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PHARMACOGNOSTICAL STUDIES ON THE WOOD OF *AQUILARIA MALACCENSIS* LAM.

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Abstract

The study claims importance in the context of some confusion and controversies relating to the authenticity and botanical diagnosis of original Agarwood/Akil, a highly valued wood in trade for its incense and medicinal properties. Due to severe scarcity and extreme endemism of the Agarwood or *Aquilaria malaccensis* Lam. (*A. agallocha* Roxb.) the traders have assorted to many trade wood samples which simulate the original Agarwood, to supply to the consumers. A perusal of literature revealed that a little fragmentary information on various perspectives of wood did not help to resolve scientific validation of the drug. The preliminary phytochemical details which are essential components in studying the microscopic identification are lacking for Agarwood and its adulterants. The present study aims at in depth pharmacognostical analysis of the Agarwood. The histochemical studies were carried out for sample tissues. The fluorescence analysis for the powder and treatment of powder with various solvents under UV was also done. The sample was subjected to qualitative phytochemical screening and physico - chemical evaluation. The results were very specific for the Agarwood which will contribute in identifying the drug from its adulterants, thus helpful for its further phytochemical and pharmacological investigations.

Keywords: Agarwood, Akil, Incense, Interxylary phloem, Volatile oil.

Introduction

In the present paper an Indian drug Agarwood/ Akil, a plant drug of controversial identity is taken for investigation. The plant *A. malaccensis ie., A*garwood is termed as true 'Akil' ascribed in Siddha text and its uses were narrated[1]. From the ancient literature[2], Akil is equated to eight different botanical binomials belonging to six families. A wood known as *Eagle wood* (Trade name), *Agaru* (Hindi), *Agil*, *Akil* (Tamil) is credited with several pharmacological properties as per the literature claims; it is also a highly priced incense wood of much popular antiquity.

The *Aquilaria malaccensis* Lam. (=*A. agallocha* Roxb.) of Thymelaeaceae[3] is a medium sized tree grown in North - Eastern parts of India and a number of countries of South - East Asia. *A. malaccensis* is reported to occur in Malaya, India, Myanmar, Sumatra, Borneo, Philippines, Hong

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Kong and New Guinea[4,5] although reports indicate that Java and Hainan are the historical sources of the product. In India *A. malaccensis* occurs in Arunachal Pradesh, Assam, West Bengal, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura. The tree flourishes well in the foot-hills of evergreen and semi evergreen forests. *A. malaccensis* is a large evergreen tree growing up to 30m high with a girth of 1.3-1.5m. The trunk is moderately straight and fluted, bearing thin pointed leaves (Fig.01). The values of the tree lie not on the timber, but on a brown or dark brown pathological product formed due to some fungal infestation on parts of the heart wood in certain trees of this species alone. It is this unique infested portion that contains concentrated amount of *oleoresin* of high commercial value. The agar formation on the wood makes it more valid in market for its aroma. It has been suggested that some fungal infection is required for formation of agar (Fig. 01c). The fungi associated with agar formation were isolated and identified as *Aspergillus* sp., *Penicillium* sp., and *Fusarium* species. The drug is used for many biological activities like in the treatment of rheumatism, as stimulant, as a liver tonic, carminative, tonic for pregnant women, palpitation of the heart, and the wood has proven for its antiallergic[6,7] and neuroleptic properties[8]. The Agarwood has been reported to have aquilochin (a coumarinolignan)[9], liriodenine (an alkaloid)[10], gmelofuran, agarol (novel sesquiterpenes)[11,12] and chromone derivatives[13], Agarospirol and jinkoh-eremal are the major chemicals reported in agarwood oil[14].

A wide lacuna in the protocol standards for the botanical identity and phytochemical parameters encourages the raw-drug dealers to market many country-woods under the name of Agarwood. The source seems to remain in controversial and confused state especially the botanical identity and genuineness of the original drug land us in still more state of bewildering and paradox. Under this backdrop, it was found worthy to contemplate on various pharmacognostic aspects of the wood and to contribute definite protocol on the Agarwood. These studies will include macroscopic, microscopic, histochemical studies, fluorescence analysis, preliminary phytochemical screening, and physico – chemical constants on the wood of the source taxon.

Materials and methods

Authentic wood sample of *A. malaccensis* was procured from Assam, through Dr. A.B.D. Selvam, Scientist, Botanical Survey of India, Calcutta. The voucher specimen studied is deposited in CSMDRIA, Chennai.

a. Macroscopic and microscopic features for the sample were carried out by standard methods[15]. The microscopic features of the sample were collected and identified using the monographs on 'Indian Woods'[16]. Botanical binomials and family details of the wood sample were traced with the help of Floras[17,18]. Certain anatomical characters relied on diagnosis of wood tissues has listed in standard texts[19,20].

Microscopic descriptions of tissue were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken using Nikon Labhot 2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the Scale – bars. Descriptive terms of the anatomical features are as given in the standard anatomy books[21].

b. The histochemical studies were carried out for the sample tissues and microphotographs were taken[22].

c. The fluorescence analysis for the wood powder and treatment of powder with various solvents under UV light (254 nm) was carried out[23].

d. The alcoholic extract of the sample was subjected for qualitative phytochemical analysis[24,25,26] using standard procedures.

e. Physico-chemical constants for the sample were also found using standard methods[27,28].

Results and discussion

External Profile of the Plant

A. malaccensis is a large evergreen tree with thin bark, 18-21m sometimes upto 40m in height, 1.5 - 2.5m in diameter with a moderately straight and often fluted stem. The tree occurs commonly on the low hills of the borders of Assam, Meghalaya and Bangladesh; it also occurs in Burma. Leaves 5-9 cm long, thinly coriaceous, oblong – lanceolate, flowers white or green or dirty yellow in terminal,

sessile or shortly peduncled, umbellate cymes[29] (Fig. 01).

a. Macroscopic and Microscopic studies

Exomorphic and Organoleptic Features of the Wood Sample

The wood is creamy white or yellow (Fig. 02). The wood is soft, light; No characteristic odour, taste, and is smooth to touch. The macroscopic features are tabulated (Table - 01).

Microscopical Observation

Bark: Bark in TS view (Fig:03.1,2) is wide with smooth surface and homocellular phellem, measuring 200 μm wide. Secondary phloem zone is also wide and continuous comprising of dilated rays and wide triangular bands of fibres and sieve elements.

Secondary xylem (wood): Growth rings wanting, vessels diffuse, thin walled, angular, in clusters or occasionally solitary or in radial multiples of 2 to many vessels (Fig. 03.2, 04.1). Diameter of the vessels varies from 40 – 70 μm.

Xylem fibres are libriform type, thin walled with wide lumen; cross sectional outline is rectangular or squarish, arranged in regular radial files, tangential diameter of the fibres 20 – 25 μm. Xylem rays thin, straight, not much prominent.

Included phloem or *interxylary phloem* is abundant forming fairly prominent tangential continuous or discontinuous bands. Some of the sieve elements in the median part of the tangential band are crushed, and those surrounding crushed cells are intact and functioning (Fig. 04.1).

TLS features of the wood

In tangential longi sectional view of the wood, the xylem rays are short, either uniseriate or in part biseriate, the two types being equal in frequency (Fig. 04.2). The ray cells are vertically oblong or squarish. The marginal cells are longer in some of the rays (rays hetero cellular) and as long as the body cells (homo cellular rays). The height of the rays ranges from 70 – 230 μm; ray frequency is 10 – 15 / mm.

Powder Microscopy (Fig. 05, 06, 07)

The wood powder (macerated sample) exhibits vessel elements, fibres and wood parenchyma. The vessel elements are narrowly cylindrical (Fig. 06.1,2 and 07) or short and broad. The perforation plate is simple and oblique. Some of the vessel elements have short, thin tails at both or one ends. The lateral wall pits are minute dense and multiseriate. The cylindrical vessel elements are up to 280 μm long; the short broad elements are 250 μm long (including the tails) (Fig. 05.1,2).

Wood fibres are thin walled and spindle shaped with tapering ends. The fibres are either wide or narrow lumened. The wide fibres are 350 - 500 μm long; the narrow fibres are 500 – 600 μm long. The fibres have no lateral wall pits.

Xylem parenchyma: The axial parenchyma cells are narrow, long and thin walled. The ray parenchyma cells are rectangular to squarish (Fig. 05, 06). The parenchyma cells also do not exhibit any prominent pits. The microscopic features are tabulated in Table - 01.

b. Histochemical Localization

Proteins, Sugar, Alkaloids, Tannins and Essential Oil were localized by histochemical studies in the sample. Results and photomicrographs are presented (Table - 02) (Fig. 08).

c. Fluorescence Analysis

The fluorescence analysis showed differences in fluorescence in both daylight and UV light (254 nm) for wood powder and treatment of powder with dilute alkalis, acids and organic solvents (Table - 03).

d. Preliminary Phytochemical Screening

The preliminary phytochemical screening of the alcoholic extract of the wood showed the presence of lignin, saponin, flavonoid, quinone, protein, tannin, terpenoid, sterol, alkaloid, sugar and absence of gum (Table - 04).

e. Physico-Chemical constants

The sample was also subjected to loss on drying at 105^{o}C, pH at 5% aqueous solution, ash value, extractive value, solubility value, volatile oil content, and inorganic chemical analysis as the standards showing the physico - chemical properties of the wood and the results are presented in Table - 05.

Table No. 01: Macroscopic and Systematic Microscopic Features of Agarwood

Macroscopic Features	Observation	Microscopic features	Observation
Colour	White creamy or yellow	Growth Rings	Not evident
Taste	Bland	GR Boundary	-
Odour	No specific Odour	Vessel element length and diameter µm	250-280µm/ 40-70 µm
Texture	Soft	Vessel aggregation	Solitary or Radial multiples
		Xylem Rays	Uni or inpart biseriate, heterocellular / homo cellular
		Size of the rays µm	70-230µm
		Axial parenchyma	Scanty
		Fibre length	350-600mm

Table No. 02: Histochemical Localization

Test for	Alkaloids	Starch	Protein	Essential Oil	Tannin
Localisation	Included phloem	Xylem Rays	Phloem parenchyma	Xylem rays, included phloem	Included phloem and xylem rays

Table No. 03: Fluorescence Analysis for Wood Powder, Powder with Dilute Alkali and Acids and its Extracts at 254 nm

Sl. No.	Treatment	Observation	Inference	Treatment	Observation	Inference
1.	Drug Powder	Day Light	Brown	Hexane extract	Day Light	Pale Yellow
		U.V. Light	Dark Brown		U.V. Light	Pale Green
2.	Drug Powder + 1N Sodium Hydroxide (Aqueous)	Day Light	Brownish Yellow	Benzene Extract	Day Light	Dull Brown
		U.V. Light	Yellowish Green		U.V. Light	Pale Greenish Brown
3.	Drug Powder + 1N Sodium Hydroxide (Alcoholic)	Day Light	Pale Yellow	Chloroform Extract	Day Light	Brown
		U.V. Light	Pale Green		U.V. Light	Green
4.	Drug Powder + 1N Hydrochloric Acid	Day Light	Yellow tint	Alcohol Extract	Day Light	Pink
		U.V. Light	Colorless		U.V. Light	Greenish Violet
5.	Drug Powder + 50% Sulphuric Acid	Day Light	Pale Yellow	Acetone Extract	Day Light	Pale Brown
		U.V. Light	Green tint		U.V. Light	Pale Greenish Black
6.				Water Extract	Day Light	Pale Orange
					UV Light	Pale Greenish Brown

Table No. 04: Preliminary Phytochemical Screening

Sl. No.	Test for	Result
1.	Gum	-
2.	Lignin	+
3.	Saponin	+
4.	Flavonoid	+
5.	Quinone	+
6.	Protein	+
7.	Tannin	+
8.	Alkaloid	+
9.	Sterol	+
10.	Terpenoid	+
11.	Sugar	+

(+) present (-) absent

Table No. 05: Physico – Chemical Constants (Mean of 3 Values)

Sl. No.	Physico – chemical Parameters	Values
I.	Loss on drying at 105^{o} C (% w/w)	13.26
II.	pH for 5% aqueous solution	6.0
III.	a. Total ash (% w/w)	1.4985
	b. Water soluble ash (% w/w)	0.1998
	c. Alkalinity for water soluble ash 'ml' in 0.1 N hydrochloric acid / 100 g	0.4850
	d. Acid insoluble ash (% w/w)	0.0999
IV.	i. n-Hexane extractive value (% w/w)	0.63
	ii. Chloroform extractive value (% w/w)	1.430
	i. Alcohol soluble extractive value (%w/w)	2.3971
	ii. Water soluble extractive value (% w/w)	1.5187
V.	Volatile oil (% v/w)	0.14
VI.	Sodium (%)	0.036
	Calcium (%)	0.038
	Phosphorous (%)	0.204
	Iron (ppm)	0.78
	Magnesium (%)	0.12
	Chloride (%)	0.17
	Sulphate (%)	0.22
	Carbonate (%)	0.18

Fig. 01: Habit Sketch and External Profile of the Plant

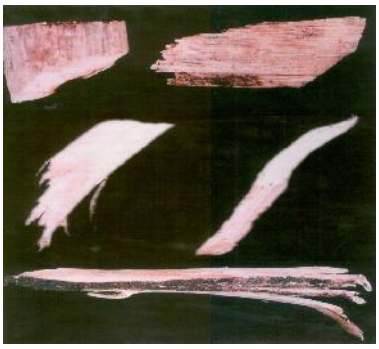


Fig. 02: Akil Wood Samples In Closer View

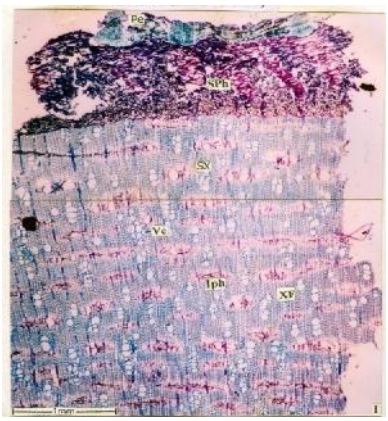
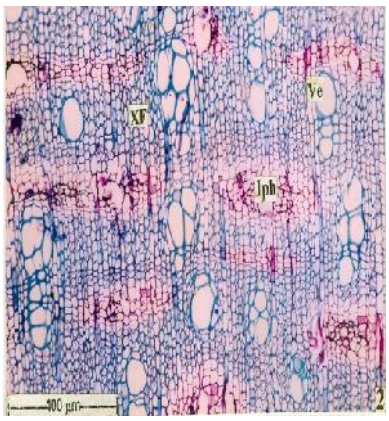


Fig. 03



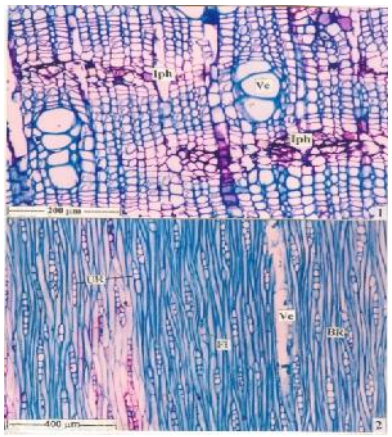


Fig. 04

Salient Microscopic Features of Agarwood

3.1. TS of wood showing bark and other portion of wood.
3.2. A portion of the wood with included phloem enlarged.
4.1. TS of wood-included phloem and vessels enlarged.
4.2. TLS of wood showing uni - seriate, partly bi – seriate rays.
IPh – included phloem, Pe – Periderm, SPh – Secondary phloem, SX – Secondary xylem,
Ve – vessel, XF – xylem fibre, BR – Bi seriate ray, Fi – Fibres, UR – uni - seriate ray.

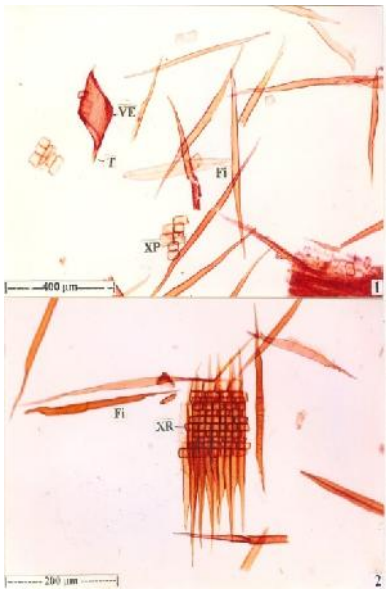


Fig. 05

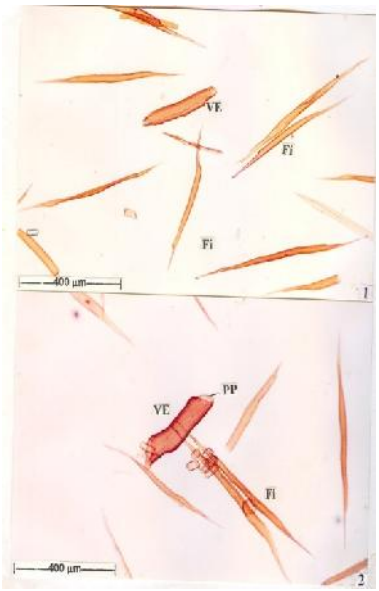


Fig. 06

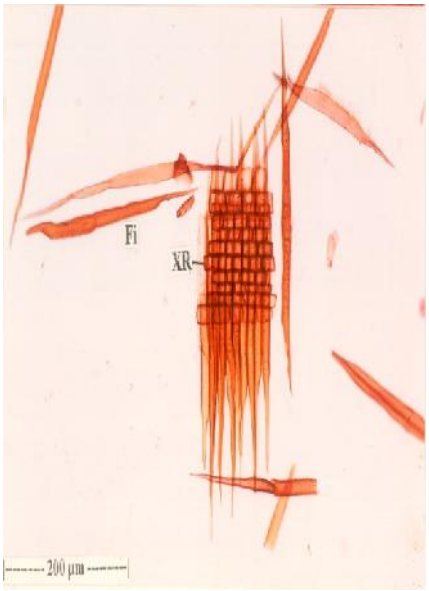


Fig. 07

Powder Microscopic Studies of Agarwood

5.1, 2. Macerated wood elements showing vessel elements, xylem fibres and xylem parenchyma.
6.1, 2. Macerated xylem elements showing cylindrical vessel elements and fibres.
7.0 Xylem ray enlarged.
Fi – Fibres, T – tail, XP – xylem parenchyma, XR – xylem ray, PP – perforation plate, VE – Vessel element.

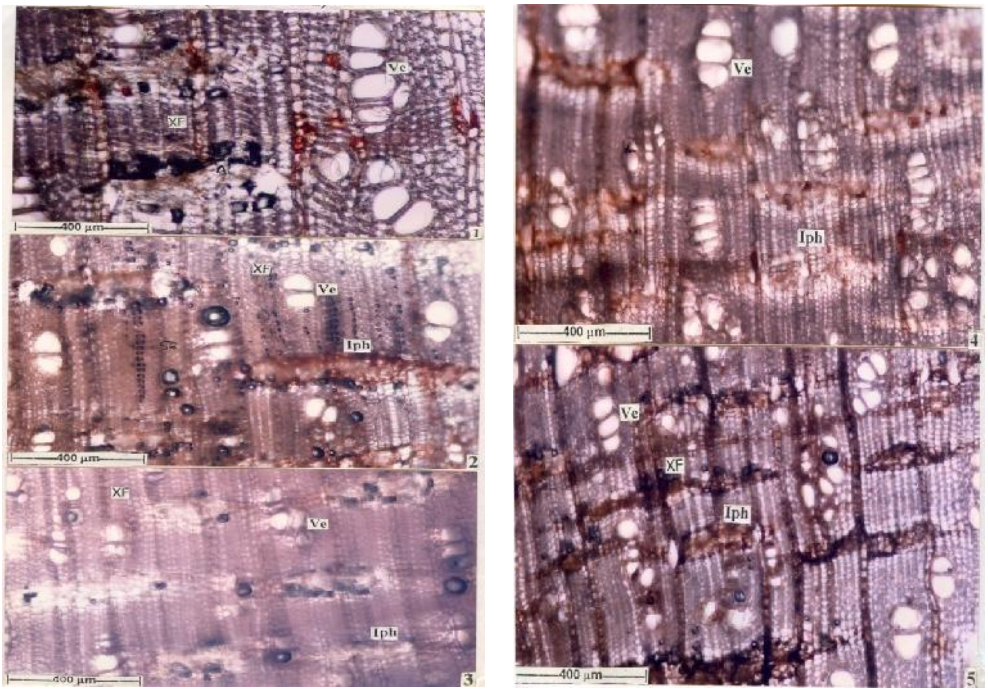


Fig. 08: Histochemical Studies of Agarwood

8.1. Alkaloids located in the parenchyma cells of the *inter xylary* phloem.
8.2. Starch grains are located in the xylem rays.
8.3. Proteins located in the parenchyma cells of the *inter xylary* phloem and in a few xylem parenchyma.
8.4. Lipids are located abundantly in the *inter xylary* phloem as well as xylem rays.
8.5. Tannins are seen fairly in dense concentration in the included phloem as well as some of the xylem rays.
Iph – Included phloem (inter xylary phloem), Ve – Vessel, XF – Xylem fibre.

Conclusion

The present study is believed to throw significant light on the pharmacognostical identification of the time-renowned drug. The original Agarwood exhibits highly specific microscopic features such as *interxylary* or *included phloem* in the wood, absence of *growth* rings, *diffused vessel* distribution, thin walled angular *clustered or solitary vessels,* short *uniseriate / biseriate heterocellular* xylem rays and *libriform* type of thin walled fibres. The microscopic features and the other constants are very specific for *Aquilaria malaccensis,* will be useful in authenticating the original Agarwood from its adulterants. The results are believed to fill in the lacuna in the Herbal Pharmacopoeia on the Pharmacognostical perspectives of Agarwood. The researchers and pharmaceutical industries may have the access of the protocol of Agarwood/Akil formulated by the present study.

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