Pharmacognostic and Preliminary Phytochemical Investigations on *Holoptelea integrifolia*

Padmaja V., Srisailam K.*, Reddy V.M.

Vaagdevi College of Pharmacy, Ramnagar, Hanamkonda, Warangal 506001, AP, India

Abstract

Various parts of *Holoptelea integrifolia*, a roadside plant, are indicated by Charaka Samhitha, Sushrutha Samhitha and other traditional systems for the treatment of inflammations, acid gastritis, dyspepsia, flatulence, colic, intestinal worms, vomiting, wounds, vitiligo, leprosy, filariasis, diabetes, haemorrhoids, dysmenorrhoea and rheumatism. The present study was aimed at pharmacognostic and preliminary phytochemical investigations of *H. integrifolia* leaves and bark. The pharmacognostic investigations were carried out in terms of organoleptic, microscopic and physical parameters. The dried leaves and bark were subjected to successive Soxhlet extraction using petroleum ether, chloroform, ethyl acetate and methanol. These solvent extracts were subjected to a preliminary phytochemical screening to detect the different chemical principles present viz., carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, tannins and phenolic compounds. The phytochemical analyses indicate that the plant contains carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, tannins and phenolics.

Keywords: *Holoptelea integrifolia*, Pharmacognosy, Phytochemistry

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***Author for correspondence:** Email: ksrisailam@yahoo.com

INTRODUCTION

Natural products are a source for bioactive compounds and have potential for developing some novel therapeutic agents. Over the last decade there has been a growing interest in drugs of plant origin and such drugs formed an important class for disease control. Among the known plant species, only a small percentage has been investigated for phytochemicals and pharmacological activities. *Holoptelea integrifolia* Planch (Syn. *Ulmus integrifolia*) is a roadside tree of the family Ulmaceae and commonly known as the Indian elm. The plant was found to be distributed in India, Sri Lanka, Myanmar, China, and Malaysia (1). The tree offers several classes of chemical constituents in which triterpenoids and sterols constitute a major portion reported so far. Various parts of the tree were found to be useful in the treatment of bronchitis and obesity (2, 3). The tree was found to possess oviposition deterrent activity and protease inhibitory activity (4, 5). Padmaja et al (6) have reviewed the phytochemical and pharmacological reports related to *H. integrifolia*.

The present investigation dealt with the pharmacognostic parameters of the leaves and bark of *H. integrifolia*, and also with preliminary phytochemical evaluation of different solvent extracts of leaves and bark. The leaves and bark were studied to know their organoleptic, microscopic, and physical parameters. The successive petroleum ether, chloroform, ethyl acetate, and methanol extracts of these two parts of the plant were examined for their phytochemical principles.

MATERIALS AND METHODS

Chemicals

All the chemicals were of highest available purity and were procured from E. Merck, Mumbai, India, HiMedia Laboratories, Mumbai, India and SD Fine Chemicals, Mumbai, India.

Procurement of plant material

The leaves and bark of *H. integrifolia* as identified by a qualified Taxonomist, were collected from the wild growing

tree in the Kakatiya University campus, Warangal, India. A specimen was deposited in the institutional herbarium. The collected plant material was made thoroughly free from any foreign organic matter and a part of the material was dried under shade.

Pharmacognostic Evaluation

Organoleptic evaluation

In organoleptic evaluation, various sensory parameters of the plant material, such as size, shape, color, odor, and taste of the leaves, bark and their powders were recorded.

Microscopic evaluation

In this study, stomatal index was determined for fresh leaves and powder analyses were performed for dried leaf and bark powders. The stomatal index was studied by using *camera lucida*, and the type of stomata present in the leaves was also recorded. Various diagnostic characters of leaf and bark powders of *H. integrifolia* were studied by microscopical analyses with or without staining.

1. Powder analysis of leaf and bark

To a little quantity of powder taken onto a microscopic slide, 1-2 drops of 0.1% phloroglucinol solution and a drop of concentrated hydrochloric acid were added and covered with a cover slip. The slide preparation was mounted in glycerol and examined under microscope. Presence of starch grains was detected by the formation of blue color on addition of 2-3 drops of 0.01M iodine solution.

2. Determination of stomatal index

Leaf fragments of about 5 × 5 mm in size were taken in a test tube containing 5 ml of chloral hydrate solution and boiled on water bath until the fragments became transparent (~15 min). These fragments were transferred onto microscopic slide and mounted in glycerol. The slide was examined with 40 × objective and 6 × eye piece to which a *camera lucida* was attached and recorded the epidermal cells and stomata lying within a selected area. Stomatal index was calculated as the percentage of number of stomata present per number of epidermal cells and each stoma was counted as one cell.

Physical evaluation

In physical evaluation, the total ash, alcohol, water, and ether soluble extractive values were determined. The determinations were performed in triplicate and the results are expressed as mean ± SD. The percentage w/w values were calculated with reference to the air-dried drug.

1. Determination of total ash

Accurately weighed powder (2 g) of both leaves and bark were taken separately in a pre-weighed ash-less filter paper and incinerated at 400°C for about 3-4 min or until the vapors completely ceased. The temperature was gradually reduced to come to normal and then the contents/ash was collected and weighed.

2. Determination of alcohol soluble extractive

Accurately weighed powder (5 g) of both leaves and bark were taken separately and macerated with 100 ml of 95% alcohol for 24 h. The contents were frequently shaken during the first 6 h and allowed to remain for 18 h. After 24 h, the extract was filtered and 25 ml of the filtrate was evaporated. The extract was dried at 105°C to a constant weight.

3. Determination of water soluble extractive

Water soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that chloroform water was used for maceration.

4. Determination of ether soluble extractive

Accurately weighed powder (5 g) of both leaves and bark were taken separately and a thimble pack was prepared for each. The crude drug in the pack was extracted with solvent ether in a continuous extraction (Soxhlet) apparatus for 6 h. The extract was filtered; the filtrate was evaporated and dried at 105°C to a constant weight.

Preliminary Phytochemical Screening

The leaf and bark powders separately were subjected to successive extraction in a Soxhlet apparatus using petroleum ether (60-80°C), chloroform, ethyl acetate, and methanol, and the extracts were evaporated to dryness, at room temperature. The dried extracts were weighed, and percentage yields were calculated. The extracts were used for preliminary phytochemical screening with a battery of chemical tests viz., Molisch's, Fehling's, Benedict's and Barfoed's tests for carbohydrates; Biuret and Millon's tests for proteins; Ninhydrin's test for amino acids; Salkowski and Liebermann-Burchard's reactions for steroids; Borntrager's test for anthraquinone glycosides; foam test for saponin glycosides; Shinoda and alkaline tests

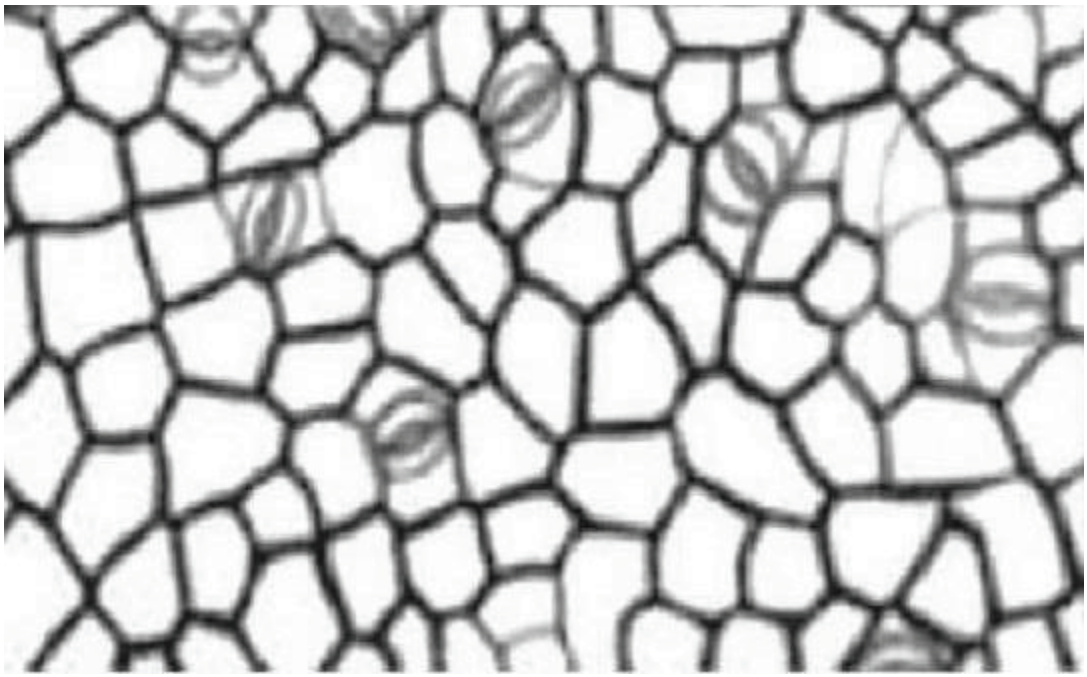

Figure 1. Stomata in lower epidermis of *Holoptelea integrifolia* leaf

for flavonoid glycosides; Dragendorff's, Mayer's, Hager's and Wagner's tests for alkaloids; and ferric chloride, lead acetate, potassium dichromate and dilute iodine tests for tannins and phenolics.

RESULTS AND DISCUSSION

Pharmacognostic Evaluation

Organoleptic and microscopic evaluation

In organoleptic evaluation, appropriate parameters like taste, odor, size, shape and color of the leaves, bark, and their powders were studied. The leaves, bark, and their powders were found to be bitter in taste with unpleasant odor. The leaves were found to be 4-8 cm in length and 2-5 cm in width. The leaves were found to be ovate-elliptic, alternate base rounded or subcordate, entire margin, short acuminate apex, subcoriaceous and glabrous in shape. The bark is of irregular stripes in nature. The leaves and their powder were found to be green in color, whereas the bark and its powder were whitish grey.

Upon microscopic evaluation, the leaves or their powder could show the presence of square, prismatic and rod shaped calcium oxalate crystals; closely packed and straight walled polygonal epidermal cells; palisade cells; thin walled polygonal cells with no intercellular spaces in the parenchyma; anomocytic stomata in lower epidermis (Fig. 1); a stomatal index of 10-15 with lower epidermis; liginified, unicellular, covering trichomes with acute apex and narrow lumen; lignified xylem fibres and scalariform xylem vessels. Similarly bark or its powder has shown the presence of prismatic and rod shaped calcium oxalate crystals; cork cells; mucilaginous parenchyma; lignified phloem fibres and, simple and concentric starch grains.

Physical evaluation of leaves

The results reveal that the leaf and bark powders produced 18.6 ± 0.2 and 7.1 ± 0.2 % w/w of total ash respectively. The ethanol, water and ether soluble extractive values of the leaf powder were found to be 15.2 ± 0.2, 18.2 ± 0.2 and 2.1 ± 0.1 % w/w respectively, and the bark powder produced 6.3 ± 0.2, 7.4 ± 0.2 and 4.1 ± 0.1 of these values respectively. Bhadauria et al. (7) reported the total ash and ether soluble extractive values for leaf powder of *H. integrifolia* as 15.34 and 2% w/w, respectively.

Preliminary Phytochemical Evaluation

The powders of leaves and bark of *H. integrifolia* were extracted with petroleum ether, chloroform, ethyl acetate and methanol and the nature and yield of the extracts were observed. The yield of the extracts was more from leaves than that of the bark. All the solvent extracts of leaves were found to be semisolid in nature. Chloroform

and methanol extracts were of green in color whereas petroleum ether and ethyl acetate produced blackish green extracts. The yields of petroleum ether, chloroform, ethyl acetate and methanol extracts were found to be 3.8, 2.89, 3.2 and 3.1 % w/w respectively. Similarly all the solvents except petroleum ether produced semisolid extracts from bark powder. Petroleum ether, chloroform, ethyl acetate and methanol produced yellow, brownish yellow, brown and reddish brown colored extracts with yields of 1.039, 0.225, 0.432 and 2.315 % w/w respectively.

The leaf and bark extracts obtained from different solvents were tested for the presence of various phytochemicals, such as: carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, tannins, and phenolics. The results indicate that all these classes of compounds were present in both leaves and bark powders. Carbohydrates were present in chloroform, ethyl acetate and methanol extracts of both leaves and bark. Proteins, amino acids, alkaloids, tannins and phenolics were found to be present in methanol extracts of leaves as well as bark. Petroleum ether, chloroform and ethyl acetate extracts of leaves could show the presence of steroids, whereas the only petroleum ether and ethyl acetate of bark were positive for steroids. Anthraquinone glycosides were present in all the extracts of leaves, and petroleum ether and methanol extracts of bark. Methanol extracts of leaves and bark were found to contain saponin and flavonoid glycosides. Presence of the flavonoid glycosides was also observed in chloroform extract of leaves.

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REFERENCES

1. Sumy O., Ved D.K. and Krishnan R., *Tropical Indian Medicinal Plants: Propagation Methods*, (Foundation for Revitalisation of Local Health Traditions, Bangalore, 2000).
2. Sudarsanam G. and Reddy M.B. Veterinary crude drugs in Rayalaseema, AP, India. *Int J Pharmacognosy* **33**: 52–60 (1995).
3. Bambhole V.D. and Jiddewar G.G. Antiobesity effect of *Iris versicolor* and *Holoptelia integrifolia* in rats. *Sachitra Ayurved* **37**: 557–561 (1985).
4. Raja N., Elumalai K., Jayakumar M., Jeyasankar A., Muthu C. and Ignacimuthu S. Biological activity of different plant extracts against armyworm, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *J Entomol Res* **27**: 281–292 (2003).
5. Rajbhandari M., Wegner U., Julich M., Schopke T. and Mentel R. Screening of Nepalese medicinal plants for antiviral activity. *J Ethnopharmacol* **74**: 251–255 (2001).
6. Padmaja V., Srisailam K. and Reddy V.M. *Holoptelia integrifolia Planch., A promising roadside medicinal tree*. In: Trivedi P.C. ed. Indian Folk Medicine. Pointer Publishers, Jaipur; 243–257 (2007).
7. Bhadauria K.K., Pailanbhadauri G.H., Das M.M., Kundu S.S., Singh J.P. and Lodhibhadauri G.N. Evaluation of shrubs and tree leaves for carbohydrates and nitrogen fractions. *Indian J Anim Sci* **72**: 87–90 (2002).