Pharmacognostic Studies of Vitex agnus-castus Linn.– Fruit

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Abstract

Fruits of *Vitex agnus-castus* Linn. (Verbaenace) is used in Homoeopathy and Indian system of medicine. Pharmacognostic studies of fruit / berry has been carried out to lay down the standards for genuine drug. Characteristic microscopical features are loculicidal drup with exalbuminous seeds; mesocarp consists of thick walled cells; endocarp stony; lignified testa cells show conspicuous scalariform folds or thickenings. It can be characterized by the presence of flavon, sterol, alkaloid, glycoside, volatile oil, sugar, tannin and starch. Other parameters also studied include TLC, UV spectrophotometry, fluorescence behavior, ash values, extractive values etc. Based on these characters, the study will help in the correct identification of this important drug.

Keywords: *Vitex agnus-castus*, Pharmacognostic, Fruit / Berry, Homoeopathy.

Introduction

Vitex agnus-castus Linn. (Fam. Verbenaceae) is commonly known as chaste tree, found in Baluchistan, Afghanistan, Western Asia, Mediterranean and it is cultivated in India (Chopra *et al.*, 1974; Anonymous, 1976). It is a woody, perennial, deciduous shrub or a small tree, 3-6 m high, with strong aromatic odour (Davis, 1982).

Fruits are reported to contain casticin and orientin (c-glycosyl compound), Homo-orientin and dark fixed oil (Mabry, 1968; Harborne *et al.,* 1975).

In Homoeopathic system of medicine fruits / berries are used specially for sexual debilities, impotence, gleet, nervous debility, melancholy and apathy from sexual abuse (Allen, 1976). It is also used for scanty emission without proper ejaculation and loss of prostatic fluid on straining (Boericke, 1976).

In Ayruvedic system of medicine fruit is useful in “Kapha” and ‘Vata’, pruritus, itching, burning sensation and thirst while seeds are prescribed for colic/ stomatic, abortifacient, diuretic, alexiteric, cause biliousness. In Unani system of medicine it is given for enlargement of spleen & liver and useful in inflammation, pains, dropsy. Seeds are astringent, purify brain and liver (Kirtikar & Basu, 1975; Nadkarni, 1976).

Decoction of berries is used for leucorrhoea, staining yellow and also shows flavourable effect on amenorrhoea (Amann, 1982).

It relieves pre-menstrual syndrom (PMS) including corpus luteum insufficiency, menopausal syndrom and insufficient milk production (Gurmeet *et al.*, 2011). Chaste tree berries has the effect of stimulating and normalising pituitary gland functions, especially its progesterone function (Anonymous (Internet), 2012). Oil from seeds have progesterone like effect on mature female rats (Anonymous, 1976).

Elaborate pharmacognostic studies on the fruit of this plant is not on record. In view of efficacy of drug the detailed pharmacognostic studies including phytochemical analysis, TLC profile and physical characters are worked out and reported in the present communication.

Materials and Methods

Fruits were collected from Central Institute of Medicinal and Aromatic Plants, (CIMAP), Lucknow and Medicinal Plants Garden, Homoeopathic Pharmacopoeial Laboratory, (HPL), Ghaziabad, preserved for macro & microscopical studies.

For histological studies microtome sections of 20 µm thickness were prepared after softening the fruit in Hydrofluoric acid; for powder study 5% aqueous KOH solution was used for tissue maceration.

For anatomical / histological characterization, Esau (1960) and Metcalfe (1950) were consulted; for powder analysis method suggested by Jackson & Snowdon (1968) were followed. Fruits were dried, powdered and extracted with ethanol at room temperature in soxhlet apparatus. Extract was filtered and filterate was used for preliminary phytochemical tests. For chemical analysis (Johnsen, 1940; Youngken, 1951; Cromwell, 1955; Trease & Evans, 1983) and for physical evaluation I.P. (1970), were followed. For fluorescence behavior of drug Harborne (1973) was consulted.

Results and Observations

Drug Evaluation

(i) Macroscopical Evaluation

Fruit a drupe, pepper corn like, hard, purple to dark brown outside & yellowish inside, spherical, obtuse, 4-celled; mostly enclosed in greenish-grey cup-shaped pubescent calyx, slightly larger than the calyx, each lobe containing an oblong seed, rich in fat. Odour aromatic spicy; taste bitter and peculiar (Fig. 1).

(ii) Microscopical Evaluation

(a) Histology

Fruit: transection shows circular in outline, locular; locules 2 to 4, bearing exalbuminous seeds (Fig. 2-A). Exocarp consists of either single layer of cuticularised cells or a zone of 5 to 9 layers of thick walled cells with cellular inclusions (fig. 2-B). Mesocarp consists of either 5 to 22 layers of thickwalled isodiametric, oval parenchymatous cells arranged tangentially in the upper half while radially in the lower half containing conducting elements or entirely of palisade parenchymatous cells with or without conducting strands (fig. 2-B). Endocarp made up of 4 to 15 layers of sclereids having elongated macrosclereids towards the inner zone extending along with septa. The inner most layer of the endocarp is thin walled, tangentially elongated, cells with cell contents. Septa made up of thickwalled, isodiametric, oval parenchymatous cells (Fig. 2-C). Seed: oval in outline; seed coat consists of single layer of lignified conspicuous sclariform folds followed by 4 to 9 layers of large tabular parenchyma cells; cotyledons 2, each consists of single layered epidermis of oval cells enclosing storage parenchyma with starch grains and oil globules, a few scattered conducting elements present (Fig. 2-D).

(b) Powder Microscopy

Powdered fruit dark brown to yellowish-brown with oily repulsive odour and oily, bitter taste. It contains elongated macrosclereids, 40 to 100 µm long, with narrow lumen and prominent pits; irregularly shaped stone cells or brachysclereid, 10 to 25 µm in length; tracheary elements with simple pits and broad lumen, 25 to 100 µm long; tracheary elements with scalariform thickenings, 20 to 55 µm in length; thick walled parenchyma cells of epicarp with prominent pits, 20 to 30 µm in diameter; cotyledonary parenchyma cells with oily globules, 10 to 25 µm or more in diameter; groups of lignified seed coat cells with scalariform folds (Fig. 2-E).

(iii) Chemical Analysis

Observations in respect of preliminary phytochemical analysis and Thin Layer Chromatography are presented in Table 1 and 2.

(iv) Physical Analysis : Observations

Fluorescence behaviour, Extractive values, Ash values, Total solids, pH (at 25°C) and UV spectrophotometry are presented in Table 3 to 5.

Table 1 : Phytochemical Tests (Preliminary colour reaction tests of fruit of *Vitex agnus-castus* Linn.)

S. No.	Reagent	Test Performed	Result
1.	Dragendroff's reagent	Alkaloids	+ ive
2.	Phloroglucinol + HCl	Lignin	+ ive
3.	$FeCl_3$	Tannin	+ ive
4.	Molish test	Sugar	+ ive
5.	Molish test after hydrolysis	Glycosides	+ ive
6.	Alc. ext. + Acetic anhydride + H_2SO_4	Saponin	– ive
7.	Mg powder + Conc. HCl	Flavones	+ ive
8.	Libermann + Conc. HCl	Steroids	+ ive
9.	Sudan IV	Oils	+ ive
10.	Borntrager reaction	Anthraquinone	– ive
11.	Weak Iodine Solution	Starch	+ ive

Table 2 : Rf values of fruit of *Vitex agnus-castus* Linn. (Mobile phase, n-Butanol : Acetic acid : water-4 : 1 : 1 v/v)

S. No.	Colour of spots	Rf values
1.	Blue	0.49
2.	Green	0.59
3.	Blue	0.73
4.	Yellow	0.92

Table 3 : Fluorescence behavior of fruit powder of *Vitex agnus-castus* Linn.

S. No.	Material taken	Colour in day light	Colour under UV light (365 nm)
1.	Entire fruit	purpulish-dark brown	dark brown
2.	Dry powder	dark brown or Yellowish-brown	dirty soil coloured
3.	Extracts		
(a)	Petroleum Ether	light brown	yellowish light green
(b)	Benzene	light green	yellowish light green
(c)	Chloroform	greenish straw	light green
(d)	Acetone	greenish straw	light green
(e)	Alcohol	greenish straw	greenish yellow
(f)	Water	reddish brown	very dark reddish brown

Table 4 : Extractive values of fruit of *Vitex agnus-castus* Linn.

S. No.	Reagents	Values (in percentage)
1.	Ethyl Alcohol	1.69
2.	Acetone	0.98
3.	Benzene	1.19
4.	Petroleum Ether	1.32
5.	Water	2.10
6.	Chloroform	1.26

Table 5 : Physico-chemical values of fruit of *Vitex agnus-castus* Linn.

S. No.	Ash	Values (in percentage)
	Total ash	3.5%
	Water soluble ash	2.3%
	Acid insoluble ash	0.98%
	Total Solids	0.20% w/v
	pH	5.60 to 6.20
	UV Spectrophotometry	λmax 268, 310 nm

A. Vetex agnus castus Linn.

B. Fruit of Vitex agnus castus Linn.

Fig. 1: (A) *Vitex agnus-castus* Linn. (Plant in flowering & fruiting)
(B) Fruit of *Vitex agnus-castus* Linn.

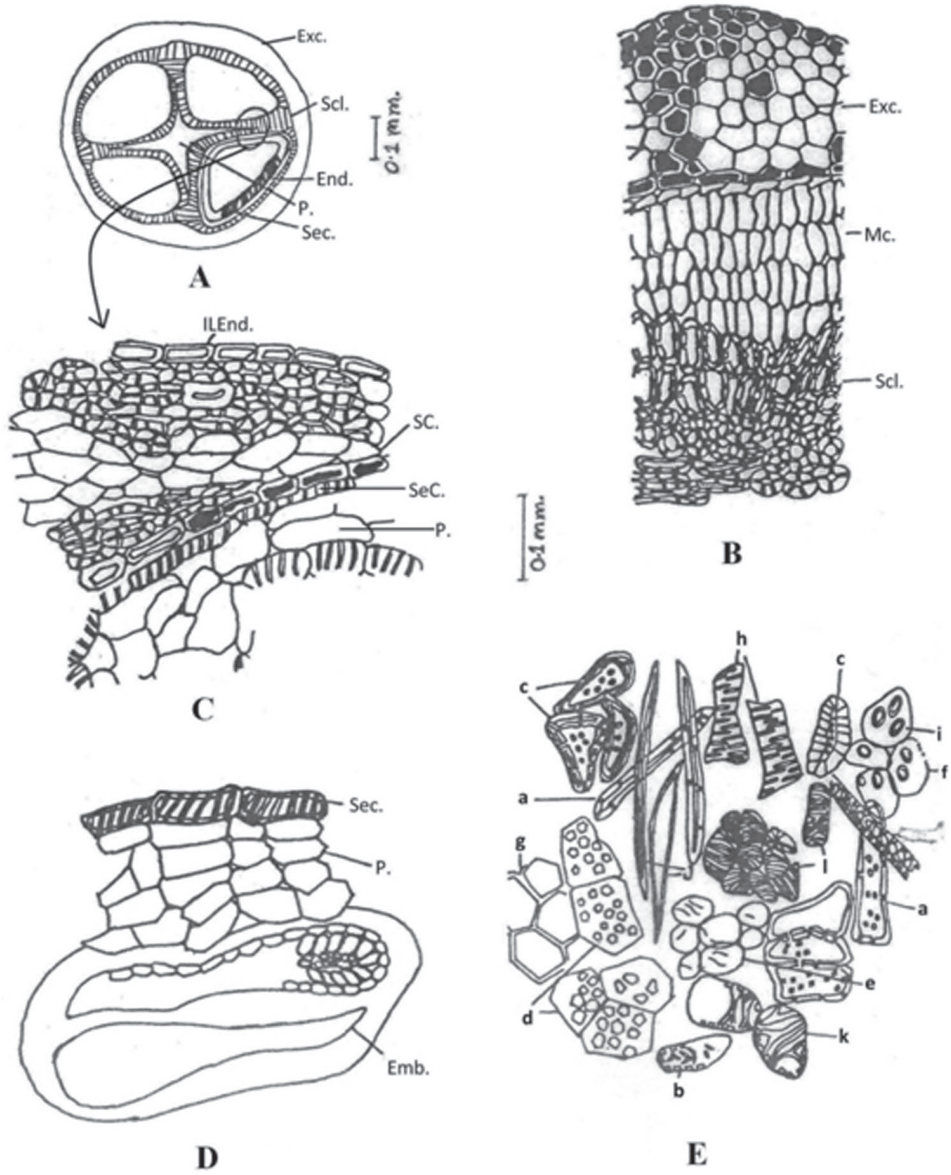

Fig. 2: (A). T.S. of Fruit (Diagrammatic); (B) T.S. of Fruit (Exocarp & Mesocarp); (C) T.S. of Fruit (Endocarp); (D) T.S. of Seed; (E) Powder Analysis of Fruit - (a) Macrosclereids, (b) Tracheary elements with simple pits, (c) Brachysclereids, (d) Epicarp parenchyma cells with pits, (e) Parenchyma cells, (f) Cotyledon parenchyma cells, (g) Thick walled parenchyma cells, (h & k) Tracheary elements with Scalariform thickenings, (i) Oil globules, (j) Fibers, (l) Lignified seed coat (Testa) cells.

Abbreviations : Emb.- Embryo; End.- Endocarp; Exc.- Exocarp; ILEnd.- Innermost Layer of Endocarp; Mc.- Mesocarp; P.- Parenchyma; SC.- Septal Cell; Se.- Seed; Scl.- Sclereids; SeC.- Seed Coat.

Discussion

The macroscopical and microscopical characters of *Vitex agnus-castus* fruit show its distinguished tissue system. Preliminary photochemical and physical

analytical data are reproducible. All these data enable the easy identification of the drug and eliminate the possibilities of adulteration and also help in achieving desired therapeutic value of drug.

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