavonoid	% Steroids
	AE	EAE	AE	EAE	AE	
Root	2.5 ± 0.03	3 ± 0.04	4 ± 0.032	3.7 ± 0.02	4.2 ± 0.03	2.8 ± 0.01
Stem	3 ± 0.02	6 ± 0.058	8 ± 0.01	8.9 ± 0.10	4.28 ± 0.04	2 ± 0.06
Leaf	2 ± 0.12	0.6 ± 0.031	7.5 ± 0.02	8 ± 0.04	16 ± 0.13	4.6 ± 0.032

n = 3, Aqueous extract- AE, Alcoholic extract- EAE.

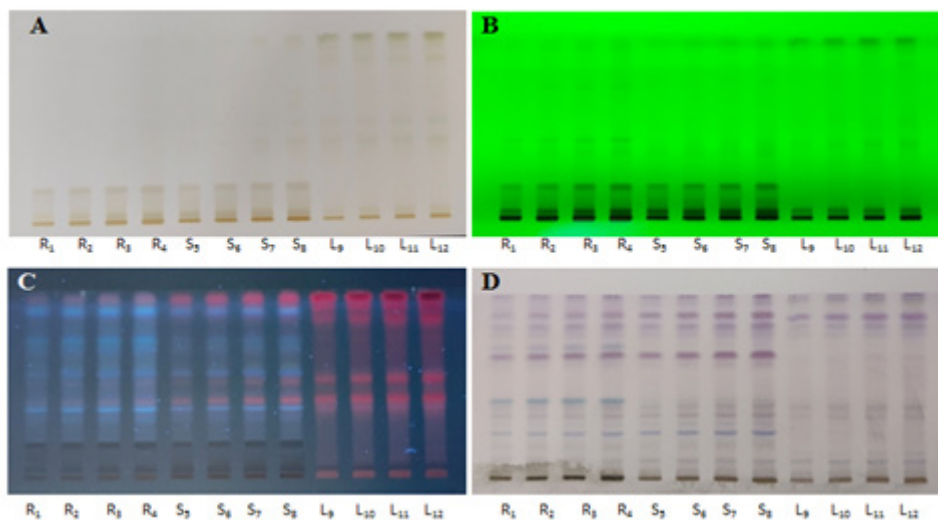


Figure 4: HPTLC fingerprint of methanolic extract of root, stem and leaf of *Pluchea wallichiana* at A) day light, B) 254 nm, C) 366 nm, D) Derivatization with anisaldehyde sulphuric acid reagent.

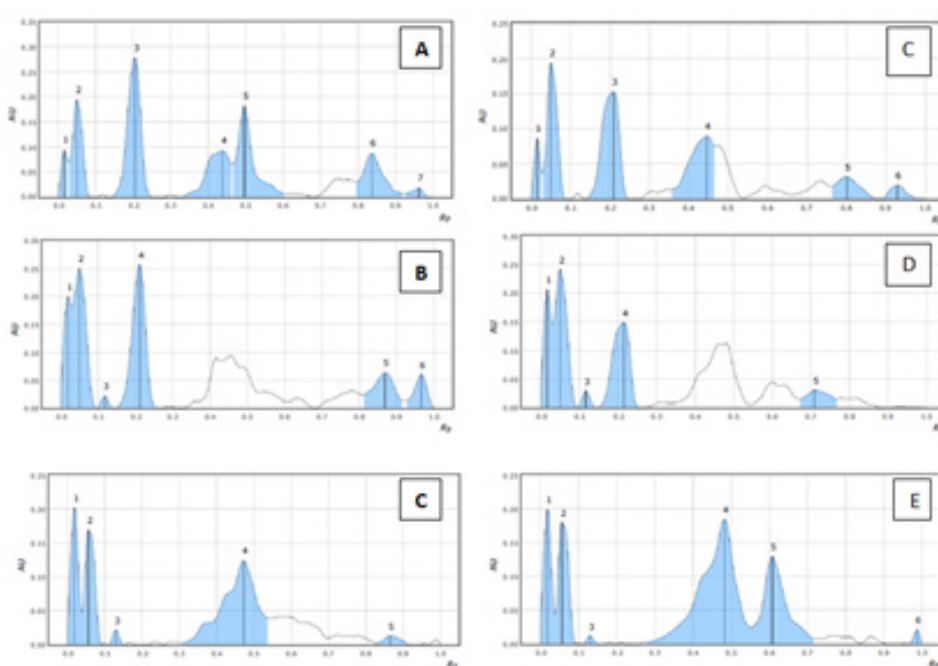


Figure 5: Chromatogram of A) Root (Track 4), B) Stem (Track 8) and C) Leaf (Track 12) at 254 nm, C) Root (Track 4), D) Stem (Track 8) E) and Leaf (Track 12) at 366 nm.

nm, the methanolic root extract showed six bands at R_f 0.015, 0.050, 0.208, 0.445, 0.802, and 0.929, and the stem extract were resolved with five spots at R_f 0.016, 0.050, 0.116, 0.215, and 0.710 while methanolic leaf extracts revealed six spots at R_f 0.018, 0.056, 0.131, 0.482, 0.608, and 0.987. Figure 4 shows the developed plate captures at day light, UV 254 and 366 nm, while Figure 5 highlights the chromatogram of root, stem and leaf extract at 254 nm and 366 nm. HPTLC fingerprint profile of ethanol extract has been documented using a suitable solvent system.

DISCUSSION

For ensure the consistent quality of natural products or their preparation, the raw materials are needed to be authentic and of desired quality. Now a day, the demand of medicinal plants is increasing, and pharmacognostic standardization plays a checkpoint role for the identification and maintenance of consistent quality and purity of the plant to fulfill the authenticity of starting material.³³ The vital step for ensuring the originality of raw material is authentication which remains a basic need even in the presence of advanced instrumental techniques. Organoleptic and microscopical features are crucial in revealing the plant's identity and detecting the source material before any other tests are carried out.¹⁴ The anatomical characteristics also serve as a diagnostic tool for the differentiation of related species of the plants. The anatomical evaluation revealed the specific arrangement of tissues like parenchyma, collenchyma, palisade cells, trichomes, vascular bundles, starch, and calcium oxalate crystals in the stem and the root part of the plant. It appears that presence of multicellular covering and glandular trichomes, pitted parenchyma and multiple vascular bundles in the leaf will serve as an identical tool. The quantitative microscopy of the leaf involves the determination of the vein islet and vein termination number, stomatal number, and stomatal index.³⁴ These characters are valuable in crude drug identification. Physico-chemical parameters are highly significant for detecting adulteration and ensuring the purity of crude drugs.¹⁴ These parameters like moisture content, ash value, and extractive value were determined for the plant parts. Detection of moisture present in the crude drug is very significant to prevent the growth of microbes and further deterioration.^{35,36} Minimal moisture level present in the dried powdered leaf, stem, and root sample ensures the good stability and quality.³⁷ Ash value determination indicates the presence of the organic impurities like carbonates, oxalates and silicates.³⁴ The amount of inorganic substance is indicated by water soluble ash measurement, while earthy matter contamination is exposed by acid insoluble ash determination.³⁵ *Pluchea wallichiana* leaf indicates a higher total ash value than stem and root part. The amount of soluble phytoconstituents in a particular solvent is established with the help of extractive value.³⁷ Determination of extractive value helps in the estimation of phytoconstituents soluble in specific solvents.³⁸ Phytochemical screening demonstrates the existence of plant secondary metabolites.³⁹ The qualitative phytochemical

screening reveals the presence of tannins, flavonoids, steroids and triterpenoids, and coumarins in the leaf. Stem powder sample has shown tannins, steroids and triterpenoids, coumarins and starch. Root powder exhibited the presence same natured constituents as stem and flavonoids additionally. HPTLC Fingerprinting is an image capture at white light, 254nm, and 366nm that indicates the phytochemical profile of a plant extract based on R_f colour and relative intensity of bands. The fingerprinting suggests that methanolic extracts of the plant stem and root parts contain similar phytoconstituent resolved at R_f with 0.05, which was found as absent in leaf. So HPTLC assist in identifying phytochemical compounds and secondary metabolites of a plant.⁴⁰ Hence the technique plays an important role in quality control of herbal medicine and is valuable to have an indication for drug discovery and development. Phenolics and flavonoids present in the plant are potent antioxidants.⁴¹ Hence, they display good antioxidant activity by DPPH scavenging effect.⁴² It is supposed to believe that the presence of polyphenols and flavonoids in extract serves as free radicals scavenging potential by donating hydrogen.⁴³ Estimation of phytochemicals suggests more amounts of total phenolic and total flavonoids in the leaf sample as compared to other plant parts. As phenolics and flavonoids are a good contributors of antioxidant potential, the leaf was further evaluated to prove the same. The leaf part shows significant anti-oxidant activities i.e., 29.49 $\mu\text{g/mL}$, comparable to standard ascorbic acid (15.42 $\mu\text{g/mL}$). ICP-MS elemental analysis of the leaf sample demonstrates the presence of calcium, sodium, potassium and magnesium in noticeable amount, along with the presence of copper, iron, manganese and zinc, which demonstrates good nutritional value of the leaf.

CONCLUSION

The pharmacognostic and preliminary phytochemical study are not reported for this *Pluchea wallichiana* plant as per available literature. Pharmacognostic evaluation of the plant can form a foundation for the correct identification and standardization of the plant or plant parts. The findings of macroscopical, microscopical, physico-chemical parameters like ash value, extractive value and leaf constants will aid the standards which will be valuable for detecting its identity and authenticity. Additionally, such reports are beneficial for monograph draft of the said plant, and for future studies, it will serve as a reference for the *Pluchea wallichiana*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

IC₅₀: Inhibitory Concentration; **DPPH**: 2, 2-diphenyl-1-picrylhydrazyl; **HCl**: Hydrochloric Acid; **KOH**: Potassium Hydroxide; **NaOH**: Sodium Hydroxide; **WHO**: World Health Organization; **Na₂CO₃**: Sodium Carbonate; **AlCl₃**: Aluminium Chloride; **NaNO₂**: Sodium Nitrite; **LOD**: Loss on Drying; **Abs**: Absorbance; **Conc.**: Concentration; **HPTLC**: High Performance thin layer chromatography.

SUMMARY

The root, leaf and stem part of *Pluchea wallichiana* was collected from the herbal medicinal garden of the Parul University campus and authenticated by a taxonomist. Macroscopical, microscopical and physico-chemical parameters are evaluated which is helpful for the standardization of crude drugs. The presence of more amount of flavonoids and phenolics and the significant antioxidant potential of the leaf reveals its medicinal values compared to root and stem when analyzed. Also, the plant leaves are found to contain a good amount of nutritional elements.

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