

PHARMACOGNOSTICAL, PHYSICO-CHEMICAL AND HISTOCHEMICAL EVALUATION OF BRIHAT PANCHAMOOLA CHURNAJunjarwad A.V^{1*}, Vyas M.K.², Harisha CR³, Shukla VJ⁴¹Ph.D. Scholar, Dept of Basic principles, Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India²Associate professor, Department of Basic principles, Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India³Head of the Department, Pharmacognosy, Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India⁴Head of the Department, Pharmaceuticals, Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

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***Corresponding author**Dr. Ashwini. V. Junjarwad, I.P.G.T.&R.A, Gujarat Ayurved University, Jamnagar -361008 Email: ashwinisavlagi@yahoo.com**ABSTRACT**

Brihatpanchamoola are among the most important drugs in Ayurveda, due to their wide range of therapeutic actions. To meet the enormously increasing demand, the procuring has been boosted significantly. This indeed resulted to the inclusion of the brihatpanchamoola in red listed plants. Hence in the present study pharmacognostical, physicochemical and histochemical evaluation was made to find out whether aerial parts can be used in place of roots with the reference of API. The pharmacognostical evaluation shows pitted vessels, Prismatic crystals, lignified fibres. The Physicochemical analysis shows the presence of alkaloids, coumarine glycosides. Histochemical tests show the presence of lignin, tannin and calcium oxalate crystals. The result shows that both stem bark and root bark are having similar pharmacognostical, histochemical and chemical properties.

KEYWORDS: Brihatpanchamoola, Pharmacognosy, Physicochemical, Histochemical**INTRODUCTION**

The brihatpanchamoola comprising of Bilwa, Agnimantha, Shyanaka, Patala and Gambhari, was first explained in bshhaja chatushka of Charaka Samhita.¹ Abundant formulations are described with these drugs, because of their wide therapeutical index, extending to almost all diseases. Globalization of Ayurveda has unfortunately resulted into consequence of counterfeit drug marketing. Hence the present study was focused to find out whether the other parts (stem & stem bark) of the same plants can be used, to prevent the adulterants, on the basis of pharmacognostical, physicochemical and histochemical study.

Charak explains regarding Bilwadi panchamoola in the Shothahara Dashemani² There the useful part is not mentioned, maybe it is left to the yukti of physician. One who is aware of samyoga and prayoga is known as Vaidya. The intelligent permutations and combinations will lead to success which is nothing but the yukti.³

Ayurvedic pharmacopeia of India Advocates that stem bark can be used instead of Root bark when it is unavailable.⁴ In some Ayurvedic texts have mentioned that the properties of the main useful part are similar with other parts of the same plant.⁵

MATERIALS AND METHODS**Collection of the drug**

Stem bark of Bilwa, Agnimantha, Shyanaka, Patala & Gambhari were collected from the pharmacy of I.P.G.T. & R.A., Jamnagar. The obtained stem barks were shade dried and made in to coarse powder separately with the help of mechanical grinder.

Organoleptic Evaluation

Various parameters of the plant materials such as size, shape, colour, odour and taste of the stem bark were observed and recorded.

Microscopic Evaluation

Microscopical examination of all the five plant stem bark powder was carried out with and without staining, by powder microscopy to determine the chemical nature of the cell wall along with the determination of the form and chemical nature of the cell contents.⁶ Microphotographs were taken using Carl Zeiss binocular microscope.

Physico-Chemical Analysis

Physico-chemical analyses were carried out by following the parameters as mentioned in Ayurvedic Pharmacopoeia of India.⁷

Preliminary Phytochemical Investigation

Preliminary phytochemical investigations are carried out by following standard procedure of API.⁸

Thin Layer Chromatography

TLC was performed as per the guidelines provided by API.⁹ Methanol extract of stem bark was used for spotting. TLC was performed using Toluene + Ethyl acetate (7:3) solvent system.

Histochemical Analysis

The powder was treated with respective reagent for the detection of chemicals in the tissues as per the method of Krishnamurthy.¹⁰

RESULTS**Pharmacognostic Study****Bilwa**

Aegle marmelos (Corr) (Fig.1.0) belonging to Family Rutaceae, is a moderate sized tree (6-7.5mt), branches armed, leaves trifoliate, flowers greenish white, fruits globose, grey, rind woody, distributed in deciduous forests of India up to the altitude of 1200 m in Himalaya. It is having many pharmacological activities like hypoglycemic, spasmogenic, antiviral, cardiac stimulant etc. Microscopic powder characters of Root bark¹¹ and stem bark are depicted in the Table - 1

Agnimantha

Premna mucronata Roxb, (Fig.2.0) belonging to Family Verbenaceae is considered as substitute for Agnimanth.¹² It is a large shrub or small tree(9m), leaves sub-rhomboid, crenate, dentate, flowers medium sized, white or pink in small dichotomous axillary cymes forming rounded terminal panicles, drupes 8mm long, black when ripe. Distributed in drier part of India, in Subhimalayan tracks of Rohilkhand, Orissa, Chota Nagpur, Bihar etc. It is having Hypoglycemic and anthelmintic pharmacological properties. Microscopic powder characters of Root bark¹³ and Stem bark are depicted in the Table - 2

Shyonaka

Oroxylum indicum vent.(Fig.3.0) belonging to Family Bignoniaceae a small to medium sized deciduous tree(12m) leaves large, bipinnate or tripinnate, flowers purple, fleshy, foetid, inlarge erect recemes, capsules flat, sword shaped, distributed throughout the greater part of India upto the altitude of 1200m including Subhimalayan tracts. It is having diuretic, spasmogenic, anti-inflammatory, antifungal pharmacological activities.

Microscopic powder characters of Root bark¹⁴ and Stem bark are depicted in the Table - 3

Patala

Stereospermum suaveolens (Roxb)DC. (Fig.4.0) belonging to Family Bignoniaceae Large deciduous tree (18m) and about 1.5m girth, bark grey or dark brown, with horizontal furrows, Leaves imparripinnate, flowers purplish yellow within, fragrant in large, capsules straight, cylindric, 30-60cm long, 1.7cm thick, with whitish specks, seeds pale yellowish, brown, with large membranous wings. Found throughout the greater part of India in mixed deciduous and sal forests. It is having anticancer, antiviral pharmacological activities.

Microscopic powder characters of Root bark¹⁵ and Stem bark are depicted in the Table - 4

Gambhari

Gmelina arborea Roxb.(Fig.5.0) belonging to Family Verbanaceae is a tall tree with young parts densely velvety tomentose, leaves opposite broadly ovate, or elliptic, cordate or subtruncate, flowers orange – yellow,brownish yellow, in dichotomously branched, velvety tomentose panicles of three flowered cymes drupes fleshy 1-1.5cm long, orange yellow or blackish 1-2 seeded. Found throughout India from foot of north-west Himalaya to Chittagang. It is having hypoglycemic and antiviral pharmacological activities.

Microscopic powder characters of Root bark¹⁶ and Stem bark are depicted in the Table - 5

Analytical Study

Results of the analytical study of Brihatpanchamoola *churna* are as follows

Organoleptic Characters

Colour	:	Pale yellow
Odour	:	Woody
Touch	:	Soft
Taste	:	Bitter & astringent
Appearance	:	Powder

Physicochemical Constants

The results are depicted in Table -6

Phytochemical Analysis

The results are depicted in Table -7

Thin Layer Chromatography (TLC)

The results are depicted in Table -8

Histochemical Analysis

The results are depicted in Table -9

DISCUSSION

Microscopic evaluation of stem bark powder of Bilwa, Agimantha, Shyonaka, Patala and Gambhari revealed the similarity of characters when compared with root bark powder as per the references of API. The root and stem bark powder of Bilwa (*Aegle marmelos* Corr) show crystal fibers, pitted vessels, xylem vessels, starch grains with concentric line. The root and stem bark powder of Agimantha (*Premna mucronata* Roxb) show aseptate lignified fibres, lignified fibres with rhomboidal crystals, starch grains, rectangular cork cells. The root and stem bark powder of Shyonaka (*Oroxylum indicum* vent.) show Stone cells, lignified cork cells, fibres of phloem, pitted vessels. The root and stem bark powder Patala (*Stereospermum suaveolens* Roxb DC.) show Fragments of thin walled rectangular cork cells, single or groups of lignified thick walled oval to polygonal stone cells having clear striations with wide lumen & pits,

no of microsphenoidal & rod shaped crystals. The root and stem bark powder Gambhari(*Gmelina arborea* Roxb.) show stone cells with pitted lumen and striations, oval starch grains, prismatic crystals of calcium oxalate.

Physicochemical analysis shows the absence of foreign matter. It may be due to the careful collection by the pharmacy. Loss on drying was very minimal (0.19%) Hence the moisture content is very low which means least susceptibility for microbial growth. The pH was slightly acidic (5.61) the maximum powder (38.27%) passed through 85#. All other parameters were found within normal limits as API guidelines. Qualitative analysis showed the presence of Alkaloids, Tannin & Phenolic compounds, Flavonoid, Saponin glycosides, Coumarine glycosides whereas, Amines, Resins, Proteins were absent.

Histochemical tests also support the results of phytochemical analysis showing the presence of lignin, starch, tannin and calcium oxalate crystals.

CONCLUSION

The above discussion reveals that the powder characters of the stem bark and root bark of Brihatpanchamoola are similar as per the API. Physicochemical analysis too shows the results within normal limits. Hence stem bark can be used instead of root bark when the later is unavailable. The study can serve as the reference for the future works on Brihatpanchamoola.

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Table -1 Powder characters Bilwa *Aegle marmelos* (Corr)

No.	Root	Stem bark
1.	Gray to grayish brown	yellowish
2.	Thick walled angular cork	Rectangular lignified cork cells.
3.	Thick walled xylem fibres	Fibres with pointed and blunt ends.
4.	Crystal fibres	Crystal fibres (Fig.1.1),
6.	Fragments of xylem vessels with bordered pits.	Lignified thick walled pitted stone cells in groups. (Fig.1.4),
7.	Starch grains simple 5 – 19 µ diameter, round to oval	Starch grains with concentric line(Fig.1.2),
8.	Numerous prismatic crystals of calcium oxalate	Prismatic crystals.()
9.		Reddish brown oleoresin content
10.		Scleroids (Fig.1.3)

Table -5 Powder characters Gambhari *Gmelina arborea* Roxb:

No	Root bark	Stem bark
1.	Yellowish brown	Brown
2.		Cork in surface view.
3.		Thin walled parenchymatous cells
4.		Fragment of a non lignified fibre. Aseptate and septate fibre with wide lumen
5.		Stone cells
6.	Lignified stone cells in groups or isolated isodiametric, circular to squarish in shape. Thick or thin walled with pitted lumen and striated walls.	Stone cells with pitted lumen and straiations (Fig.5.3), stone cells in groups (Fig.5.4),
7.	Abundant starch grain simple and compound with central hilum	Oval starch grains
8.	Cone shaped scleroids	Cone shaped scleroid (Fig.5.2)
9.	Acicular and rod shaped prismatic crystals of calcium oxalate.	Cigar and prismatic crystals (Fig.5.1).

Table -2 Powder characters Agnimantha - *Premna mucronata* Roxb

Sl no	API(root)	Stem bark
1.	Dull yellow	yellow
2.	Fragments or cork cells	Thick walled yellowish brown rectangular cells of cork (Fig.2.2),
3.	Small pointed aseptate lignified fibres	aseptate lignified fibres (Fig.2.1)
4.	Simple pitted fibres	Lignified fibres (Fig.2.3) with rhomboidal crystals (Fig.2.4)
5.	Lignified cells packed with rhomboidal crystals of calcium oxalate	Acicular crystals
6.	Numerous simple, round to oval starch grains with narrow hilum measuring 6-11 µ in diameter	Simple starch grains round to oval
7.		Yellowish brown content

Table - 6 Showing physicochemical constants

Sl. No	Name of the Parameter	Results
1	Foreign matter	Nil
2	Loss on Drying	0.19 %w/w
3	Total Ash content	8.8 % w/w
4	Acid insoluble ash	0.08 % w/w
5	Water soluble ash	2.43 % w/w
6	Alcohol soluble extractive value	8.32 %w/w
7	Water soluble extractive value	10 %w/w
8	pH Value	5.61
9	Particle size assessment i) Fraction of powder remained on 60# ii) fraction of powder passed from 60# iii) fraction of powder passed from 85# iv) fraction of powder passed from 120#	15.92% 33.68% 38.27% 10.72%

Table -3 Powder characters Shyonaka *Oroxylum indicum* vent :

No	Root	Stem bark
1.	Brownish Cream	Yellow cream
2.	Groups stone cells	Stone cells
3.	Fragments of cork	Lignified Cork cells
4.	Phloem fibres with wide lumen and pointed tips	Fibres of phloem with narrow lumen(Fig.3.1)
5.	Reticulate vessels and tracheids	Pitted vessels (Fig.3.3),
6.		Pitted Parenchyma (Fig.3.2),
7.		rod shaped crystals(Fig.3.4)

Table -4 Powder characters Patala- *Stereospermum suaveolens* (Roxb)DC:

Sl.No	Root	Stem bark
1.	Dark brown	Dark Brown
2.	Fragments of rectangular cork and phloem parenchyma cells	Fragments of thin walled rectangular cork cells
3.	Stone cells thick walled, cubical to rectangular, lignified stone cells having striation and wide lumen.	Single or groups of lignified thick walled oval to polygonal stone cells (Fig.4.3) having clear striations with wide lumen & pits
4.	A no of microsphenoidal crystals of calcium oxalate intact and scattered outside	no of microsphenoidal & rod shaped crystals of calcium oxalate (Fig.4.2)
5.		Fibres with small tapering & pointed ends scleroids (Fig.4.1),
6.		Yellowish brown content cells (Fig.4.4),
7.		Parenchyma cells with Starch grains

Table -7 Showing results of phytochemical analysis

Sl. No	Components	Results
1.	Alkaloids	+
2.	Tannin & Phenolic compounds	+
3.	Flavonoid	+
4.	Saponin Glycosides	+
5.	Coumarine glycosides	+
6.	Steroids	+
7.	Carbohydrates	+
8.	Anthicyanins	-
9.	Terpinoids	+
10.	Protein	-
11.	Resin	-
12.	Amines	-

+ Present, - Absent.

Table -8 Showing the results of TLC Study

TLC	254nm	366nm	After Anisaldehyde spray	After Wagners spray
No of spots	4	7	2	2
Rf values	0.09 0.27 0.36 0.45	0.09 0.16 0.27 0.36 0.45 0.63 0.8	0.36 0.45	0.72 0.81

Table - 9 Showing the results of histochemical analysis

No	Test	Chemicals	Result
1	Lignin	Phluroglucin + ConCHCl	Dark red
2	Ca oxalate crystals	Phluroglucin + ConCHCl	Effervescence
3	Starch	Iodine solution	Dark blue to black
4	Saponin	Conc. H ₂ SO ₄	Yellow
5	Tannin	Acidic FeCl ₃ solution	Dark blue to black
6	Glycisides	Guignards's test	Brown
7	Alkaloids	Mayer's reagent Wagner's reagent Dragendorff's reagent Tannic acid Hager's reagent	Brown Colourless Orange -Dark brown Buff colour Yellow