

PHARMACOGNOSTIC STUDIES ON FRESH MATURE LEAVES OF *HOLOPTELEA INTEGRIFOLIA* (ROXB) PLANCH

SHAUKAT MAHMUD, HUMA SHAREEF, MARYAM AHMAD,
SHAHNAZ GOUHAR AND GHAZALA H. RIZWANI

Department of Pharmacognosy,
Faculty of Pharmacy, University of Karachi, Karachi, Pakistan.

Abstract

Medicinal plants which are found on earth have renowned medicinal significance and their usage are increasing day by day in our daily life. Different researches are going on to explore the beneficial, pharmacological and medicinal properties of herbal drugs. The present investigation has been carried out on fresh mature leaves of *Holoptelea integrifolia* (Roxb) Planch., used traditionally for the treatment of inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhoea and rheumatism. The present studies deals with the pharmacognostic and preliminary Phytochemical investigations of mature leaf *Holoptelea integrifolia* (Roxb) Planch. The pharmacognostic studies were carried out in terms of organoleptic, microscopic and physical parameters. Preliminary Phytochemical screenings of the extracts of leaf have been performed and results are recorded and discussed. In conclusion the macroscopic and microscopic characters, physico- chemical determination and preliminary phytochemical screenings have been used as a diagnostic tool in the identification of the *Holoptelea integrifolia* (Roxb) Planch and also to establish the quality and purity of this valuable drug.

Introduction

Medicinal plants are a source of bioactive compounds and are of great value for developing some novel therapeutic agents. Due to this tremendous potential they offered new drugs against diseases that effect the health of man kind (Shrinivas, 2008). Most of the medicinal plants are being utilized since centuries in various formulations specially in Pakistan and India as Unani medicines and they are also the part of Allopathic medicine too.

Holoptelea integrifolia belongs to the family Ulmaceae, having 15 genera and about 200 species, distributed over tropical and temperate regions of Northern hemisphere including Indian peninsula to Indo China and Srilanka (Parrotta, 2001). It is found mostly in the sub Himalayan hills to Assam. Ulmus also occurs in different areas especially in Karachi and some adjoining parts of Sindh. This is locally called as *Chilhil papri* while in English this known as *Indian Elm*. Many chemical constituents such as terpenoids, sterols, saponins, tannins, proteins, carbohydrates and alkaloids have been reported from various species of this plant (Minetal, 2000, K. Machida *et al.*, 2005). The plant is useful in the treatment of obesity, edema, bronchitis (Nasir & Ali, 1985, Baquar, 1995), and also possess ovipositor deterrent activity and protease inhibitory activity (Sastri, 1950, Singetal, 1992). Its bark and leaves are also known for its medicinal importance. The mucilage and juice obtained from the boiled bark is reported to be useful as an external application against rheumatism (Bajapi *et al.*, 1995), intestinal tumors (Sabnis & Bedi, 1983) and is oxytoxic in pregnancy (Tiwari & Padhye, 1993). The paste of seeds and stem bark is externally applied against ringworm, eczema and cutaneous affections (Sharma *et al.*, 1992). Paste of the stem bark is externally applied to treat the inflammation of lymph gland, common fever (Singh & Ali, 1994) ringworm and scabies. Stem bark acts as anti-inflammatory agent, specially for eye (Mudgal & Pal, 1980). Bark and leaf paste are applied externally on leucoderma (Maheswari & Singh, 1990).

Material and Method

The fresh mature leaves of *Holoptelea integrifolia* (Roxb Planch) Synonym *Ulmas integrifolia* (Roxb), were collected from university garden and authenticated by Prof. Dr. Ghazala.H. Rizwani, Dean Faculty of Pharmacy, University of Karachi. Specimen No 014 was deposited in the museum of the Department of Pharmacognosy, Faculty of Pharmacy for histological studies. Microtome as well as free hand sectioning was done using standard methods (Parajaput *et al.*, 2003, Baquar, 1989) and for preliminary Phytochemical studies (Jackson, 1992, Brain, 1975).

Preliminary phytochemical studies: The sample was weighed extracted and percolated in absolute ethanol followed by partitioning with ethyl acetate and water using various solvents and were tested for tannins, saponins, flavonoids, terpenes, sterols, alkaloids, ketones, sugar, protein, (Brindha *et al.*, 1981; Eagan Sthal, 1969).

Results

Organoleptic evaluation: The organoleptic features of the plant were examined by using sensory organs and was noted as green colour, odour; slightly aromatic; herbal taste, tingling and burning, external margin of leaf some what rough; venation particulate, sinuate; apex acute, base symmetrical, with curved small petiole, subribis alternate, lamina broad; the texture, rough, external marking: internal marking: not prominent; fracture; soft and fibrous; shape; some what broad, lanceolate; size 2-3cm.

Microscopic studies: The leaf lamina showed numerous scattered stomata and radial lining through out the lamina, which consist of 1-2 cells. Spongy parenchymatous cells small rounded and loosely arranged. Upper and lower epidermal cells consist of isodimetric thin walled parenchyma cells. Lower epidermis has abundant protective layer of trichome, which are unicellular and conical in shape. The transverse section of plant leaf shows that the upper epidermal layer is composed of three main fragments of cells (i) thick wall with stomata, (ii) epidermal cells having small barrel or isodiametric shaped parenchyma, (iii) The most abundant epidermal trichome of protective type. Lamina of the leaf has two zones (upper and lower) of chloroplast, containing elongated column of cell in rows, on the mid rib having two zones of spongy cells. Lower zone consists of smaller cells while the upper region shows slightly larger spongy cells. Some oil glands are present in the lower epidermis. The vascular bundles were closed, radial type. Both xylem and phloem fragments were found in their own axis. The lower region occupied 7-8 longitudinal rows of xylem parenchyma while phloem vessels found in the upper region were arranged in clusters. Below the vascular bundle a zone of sclerenchymatous tissues were present. In between the upper epidermis and vascular bundles 6 to 7 layer of irregular shaped collenchymatous cells were present. The vascular bundle is collateral and open. Cambium cells are present in between the xylem and phloem. Pith is broad with rounded cells, containing radial fragments of unicellular structure.

Physico chemical evaluation: Physico-chemical analysis is helpful in determining the quality and purity of crude drugs, especially in the powder form. The results showed that the mature leaf powder of *H. integrifolia* (Roxb) Planch produced $18.4 \pm 0.2\%$ w/w of total ash value. The ethanol and water soluble extract values of the mature leaf powder was found to be 15.4 ± 0.2 , $18.01 \pm 0.2\%$ w/w respectively.

Preliminary phytochemical analysis: Preliminary phytochemical screening of *H.integrifolia* (Roxb) Planch showed significant result. Which are presented in Table 1

Table 1. Preliminary phytochemical analysis of *Holoptelea integrifolia* (Roxb) Planch.

S. No.	Test for	Colour indication	Observation
Tannins			
1.	(a) Ferric chloride test	(a) Bluish Black Precipitation	+
	(b) Lead acetate test	(b) Precipitation	+
Saponins			
2.	Frothing test	Frothing Persistence	+
Flavonoids			
3.		Pink tomato red colour	+
Terpenes / Steroids			
4.	Liebermann's Burchard test	Bluish green ring for steroid and pinkish purple ring for tritertenoids	+
Alkaloids			
5.		Orange precipitate	+
Ketones 60% H₂SO₄			
6.		Bottle green colour	+
Sugar α - naphthol			
7.		Purple colour	+

+ = Presence
- = Absence

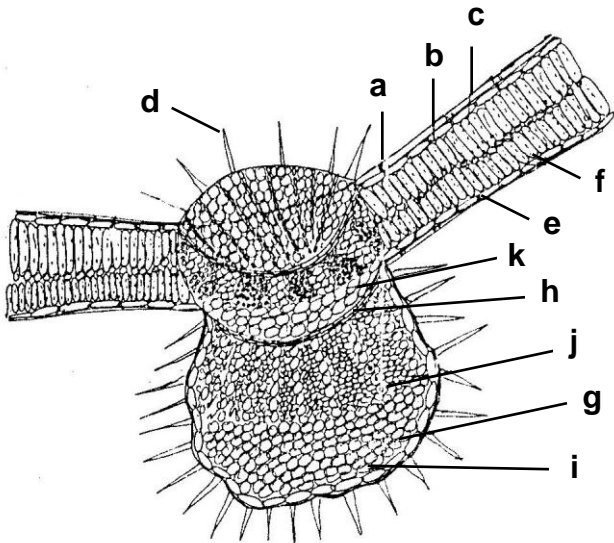


Fig. 1. Transverse section of leaf of *Holoptelea Integrifolia* (Roxb) Planch. (a) Parasitic stomata with cuticle; (b) Upper eqidermis; (c) Lower eqidermis; (d) Upper epidermal trichome; (e) Upper palisade zone; (f) Lower epidermis; (g) Spongy Parenchyma; (h) Pericycle fibre; (i) Lower zone spongy parenchyma; (j) Xylem vessel; (k) Phloem vessel.

Conclusion

Holoptelea integrifolia (Roxb) Planch is indigenous to Australia and is cultivated in many parts of the world, including Pakistan, for ornamental purposes only. The multidisciplinary approach to the study of fresh mature leaf of *H. integrifolia* does help in understanding their identification, taxonomical features and medicinal importance. The chromogenic testing was performed to confirm the presence of chemical compounds in ethanolic extract of the leaves and reported as *titer active*. These compounds were also reproduced in ethanolic extract and different colour pattern were observed. The chromogenic testing showed the presence of terpenoid, steroids, tannins, saponins, carbohydrates and protein in the leaves. These identification standards suggest that it

could be helpful in utilization of this plant in herbal formulations, as a medicine and as pharmaceutical raw material.

References

- Bajpai, A., J.K. Ojha and H.R. Sant. 1995. Medico botany of the varanasi District. *Int. J. Pharmacy*, 33: 172-176.
- Baqar, S.R. 1989. *The scope of Herbal Medicine in Pakistan. J. Baqai Medical University*, 1: 27-30.
- Baqar, S.R. 1995. *Trees of Pakistan* their natural history, characteristics and utilization. Royal Book Co, Karachi.
- Brain, K.R. and T.D. Turner. 1975. *The Practical Evaluation of Phyto. Pharmaceuticals*. Wrieght Sciencetchnica Bristot, 18-35.
- Brindha, P., B. Sasikala and K.K. Purushothaman. 1981. Pharmacognostic Studies on Merugan Kizhangu. *Medico Ethno Botanical Res.*, 3: 84-86.
- Eagan Sthal. 1969. Thin Layer Chromatography. A Laboratory handbook, Springer International Student Edition, New York.
- Jackson, B.P. and D.W. Snowdon. 1992. *Atlas of Microscopy of Medicinal Plants, Culinary Herbs and Species*. CBS Publisher and Distributor (Pvt) Ltd, Daryaganj, New Delhi, 8-11.
- Machida, K., E. Matsuoka, T. Kashahera and M.K. Kuchi. 2005. Studies on the Constituents of *Juglans* Species. I. Structural determination of (4S)- and (4R)-4-Hydroxy- α -tetralone derivatives from the fruit of *Juglans mandshurica* MAXIM. var. *sieboldiana* MAKINO. *Chem. Pharm. Bull.*, 53(8): 934-937.
- Maheswari, J.K. and Harish Singh. 1990. Herbal remedies of Nainital district, UP Aryavaidyan, 4: 30-34.
- Min, B.S., N. Nakamura, H. Mizashiro, Y.H. Kim and M. Hattori. 2000. Inhibition of human immunodeficiency Virus Type 1 reverse transcriptase and ribonuclease H activities by constituents of *Juglans mandshurica* ,*Chem.Pharm. Bull.*, 48: 194-200.
- Mudgal, V. and D.C. Pal. 1980. Medicinal plants used by tribals (Orissa). *Bull. Bot. Survindia*, 22: 59-62.
- Nasir, E. and S.I. Ali. 1985. *Flora of West Pakistan*. Ulmaceae compiled by Rubina Akhtar, No, 170, 1-11.
- Parjaput, N.D., S.S. Purohit, A.K. Sharma and T. Kumar. 2003. A Hand Book of Medicinal Plants. *Agribios India*, 1: 151-157.
- Parrott, J.A. 2001. *Healing Plant of Peninsular India* (AB), New York, U.S.A, 18-719.
- Sabnis, S.D. and S.J. Bedi. 1983. Ethnobotanical Studies in Dadra-Nager Haveli and Daman. *Indian J. For.*, 6: 65-69.
- Sharma, M.P., J. Ahmad, A. Hussain and S. Khan. 1992. Folklore medicinal Plant of Mewat (Gujrat District), Haryana, India, *Int. J. Pharmacog*, 30: 135-137.
- Sharma, S., K. Ravindra, M. Anadhana and B. Kalish. 2008. Ethan tic leaf extract of *Holoptelea integrifolia* (Roxb) Planch decreases cisplatin-induced pica in rat. *Pharmacognosy Magazine*, 4(16): 239-297.
- Shastri, B.N. 1950. *The Wealth of India A Dictionary of Raw Material and Industrial Products*. Publication and Information Directorate, CSIR, New Delhi, 100-110.
- Sing, A.B., P. Malik, D. Parkash and C.K. Gupta. 1992. Biological standardization of pollen allergens from India. *Asian-Pac. J. Allergy-Immunol*, 10(2): 103109.
- Singh, V.K. and Z.A. Ali. 1994. Folk medicine in primary health care, common plants used for the treatment of fever in India. *Fitoterpia*, 65: 68-74.
- Tiwari, V.J. and M.D. Padhy. 1993. Ethnobotanical study of Gond tribe of Chandrapur and Gadchiroli district of Maharashtra state India, *Fitoterapia*, 64: 58-61.