AYU

Access this article online
Website: www.ayujournal.org
DOI: 10.4103/0974-8520.115441
Quick Response Code:

Pharmaceutical Standardization

A comparative pharmacognostical and preliminary physico-chemical analysis of stem and leaf of *Bacopa monnieri* (L.) Pennel and *Bacopa floribunda* (R.BR.) Wettst

Jyoti S. Gubbannavar, H. M. Chandola[1], C. R. Harisha[2], Komal Khanpara[3], V. J. Shukla[4]

M.D. Scholar, [1]Director, Chaudhary Brahm Prakash Ayurved Charak Sansthan, Khera Dabar, Najafgarh, New Delhi, India, [2]Head, Pharmacognosy Laboratory, [3]Ph.D Scholar, [4]Head, Pharmaceutical Chemistry Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

Abstract

Brahmi is a well-known herbal drug having an effect on brain as a memory enhancer. *Bacopa monnieri* (L.) Pennel and *Bacopa floribunda* (R.Br.) Wettst are both marketed in the name of *Brahmi*. The present study differentiates *Bacopa monnieri* and *Bacopa floribunda* in morphology, transverse section (T.S.) of root and leaf, powder microscopy, and chemical constituents. Morphological characters show color difference in flower, stem and leaf and differences in microscopic study, organoleptic study, and powder characteristics. Morphologically, *Bacopa monnieri* leaf is fleshy and more succulent than *Bacopa floribunda* leaf. There is also a difference in the interval of the stem internodes of the two. Physico-chemical analysis revealed presence of 26% bacoside A in *Bacopa floribunda* leaf and 27% in *Bacopa floribunda* stem, which is higher than the bacoside A content in leaf (22%) and stem (18%) of *Bacopa monnieri*. However due to the hemolytic action of bacoside A, which is the toxic effect of the chemical constituent, it seems likely that *Bacopa monnieri* is more popular in regular use than *Bacopa floribunda*.

Key words: *Brahmi, Bacopa floribunda, Bacopa monnieri,* bacoside A, HPTLC, pharmacognosy

Introduction

Materia medica or Pharmacognosy, may be defined as science that aims at a complete and systematic knowledge of crude drugs of animal or vegetable origin. A complete knowledge of a drug must include knowledge of the morphological nature and the structure, both macroscopic and microscopic, with active constituents, their nature, quantification and the relation they bear to the constituents of other drugs. To attain this knowledge of chemistry and chemical methods for investigation, or at least the results of other investigations in these respects utilized, and the information thus obtained, combined to form a complete knowledge of each drug.[1] The herb *Brahmi* is reputed as a brain tonic for promoting memory and various mental faculties. The leaves and whole plant are used in psychological disorders, skin diseases and used as a general restorative or rejuvenating drug. The leaf juice is applied to swellings and is a good liniment for rheumatism. The juice of leaves is given to infants suffering from catarrh or severe bronchitis.[2] Two plants *Bacopa monnieri* and *Bacopa floribunda* are used in Ayurveda for *Brahmi*. Till date, no study has been carried out to differentiate the characters between leaf and stem of both the plants. Considering this, the present study is designed to evaluate comparative pharmacognostical profiles of stem and leaf powder of *Bacopa monnieri* and *Bacopa floribunda*. The study also attempts to evaluate bacoside A quantitatively in the stem and leaf powder of *Bacopa monnieri* and *Bacopa floribunda*.

Address for correspondence: Dr. Jyoti Gubbannavar, D/O Shree. S.Y. Gubbannavar, Rajendra Nagar, Haveri - 581 110, Karnataka, India.
E-mail: jyoti.gubbannavar@gmail.com

Materials and Methods

Collection of the drug

Whole plant of *Bacopa monnieri* was collected from the Foundation for Revitalization of Local Health Traditions (FRLHT), Bangalore, in the month of December 2011 and *Bacopa floribunda* was collected from Jamnagar in December 2011. Both the plants were identified, authenticated by a pharmacognosist and preserved in the Pharmacognosy Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurveda University, Jamnagar, vide no.

Phm. 6037 and 6038, respectively. The reference standard of bacoside A was procured from M/s Natural Remedies Pvt. Ltd. Bangalore.

Macroscopic evaluation
Macroscopic characters of fresh leaves like size, shape, color, surface, venation, presence or absence of petiole, apex, margin, base, lamina and texture were noted.[3,4]

Microscopic evaluation
Thin, free hand sections of the stem and leaf were made and washed with chloral hydrate. Diagnostic characters of the sections and powders of both stem and leaf were studied with and without staining. The microphotographs were taken under a Carl-Zeiss binocular microscope attached with a camera.[5,6]

Preparation of sample solution
The leaf and stem powder samples were accurately weighed (1 mg/mL) and extracted with methanol for 24 h and filtered and evaporated off. The dried material was again dissolved in methanol and used for Thin Layer Chromatography (TLC) identification.

Preparation of standard solution
The reference standard stock solution (1 mg/mL) of bacoside A was prepared in methanol. From the stock solution, further dilutions were performed to procure the lower concentrations.

Chromatographic conditions
To quantify bacoside A-stationary phase: Precoated silica gel GF 254 (E. Merck), mobile phase: Dichloromethane: Methanol: water (4.5:1.0:0.1 v/v/v), sample volume: 5 μL, sample for HPTLC:methanol extracts of leaf and stem powder of the plants and standard bacoside A solution, spray reagent-Vanillin-sulfuric acid.

Instrumental conditions
Application mode: Camag Linomat V, development chamber: Camag Twin trough chamber, plate: Precoated Silica Gel GF254, chamber saturation: 30 min, development time: 30 min, development distance: 7 cm, scanner: Camag Scanner III, detection: Deuterium lamp and Tungsten lamp, data system: Win cats software.

Procedure
The TLC plate was washed with methanol and then the standard as well as the sample solutions were applied to the plate as sharp bands (6 mm) by means of a Camag Linomat V sample applicator. Then, the plate was dried in a current of air. The mobile phase (10 mL) was poured into a twin trough glass chamber and the whole assembly was left to equilibrate for 30 min. The plate was then placed in the chamber. Later, the plate was developed until the solvent front had travelled a distance of 80 mm above the base of plate. Then, the plate was removed from the chamber and dried in a current of air. Later, detection and quantification were performed with a Camag TLC scanner 3 at short UV (254 nm), long UV (366 nm) and derivitisation with Vanillin sulfuric acid.[7]

Assay
Standard and sample solutions were spotted on the HPTLC plate (E. Merck). The percentage of bacoside A present in the leaf and stem powder of both the plant extracts was calculated by comparing the areas measured for the standard solution.

Linearity
Linearity was performed by applying the standard solution at different concentrations ranging from 2.5 to 7.5 $\mu g/\mu L$ spot on a 10 cm × 10 cm HPTLC plate precoated with silica gel GF 254 (E. Merck) in the form of sharp 6 mm bands. The distance between two adjacent bands was 9 mm. The plate was developed in a solvent system of dichloromethane: Methanol: Water (4.5:1.0:0.1 v/v/v) up to a distance of 80 mm at room temperature. The plate was dried in air. The detector response for bacoside A was measured for each band at a wavelength of 254 nm using the Camag TLC scanner and win CAT software Camag HPTLC instrument catalog No 0276481(Switzerland).[8] The peak areas of bacoside A were obtained by plotting a graph of peak versus applied concentration of bacoside A (5 μL) [Figure 1].

Observations and Results

Pharmacognostical study
Organoleptic characters: Various parameters of the plant material, such as odor, color, and taste of stem and leaf were recorded [Table 1].

Bacopa monnieri
Plant morphology
A creeping herb with ascending branches, rooting at nodes. Leaves are sessile, alternate to spiral up to 2.5 cm × 0.6 cm, oblong or obovate, entire, obtuse at apex, fleshy succulent. Flowers solitary, axillary, pedicelled. Calyx - 6 mm long; upper sepal broadly ovate, lateral sepals linear lanceolate. Corolla is 5-6 mm long, white or pale violet-blue. Capsules are ovoid, apiculate. The plant is succulent when fresh but becomes shriveled on drying, slightly bitter in taste, without any characteristic odor, and is composed of crumpled, matted broken pieces of roots, branching stems, leaves, flowers and few tender fruits [Figure 2].

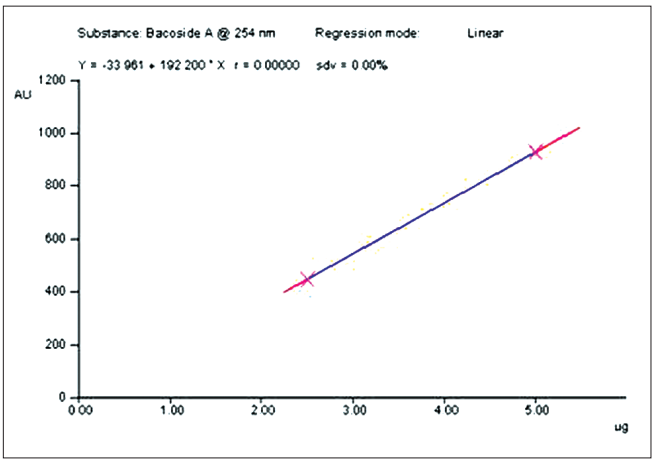

Figure 1: Linearity graph bacoside A (Range 2.5 µg-7.5µg)

Transverse section of stem

Transverse section (T.S.) of the stem shows epidermis, hypodermis and cortex with parenchyma with large airspaces, endodermis, pericycle, vascular bundle and centrally large pith [Figure 3a]. Detailed T.S. shows an outermost single-layered barrel-shaped compactly arranged epidermis with cuticle with few stomata, two to three layers of hypodermis consisting of parenchyma cells with chlorophyll pigments and tannin content cells. One-third of the section is occupied by the loosely arranged large intercellular spaces (aerenchyma) of parenchyma cells, each cell containing tannin at the place of connection between adjacent cells, giving a Y-like appearance [Figure 3b]. Some of the cells are loaded by starch grains and prismatic crystal of calcium oxalate crystals [Figure 3c]. A single-layered endodermis separates cortex from vascular tissue forming a ring. The pericycle is made of barrel-shaped cells that are compactly arranged and thin walled. The vascular tissue system consists of radially arranged vascular bundles. The phloem is situated above the xylem. Xylems are numerous in number, metaxylem toward the pericycle, protoxylem toward the pith with xylem parenchyma and fibers, centrally situated pith containing compactly arranged parenchyma cells with some prismatic crystals [Figure 3d].

T.S. of leaf

T.S. of the leaf shows typical dorsi ventral structure. Epidermis is single layered with cuticle. Lamina is differentiated into two layers; i.e. spongy and palisade. There are three to five layers of palisade cells found below the upper epidermis. The pallisade and spongy parenchyma cells are filled with chlorophyll pigments. The midrib portion consists of vascular bundle with loosely arranged xylem, which is in three to six groups. Phloem is with few elements of companion cells. Glandular sessile trichomes, diacytic and anisocytic stomata, prism of calcium oxalate crystals found in the lamina and midrib portion of leaf [Figure 4a-e].

Powder microscopy

Stem: Diagnostic powder microscopy of the stem shows lignified fibers, prismatic crystals of calcium oxalate, tannin contents, pitted vessels, simple and compound starch grains and parenchyma cells filled with starch grains [Figure 5a-e].

Leaf: Diagnostic powder microscopy of the leaf shows diacytic and anisocytic stomata from epidermis, epidermal cells, prismatic crystals of calcium oxalate, lignified fibers, oil globules, fragments of spiral and annular vessels from vascular bundle [Figure 6a-f].

Bacopa floribunda

Plant morphology

It is a sub creeping herb with ascending branches, and rooting at nodes. Leaves subsessile, up to 3 cm × 0.4 cm, oblong or obovate, entire, obtuse at apex, flattened not much succulent. Flowers 1-3 axillary, pedicelled. Calyx - 7 mm long; upper sepal broadly ovate, lateral sepals linear lanceolate. Corolla is 7-9 mm

Figure 2: ***Bacopa monnieri***

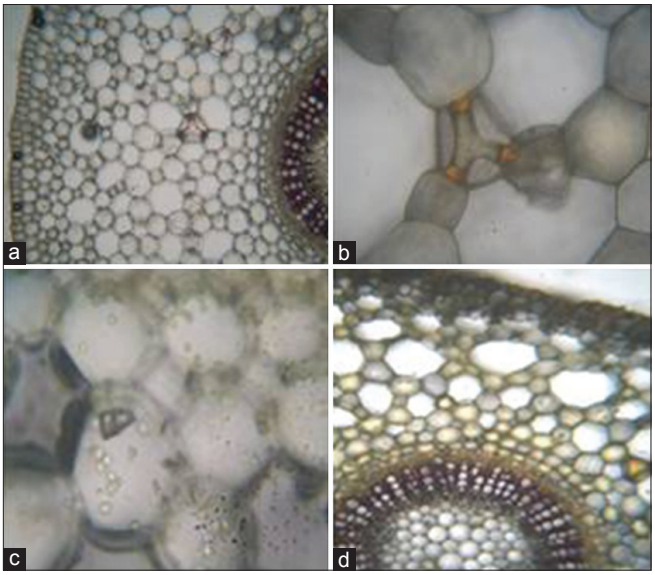

Figure 3: T.S. of Bacopa *monnieri stem* (a) Vascular bundle and centrally large pith, (b) Y linked parenchyma cells, (c) Prismatic crystal of calcium oxalate, (d) Xylem phloem

Table 1: Comparative organoleptic characters

Character	*Bacopa monnieri*		*Bacopa floribunda*	
	Leaf	**Stem**	**Leaf**	**Stem**
Color	Light greenish	Light greenish brown	Greenish	Light brownish to pale green
Odor	Slightly aromatic, bitter	Slightly aromatic	Specific	Nil
Taste	Bitter ends in strongly mucilage	Strongly bitter in taste	Bitter, ends in mucilage	Bitter taste

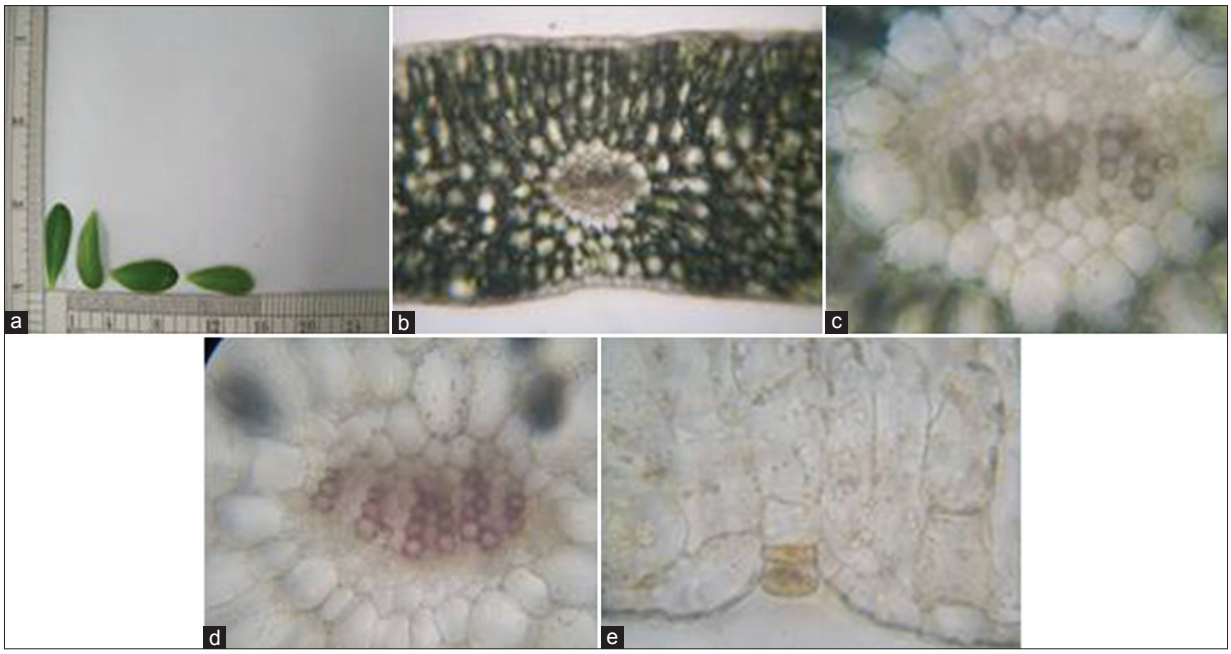

Figure 4: T.S. of *Bacopa monnieri* leaf (a) Leaf measurement, (b) T.S. of leaf through mid-rib, (c) Vascular bundle, (d) Xylem phloem, (e) Sunken stomata

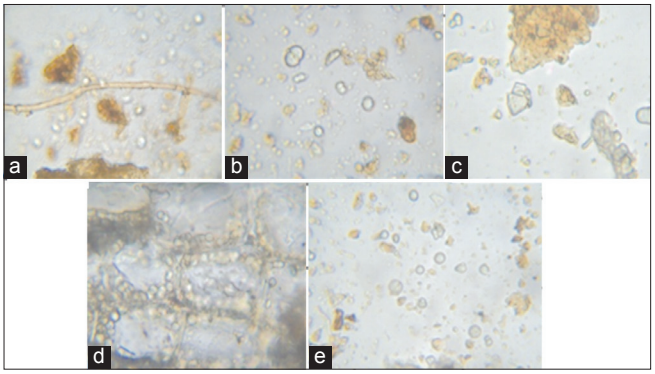

Figure 5: Powder microscopy of *Bacopa monnieri* stem (a) Single fibre, (b) Simple and compound starch grains, (c) Prismatic crystals, (d) Parenchyma with starch grains, (e) Oil globule

long, violet-blue, with purplish strips on the upper lip. Capsules are sub-globose [Figure 7].

T.S. of stem

T.S. of the stem shows epidermis, hypodermis and cortex with parenchyma containing less airspaces, endodemis, pericycle, vascular bundle and centrally large pith [Figure 8]. Detailed T.S. shows outermost single-layered barrel-shaped compactly arranged epidermis with cuticle rarely with some stomata, followed by two to three layers of hypodermis consisting of parenchyma cells with chlorophyll pigments and tannin content cells. One-third of the section is occupied by loosely arranged large intercellular spaces (aerenchyma) and parenchyma cells. Some of the cells are loaded by starch grains and prismatic crystal of calcium oxalate crystals. A single-layered endodermis separates cortex from the vascular tissue, forming a ring. Pericycle made up of barrel-shaped cells that are compactly arranged and thin walled. The vascular tissue system consists of radially

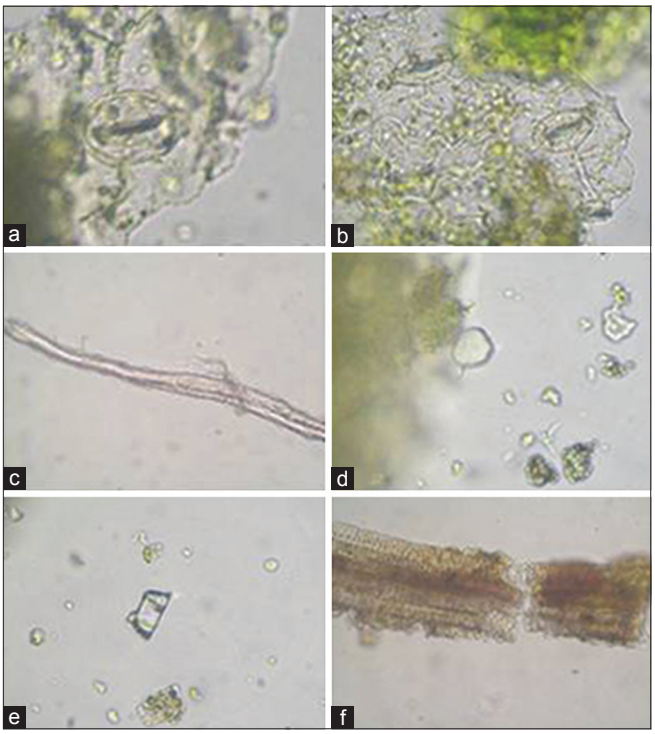

Figure 6: Powder microscopy of *Bacopa monnieri* leaf: (a) Anicocytic crystal, (b) Diacytic stomata, (c) Lignified fibre, (d) Oil globules, (e) Prismatic crystals, (f) Annular vessels from vascular bundle

arranged vascular bundles. Phloem situated above the xylem. Xylems are numerous in number, metaxylem toward pericycle, protoxylem toward pith with few numbers of xylem parenchyma and fibers, centrally situated pith containing compactly arranged parenchyma cells with some prismatic crystals [Figure 8a-f].

T.S. of leaf

It shows a typical dorsi ventral structure. Epidermis is single layered with cuticle. Lamina is differentiated into two layers, i.e. spongy and palisade. There are two to three layers of palisade cells found below the upper epidermis. Palisade and spongy parenchyma cells filled with chlorophyll pigments. Midrib portion consists of vascular bundle with compactly arranged xylem, which is in three to five groups. Phloem with few elements of companion cells. Diacytic and anisocytic stomata, prism of calcium oxalate crystals found in lamina and midrib portion of the leaf [Figure 9a-c].

Powder microscopy

Stem: Diagnostic powder microscopy of stem shows simple fibers, prismatic crystals of calcium oxalate, tannin contents, pitted vessels, simple and compound starch grains and parenchyma cells filled with starch grains [Figure 10a-d].

Leaf: Diagnostic powder microscopy of leaf shows diacytic and anisocytic stomata from epidermis, epidermal cells, prismatic crystals of calcium oxalate, lignified fibers, fragments of spiral and annular vessels from vascular bundle and simple fibers [Figure 11a-f].

Physico-chemical evaluation

The percentage of bacoside A in the stem and leaf of both the plants noted is presented in Table 2.

Figure 7: ***Bacopa floribunda***

Discussion

Comparative characters of both the plants, like powder microscopy, T.S. of stem, T.S. of leaf and morphological characters, are discussed in Tables 3-6, respectively. The method applied in the present study utilizes silica gel GF 254 HPTLC plates as stationary phase and dichloromethane: methanol: water (4.5:1:0.1 v/v/v) as mobile phase, which gives fine tune separation of bacoside A (Rf. =0.58)

Table 2: Percentage of bacoside A in stem and leaf of both the plants

	***Bacopa monnieri* (%)**	***Bacopa floribunda* (%)**
Leaf	22	26
Stem	18	27

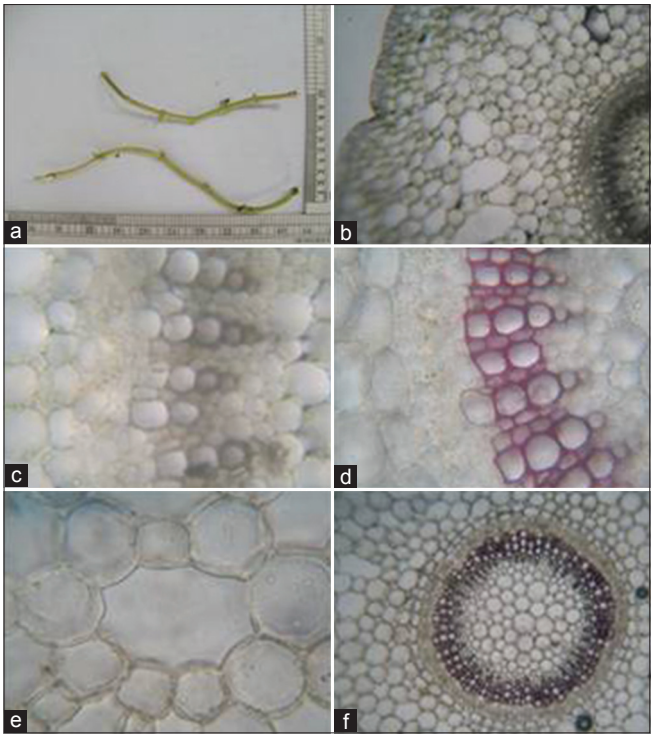

Figure 8: Figure 8: T.S. stem of *Bacopa floribunda* (a) Stem measurement, (b) Ground TS, (c) Vascular bundles, (d) Xylem phloem, (e) Aerencyama, (f) Vascular bundle with pith

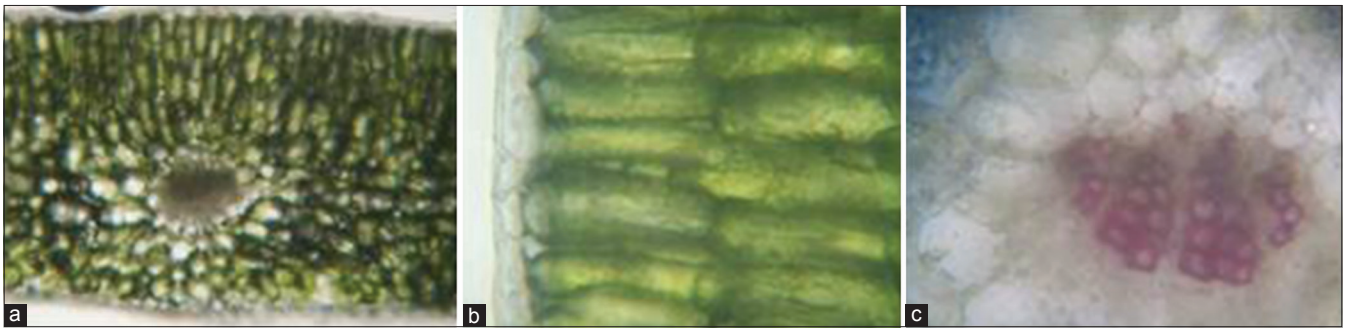

Figure 9: T.S. leaf of Bacopa floribunda, (a) Vascular bundle through mid rib, (b) Pallicyde parenchyma, (c) Xylem phloem

standard, although the powder sample was found loaded with a number of components [Table 7]. The identified band of bacoside A in the sample extract was confirmed by overlapping the UV absorption spectra of sample with that of reference standard at 366 nm [Figures 12-14]. The calibration curve was linear in the range of 2.5-7.5 μg/spot and the correlation coefficient was determined. 3-D densitogram at all wavelengths shows comparative Rf value of sample with standard [Figures 15 and 16] and derivatisation with vanillin sulfuric acid of the powder sample as that of standard at Rf value 0.94 for bacoside A.

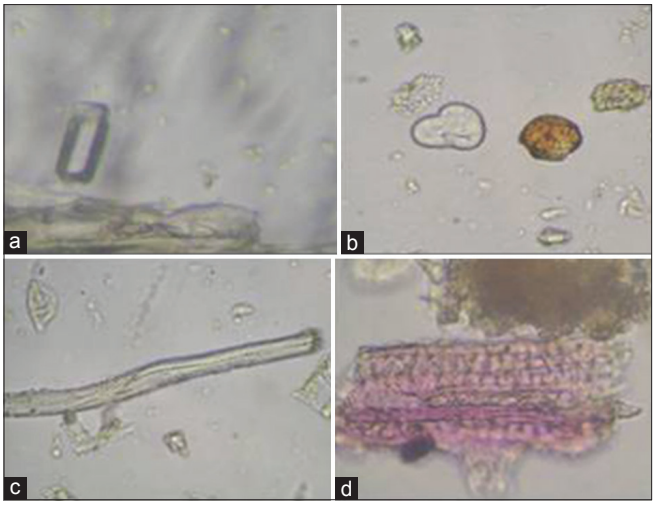

Figure 10: Powder microscopy stem of *Bacopa floribunda* (a) Prismatic crystals, (b) Oil globules (c) Fibre, (d) Pitted vessels

Table 3: Comparative powder microscopy characters

	Bacopa monnieri	*Bacopa floribunda*
Leaf	Lignified fiber	Simple fiber
Stem	Oil globule	-
	Lignified fiber	Simple fiber

Table 4: Comparative transverse section of stem characters

Bacopa monnieri	*Bacopa floribunda*
Parenchyma with large air spaces	Less air spaces
Each cell containing tannin at place of connection between adjacent cells shows Y like appearance	Absent
Both cortex and pith region consists of prismatic crystals	Only cortical region contain prismatic crystals

Conclusion

Morphologically, the leaves of *Bacopa monnieri* are fleshy and succulent (yields more extract) than *Bacopa floribunda* leaf. The taste of the leaf and stem powders was strong, with bitter ends in mucilage, while *Bacopa floribunda* was only bitter in taste. The *Bacopa monnieri* leaf and stem showed more lignified fibers, oil globules, tannin material and prismatic crystals of calcium oxalate than *Bacopa floribunda*. Study revealed presence of higher bacoside A content in *Bacopa floribunda*, i.e. 26% in leaf and 27% in stem, compared with *Bacopa monnieri*, i.e. leaf 22% and stem 18%. Considering the hemolytic action of bacoside A, it seems that *Bacopa monnieri* is more popular in regular use, than *Bacopa floribunda*.[9]

Table 5: Comparative transverse section of leaf characters

Bacopa monnieri	*Bacopa floribunda*
Contain 3-5 layers of palisade cells	2-3 layers of palisade cells
Midrib portion contain vascular bundle which contains compactly arranged xylem	Midrib portion consists vascular bundle which contains loosely arranged xylem
Contain glandular sessile trichome	Absent

Table 6: Comparative morphology characters

Characters	*Bacopa monnieri*	*Bacopa floribunda*
Herb	Creeping	Sub creeping
Leaves	Sessile	Sub sessile
	Fleshy, succulent	Not much succulent, flattened
Flowers	Solitary, axillary, pedicelled	Axillary 1-3 flowers, pedicelled
Calyx	6 mm long	7 mm long
Corolla	5-6 mm long	7-9 mm long
Corolla color	White/pale violet blue	Violet-blue with purplish strips on upper lip
Fruit	Capsule, ovoid	Capsule, subglobose

Table 7: Chromatographic separation

Sample	254 nm	366 nm	After spray
Bacopa monnieri leaf	0.01	0.01, 0.05, 0.13	0.12, 0.37, 0.43, 0.50
Bacopa monnieri stem	0.01, 0.41, 0.93	0.01, 0.04, 0.14, 0.93	0.11, 0.23, 0.26, 0.49
Bacopa floribunda leaf	0.01, 0.57, 0.95	0.01, 0.95	0.12, 0.23, 0.28, 0.32, 0.39, 0.54
Bacopa floribunda stem	0.01, 0.94	0.01, 0.04, 0.13, 0.94	0.10, 0.20, 0.26, 0.39, 0.44, 0.49
Standard bacoside	0.00	0.00, 0.84	

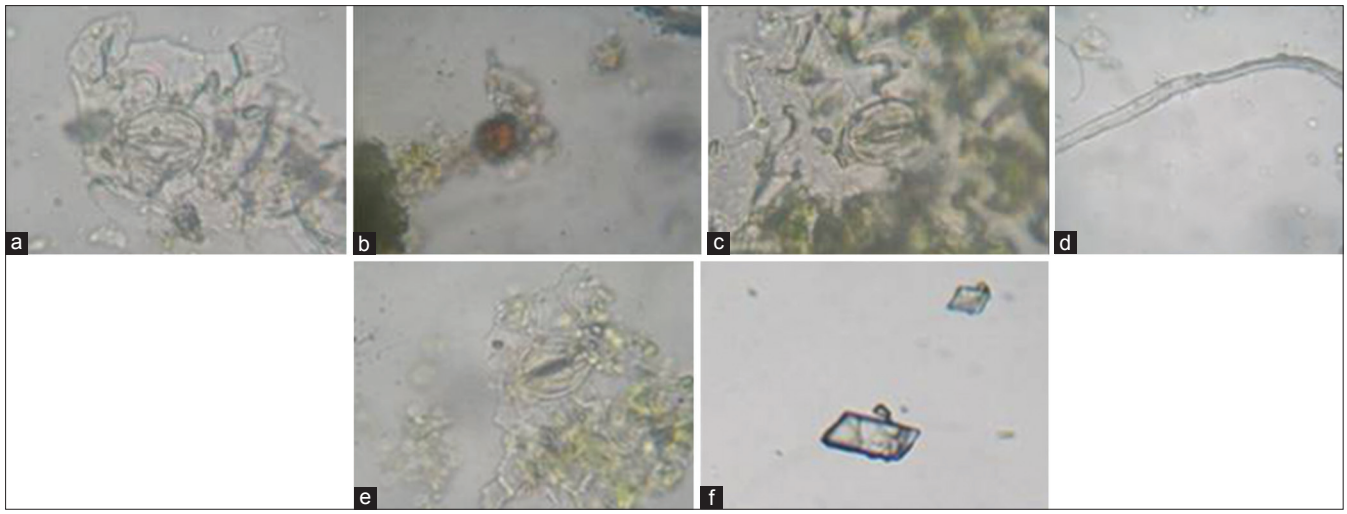

Figure 11: Powder microscopy leaf of *Bacopa floribunda* (a) Anicocytic stomata, (b) Brownish yellow content, (c) Diacytic stomata, (d) Fibre, (e) Palliside parenchyma, (f) Prismatic crystals

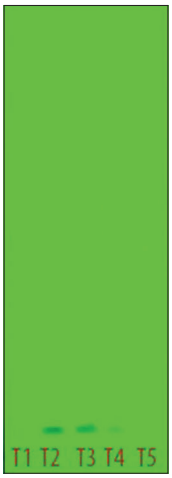

Figure 12: HPTLC picture at 254 nm

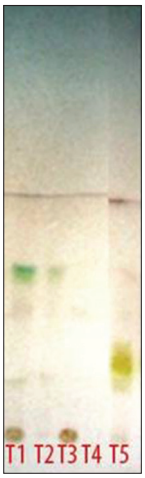

Figure 13: HPTLC picture at 366 nm

Figure 14: After spray. T1 - *Bacopa floribunda* leaf powder, T2 - *Bacopa monnerie* leaf powder, T3 - *Bacopa floribunda* stem powder, T4 - *Bacopa monnerie* stem powder, T5 - Standard bacoside A

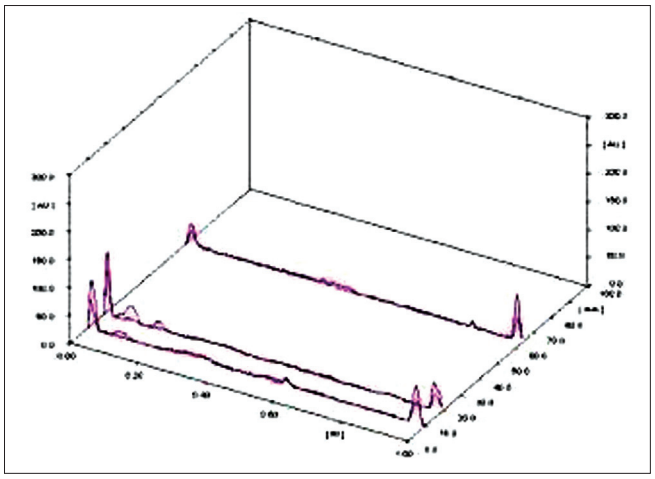

Figure 15: 3D densitogram at all wavelengths-Leaf

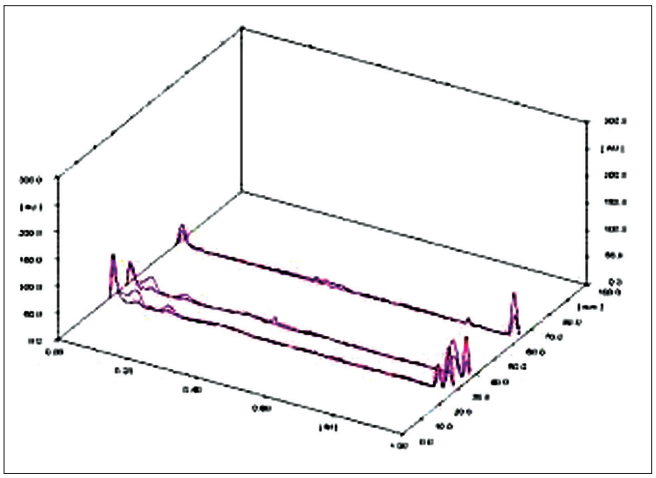

Figure 16: 3D densitogram at all wavelengths Stem

Acknowledgments

The author acknowledges the Director, IPGT and RA, Gujarat Ayurved University, Jamnagar, and staff of Pharmacognosy and Pharmaceutical Chemistry Laboratory for their kind support. The author is also thankful to FRLHT, Bangalore, for providing a genuine sample of *Bacopa monnieri*.

References

1. Kumar N. Introduction to Pharmacognosy. The Textbook of Pharmacognosy. 1st ed., Ch. 1. New Delhi: A.I.T.B.S. Publishers; 2010. p. 3.
2. Pandey G. Brahmi Dravyaguna Vijnana. Vol. 2. Varanasi: Chowkhamba Krishnadas Academy; 2004. p. 434-5.
3. Evans WC. Deterioration of stored Drugs Trease and Evans Pharmacognosy. 14th ed. London: W.B. Saunders Ltd; 1996. p. 119-59.
4. Wallis TE. Drug Descripton and Habitats Text Book of Pharmacognosy. 5th ed. New Delhi: CBS Publishers; 1985. p. 572-5.
5. Khandelwal KR. Preliminary Phytochemical Screening. Practical Pharmacognosy. Pune: Nirali Prakashan; 2008. p. 149-66.
6. Kokate CK, Purohit AP, Gokhale SB. Analytical Pharmacognosy. 42nd ed. Pune: Nirali Prakashan; 2008. p. 6.1-6.13.
7. Stahl E, Ganshirt H. Documentation of Thin Layer Chromatograms Thin Layer Chromatography: A Laboratory Hand Book. Berlin: Springer-Verlag; 1969. p. 124-241.
8. Barwick V. Preparation of calibration of curves: A guide to best practice, 2003. p. 3. Available from: http://www.nmschembio.org.uk/dm./LGCVAM2003032_xsJGL.pdf. [Last accessed 2012 Sep 14].
9. Khare CP. Bacopa monnieri (Linn.) Penn. Indian Medicinal Plants. New york: Springer Science and Buisness Media, LIC.; 2007. p. 77.

हिन्दी सारांश

Bacopa monnieri (L.) और *Bacopa floribunda* के पत्र एवं काण्डका नामरूपविज्ञानीय एवं रासायनिक विश्लेषण – एक तुलनात्मक अध्ययन

ज्योति एस. गुब्बण्णवर, एच. एम. चन्दोला, सी. आर. हरीशा, कोमल खानपरा, वी. जे. शुक्ला

Bacopa monnieri (L.) और *Bacopa floribunda*, दोनों को ब्राह्मी के नाम से औषध बाजार में प्रयोग किया जाता है । ब्राह्मी, मस्तिष्क की क्रियाशीलता एवं स्मृतिवर्धन के लिए प्रख्यात औषध है। प्रस्तुत अध्ययन में ब्राह्मी की इन दोनों प्रजातियों में भिन्नताका अध्ययन परिचयात्मक लक्षण, काण्ड, पत्र, चूर्ण का सूक्ष्मदर्शीय परीक्षण एवं रासायनिक संघटन के आधारपर किया गया। परिचयात्मक लक्षणों में दोनों औषध वनस्पतियों के पुष्प के रंग, पत्र, काण्ड में भिन्नता पाई गई । *Bacopa monnieri* (L.) के पत्र ज्यादा मांसल एवं रसयुक्त होते है। दोनों के काण्ड में internode के अंतर में भी भिन्नता प्राप्त हुई । इस अध्ययन से यह निश्चित हुआ कि - bacoside A की मात्रा *Bacopa floribunda* के पत्र में २६%, और काण्ड में २७% पाई गई, जबकि *Bacopa monnieri* (L.) के पत्र में २२%, और काण्ड में १८% पाई गई। रासायनिक घटक - bacoside A में hemolytic action जैसा विषाक्त प्रभाव होने से शायद *Bacopa monnieri* (L.) वनौषधि अधिक प्रचलित है क्योंकि इसमें bacoside A, *Bacopa floribunda* की अपेक्षा कम पाया जाता है ।