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Website: www.aarf.asia Email : editor@aarf.asia , editoraarf@gmail.com

PHYSICO-CHEMICAL STANDARDIZATION OF STEM BARK OF AVARTTANI (*HELICTERES ISORA* LINN.) -AN AYURVEDIC DRUG

Jaly Shashikanth, Dorcas. M
Plant Anatomy and Taxonomy Laboratory,
Department of Botany, University College For Womens, Koti, Hyderabad, Telangana, India.

ABSTRACT:

Helicteres isora L. belonging to family Sterculiaceae is one such plant which is extensively used in Indian system of medicine for skin diseases, dysentery, piles and diabetes. Studies of physicochemical, High performance thin layer cromatography profile, fluorescence and quality evaluation of 'Avarttani' is presented. Qualitative phyto-chemical screening reveals the presence of alkaloids, phenolic compounds and tannins, saponins, flavonoids and carbohydrates.

Key words: *Helicteres isora*, stem bark, standardization, physico-chemical, High performance thin layer cromatography.

1. Introduction

Helicteres isora Linn. (Sterculiaceae) is a small tree or large shrub growing gregariously throughout in dry deciduous forests of India. It is commonly called as "Mrigashringa" and " Avarttani" in Sanskrit. In English it is known as " East Indian Screw Tree" and in telugu 'thada'. It is an important medicinal plant described in various indigenous system of medicine (Anonymous, 1967). In Ayurveda stem bark is useful as an expectorant, demulcent, astringent, antigalactogogue and in diarrhoea, dysentery andbiliousness (Prajapati and Kumar. 2005; Chopra et al, 1969). Most parts of the india shrub are used as medicine. It is used by yanadi tribe in Mamandur forest as fiber (Basha et al., 2011) and gond tribe as fish poison of Kawal wildlife sancutury, Andhra Pradesh (Murthy et al., 2010). The root and stem barks are considered to be expectorant are useful in colic, scabies, emphysema, gastropathy, diabetes, diarrhoea and

dysentery (Kashmir et al., 2010). The literature survey reveals the presence of flavones, triterpenoids, cucurbitacin, diosgenin, isocucurbitacin, phytosterols, saponins, sugars and phlobatannins. (Badgujar, 2009). The aqueous extract of the bark showed significant hypoglycemic effect and lowering effect in hepatic enzymes (Kumar et al., 2006 a, 2006 b), heart antioxidant and lipid peroxidation in diabetic rats (Kumar et al., 2008). Physico-chemical standardization of stem bark is lacking. Hence present investigation is taken up to establish Physico-chemical profile which will help in identification of crude drug as well as to establish standards for quality and purity.

2. **Materials and Methods**

2.1 Collection of stem bark

The stem bark material was collected from Narsapur forest, Medak district,Telangana state, India..The plant specimens was identified and authenticated and deposited in Herbarium Hyderabadense (HY), Osmania University and crude drug samples are also preserved in Crude Drug Repository of Plant Anatomy Laboratory, Department of Botany, Osmania University. Stem bark was powdered, passed through 80 mm sieve; stored in airtight container at 25^0C and used for further studies.

2.2 Physico-chemical studies

Physico-chemical values as percentage 0f loss on drying (Moisture content), foreign organic matter, total ash, acid insoluble, water-soluble ash and petroleum ether, chloroform, ethanol, methanol and water soluble extractives were calculated as per Ayurvedic pharmacopoeia (1989 – 2005) and Homoeopathic pharmacopoeia (1971). Preliminary phytochemical screening was done by standard procedures (Khandelwal, 2005; Trease and Evans, 1989; Harborne, 1998). Fluorescence analysis of powdered drug with various chemical reagents was also carried out standard procedures (Kokoski, 1958; Chase and Pratt, 1949).

2.3 Hptlc studies

A densitometric HPTLC analysis was performed for the development of characteristic finger print profile using different solvents according to polarity viz. benzene, pet. ether, ethanol, methanol and water, which may be used for quality evaluation and standardization of the drug.

Powdered stem bark material 1gram was extracted with 3x10ml methanol. The extract was concentrated under reduced temperature and pressure using rotary evaporator. The plates were then developed in glass trough chamber presaturated with mobile phase. HPTLC studies of stem bark were carried out as per standard procedure (Sethi, 1996).

Chromatographic conditions

Make of Instrument	:	Desaga Sarstedt Gruppe (Germany)
Photodocumentation System	:	UV cabinet with imaging and camera set up with remote shooting
Development Chamber	:	10X10, Twin-trough chamber
Stationary phase	:	Pre coated silica gel 60 F_{254} Alluminium plates (Merck, KgaA, Germany)
Plate thickness	:	0.2 mm
Plate size	:	10 x 10 mm
Distance from starting	:	9 mm
Distance from bottom	:	99 mm
Volume applied	:	5 – 10 µl
Band length	:	5 mm
Distance between tracks	:	10 mm
Development distance	:	90 mm
Solvent used	:	HPLC grade
Extract storage vials	:	5 ml glass vials
Mobile phase preparation	:	Chloroform: Methanol (9: 1)

3. **Results**

3.1 Physico-chemical studies

Results of physico-chemical parameters of the stem bark of *Helicteres isora* Linn. are tabulated in Table 1. The results of petroleum ether, chloroform, ethanol, methanol and water soluble

extract values were presented in Table 2. Preliminary phytochemical results showed the presence or absence of certain phytochemicals in the drugs. The test performed using petroleum ether, chloroform, ethanol, methanol and water extracts. Phytochemical test revealed the presence of alkaloid, phenolic compounds and tannins, saponins, flavonoids and carbohydrates and results of Preliminary phytochemical screening of the different extracts are given in Table 3. The result of fluorescence studies of stem bark using different reagents are shown in Table 4.

3.2 Hptlc studies

TLC of the methanol extract developed in the mobile phase of Chloroform: Methanol (9: 1) after drying the plate was dipped in anisaldehyde sulphuric acid and heated in hot air oven at 105^0 c till the spots are developed under 540 nm, 8 spots were observed at Rf values 0.03, 0.15, 0.23, 0.45, 0.54, 0.60, 0.67, 0.74, 0.81, 0.86 and 0.92. Hptlc fingerprints, densitogram and peak data is tabulated Figure 1, 2 and Table 5 resppectively.

4. **Discussion**

Physico-chemical parameters like foreign matter,moisture content, ash values andetractive values are used to determine quality and purity (WHO,1998). In the present study drug containing the foreign organic matter is indicate purity of drug. Deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, it can be easily deteriorated due to fungus. The loss on drying at 105^0 c in stem bark was found to be 10.75%, total ash value of plant material indicated the amount of minerals and Earthy materials attached to the plant material. Analytical results showed total ash value content was 11.95%. The negligible amount of acid insoluble ash with siliceous matter was 0. 25% and water soluble ash found to be 4.8%.

Extractive values give an idea about the nature of the chemical constituents present in the drug and primarily useful for the identification of exhausted and adulterated drugs. The chloroform and petroleum ether extractive values served as the standards for the specific drug. The water soluble extract value is indicative of the present of sugar, acids and inorganic compounds. The alcohol soluble extractive values indicated the presence of polar constituents like phenols alkaloids etc. The preliminary phytochemical studies revealed the presence of

diverse types of primary and secondary metabolites which will be useful in management of various diseases. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Many phytochemicals exhibit fluorescence when suitably illuminated. The fluorescence color is specific for each compound. A non fluorescent compound may fluorescent if mixed with impurities that are fluorescent (Pimenta et al., 2006). Some constituents show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural compounds (e.g alkaloids like Berberine), which is not visible in day light. Hence some crude drugs are assessed qualitatively in this way which is an important parameter of pharmacognostical evaluation (Ansari, 2006). The results of fluorescence analysis of stem bark powder showed their characteristic fluorescent color. High performance thin layer chromatographic technique was used to separate the chemical compounds present in the drug and may play a significant role in the identification and quality evaluation of the drug.

Preliminary phytochemical as well as other aspects of the stem bark was studied and detailed along with physico-chemical parameters and hptlc studies. Presently found standards helps in the authentication and quality control of raw drugs. The stem bark of *Helicteres isora* Linn. exhibits diagnostic characters which help to identify the raw drug. The present results may serve and supplement chromatographic and Fluorescence of powdered crude drug with different chemical reagents.

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Table 1. Physico-chemical standards of stem bark of *Helicteres isora.*

No.	**Evaluation Parameters**	**Quantitative values**
1.	Moisture content (Loss on drying at 105^0 C)	10.7% w/w
2.	Foreign organic matter	Nil
3.	Total ash	11.95% w/w

4.	Acid insoluble ash	0.25% w/w
5.	Water soluble ash	4.8% w/w

Table 2. Extractive values of stem bark of *Helicteres isora.*

No.	Solvents	Extractive values
1.	Petroleum ether (60-80)^{o}	3% w/w
2.	chloroform	3.75% w/w
3.	Ethanol	5.5% w/w
4.	Methanol	4.75% w/w
5.	water	15.75% w/w

Table 3. Preliminary pytochemical screening of extracts stem bark of *Helicteres isora.*

No.	Test for (or) Constituents	Water extract	Petroleum ether extract	Chloroform extract	Ethanolic extract	Methanolic extract
1.	Alkaloids	+	+	+	_	_
2.	Phenolic compounds and Tannins	+	_	_	+	+
3.	Phytosterols	_	_	_	_	_
4.	Fixed oils and Fats	_	_	_	_	_
5.	Saponins	+	_	_	+	+
6.	Flavonoids	_	_	_	+	_
7.	Amino acids	_	_	_	_	_
8.	Terpenoids	_	_	_	_	_
9.	Carbohydrates	+	_	_	+	+

" + " indicates presense of constituents "_" indicates absence of constituents

Table 4: Fluorescence behaviour of stem bark of *Helicteres isora*

No.	Powder + Reagents used	Light source	Colour
1.	Powder as such	Visible	Limon
		Short UV(254 nm)	Pista
		Long UV (366 nm)	English elms
2.	Powder as such	Visible	Gold rush
		Short UV(254 nm)	Leaf green
		Long UV (366 nm)	Green
3.	Powder + 50%H_2SO_4	Visible	Sporty yellow
		Short UV(254 nm)	Pastel green
		Long UV (366 nm)	Green
4.	Powder + 1N HCl	Visible	Black brown
		Short UV(254 nm)	Black brown
		Long UV (366 nm)	Black brown
5.	Powder + 1N HCl	Visible	Thar desert
		Short UV(254 nm)	Light green
		Long UV (366 nm)	Pastel green
6.	Powder + 1N NaOH IN Ethanol	Visible	Thar desert
		Short UV(254 nm)	Light green
		Long UV (366 nm)	Wild yellow
7.	Powder +1N NaOH IN methanol	Visible	Casablanca
		Short UV(254 nm)	Green
		Long UV (366 nm)	Daffodil
8.	Powder + Pet.ether	Visible	White
		Short UV(254 nm)	White
		Long UV (366 nm)	White
9.	Powder + Methanol	Visible	Autumn gold
		Short UV(254 nm)	Green
		Long UV (366 nm)	Limon yellow
10.	Powder + Acetone	Visible	Brazgen gold
		Short UV(254 nm)	Pastel green
		Long UV (366 nm)	Milky white

Table 5. Paek data of of Stem bark of *Helicteres isora* scanned at 580 nm

No.	Y-Pos	Area	Area (%)	Height	Rf values
1	10.2	2351.00	54.8	527.89	0.03
2	19.9	57.42	1.3	22.03	0.15
3	26.6	445.85	10.4	168.47	0.23
4	45.1	171.35	4.0	36.93	0.45
5	51.9	112.30	2.6	30.67	0.54
6	56.9	16.48	0.4	11.33	0.60
7	63.3	24.24	0.6	9.92	0.67
8	69.0	115.11	2.7	39.75	0.74
9	74.4	22.16	0.5	12.10	0.81
10	78.4	543.22	12.7	191.07	0.86
11	83.3	429.01	10.0	117.66	0.92

Figure 1. HPTLC fingerprints of *Helicteres isora* Stem bark of methanol extract after derivatization at 580 nm.

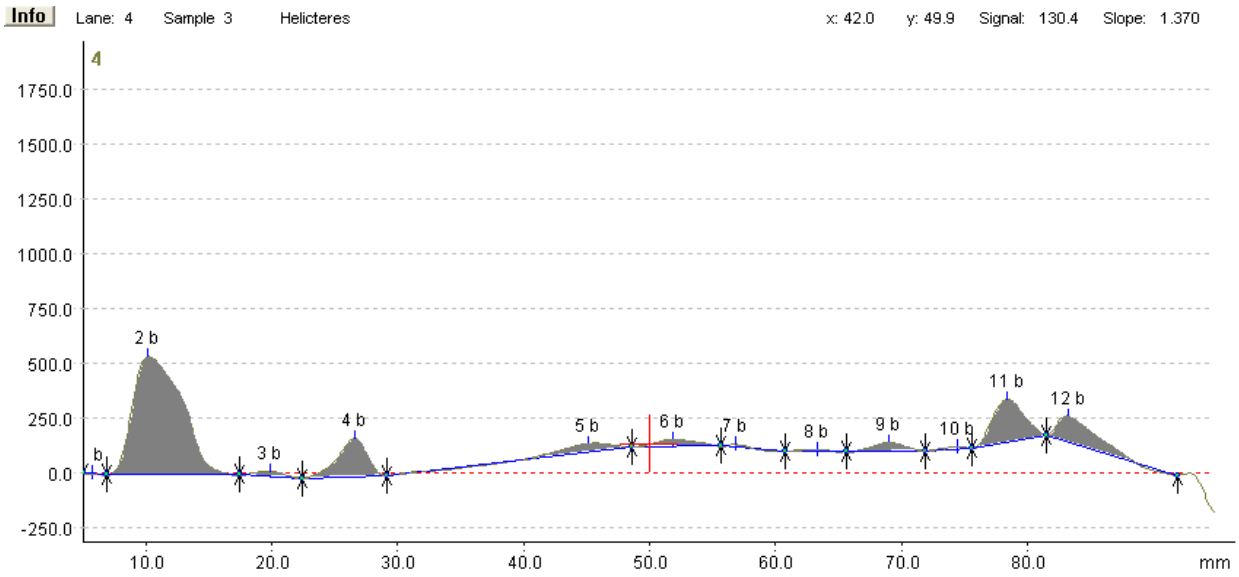

Figure 2. HPTLC densitogram of Stem bark of *Helicteres isora* at 580 nm after derivatization.

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