Research & Reviews

ISSN: 2320-0189

Research and Reviews: Journal of Botanical Sciences

Phytochemical Screening of Leaf and Stem Bark of *Holoptelea Integrifolia* Roxb.

Banshidhar Behera*, Deepmala Yadav, and MC Sharma

National Institute of Ayurveda, Jaipur -302002, Rajasthan, India.

Article

Received: 04/01/2013
Revised: 21/01/2013
Accepted: 11/02/2013

For Correspondence:

Department of DravyaGuna, Gaur Brahman Ayurdedic College, Rohtak, Haryana - 124001.
Phone: 09414458895

Keywords: Phytochemical, Chromatography, *Holoptelea integrifolia,*Traditional Use

ABSTRACT

Over the centuries societies around world have developed their own traditions to make the sense of medicinal plants and their uses for attempt to overcome illness and suffering and enhance the quality of life. Plants have a great potential for producing new drugs of great benefit to mankind & medicinal properties of plants depend upon the presence of the different physiologically active component which lead to the search for new biologically active principles for therapeutic efficacy. The present phytochemical screening has been carried out on fresh mature leaves and Bark of *Holoptelea integrifolia* (Roxb) Plant for it's different phyto constituents along with chromatographic profile. The results of the study could be useful in diagnostic tool of the identification of the *Holoptelea integrifolia* (Roxb) Plant and also to establish the quality and purity of this valuable drug.

INTRODUCTION

Holoptelea integrifolia (Roxb.) is a large deciduous tree, 15-18 m high commonly known as Indian Elm belongs to family Ulmaceae found throughout the greater part of India [1,8] and is one among the variety of Karanja (Chirbilwa) described in Classical Ayurvedic Texts like Charak samhita[2] and Sushrut samhita [3]. Bark of the tree remains grey, pustular, exfoliating in somewhat corky scales. Leaves are elliptic-ovate, acuminate, base rounded or subcordate in shape, flowers greenish yellow, polygamous and found in short racemes or fascicles. Fruits are sub-orbicular with membranous wing. The flowering time of the tree is January to February, whereas, fruiting is seen in April to May [4].

The plant *Holoptelea integrifolia* is used traditionally for the treatment of inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhoea and rheumatism [5]. Bark and leaves are used as bitter, astringent, thermogenic, anti-inflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism [6,7].

MATERIALS AND METHODS

Plant Material

Fresh bark of main trunk and leaf of *Holoptelea integrifolia* was collected from National Institute of Ayurveda campus, Jaipur in the month of January. The plant material was identified and authenticated by Prof. M.C. Sharma, Deptt. of DavyaGuna, National Institute of Ayurveda, Jaipur (Rajasthan). After the collection of plant material they were cut into small pieces and shade dried.

Research & Reviews

ISSN: 2320-0189

Phytochemical Screening

All Phytochemical screening were carried out in the Drug Standardization Laboratory of National Institute of Ayurveda, for the analysis of various phyto-constituents as per standard procedure described in A.P.I. [9,10]. In the plants, two types of phytochemicals are present, i.e.Inorganic matters & Organic matters. Inorganic Matters are those which are free from carbon i.e. all electrolytes comes under inorganic matter. Organic Matters are secondary metabolite products in the plants. Role of these components for medicinal purposes are important and examinations of both Organic and Inorganic matters are carried out quantitatively & qualitatively.

Qualitative Analysis for Inorganic Matter (Minerals)

The drug sample of leaf and bark are examined qualitatively in order to determine the presence of various electrolytes. The test methods for this examination carried out as per the methods of Ayurvedic Pharmacopeia of India.

Qualitative Analysis of Minerals

S.No.	Name of Drug	Name of Minerals/Electrolytes				
		Calcium	Iron	Phosphorus	Manganese	Sulphar
1.	Leaves	–	+	+	–	+
2.	Bark	–	+	+	–	+

+ stands for present & – stands for absent

Determination of Extractive Values

The organic substance of the drug sample shows different solubility in different solvents. Extractive value of both the bark and leaves of *Holoptelea integrifolia* in various solvents were observed.

Extractive value of Leaves of *Holoptelea integrifolia*.

Solvent	Water	Methanol	Ethanol	Ether	Acetone	Chloroform	E. Acetate	Hexane
Result in %	24.22	15.07	11.31	4.1	5.42	5.8	5.86	4.2

Extractive value of Bark of *Holoptelea integrifolia*

Solvent	Water	Methanol	Ethanol	Ether	Acetone	Chloroform	E.Acetate	Hexane
Result in %	18.84	13.46	3.34	2.6	4.06	1.24	2.97	1.68

Determination of Moisture contents (Loss on Drying)

Sl. no	Sample	X	W3	W2	W3-W2	W x 100 / X
1	LEAVES	5 gms	37.4180	37.3280	0.09	1.8%
2	BARK	5 gms	41.8992	41.5752	0.143	2.86%

W3 = wt. of silica crucibles with drug sample before drying

W2 = wt. of silica crucibles with drug sample after drying

Determination of Total Ash

Drug	Weight of the sample (X)	Weight of empty crucible (A_1)	Weight of Crucible with ash (A_2)	Percentage of total ash $[(A_2-A_1)/X] \times 100$
Bark	5.0 gm	30.914gm	31.408gm	9.88%
leaves	5.0 gm	33.1372gm	32.1933gm	19.678%

Organic Matter

These are the secondary metabolic products of the plants which held responsible for the different pharmacological activity and plays an important role for their medicinal value.

Research & Reviews

ISSN: 2320-0189

Qualitative Analysis for Organic Matter

S.No.	Chemical Constituents	Test Applied	Name of the Drug	
			Bark	Leaves
1.	Carbohydrates	Molisch's Reagent	+	+
2.	Reducing Sugar	Fehling solution	+	+
3.	Alkaloids	Dragon droff Reagent	+	-
4.	Protein	Ninhydrin Reagent	+	+
		Xanthoprotic test	+	+
5.	Glycoside	Killer killiani Test	+	+
6.	Phenol	Ferric chloride solution	+	-
7.	Tanin	Vanillin solution	+	-
8.	Saponin	Shaking with water	+	+
9.	Fixed Oil/Fat	Aceto Nitrite	-	-
10.	Starch	Iodine Solution	-	+

+ stands for present & - stands for absent

Determination of Acid Insoluble Ash

% of Acid Insoluble Ash of Leaves of Chirbilwa was 6.56%
% of Acid Insoluble Ash of Bark of Chirbilwa was 0.4%

Determination of Water Soluble Ash

% of water-soluble Ash of Bark of Chirbilwa was 3.88
% of water soluble ash of Leaves of Chirbilwa was 1.593%

Organoleptic Examinations of *Holoptelea integrifolia*

Drug	Colour	Taste	Odour	Texture
Bark Powder	Pale yellow	Bitter	Characteristic	Smooth
Leaf Powder	Green	Bitter	Characteristic(foul)	Smooth

Reaction of different samples of drugs with different regents

S.No.	Reagents	Bark Powder	Leaves Powder
1.	Nitric Acid (Conc.)	Formation of foaming turns to deep orange.	Formation of Forming, deep orange
2.	Nitric Acid (Dil.)	Light - Yellow	Brownish - Yellow
3.	Sulphuric Acid (Conc.)	Deep Brownish - Black	Deep greenish Black
4.	Sulphuric Acid (Dil.)	Light Yellow	Greenish - Brown
5.	Hydrochloric Acid (Conc.)	Yellowish - Brown	Deep Yellowish - Green
6.	Hydrochloric Acid (Dil.)	Very Light Yellow	Light Greenish - Yellow
7.	Iodine Solution	Light Redish Brown	Darker Redish - Brown as compare to root
8.	Ethyl Methyl Ketone	Faint yellow	Green

Thin Layer Chromatography (TLC)

Thin layer chromatography is a technique to separate the compounds from a mixture based on adsorption principle. It has the advantage of faster runs, better separations, and the choice between different adsorbents. Different compounds in the sample mixture travel different distances according to how strongly they interact with the adsorbent. This allows the calculation of an R_f value and can be compared to standard compounds to aid in the identification of an unknown substance.

Calculation of Rf Value

$$Rf = \frac{\text{Distance travelled by solute from origin line}}{\text{Distance travelled by solvent from origin line}}$$

ISSN: 2320-0189

Chromatography Plates:

TLC plates coated with 0.25 mm layer of silica gel GF 254 with fluorescent indicator, (Mercks) were used. Each plate having dimension 10 cm long and 1 cm width.

Visualization

Long wave and short wave of U.V.radiation &Iodine vapour

Solvent System – According to their increasing order of polarity.

TLC of the Leaf of *Holoptelea integrifolia*

Ethanolic Extract

Mobile phase – Chloroform: Ethyl acetate (7:3)
Exposure – short wave & iodine vapour

Spot. no	Distance traveled by solvent	Distance traveled by solute	Rf value
1	8.1	2.2	0.27
2	8.1	4.6	0.57
3	8.1	6.3	0.77
4	8.1	7.5	0.92
5	8.1	7.8	0.96

Methanolic Extract

Mobile phase – Chloroform: Ethyl acetate (7:3)
Exposure – short wave & iodine vapour

Spot. no	Distance traveled by solvent	Distance traveled by solute	Rf value
1	7.9	2.5	0.32
2	7.9	4.8	0.61
3	7.9	5.6	0.70
4	7.9	7.3	0.92
5	7.9	7.6	0.96

Water Extract

Mobile phase – Chloroform: Ethyl acetate (7:3)
Exposure – Iodine vapour

Spot. no	Distance traveled by solvent	Distance traveled by solute	Rf value
1	5.8	1.4	0.24
2	5.8	1.9	0.33
3	5.8	4.1	0.70
4	5.8	4.4	0.76

TLC of fraction obtained from column chromatography Bark)

Ethyl Acetate Fraction

Mobile phase – Acetone: Methanol (9.5 : 0.5)
Exposure – naked eye/short wave/Iodine vapour

ISSN: 2320-0189

Spot. no	Distance traveled by	solvent	Distance traveled by solute	Rf value
1	6.9		4	0.58
2	6.9		4.9	0.71
3	6.9		6.6	0.95

Chloroform Fraction

Mobile phase – Benzene : Ethyl acetate (8:2)
Exposure – short wave

Spot. no	Distance traveled by	solvent	Distance traveled by solute	Rf value
1	8.4		2.4	0.29
2	8.4		6	0.71
3	8.4		6.9	0.82
4	8.4		7.5	0.89
5	8.4		8	0.95

Acetone Fraction

Mobile phase – Chloroform
Exposure – short wave

Spot. no	Distance traveled by	solvent	Distance traveled by solute	Rf value
1	5.7		1.2	0.21
2	5.7		2	0.35
3	5.7		2.5	0.43
4	5.7		3.8	0.66
5	5.7		4.4	0.77

Ethyl acetate 85% +Methanol 15% fraction – dry + Ethyl acetate – filter – ethyl acetate fraction

Mobile phase – Ethyl acetate: Chloroform (6:4)
Exposure – short wave/ iodine vapor

Spot. no	Distance traveled by	solvent	Distance traveled by solute	Rf value
1	5.3		0.8	0.15
2	5.3		1.1	0.21
3	5.3		1.9	0.35
4	5.3		2.4	0.45
5	5.3		4.7	0.88

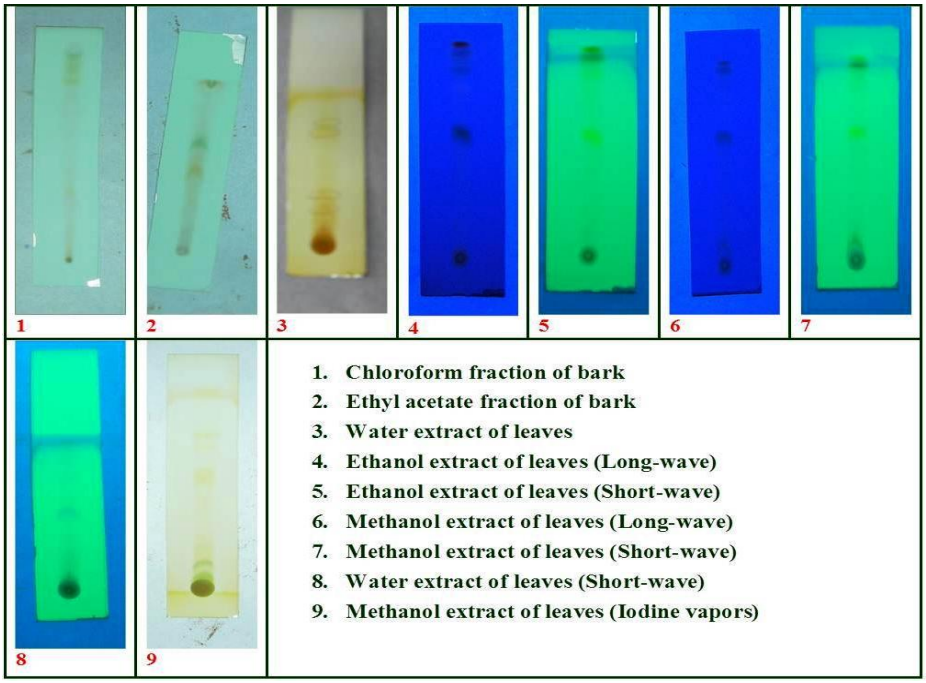
Ethyl acetate 70% + 30%methanol fraction– dry

This fraction (dry) mixed with 100% methanol and then subjected to filter; white colored precipitation was obtained as residue. This residual part was salty in taste which remains insoluble in organic solvent but soluble in water.

DISCUSSION

From the Earliest times, herbs have been prized for their pain reliving and healing abilities and today we still relay on the curative properties of plants in about 75% of our medicines [11]. Now globally the mindsets of people is changing towards the use of safer or less toxic natural products against chemically synthesized medicine in laboratory with lots of side effects. The curative properties of medicinal plants per are mainly due to the presence of different biologically active compound [12] such as Alkaloids, Protein, Glycoside, Phenol, Tanins, Carbohydrates, Reducing Sugar, Saponin, Flavonoids etc. As crude extract of the leaves and stem bark of this plant were used traditionally for the treatment of various ailments without standardization hence phytochemical screening is necessary in order to establish their identity, purity, safety and quality.

ISSN: 2320-0189



TLC Profiles

CONCLUSIONS

The preliminary phytochemical screenings, physico- chemical evaluation and chromatogram profile can be used as a diagnostic tool in the identification of the *Holoptelea integrifolia* (Roxb) as well as to establish the quality and purity of this valuable drug which may be helpful in utilization of this plant in herbal formulations, as a medicine and as pharmaceutical raw material.

REFERENCES

1. The Ayurvedic Pharmacopoiea of India. Deptt. of ISM&H, Ministry of Health and Family Welfare, Govt. of India..2001; Part-1,vol-3: 39-40.
2. Anonymous (1970),Charak Samhita, commentary by Shastri,K.N. et al., Chaukhamba vidya Bhavan, Varanasi, Su.4.9(3,9(4), Sa.8.34,38;Ci.3.267
3. Anonymous (1980), Sushrut Samhitaed Acharya JT, et al. Chaukhamba Orientalia, Varanasi, Su.37.10; Ci.2.89; Ka.6.3; U.42.90.
4. The Wealth of India, 1959. Council of Scientific and Industrial Research, New Delhi, pp: 109-110.
5. Warner PK, VPK Nambiar and C Ramakutty. 1995. Indian Medicinal Plants a compendium of 500 species, Orient Longman private Limited, 3: 162.
6. Prajapati ND, SS Purohit, AK Sharma. 2003. A Handbook of Medicinal Plants a Complete Source Book. Agrobias India Jodhpur, 273.
7. Database on Medicinal Plants used In Ayurveda, CCRAS, Deptt. of ISM&H, Ministry of Health and Family Welfare, Govt. of India.2005,Part-2:171-176
8. Anonymous (1987),Medicinal Plants of India, Indian Council of Medical Research, New Delhi, Vol.2 p.48-50.
9. The Ayurvedic Pharmacopoeia of India. Dept. of ISM&H, Ministry of Health and Family Welfare, Govt. of India..2001; Determination of Quantitative Data of Vegetable Drugs, Part-1, vol-1, Appendix 2.2: 158-165.
10. The Ayurvedic Pharmacopoeia of India. Dept. of ISM&H, Ministry of Health and Family Welfare, Govt. of India..2001; Reagents and Solutions, Part-1, vol-1, Appendix 4: 178-230.
11. Chevallier, A. Encyclopedia of medicinal plants. Published by D.K. ADULT. 2002.
12. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. Published by Springer; 3rd ed. 1998.