

Standardisation of Homoeopathic Drug – *Hypericum perforatum* L.

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Abstract

Hypericum perforatum L., a perennial herb belonging to family Hypericaceae is a potential drug in Homoeopathy. It is used for nerve injuries, excessive pain, spasms, hemorrhoids, asthma, tetanus, neuritis, burning pain and numbness, pulmonary and urinary troubles besides as an astringent, expectorant and diuretic .

In the present paper pharmacognostic and physico-chemical studies of leaves and stem of the plant have been presented. Morphological characters of leaf and stem and their transverse sections and longitudinal sections are detailed. Physico-chemical parameters of raw drug viz., extractive values, ash values, besides preparation of formulation weight. per ml., total solids, alcohol content and T.L.C. studies are given for mother tincture.

INTRODUCTION

Hypericum perforatum L., belonging to the family Hypericaceae, is popularly known in English as St. Johns wort and in Hindi as bassant. It is a well known drug mentioned in the Materia Medica's by Allen and Clarke and has the status of an official drug in Homoeopathy. The drug was first introduced by Dr. George F. Mueller (Allen, 1982).

It is a great remedy for injuries to nerves especially of fingers and toes. Excessive pain is one of the guiding symptoms for its use. It is indicated in punctured wounds, prevents lockjaw, relieves post-operative pains and spasms after injury and has an important action on the rectum, hemorrhoids, spasmodic asthmatic attacks with changes of weather, besides bites of animal, tetanus, neuritis, tingling, burning and numbness (Boericke, 1985). It is useful as astringent, expectorant, diuretic and in diarrhea, pulmonary and urinary troubles (Anon. 1985).

The plant is reported to contain volatile oil, tannins and a red pigment hypericin, besides rutin, alkaloids, fixed oils, Vit. C, pro-vitamin A (Anon. 1985 and Rastogi, 1991), 3,5,7-trihydroxy anthrone (IV), 2,5-dimethyl 7-hydroxyl chromone VII and vanillic acid VIII (Yin Zhiqi, 2002).

In view of its immense importance as a homoeopathic drug, pharmacognostic and physico-chemical studies have been undertaken to validate the results of earlier studies (Anon. 2000).

MATERIAL AND METHODS

Pharmacognosy

The shade dried plant material of *Hypericum perforatum* L. was supplied by Survey of Medicinal Plants and Collection Unit, Udhamandalam, Tamilnadu. The leaves and stems were fixed in F.A.A. (formaldehyde-acetic acid-alcohol) and further processed for microtoming following Johansen (1940). Epidermal peels were obtained by gently peeling with a razor blade. The transection of leaf and stem at 8-10 µm thickness were taken on Spencer's rotary microtome and stained following Johansen (1940).

The powder microscopic characters were studied by boiling the powdered material in distilled water, stained in safranin and mounted with glycerine. Photomicrography was done with Olympus CH-2 trinocular research microscope.

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Physico-chemical

The coarsely powdered plant drug material was subjected to determination of moisture (loss on drying at 105° C), total ash, water soluble ash, acid insoluble ash and sulphated ash, extractability in water and alcohol. The above parameters were determined as per procedure recommended in Homoeopathic Pharmacopoeia of India (H.P.I. 1971). The mother tincture was prepared as per H.P.I. (1971).

RESULTS AND OBSERVATIONS

Morphology

A perennial herb, 30-60 cm., stem 2-angled, leaves oblong, 8-25 mm., obtuse, pellucid punctate, lower surface pale. Flowers 2.5 cm in diameter, in terminal corymbs. Sepals narrowly lanceolate, acute, 6 mm, margins often dotted. Petals black dotted on margins. Stamens in 3 bundles, anthers black dotted. Ovary 3-celled, styles 3, twice as long as the ovary, capsule 8 mm.

Leaf

Leaves small, amplexicauline, elliptic and ovate, pellucid punctate. Epidermis of the abaxial surface shows polygonal isodiametric and anisodiametric cells with walls septate, curved to wavy and sinuate; 1440 per sq. mm. Adaxially cells are frequently papillate (Fig. 1.7), isodiametric, sides septate, straight to curved to slightly wavy; 1700 per sq. mm. Palisade ratio : 9-10.25. Stomata anisocytic, a few tetracytic and anomocytic with 3 or 4 indistinct subsidiary cells. Stomata 400 per sq. mm on abaxial and 60 per sq. mm on adaxial surface. Stomatal Index 21.7 (on abaxial) and 3.4 (on adaxial). Stomata 19-27 µm long and 14-19 µm wide (Fig. 1.1,2), trichomes absent. Surface is provided with pellucid glandular punctae (Fig. 1.4,5).

In transection, the leaf at midvein and secondary veins is grooved adaxially and ribbed on the abaxial. Midvein 183-194 µm and lamina wings 151-162 µm in thickness (Fig.1.3). Epidermis shows papillae predominantly on the adaxial side; cells barrel shaped, few oval to spherical with sunken stomata occurring rarely. Abaxially epidermal cells are smaller.

Mesophyll dorsiventral, often interrupted with secretory canals, 45-70 µm in diameter. Palisade 1-layered, cells cylindrical 24-55 µm long and 11-17 µm wide, with dense chloroplasts; interrupted at midvein. Spongy parenchyma loose with cylindrical, oval to oblong cells, 19-33 µm long and 8-14 µm wide, isodiametric ones 11-22 µm in diameter, filled with chloroplasts.

Ground tissue of midvein solely with parenchyma as a group of few cells on the adaxial surface and 5 or 6 layered on the abaxial surface, cells 8-29 µm in diameter, walls thin. (Fig. 1.3).



Flowers of Hypericum perforatum

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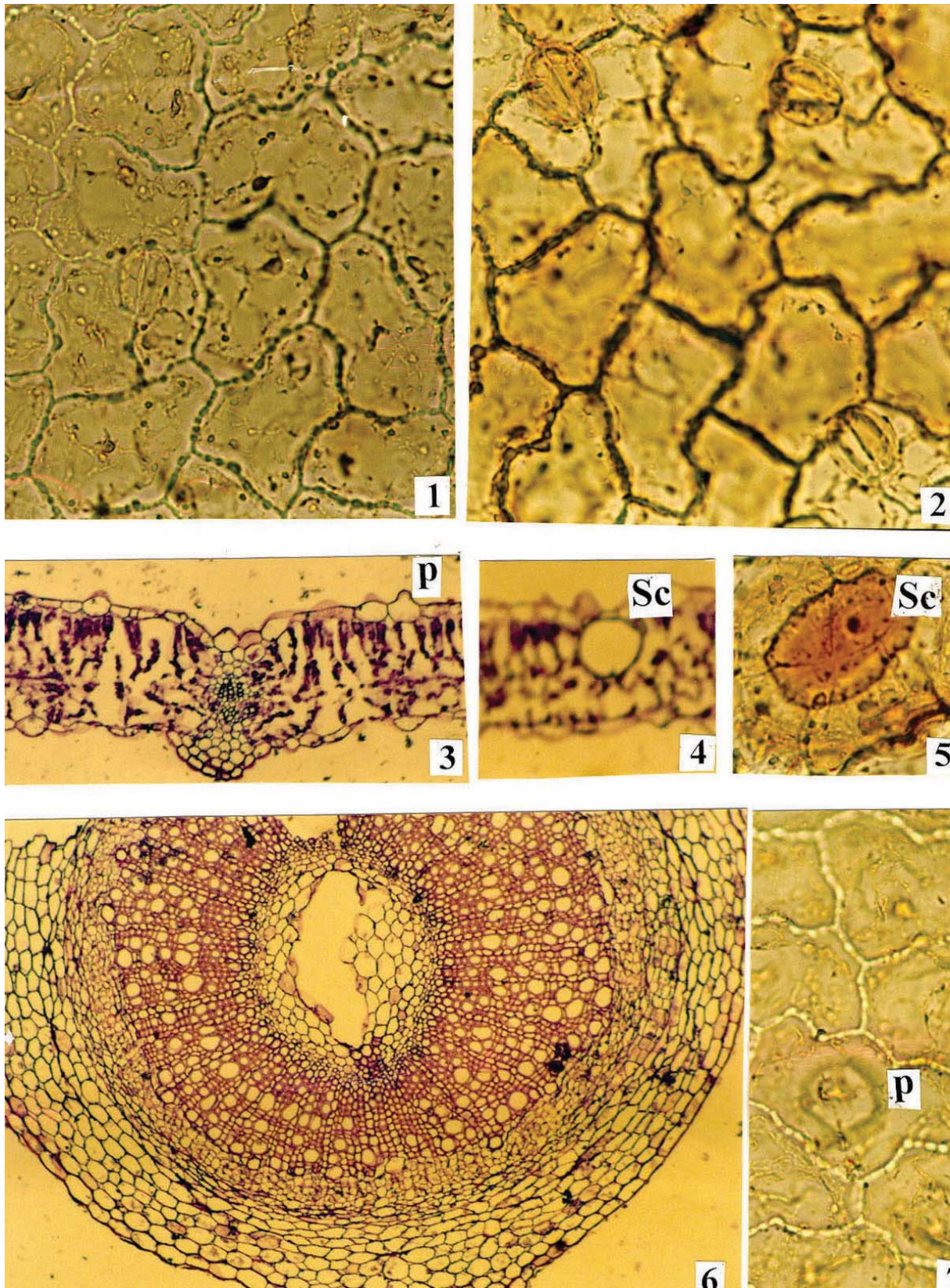


Fig. 1

Vascular tissue at midvein of a single ovate bundle, endarch, conjoint, collateral, open and apercyclic, 49-63 μm and lateral bundles 38-47 μm in diameter (Fig.1.3). Xylem elements in radial rows, few clusters; in T.S. 5.5-11 μm in diameter, secondary walls mostly helical, few scalariform and reticulate, phloem scanty with phloem parenchyma, fibers, sieve tubes with companion cells. Secondary veins also exhibit similar pattern.

Stem

Rounded to slightly angular, erect or procumbent, 990-1075 μm in diameter (Fig.1.6). Epidermal cells in surface anisodiametric to linear, sides slightly thick, straight to curved, septate, surface striated; stomata and trichomes absent (Fig.1.6).

In T.S. epidermis 1-layered, cells barrel shaped; cortex 8-10 layered, cells tangentially elongated, 25-55 μm long and 19-33 μm wide. Inside cortex the phloem occurs as a continuous layer consisting of phloem parenchyma, fibers and sieve cells accompanied with companion cells; the phloem is interspersed with secretory canals. Xylem is in the form of continuous cylinder consisting of vessels or tracheids, fibers and xylem parenchyma. Secondary walls of tracheary cells in L.S. possess bordered pits, helical and reticulate thickenings. Pith parenchyma is present at the center (Fig.1.6).

Powder Microscopy

The powder shows fragments of:

1. Epidermis with curved to wavy sides with anisocytic stomata.
2. Leaf, dark greenish to yellowish brown epidermis with papillate cells.
3. Cortical cells and attached xylem cells with helical thickenings.
4. Hyaline resinous masses appearing translucent.
5. Epidermis with pellucid punctate glands.

Organoleptic Characters

Colour	:	Mustard green
Touch	:	Coarse
Taste	:	Sharply pungent, tingling
Odour	:	Choking

Physico-chemical studies

The physico-chemical data pertaining to raw drug, mother tincture preparation and its standardization along with TLC studies are provided in the following tables:

Table-1: Standardisation of Raw Drug

S.No.	Parameters	Quantitative Value % w/w
1.	Moisture content (L.O.D. at 105°C)	13.75
2.	Alcohol soluble extractive	29.85
3.	Water soluble extractive	23.3
4.	Total ash	8.2
5.	Water soluble ash	3.15
6.	Acid insoluble ash	2.0
7.	Sulphated ash	11.05

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Table-2: Formulation of Mother Tincture

Drug Strength	1/10
<i>H. perforatum</i> in coarse powder	100 g
Strong alcohol	684 ml
Purified water	350 ml

to make one thousand milliliters of the mother tincture.

Table-3: Physico-chemical constants of Mother Tincture

S.No.	Parameters	Observations
1.	Organoleptic Characters a) appearance b) colour c) odour	Clear, foam formed on shaking Dark maroon Fruity and aromatic
2.	Sediments	Absent
3.	Weight per ml	0.93 g
4.	Total solids	2.6% w/v
5.	pH at room temperature	3.0-3.5
6.	Alcohol content	60-65% v/v
7.	ëMax	664 and 590.5 nm

Table-4: Chromatographic results of *H. perforatum*

Extract	Concentrated mother tincture
Adsorbent	Silica gel 'G'
Layer thickness	0.4 mm on wet conditions (0.25 mm after drying and activation)

Solvent system	Detecting agent	No. of spots	Rf vlues
n-butanol : acetic acid : water [4 : 1 : 1 v/v]	Vanillin-sulphuric acid	6	0.28 0.35 0.71 0.79 0.85 0.91 all violet color

DISCUSSION

Pharmacognosy

Hypericum perforatum L. is a perennial herb, belonging to the family Hypericaceae. It is a potential medicinal herb being used for various ailments (as cited elsewhere) in Homoeopathy.

The leaves are small, amplexicauline, elliptic to oval and pellucid punctate. The stem is rounded with slight edged angles. The leaves and stems being medicinally important are pharmacognostically studied. The review of earlier literature reveals its description in pharmacopoeia (Anon.2000).

In surface, epidermis shows polygonal iso- and anisodiametric cells with curved to wavy and sinuate sides on the abaxial while straight to curved to slightly wavy and papillate on the adaxial surface (Fig. 1.2,7). The epidermal cell frequency is more (1700 per sq. mm) on the adaxial and less (1440 per sq. mm) on the abaxial surface. Further the palisade ratio was reported 5.0-7.9 (Anon. 2000), but presently it is found as 9-10.5. The adaxial surface also shows punctate glandular pores (Fig. 1.5). The stomata are reported as anomocytic and confined to lower surface (Anon. 2000). But presently the leaves are found to be amphistomatic with anisocytic, anomocytic and tetracytic types of stomata (Fig. 1.1,2). The stomatal index on the lower side was reported as 11.63-28.24 (Anon. 2000), while presently it is 21.7 (average) and conforms with earlier report. Besides the S.I. on the adaxial side is 3.4. The stomata are 19-27 μ m long and 14-19 μ m wide.

In T.S. the leaf at midvein and secondary veins is grooved adaxially and slightly ribbed on the abaxial surface and nearly triangular as reported (Anon.2000). The midvein is 183-194 μ m and lamina wings 151-162 μ m in thickness. Epidermis is 1-layered and shows papillae predominantly towards adaxial surface confirming the earlier studies (Anon.2000).

Mesophyll is dorsiventral with adaxial 1-layered palisade consisting of cylindrical cells 24-55 μ m long and 11-17 μ m wide. Spongy parenchyma is loose with oval to oblong and elongated cells. The mesophyll possesses secretory oil canals 45-70 μ m in diameter mostly oriented towards adaxial side (Fig.1.4).

Ground tissue of midvein was reported to possess single layered collenchyma hypodermally, but presently the ground tissue is found to be composed solely parenchyma, as a group of a few cells on the adaxial and 5-6 layered on the abaxial side.

The vascular tissue at midvein was reported as an arc shaped bundle (Anon.2000) and presently it is found as single ovate bundle 49-63 μ m in diameter, endarch, conjoint, collateral and open (Fig.1.3). The lateral bundles are 38-47 μ m in diameter. The secondary walls of xylem elements are mostly helical, a few scalariform or reticulately thickened.

Young Stem

Epidermis exhibits anisodiametric to linear cells with straight to curved sides, septate walls and striated surface. In T.S. the stem was reported as quadrangular with four prominent ridges (Anon.2000) and presently it is observed to be rounded with slight angles, 990-1075 μ m in diameter. Epidermis is 1-layered made up of barrel shaped cells. The hypodermis is reported as 1-2 layers of collenchyma followed by 3 or 4 layered parenchymatous cortex and interrupted by secretory cavities (Anon.2000). Presently the hypodermis consists of 8-10 layered cortex with tangentially elongated cells, followed by phloem, interspersed with secretory cavities. The xylem is in a continuous cylinder surrounded by phloem. The secondary walls of xylem cells possess bordered pits, helical and reticulate thickenings. Pith parenchyma is present at the center (Fig.1.6).

Physico-chemical Studies

Preliminary phytochemical screening indicates the presence of alkaloids, tannins and saponins. The physico-chemical data for the raw drug study has been summarized in Table-1 and that of mother tincture preparation in Table-2, while the data for the mother tincture standardization and TLC study is given in Tables 3&4 respectively. The TLC study confirms the presence of 6 spots as stated earlier (Anon.2000). The slight deviation in the values in decimal level may be due to difference in the TLC plate used.

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