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Preliminary Phytochemical and Pharmacognostic Studies of *Holoptelea integrifolia* Roxb.

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Abstract

Systematic and detailed pharmacognostical studies have been performed on an important medicinal plant, *Holoptelea integrifolia* Roxb. (Ulmaceae). This species is used traditionally for the treatment of edema, diabetes, leprosy, skin diseases, intestinal disorders and piles. The present investigation deals with the internal structures of the leaf and stem, which have been studied and photographed. Fluorescence characteristics of the leaf and stem powder and the extracts of the leaves in various solvents have been compared along with quantitative values, ash values and extractive values. Preliminary phytochemical screenings of the extracts leaf and stem have also been performed and results are recorded and discussed. In conclusion, the macroscopic and microscopic characters, fluorescence analysis, physico-chemical determination and preliminary phytochemical screening can be used as a diagnostic tool in the correct identification of the plants. The adulterants if any present in these plants can also easily identified by the above studies.

Key words: *Holoptelea integrifolia,* Phytochemical and Pharmacognostical studies.

Introduction

Medicinal plants and herbal plants has assumed greater importance in recent days, due to the tremendous potential they offer in formulating new drugs against many disease and illness that affect the human kind. Plants have been used in the traditional health care system from time immemorial, particularly among tribal communities. *Holoptelea integrifolia* belongs to the family Ulmaceae. It is commonly known as Indian Elm Tree. It is a large deciduous tree distributed throughout the greater part of India up to an altitude of 2,000 feet. Bark of the tree remains grey, pustular, exfoliating in somewhat corky scales. The common vernacular names are Aya, Ayil, Kanci, and Vellaya. The plant is being used by tribal people for their medicinal properties. The decoction of the bark is externally used in rheumatism (Bajpai et al., 1995), intestinal tumors (Sabnis and Bedi, 1983) and oxytocic in pregnant ladies (Tiwari and Padhye, 1993). Decoction of the leaves is used to regulate fat metabolism (Trivedi and Mann, 1972), treat ringworm eczema and cutaneous diseases (Sharma et al., 1992). Paste of the stem bark is externally applied to treat the inflammation of lymph glands, common fever (Singh and Ali, 1994) and ringworm and scabies. Stem bark acts as an anti-inflammatory agent specifically for eyes (Mudgal and Pal, 1980). Bark and leaf paste of the plant are applied externally on the white patches or leucoderma (Maheswari and Singh, 1990).

Materials and Methods

The specimen plant for the study was collected from Singikulam hills near Palayamkottai. The taxonomic features collected from the species have been confirmed with the 'Flora of Presidency of Madras' (Gamble 3:1348.1928) and the 'Flora of the Tamilnadu Carnatic' (Matthew 342.1981). It was subjected to the phytochemical and pharmacognostical studies.

Macroscopic studies

The morphological and taxonomical observations were made by using compound light microscope and the characters were described in technical terms.

Microscopic studies

Fresh stem and leaf was collected and fixed in FAA (formalin 40% - 5ml, acetic acid - 5ml, alcohol 70% - 90ml). Free hand sections of the stem and leaf were taken and stained by double stain method. The photomicrographs were taken using Motic digital research microscope.

Leaf constant studies

Stomatal index was studied using samples treated in 5% potassium hydroxide solution. Stomatal index value is then calculated by using the S/(S + E) x 100. Where E and S represent the number of epidermal cells and stomata per unit area respectively (Salisbury, 1928)

Powder analysis

The powered plant material was sieved and the macroscopic and microscopic characters were analyzed. The powder was sieved and was used for the organoleptic study. The behavior of the solvents/reagent and the fluorescence analysis was also studied. (Chase and Pratt, 1949)

Physico - chemical analysis

The percentage of loss of weight on drying, total ash, water soluble ash, acid insoluble ash, water soluble extractive value, sulphated ash, residue on ignition, moisture content were obtained by employing standard methods of analysis as described in Pharmacopoeia of India (1996).

Preliminary phytochemical studies

The powdered sample was weighed and extracted with Soxhlet apparatus using different solvents. The different solvent extracts were tested for reducing sugar, protein, phenolic groups, alkaloids, steroid, triterpene, flavonoid, catachin, tannin and anthroquinone (Brindha et al., 1981).

Observations and Results

Macroscopic study

Habit: polygamous, deciduous tree to 15 (25) m, tender parts pubescent. Leaves: simple, distichous, elliptic-ovate, 8-14 cm, (sub) coriaceous, pen - ninerved, appressed - pubescent below, base rounded to (sub) cordate, apex, acute, lateral nerves 8 -10 pairs, flattened above, raised below, petiole to 1 cm, stipules lateral, scarious, fascicles at the scars of the old shoots, bracts scaly. Flowers: (sub) sessile, male and bisexual mixed, tepals

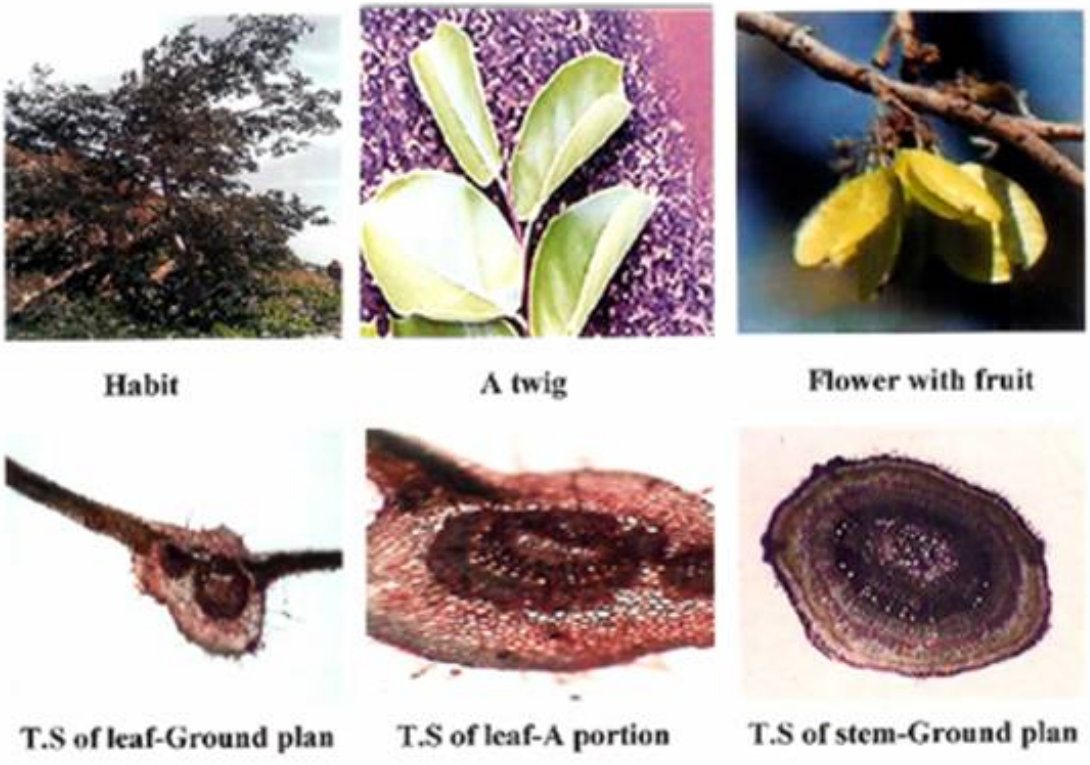
4, free, concave, 1.5 mm, imbricate, unequal, ciliate, and obtuse. Stamens: 7-9, (sub) biseriate, filaments 1.7 mm, anthers slightly curved, 1.3 mm, hairy, introrse. Ovary: compressed - ovate, 2 - 1.5 mm, stalk elongating in fruit, style-arms 2, stigmatose. Fruit: dry, winged, compressed samara, 1 - 0.7 cm, wings membranous, 3 cm across, reticulate, seed flat, exalbuminous. Densely foliaceous tree, girth to 2.34 m. Leaf-fall February gradual, flowers February-March, fruits March onwards. New foliage April. Distribution: Sri Lanka, India, Himalaya, Burma, Indo – China.

Microscopic characters

LEAF

The upper epidermis consists of small barrel shaped paranchymatous cells. In surface view they appear wavy in outline and trichomes are present on both the surfaces of leaf, more along the midrib region and less along the lamina. The covering trichomes are simple, unbranched, uniseriate, unicellular structures, apex blunt and walls smooth. Stomata are present on the lower surface. Leaf type is dorsiventral. The palisade consists a single layer of regular, long, columnar cells, beneath which is a 3 to 4 layered mass of closely packed cells filled with chloroplast. Stomata represented by anomocytic type. Some oil glands are present in the lower epidermis.

In the midrib region cortex consists of 5 to 7 layers of paranchymatous cells. The vascular bundle is ovoid in shape. Mass of xylem and phloem shows different structures. Below the vascular bundle a zone of sclerenchymatous tissues are present. In between the upper epidermis and the vascular bundle 6 to 7 layer of irregular shaped collenchyma cells are present. The vascular bundle is collateral and open endark. There are few layers of cambium in between the xylem and phloem. The phloem consists of sieve tubes, companion cells and phloem parenchyma. Xylem consists of xylem vessels, tracheids and parenchyma. Xylem is seen on the upper side whereas phloem is seen towards the lower side of the epidermis.



Habit | A twig | Flower with fruit

T.S of leaf-Ground plan | T.S of leaf-A portion | T.S of stem-Ground plan

Plate 1. *Holoptelea integrifolia* RFoxb.

STEM

The outline of the transverse section of the stem is nearly circular covered with many unicellar, uniseriate trichomes. The outermost multilayered periderm consists cork cambium and secondary cortex. The cork layer is interrupted at many places due to the presence of lenticels. The cortex is multilayered consists of parenchymatous cells. The primary phloem remains as patches of crushed tissue. The secondary phloem consists of sieve tubes, companion cells, phloem parenchyma and phloem rays. Vessels are present in broken conditions and crushed form. The xylem is represented by both primary and secondary xylem tissue. It consists of vessels and tracheids. The primary xylem towards pith. The secondary xylem consists of large vessels and xylem parenchyma. Xylem is found in the form of continuous medullary rays. The pith is large and remains to the central part of the stem. It consists of thin walled parenchymatous cells having many intercellular spaces. The pith regions have oil droplets. The vascular bundle is collateral and open endark.

Leaf constant studies

The stomata are present only in lower surface of leaf. They are anomocytic type. The stomatal index is 14.83mm2 and the stomatal frequency range is 34.25

Powder analysis

The organoleptic evaluations means conclusions drawn from studies resulted due to impression on organs of senses. The color, texture, odour and taste of the plant powder were analyzed. It is presented on a Table 1.

Table 1: Powder analysis.

sl.no	Particulars	Plant part	
		Leaf	Stem
1	Color	Green	Brown
2	Odour	Disagreeable smell	Agreeable smell

3	Taste	Slightly bitter	Bitter
4	Texture	Coarse	Smooth

Table 2: Fluorescence analysis of the extracts.

Sl.NO	Treatment	Plant part	Under visible light	Under UV light
1	Petroleum ether extract	Leaf	Dark green	Dark green
		Stem	Light green	Light green
2	Benzene extract	Leaf	Black	Black
		Stem	Light dark	Light dark
3	Chloroform extract	Leaf	Black	Dark green
		Stem	Black	Dark brown
4	Methanol extract	Leaf	Dark brown	Black
		Stem	Light brown	Green
5	Powder + ethyl alcohol	Leaf	Pale green	Pale green
		Stem	Brown	Brown
6	Powder + acetone	Leaf	Light green	Dark green
		Stem	Pale green	Light green
7	Powder + 1N HCl	Leaf	Light yellow	Pale green
		Stem	Light yellow	Pale green
8	Powder + 1N NaOH	Leaf	Yellowish green	Pale green
		Stem	Brown	Light brown
9	Powder + 50% HNO_3	Leaf	Light yellow	Pale green
		Stem	Light brown	Pale green
10	Powder + 1N NaOH	Leaf	Light red	Dark red
		Stem	Reddish brown	Dark green
11	Powder + distilled water	Leaf	Light red	Dark green
		Stem	Pale yellow	Pale green

Fluorescence analysis

Fluorescence analysis of the leaf and stem power in various solvents have been studied and presented in Table 2. It can be as a diagnostic tool for testing the adulterations.

Physico - chemical analysis

Ash values are helpful in determining the quality and purity of crude drugs, especially in the powered form. The different physico-chemical standards and solvent extractive value were presented in Table 3.

Extractive value of leaf is high in methanol extract whereas very low in benzene extract. Extractive value of stem is high in methanol extract while very low in chloroform extract.

Preliminary phytochemical screening

Phytochemical screening of this plant of various extracts showed significant results The results are presented in Table 4. Reducing sugar present only in petroleum ether extract. Steroids and protein were resulted in all extracts except distilled water. Phenol present only in chloroform, methanol and distilled water extract. Alkaloid present only in methanol extract. Triterpenoid and anthroquinone present in methanol and distilled water extract. Flavones and amino acid present all extracts expect distilled water. Cathacin present in benzene and methanol extract. Tannin was resulted in petroleum ether, benzene, and distilled water extract. Saponin is absent in all extracts.

Table 3: Physico - chemical analysis.

Sl.No	Parameter	Percentage of W/W	
		Leaf	Stem
1	Total ash	10.0	4.0
2	Sulphated ash	10.5	19.3
3	Water soluble ash	5.5	1.98
4	Acid insoluble ash	8.0	2.4
5	Water soluble extractive value	12.7	13.3
6	Moisture content	37.0	39.2
Extractive value in different extracts			
7	Petroleum ether	2.16	1.25
8	Benzene	0.21	1.03
9	Chloroform	0.12	0.25
10	Methanol	2.75	2.13

Table 4: Preliminary phytochemical screening

Sl. No	Solvent	Plant part	Reducing sugar	Protein	Phenol	Alkaloid	Steroid	Tri terpenoid	Flavones	Cathacin	Anthroquinone	Tannin	Amino acid	Saponin
1	Petroleum ether	Leaf	+	+	-	-	+	-	+	-	-	+	+	-
		Stem	-	+	-	-	+	-	-	-	-	+	+	-
2	Benzene	Leaf	-	-	-	-	+	-	+	+	-	+	-	-
		Stem	-	+	-	-	+	-	+	-	-	+	+	-
3	Chloro form	Leaf	-	+	+	-	+	-	-	-	-	-	+	-
		Stem	-	+	-	-	+	-	+	-	-	-	+	-
4	Methanol	Leaf	-	+	+	+	+	-	+	-	+	-	+	-
		Stem	-	+	+	+	+	+	-	+	+	+	+	-
5	Distilled water	Leaf	-	-	-	-	-	+	-	-	+	+	-	-
		Stem	-	-	+	-	-	+	-	-	+	+	-	-

(+) denote present, (-) denote absent

Conclusion

The comparative and multidisciplinary approach to the study of *Holoptelea integrifolia* Roxb. does help in understanding their identification taxonomical determination, and medicinal importance in depth. The adulterants in drugs obtain from *Holoptelea integrifolia* Roxb. can be identified by this investigation. Adulterants if any can be easily identified using these parameters.

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