Original Article

Pharmacognostical and Phytochemical Studies to Resolve the Identity of Agnimantha, An Ingredient in Dasamoola

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Chettinad Health City Medical Journal 2021; 10(1): 10 - 20

DOI: https://doi.org/10.36503/chcmj10(1)-03

Abstract

Ayurvedic medicines will be effective only if they are prepared using genuine medicinal herbs. The major drawback, however, is the difficulty in identifying the genuine medicinal herb prescribed by the founders of the system. Their descriptions of medicinal plant are more poetic than scientific and lack precision because the language that was used is not technical. Agnimantha is one such controversial herb in Ayurveda. It is one of the potent drug prescribed singly as well as in various formulation like Dasamoola and Brihat panchamoola, since ancient time. But there is existing controversy regarding this herb. This study deals with resolving this controversy by doing pharmacognostical and phytochemical studies. Pharmacognostical studies includes morphology, microscopical analysis and proximate analysis. Phytochemical studies includes qualitative evaluation of phytochemicals. Results of this study will be helpful in identifying genuine source. But from all these analysis it is difficult to conclude which is authentic Agnimantha and which can be used as substitute. Hence need further analysis to resolve this existing controversy.

Keywords: Agnimantha, controversy, dasamoola.

Introduction

Sandigha dravays is the term used for medicinal plants having controversial botanical as source.[1] Agnimantha is one such controversial herb in Ayurveda. Agnimantha is an important drug of Ayurvedic system of medicine. The word Agnimantha means “agnim manthyte iti” – its sticks or dried stem or wood were used to produce fire by rubbing together. It is one of the ingredients of the popularly used Ayurvedic formulation “Dasamoola” which literally means the group of ten roots, in which Agnimantha is mainly used for treating inflammation, hyperglycemia, anemia and post natal complaints. Agnimantha is one of the major ingredients of popular Ayurvedic medicine like Chyavanaprasha, Brahma rasayana, Narayana taila, Gorocanadi Vati and around 25 similar products in which Dasamoola is included.[2]

In some Nighantus (Materia Medica) of Ayurveda, only one type of Agnimantha is described but in some cases, two types of Agnimantha have been mentioned i.e. Laghu or Kshudra (small) and Brihad (Large). Some texts refer to the source of Kshudra Agnimantha or Laghu Agnimantha as dried mature roots of Clerodendrum phlomidis and Premna serratifolia as Brihad Agnimantha. Some consider the smaller type as Premna serratifolia and larger one as Clerodendrum phlomidis. Some refer to Agnimantha as Premna serratifolia and Arani as Clerodendrum phlomidis but others quote both as same. Though Tarkari is regarded as synonym of Agnimantha, Charaka and Sushrutha have enumerated both Agnimantha and Tarkari side by side in one group, while Dravyaguna (Science of treatment) considers Agnimantha to be a valiya munna (Premna serratifolia) and Tarkari to be a ceriya munna (Clerodendrum phlomidis). The Ayurvedic Pharmacopoeia of India correlates the Latin name for Agnimantha as Clerodendrum phlomidis and it also states that Premna species can be used as substitute.[3]

Thus there is lack of clarity regarding the genuine Agnimantha that has been referred to in the ancient texts. In South India Premna serratifolia are used as Agnimantha, whereas in North India Clerodendrum phlomidis is considered as Agnimantha.[4] Hence several varieties of raw drug material are traded as Agnimantha in the market. These include Clerodendrum phlomidis, Premna serratifolia and Premna latifolia. Both Clerodendrum phlomidis and Premna

serratifolia are traded in high volume.[5] Current practice of Ayurvedic physician and industry is to use one of the species as Agnimantha depending on the availability in a particular area. But for global acceptance as well as for providing safe and effective Ayurvedic products, it is imperative to identify the authentic botanical entity to have regulatory compliance. Authenticity is one of attributes for standardization. It relates to proving that the material is the correct one i.e. it corresponds to right identity. Proper identity of herbs leads to detection of adulterants, which causes low quality of herbals.[6] Thus establishing genuine raw material helps in production of good quality herbal products.

The present study is an attempt to throw light on the various species being accepted as Agnimantha and to identify the genuine Agnimantha. This study hopes to create a right platform for recognition of indigeneous medicine in the global market. This will help us to deliver the best of Ayurvedic medicine from our country globally which in turn will also boost our Indian economy.

Materials and Methods

Collection and authentication of samples

Dried and fresh roots of Clerodendrum phlomidis, Premna serratifolia and Premna latifolia were used for the study. Seven samples of Clerodendrum phlomidis, two samples of Premna serratifolia and five samples of Premna latifolia were collected by qualified field botanists from different locations. The samples were authenticated by Dr. Ravi Kumar, Herbarium division of FRLHT. Voucher specimens were deposited at Raw Drug Collection Centre, FRLHT, Bengaluru. Details of the samples collected are tabulated in Table 1.

Pharmacognostical Evaluation

i) MORPHOLOGY: All root samples were subjected to morphological identification based on colour, odour, and taste.

ii) MICROSCOPICAL ANALYSIS: Transverse section, powder microscopy and histochemical studies were done for all three roots (one accession in each species was used)[7]

iii) PROXIMATE ANALYSIS: Proximate values like foreign organic matter, moisture content, total ash value, acid insoluble ash value, alcohol soluble extractive values and water soluble extractive values were determined[7]

Sl.no	Species	Lab ID	Source
1	*Clerodendrum phlomidis*	L/10/11/08	Gingee raja fort, Villupuram
2	*Clerodendrum phlomidis*	L/10/11/09	Kanichikudi temple, Krishnagiri
3	*Clerodendrum phlomidis*	L/10/11/10	Nellikupam, Cudallore
4	*Clerodendrum phlomidis*	L/10/11/14	Padmanadhamangalam village
5	*Clerodendrum phlomidis*	L/10/11/16	Maniyakaranpallayam, Coimbatore
6	*Clerodendrum phlomidis*	L/10/11/17	Karunya nagar, Coimbatore
7	*Clerodendrum phlomidis*	L/10/11/21	Savandurga forest, Karnataka
8	*Premna serratifolia*	L/10/11/01	Pichavaram, Cuddalore
9	*Premna serratifolia*	L/10/11/05	Alapakam, Cuddalore
10	*Premna latifolia*	L/11/07/22	Kathibari forest, Odisha
11	*Premna latifolia*	L/11/07/23	Kathibari forest, Odisha
12	*Premna latifolia*	L/11/07/24	Kathibari forest, Odisha
13	*Premna latifolia*	L/11/07/25	Kathibari forest, Odisha
14	*Premna latifolia*	L/11/07/26	Kathibari forest, Odisha

Table 1: Details of sample collected for comparative studies.

Phytochemical Evaluation

Extracts were prepared using five grams of dried coarse powders of species used as Agnimantha were successively extracted using a soxhlet apparatus with solvents of increasing polarity: hexane, chloroform, ethyl acetate, methanol and water for 3 complete cycles. All extracts were concentrated at 30-40°C on rotavapour to 25ml and tested for the presence of chemical constituents.[8]

Results and discussion

Pharmacognostical Evaluation

i) MORPHOLOGY: The dried and fresh roots of Clerodendrum phlomidis, Premna serratifolia, and Premna latifolia were subjected to morphological study. Morphology of all the species was compared and tabulated in Table 2. The photographs of the sample are shown in Fig 1. The roots are characteristically different. However, there is no morphological difference in the powders of the three samples.

Characteristics	Clerodendrum phlomidis	Premna serratifolia	Premna latifolia
Colour	Yellowish brown	light brownish with whitish inner part	Brown
Odour	Aromatic pleasant	Aromatic pleasant	Agreeable
Taste	Slightly astringent	Bitter and pungent	Astringent

Table 2: Morphological features of the roots of three species used as Agnimantha

Premna serratifolia Clerodendrum phlomidis Premna latifolia

Figure 1: Photographs of roots of three species used in the name of Agnimantha

S.no	Characters	*Clerodendrum phlomidis*	*Premna serratifolia*	*Premna latifolia*
1	Pith	Present	Present	Present
2	Wood	Diffuse porous & scattered	Diffuse porous& dense	Diffuse porous& scattered
	a. Axial parenchyma	Scanty vasicentric	Scanty paratrachial	Abundant aliform or aliform confluent
	b. Ray parenchyma	2-3 seriate, cell walls are thick and lignified	Biseriate, cell walls are thick and lignified	2-3 serriate, cell walls are thin and not lignified
	c. Fibres	Occurs in radial lines	Occurs in groups	Occurs in bands
3	Phloem	Continuous and discontinu-ous masses of fibres	Wide & compact radial files of phloem element	Phloem elements are tabular in shape and occur in radial files.
4	Periderm	Wide and rectangular arranged in radial files	Wider deeply fissured tabular thin walled cells	Cells are thick, tabular in shape and suberized

Table 3: : Comparison of the features seen in the transverse sections of the three species used as Agnimantha

S.no	Characters	*Clerodendrum phlomidis*	*Premna serratifolia*	*Premna latifolia*
1	Starch	Simple,spherical to ovaidal or oval	Simple, spherical have concentric hilum	Simple, spherical, elliptical or ovoidal
2	Vessels	Simple, circular or elliptical pits	Simple, circular pits	Simple, circular or elliptical pits
3	Fibres	Libriform fibres, tips blunted	Libriform fibres, tips sharp	Libriform fibres, tips sharp

Table 4: Comparison of the features seen in the powder microscopy of the three species Used as Agnimantha

ii) MICROSCOPICAL ANALYSIS : Transverse section of roots of Clerodendrum phlomidis, Premna serratifolia and Premna latifolia were done and results are tabulated in table 3 and figures in 2, 5 and 8 respectively.

iii) POWDER MICROSCOPY: Results are tabulated in table 4 and figures in 3, 6 and 9.

iv) HISTOCHEMICAL STUDIES: Histochemical studies revealed that starch and lignin is present in all three species but calcium oxalate and alkaloids were present in Clerodendrum phlomidis alone and results tabulated in table 5 and figures in 4, 7 and 10.

v) PROXIMATE ANALYSIS: All the three species Clerodendrum phlomidis, Premna serratifolia and Premna latifolia which are used as Agnimantha were subjected to proximate analysis to evaluate their foreign organic content, moisture content, total ash, acid insoluble ash, alcohol soluble extractive value and water soluble extractive value. The data along with the limits for proximate analysis of Agnimantha as per Ayurvedic Pharmacopoeia of India are tabulated.

S.no	Chemical constituent	Test	*Clerodendrum phlomidis*	*Premna serratifolia*	*Premna latifolia*
1	starch	Lugol's iodine	+	+	+
2	Lignin	Phloroglucinol and sulphuric acid	+	+	+
3	Total lipid	Sudan red	-	-	-
4	Calcium oxalate	Rubeanic acid- nitric acid	+	-	-
5	Tannin	Ferric chloride	-	-	-
6	Alkaloid	Dragendroff reagent	-	-	-

Note : + detected, – not detected

Table 5: Comparison of the features seen in histochemical studies of the three species used as Agnimantha

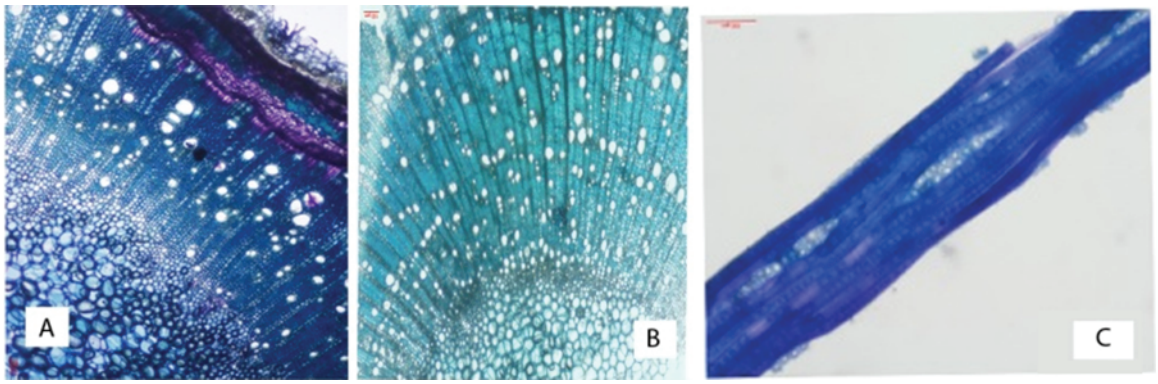

Figure 2: . TRANSVERSE SECTION OF ROOTS OF CLERODENDRUM PHLOMIDIS
A) TS of root shows the wood region stained with TBO
B) TS of root shows the secondary xylem and pith stained with TBO
C) TLS view of medullary rays

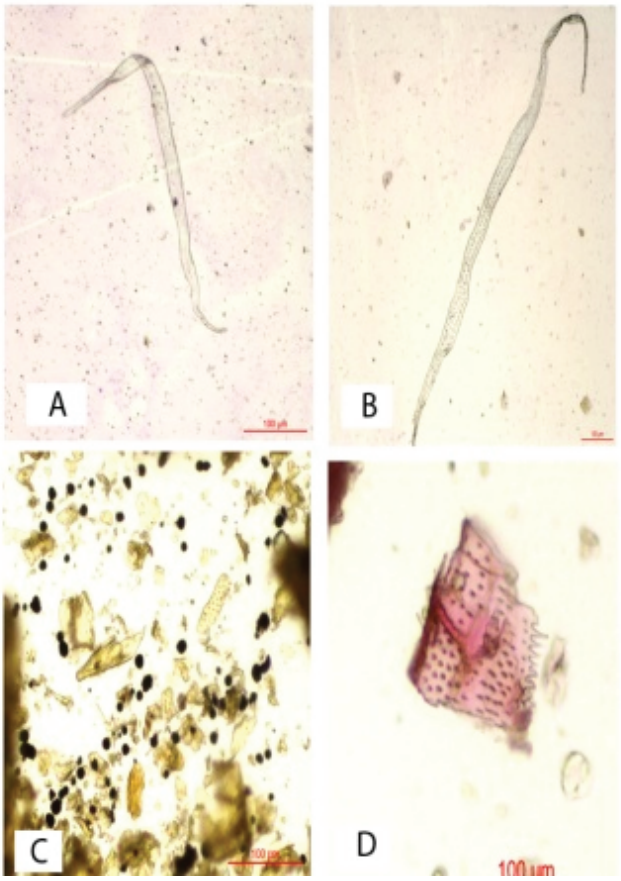

Figure 3: POWDER MICROSCOPY OF ROOTS OF CLERODENDRUM PHLOMIDIS SHOWING
A) Libriform fibre B) Trachied C) Starch grain D) Xylem vessel

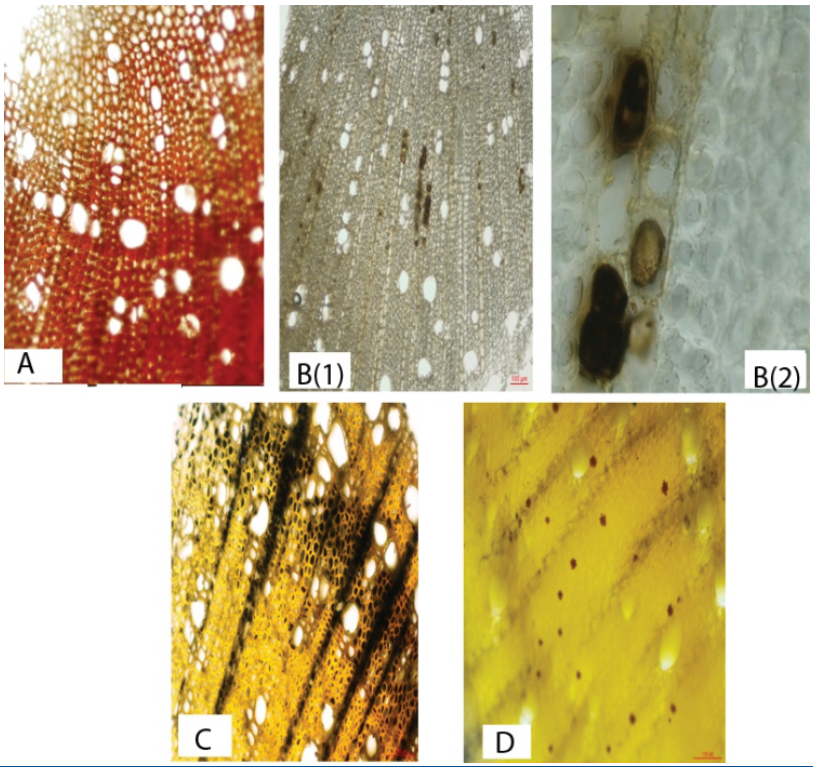

Figure 4: HISTOCHEMICAL STUDIES OF ROOTS OF CLERODENDRUM PHLOMIDIS
A) TS of root shows the presence of lignin in medullary rays
B(1) TS of root shows the presence of calcium oxalate in medullary rays
B(2) TS of root shows the presence of calcium oxalate in medullary rays(SECTOR ENLARGED)
C) TS of root shows the presence of starch in medullary rays
D) TS of root shows the presence of alkaloids in medullary rays

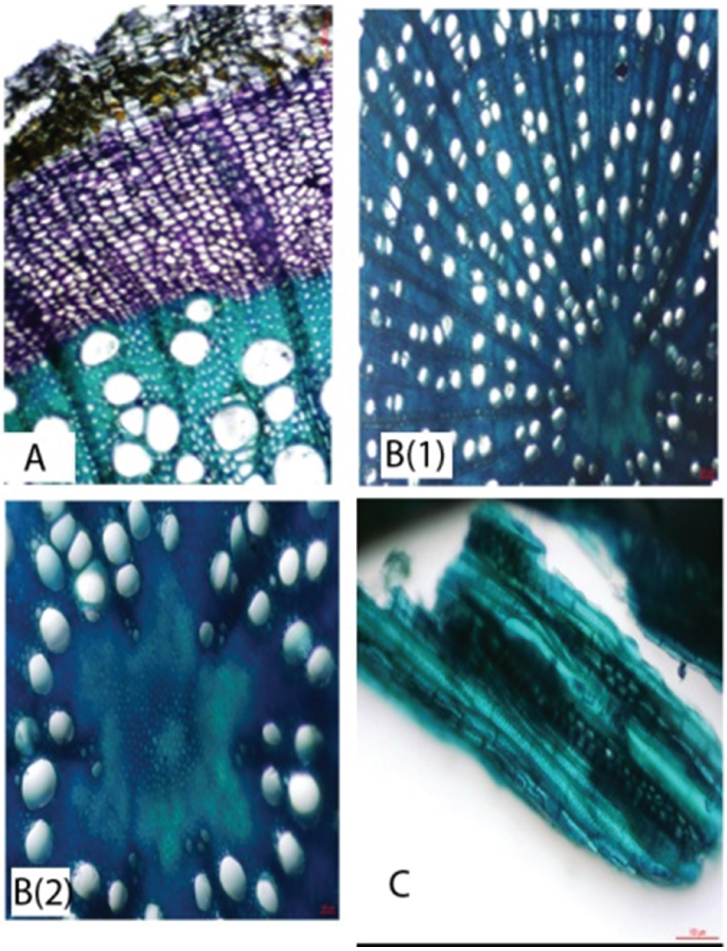

Figure 5: TRANSVERSE SECTION OF ROOTS OF PREMNA SERRATIFOLIA.
A) TS of root shows the wood region stained with TBO
B(1) TS of root shows the secondary xylem and pith stained with TBO
B(2) TS of root shows the secondary xylem and pith stained with TBO(SECTOR ENLARGED)
C) TLS view of medullary rays

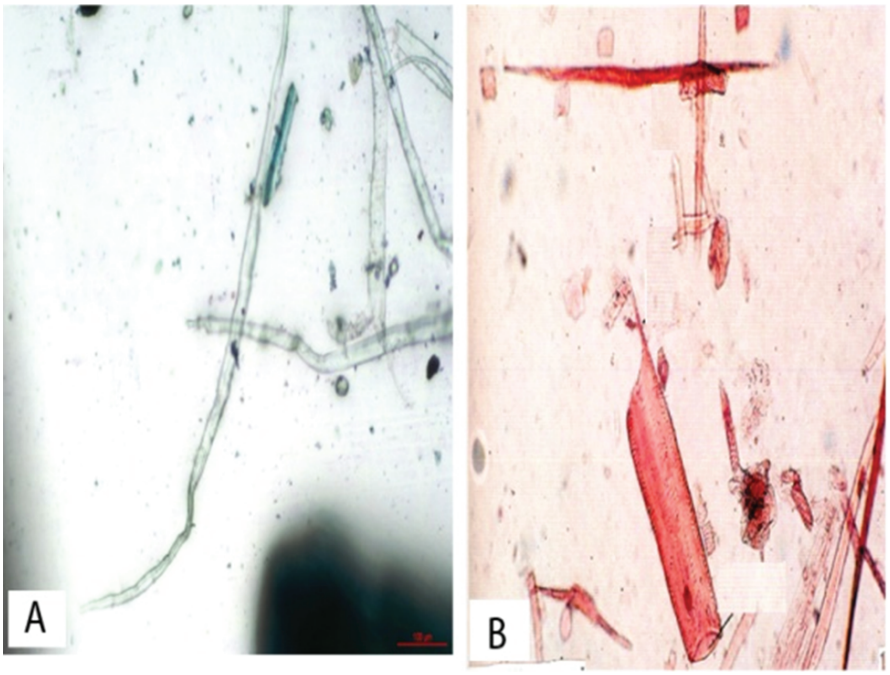

Figure 6: POWDER MICROSCOPY OF ROOTS OF PREMNA SERRATIFOLIA SHOWING
A) Libriform fibre and starch grains B) Xylem vessels

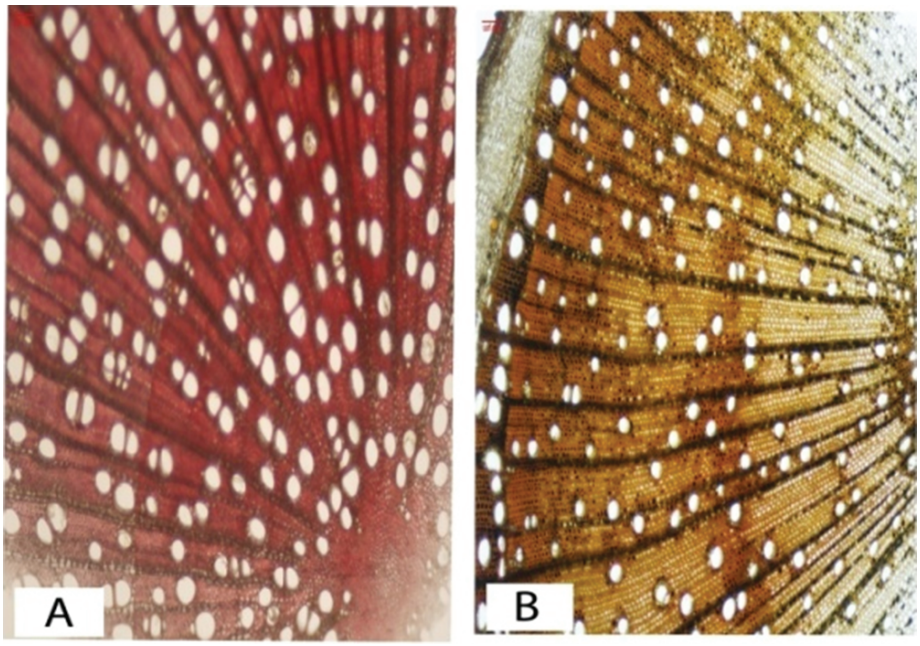

Figure 7: HISTOCHEMICAL STUDIES OF ROOTS OF PREMNA SERRATIFOLIA
A) TS of root shows the presence of lignin in medullary rays
B) TS of root shows the presence of starch in medullary rays

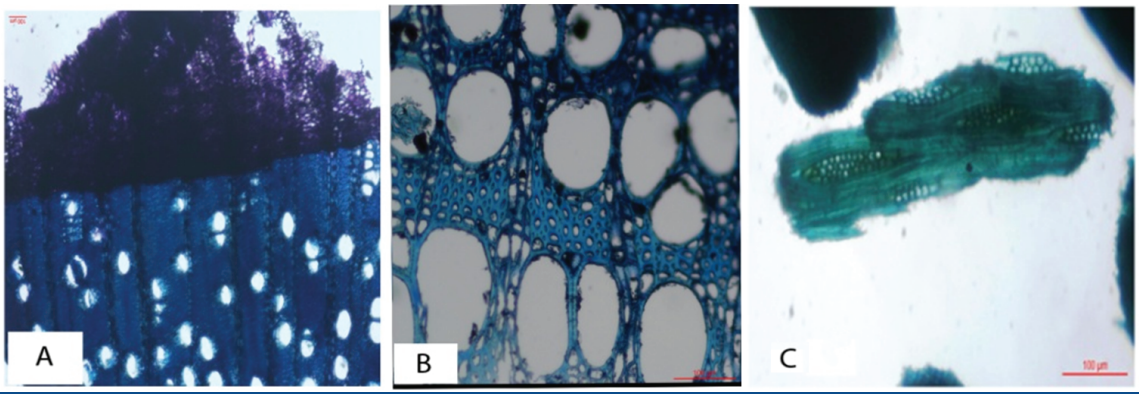

Figure 8: TRANSVERSE SECTION OF ROOTS OF PREMNA LATTIFOLIA.
A) TS of root shows the wood region stained with TBO
B) TS of root shows the secondary xylem stained with TBO
C) TLS view of medullary rays

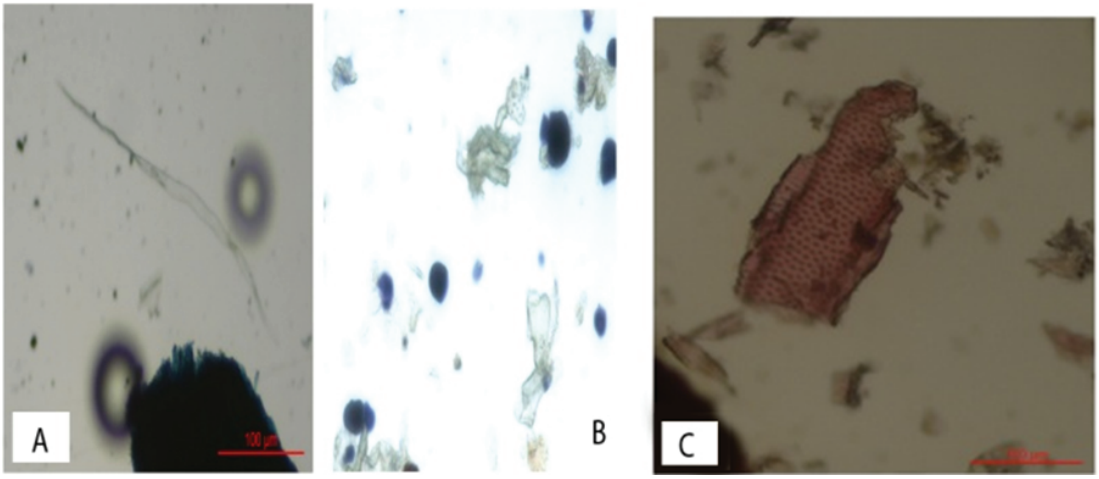

Figure 9: POWDER MICROSCOPY OF ROOTS OF PREMNA LATTIFOLIA SHOWING
A) Libriform fibre B) Starch grains C) Xylem vessel

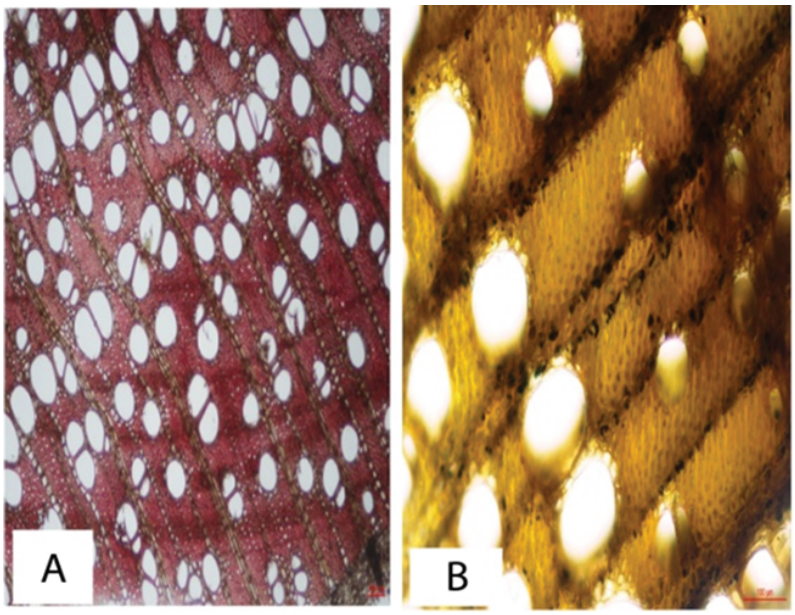

Figure 10: HISTOCHEMICAL STUDIES OF ROOTS OF PREMNA LATTIFOLIA
A) TS of root shows the presence of lignin in medullary rays
B) TS of root shows the presence of starch in medullary rays

Parameters	Accession. no						Limits (as per API)
	L/10/11/10	L/10/09/14	L/10/10/16	L/10/11/9	L/10/10/17	L/10/07/21	
Foreign matter	NIL	NIL	NIL	NIL	NIL	NIL	NMT 2
Moisture content	6.4%	6.2%	6.2%	6.8%	6.2%	6.2%	NIL
Total Ash	10.6%	6.4%	8.9%	10.1%	9.5%	7.5%	NMT 6
Acid insoluble ash	3.3%	1.2%	1.1%	3.07%	1.5%	0.8%	NMT 1
Alcohol soluble extractive value	2.5%	6.2%	4.9%	4.6%	3.8%	9.2%	NLT 2
Water soluble extractive value	9.6%	15.4%	12.7%	12.3%	14.6%	27.05%	NLT 5

Table 6: Data showing proximate analysis of Clerodendrum phlomidis

Parameters	Accession. no	Limits(as per API)
	L/10/11/05	
Foreign matter	5.8%	NMT 2
Moisture content	3.2%	NILL
Total Ash	0.8%	NMT 6
Acid insoluble ash	8.6%	NMT 1
Alcohol soluble extractive value	8.6%	NLT 2
Water soluble extractive value	12.4%	NLT 5

Table 7: Data showing proximate analysis of Premna serratifolia

Parameters	Accession. no					Limits (as per API)
	L/11/7/22	L/11/7/23	L/11/7/24	L/11/7/25	L/11/7/26	
Foreign matter	NIL	NIL	NIL	NIL	NIL	NMT 2
Moisture content	4.2%	5.2%	4.4%	3.8%	4.4%	NILL
Total ash	3.7	1.8	2.3	2	4.4	NMT 6
Acid insoluble ash	0.2	0.2	0.1	0.1	1.4	NMT 1
Alcohol soluble extractive value	7.9	11.9	11.7	11.6	8.2	NLT 2
Water soluble extractive value	18	16.8	16.9	15.9	20.0	NLT 5

Table 8: Data showing proximate analysis of Premna latifolia

Chemical constituents	Tests	Hexane Extract	Chloroform Extract	Ethylacetate Extract	Methanol Extract	Water Extract
Alkaloids	Mayers	-	-	-	-	+
	Dragendroff	-	-	-	-	-
	Wagner	-	-	-	-	+
	Hagner	-	-	-	-	-
Carbohydrates	Molisch	-	+	+	+	+
	Benedict	-	-	-	+	-
	Fehling	-	+	+	+	-
Glycosides	LibermannBuchard	+	+	+	-	-
	Modified Borntrager	-	-	-	-	-
	Legals	-	-	-	-	-
Saponins	Foam test	-	-	-	-	+
Phytosterol	Salkowski	-	-	-	-	-
	LibermannBurchard	+	+	+	-	-
	Tshugajeu	-	-	-	-	-
Fixed oils and fat	Stain test	-	-	-	-	-
Resins	Acetone-water test	-	-	-	-	-
Phenolic acids and tannin	Ferric chloride	-	-	-	-	-
	Gelatin	-	-	-	-	-
Proteins and amino acids	Xanthoprotein	-	-	-	-	+
	Ninhydrin	-	-	-	-	-
	Biuret	-	-	-	-	-
Flavonoids	Alkaline reagent	-	-	-	-	-
	Lead acetate	-	-	-	+	+
	Shinoda	-	-	-	-	-
Gums and Mucilage	Alcohol precipitation	-	-	-	-	-

Note : + detected, – not detected

Table 9: Qualitative Phytochemical analysis of Clerodendrum phlomidis

Ayurvedic pharmacopoeia of India has provided physicochemical values for Agnimantha. The physicochemical values were found to be within prescribed limits for all three species except in the case of ash values of Clerodendrum phlomidis. All three species are found free from foreign matter. The moisture content of Clerodendrum phlomidis is 6%, Premna serratifolia 3% and Premna latifolia 4%. The total ash content was found to be highest in the case of Clerodendrum phlomidis (10.6%) and lowest in Premna latifolia (1.8%). The acid insoluble ash content was found to be highest in the case of Clerodendrum phlomidis (3.3%) and lowest in the case of Premna latifolia (0.1%).

Premna latifolia was found to have maximum alcohol soluble extractive value (12%) indicating that this contains high amount of lipophilic active constituents or non-polar constituents compared to Clerodendrum phlomidis and Premna serratifolia. The roots of Premna latifolia were found to have maximum water soluble extractive value (20%) indicating presence of larger amount of water soluble active constituents compared to the other two species.

Phytochemical Analysis

Qualitative evaluation of phyto-constituents in extracts

Extracts were subjected to qualitative evaluation in order to find out the chemical constituents. Results are tabulated in Tables 9, 10 &11.

Qualitative preliminary phytochemical analysis was performed initially with different respective chemical detecting agents to detect the presence of various phytoconstituents in successive extracts of all the three species. Hexane, ethyl acetate and methanol extracts of all three species shows presence of phyto-sterols. Methanol and water extract of all three species show presence of flavonoids. Carbohydrates are present in ethyl acetate, methanol and water for all three species. Alkaloids are present in water extract for Clerodendrum phlomidis whereas for Premna serratifolia and Premna latifolia in methanol extract. Tannins are present in water extracts of Premna serratifolia and Premna latifolia but it is absent in the case of Clerodendrum phlomidis. The qualitative analysis result shows that there are some differences among three species.

Chemical constituents	Tests	Hexane Extract	Chloroform Extract	Ethylacetate Extract	Methanol Extract	Water Extract
Alkaloids	Mayers	-	-	-	+	-
	Dragendroff	-	-	-	+	-
	Wagner	-	-	-	+	-
	Hagner	-	-	-	-	-
Carbohydrates	Molisch	-	-	-	+	+
	Benedict	-	-	-	+	+
	Fehling	-	-	+	+	+
Glycosides	LibermannBuchard	+	+	-	+	-
	Modified Borntrager	-	-	-	-	-
	Legals	-	-	-	-	-
Saponins	Foam test	-	-	-	-	-
Phytosterol	Salkowski	-	-	-	-	-
	LibermannBurchard	-	-	+	+	-
	Tshugajeu	-	-	-	-	-
Fixed oils and fat	Stain test	-	-	-	-	-
Resins	Acetone-water test	-	-	-	-	-
Phenolic acids and tannin	Ferric chloride	-	-	-	+	+
	Gelatin	-	-	-	-	-
Proteins and amino acids	Xanthoprotein	-	-	-	-	-
	Ninhydrin	-	-	-	-	-
	Biuret	-	-	-	-	-
Flavonoids	Alkaline reagent	-	-	-	-	-
	Lead acetate	-	-	-	+	+
	Shinoda	-	-	-	-	-
Gums and Mucilage	Alcohol precipitation	-	-	-	-	-

Note : + detected, – not detected

Table 10: Qualitative Phytochemical analysis of Premna serratifolia

Chemical constituents	Tests	Hexane Extract	Chloroform Extract	Ethylacetate Extract	Methanol Extract	Water Extract
Alkaloids	Mayers	-	-	-	-	-
	Dragendroff	-	-	-	+	-
	Wagner	-	-	-	+	-
	Hagner	-	-	-		
Carbohydrates	Molisch	-	-	-	+	+
	Benedict	-	-	-	+	-
	Fehling	-	+	-	+	-
Glycosides	LibermannBuchard	+	-	-	+	_
	Modified Borntrager	-	-	-	-	-
	Legals	-	-	-	-	-
Saponins	Foam test	-	-	-	-	-
Phytosterol	Salkowski	-	-	-	-	-
	LibermannBurchard	+	-	-	+	-
	Tshugajeu	-	-	-	-	-
Fixed oils and fat	Stain test	-	-	-	-	-
Resins	Acetone-water test	-	-	-	-	-
Phenolic acids and tannin	Ferric chloride	-	-	-	-	+
	Gelatin	-	-	-	-	-
Proteins and amino acids	Xanthoprotein	-	-	-	-	+
	Ninhydrin	-	-	-	-	-
	Biuret	-	-	-	-	-
Flavonoids	Alkaline reagent	-	-	-	-	-
	Lead acetate	-	-	-	+	-
	Shinoda	-	-	-	+	-
Gums and Mucilage	Alcohol precipitation	-	-	-	-	-

Note : + detected, – not detected

Table 11: Qualitative Phytochemical analysis of Premna latifolia

Conclusion

Proximate analysis show some differences in ash values of Clerodendrum phlomidis. Microscopical studies shows similarities in case of powder microscopy whereas many dissimilarities in case of transverse section and histochemical studies. Qualitative analysis showed presence of chemical constituents viz., sterols, alkaloids, carbohydrates and flavonoids in the three drugs. Phytochemical screening showed slight differences in the presence of tannins, which is present in both Premna species and not in Clerodendrum phlomidis. But from all these analysis it is difficult to conclude which is authentic Agnimantha and which can be used as substitute. There was need for future analysis to elicit the existing controversy, such as quantification of a marker compound which is more specific to the genuine species Clerodendrum phlomidis, phylogenetic studies may also be carried out to further substantiate the identification process.

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