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DRUG STANDARDISATION

Standardisation of Homoeopathic Drugs – *Castanea Sativa* Mill.

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Abstract

Castanea sativa Mill. A tree belonging to the family Fagaceae is a potential drug in Homoeopathy. The leaves are used as tonic, astringent, In paroxysmal coughs and diarrhoea.

The leaves are large, lanceotate with deep serrate margins and covered by indumentum on the lower side. The epidermis has cells with thick straight to curved or wavy sides. Stomata are actinocytic or anomocytic. The abaxial side has dense hairy cover consisting of 2-6 armed and stellate hair. In T.S. the ratio of midvein to lamina is 6:1.

Sphaeraphidal idioblasts are present in the cortical perenchyma and pith of midvein and petiole. The vascular tissue appears in the form of a plectostele in the midvein end petiole.

Besides, powder microscopical and organoleptic characters are presented. Physico-chemical parameters of raw drug viz., extractive values, ash values, formulation, besides. Wt. per ml, total solids, alcohol content and T.L.C. and U.V. studies are given for mother tincture.

Keywords: homoeopathy, castanea sativa mill., standardisation, pharmacognosy, physico-chemical, pharmacopoeia.

INTRODUCTION

Castanea sativa Mill., popularly known as chestnut, is a tree belonging to the family Fagaceae. It is a native of Southern Europe, North Africa and West Asia and cultivated in parts' of Himalayas, especially in Punjab North West Frontier and Darjeeling[1].

The leaves of the tree are used, as tonic and astringent; in paroxysmal cough, and other irritable conditions of the respiratory system besides diarrhoea[1]. Castan has been proved by Dr. H.C. Houghton and three others.

The leaves are reported to contain ursolic acid, lupeol, betulin, myricetol, rutoside, myricitroside besides protein, tannins and fatty acids[1,2] 1,4 rhamnogalacturonans, uronic acid, castamollin, g-terpinene, furfural and benzaldehyde[3,4]. A new pyrrole alkaloid, Methyl - (5-formyl)-1H-pyrrole-2-yl)--hydroxybutyrate (1)[5]. Antibacterial activity was found in the extract of leaves[6].

A review of literature reveals no pharmacognostic standards recorded for the drug. In view of the importance of the drug in Homoeopathy, pharmacognostic and physicochemical studies of leaves are carried out for their standards.

MATERIAL AND METHODS

Pharmacognosy

The plant material of *Castanea sativa* Mill was supplied by Survey of Medicinal Plants and Collection Unit, Udhagamandalarn, Tamilnadu (SMPCU Herbarium specimen No. 7872 SR Dt. 24.06.2007). The leaves were fixed in F.A.A. (Formaldehyde--acetic acid-alcohol), processed for microtoming (usual paraffin method), sectioned, stained and permanent slides prepared following usual methods. The epidermal peels were obtained by gently scraping and peeling with razor blade. The powder microscopic characters were studied by boiling the powder drug in distilled water, stained in saffranin and mounted with glycerine. Photomicrography was done with Olympus CH-2 trinocular research

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microscope.

Physico-chemical

The air-dried leaves of the drug was coarsely powdered to 10/44 seive size and was subjected to determination of moisture content (loss on drying at 105^0), total ash, acid insoluble ash, sulphated ash and extractability in water and alcohol following official methods[8,9]. Mother tincture was prepared as per H.P.I.[9]. In this method 100 gm of coarse powder of the drug was suspended in 475 ml of 95% alcohol and 550 ml of purified water for 24 hrs. at room temperature. It was filtered and made up to 1000 ml using the same solvent, ratio. Percolation method[9] was used for the preparation of mother tincture.

Observations and Results

Morphology: The chestnut is large tree with massive trunk and rough bark. Leaves bright green, large, lanceolate with saw-like teeth. The male catkins are perfumed. Fruits (chestnuts) encased in a thick and very spiny cupules[10].

Anatomy

Leaf

Epidermal Surface view : Cells polygonal anisodiametric or isodiametric; sides thick (adaxially), straight to curved and also wavy on abaxial (Fig.1.1,2); 4220 per sq. mm on adaxial and 10220 per sq. mm on abaxial. Stomata towards abaxial, actinocytic and anomocytic, subsidiaries 7 to 9, slightly sunken, 1180 per sq. mm. Stomatal index-10.35 (average), Palisade ratio is 6 to 8.5, Vein Islet number is 3.5 to 5.

Transection : Ribbed slightly on adaxial and prominently on abaxial side, dorsiventral; covered by 2-

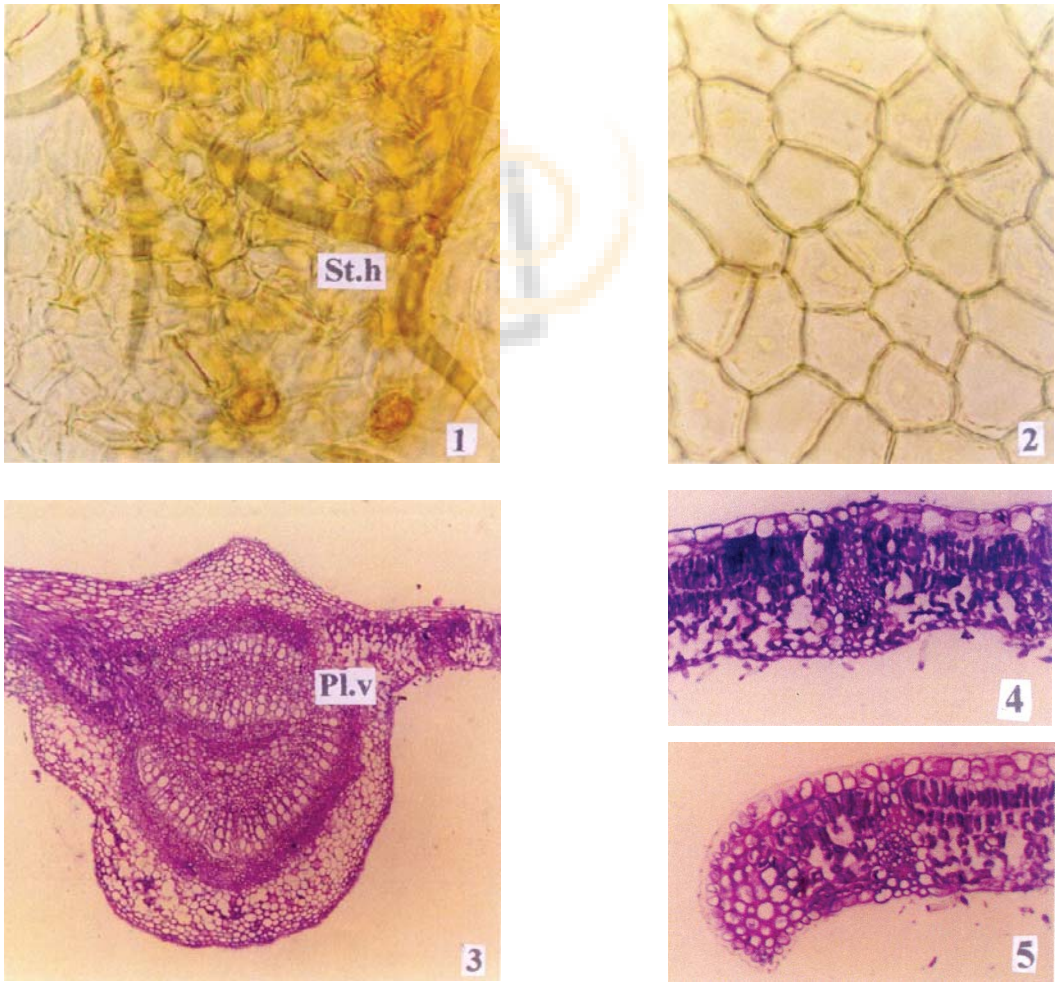

Fig. 1: (1) Abaxial epidermis (surface view) x 486; (2) Adaxial epidermis (surface view) x 545; (3) T.S. of leaf midvein x 51; (4) T.S. op leaf lamina x 81; (5) T.S. of leaf margin x 123, St. h – stellate hair; Pl. v.-plectostele vascular bundle

6 armed hairs on abaxial (Fig 1.1) Midvein 1.3-1.7 mm thick, lamina wings 259-291 mcm (276) thick. Ratio of midvein to lamina 6:1, margins pointed and incurved (Fig 1.4,5).

Epidermis 1-layered throughout, except, 2-layered near the midvein and covered by a thick cuticle; hypostomatic, with slightly sunken stomata. Mesophyll dorsiventral, palisade 2-layered (Fig.1.4,5). Cells 19-60 mcm (37) long and 5.5-10 mcm (7) wide, filled with chloroplasts and tannins, interrupted at veins; spongy tissue with loosely dispersed cells, interspersed with sphaeraphidal cells.

Collenchyma 3-4 layered in the adaxial region and 2-3 layered on the abaxial, lamellar thickened. Parenchyma 6-9 layered, interspered with sphaeraphidal idioblasts near the pericycle; centrally pith parenchyma is present between vascular plates and contain sphaeraphldes, starches or inulin in some; sclerenchyma is 4- layered and often contain, tannins and crystals. (Fig. 1.3).

Vascular system is shield-like plectostele, with 3 vascular plates in a row; 2 plates large, arcuate and overlapping smaller saucer shaped bundles on the abaxial and 1 single inverted arc shaped on adaxial side (Fig.1.3). V. bundles endarch, collateral, open. The vessels and tracheids in radial rows, secondary walls bordered pitted, scalariform and helically thickened. The minor vein bundles with sheath extensions.

Petiole

Epidermal cells similar as on leaf adaxial in surface and parallelly oriented, contents with crystals of calcium oxalate in few. Covered with 2-6 armed stellate hairs all over.

In transaction, 2.3-2.5 mm thick, shield-like (Fig. 2.1). Epidermis 1-layered, with dense contents. Collenchyma is 1-2 layered, lamellar with scanty contents. Parenchyma 10-16 layered, Interspersed with sphaeraphidal idioblasts and tanniniferous cells. Sclerenchyma 5-12 layered, surrounding the vascular bundle (Fig.2.1).

Vascular tissue Is characterized by the presence of 4 central oval bundles in an arc, enveloped by a vascular continuous cylinder. V. bundles endarch, conjoint, collateral and closed. Phloem is interspersed with sphaeraphidal idioblasts. A 3-4 layered vascular cambium is present.

Powder Microscopy

Pieces of adaxial epidermis with thick anticlinal walls and straight sides. Fragment of armed stellate hair. Pieces of abaxial epidermis with wavy sides and actinocytic stomata Vascular tissue with helical and reticulate thIckenings. Pieces of ground tissue with sphaeraphidal idioblasts 19-35 mcm in diameter. Isolated sphaeraphides 16-27 mcm in diameter, either whole or fragments. Few dispersed starch and inulin grains.

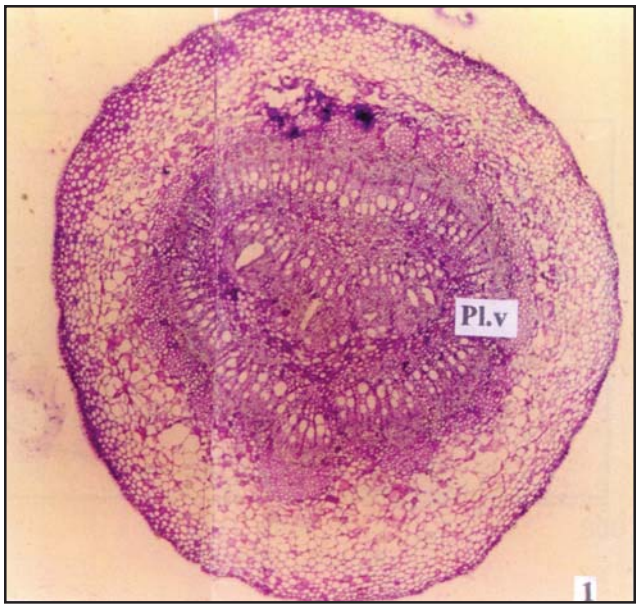

Fig. 2: 1. T.S. of petiole x 57
Pl.v – plectostele vascular bundle

Organoleptic characters: Powder is mustard green in colour. Coarse to touch; pungent to slightly choking to smell and sour to taste.

Physico-chemical

The determined data under the physico-, chemical study for the raw drug is summarized in table I and that of mother tincture preparation and its standardization in Table II and III respectively and the results of T.LC. studies are presented in Table IV.

Table I: Physic-Chemical Standards of Raw Drug

S. No.	Parameters	Quantitative Values % w/w
1.	Moisture content (L.O.D. at 105^0)	13.7
2.	Alcohol soluble extractive	31.25
3.	Water soluble extractive	25.5
4.	Total ash	4.0
5.	Acid insoluble ash	1.5
6.	Water soluble ash	0.75
7.	Sulphated ash	5.05

Table II: Formulation and Preparation of Mother Tincture

Alcohol content	=	45% v/v
Drug strength	=	1/10
Percolation technique as per H.P.I.[9]		
Preparation		
Castanea sativa in coarse powder	:	100 g
Strong alcohol	:	475 ml
Purified water	:	550 ml

To make one thousand milliliters of the mother tincture

Table III: Standardization of Mother Tincture

S. No.	Parameters	Observations
1.	Organoleptic properties	
	a. Appearance	Clear, non-viscous
	b. Colour	maroon
	c. Odour	Charecteristic
2.	Sediments	absent
3.	Wt. per ml.	0.99 g
4.	Total solids	2.73% w/v
5.	pH at room temperature	3.5-4.0
6.	Alcohol content	40 – 45% v/v
7.	I max (Absorbance) in MeOH	203.0 and 270.0 nm
8.	I max of Rf 0.82	270nm

Table IV: Chromatographoc Results of *Castanea sativa* Mother Tincture

Extract : Chloroform extract of the mother tincture
Adsorbent : Silica gel 'G'
Layer thickness : 0.4 mm tickness (wet conditions)

Solvent system	Detecting Agen	No. of Spots	Rf values
Chloroform : Acetic acid (9 : 1) v/v	10% Ferric chloride	2	0.82 0.97 (Black)

DISCUSSION

General features

Castanea sativa Mill., is popularly known as chestnut belongs to, the family Fagaceae. Morphologically the leaves are bright green, large, lanceolate with an indumentum on lower side. Margins are with saw-like teeth.

Epidermal cells in surface have thick, straight to curved sides and also wavy towards abaxial side (Flg.1.1,2)-. Leaves possess actinocytic and anomocytic types of stomata (Fig.1.1). The stomatal, index (S.1) is 10.35. The palisade, ratio is 6-8.5 and the vein islet No. is 3.5 to 5.

In transection leaf abaxially is covered by 2-6 armed stellate hairs (Fig. 1.3). The ratio of midvein to lamina thickness is 6:1. The palisade is 2-layered. The mesophyll is interspersed with sphaeraphidal idioblasts.

The vascular system is a plectostele characterized by 3 vascular plates in a row (Fig.1.3). The secondary walls of vessels have bordered pits, helical and scalariform thickenings.

Petiole

The epidermal cells in surface are polygonal with parallelly oriented cells. Few armed stellate hairs (i.e.) occur all over. In T.S. epidermis is 1-layered while collenchyma is 1-2 layered. Cortex is interspersed with sphaeraphidal idioblasts and tannin cells. A sclerenchymatous layer encloses the vascular bundles (Fig. 2.1).

The vascular tissue is characterized by 4 central oval bundles, enveloped by a continuous vascular cylinder (Fig. 2.1). A 3-4 layered vascular cambium is present.

The salient pharmacognostic features along with powder microscopic and organoleptic characters presented are helpful in authentification of the drug.

Physico-chemical

The observed physico-chemical data for the raw drug and finished product are summarized in tables 1-3.

The results of TLC studies are presented in Table IV and reveal 2 distinct black spots in 9:1 (Chloroform-acetic acid) solvent system when sprayed with 10% Ferric chloride.

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