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Comparative pharmacognosy of two medhya dravyas, Brahmi (*Bacopa monnieri* Linn.) and Mandukaparni (*Centella asiatica* Linn.)

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Abstract

Background: Brahmi (*Bacopa monnieri*) and Mandukaparni (*Centella asiatica*) are the two important distinct Medhya (Nootropic) drugs mentioned in Indian systems of medicine. Lack of morphological description and attribution of similar properties led towards confusion in identity of these two sources. Pharmacopoeias suggest macro-microscopic characterization and chemical profiling of botanical material forms a pilot step in drug standardization. Hence the detailed macro-microscopic records, along with phytochemical documentation of these two plants were planned in the present investigation. **Methodology:** Matured, authenticated plants were collected from its natural habitat. Macro-microscopic and preliminary phytochemical studies were carried out. **Results:** Transverse section of *Bacopa monnieri* passing through midrib is isobilateral in histology whereas that of *Centella asiatica* is dorsiventral. Striated cuticle and few layered spongy parenchyma are the features of *Centella asiatica*. Plenty of air cavities with stomatal opening are specific to *Bacopa monnieri*. Phytochemical analysis of these two drugs has revealed the presence of alkaloids, saponins, glycosides and tannins. In addition to this *Centella asiatica* has shown the presence of flavanoids. **Conclusion:** The macro-microscopic and phytochemical tests can be used to differentiate the botanical source of Brahmi/Mandukaparni.

Keywords: Brahmi, *Bacopa monnieri*, *Centella asiatica*, Macro-microscopic, Mandukaparni, Phytochemical.

Introduction

The main object of *Ayurveda* is to live full length of life which is free from physical and mental disorders. *Medhya rasayana* drugs have been claimed to exert a pronounced effect on the mental capability of a person.[1] The potential role of these drugs is on intellectual function and mental performance. *Brahmi* and *Mandukaparni* are the two most popular drugs which are restorative with a specific effect on the intellectual functions.[2] *Charaka* considers both these drugs as promoters of general mental ability (medhya).[3] Inclusion of these two drugs in *Ashtanga ghrita* a formulation mentioned in *Asthanga hridaya* clearly implies these as two distinct drugs.[4] *Brihatrayees*, the three classical texts of *Ayurveda* even supports the same.

Bacopa monnieri of *Scrophulariaceae* is the accepted source of *Brahmi*. It is a weak, creeping herbaceous plant common in marshes and aslong back water and is called *Brahmi* or *Nirbarhmi* in vernacular language.[5] The drug is reported to be cold, sweet, astringent, diuretic, laxative and tonic for the heart and nerves. The whole plant is used in a variety of preparations like *Brahmighritam*, *Mishrakasneham* etc.[6]

The literal meaning of the term *Mandukaparna* is a plant having leaves resembling the shape of frogs. And it also means, the stolons grow in the manner of jumping frog. *Centella asiatica* is the accepted source of this herb.[3] It is a stoloniferous creeping herb, rooting at nodes. The whole plant is reported to be a nervine and cardiotonic, astringent and diuretic.[7] *Charaka* includes this under *Vayasthapana varga*[8], the group of drugs that are capable of maintaining the youthful vigour and strength.

There is, however, some confusion with regard to the drugs *Mandukaparni* and *Brahmi*. This may be due to the lack of description of the two drugs in the texts, attribution of similar properties to them and also application of the same synonyms.[3]

Drug standardization or quality assurance forms an essential step before its therapeutic utility.[9] The word standardization should encompass the entire field of study from the birth of a plant to its clinical application. Most of the pharmacopoeias suggest macro-microscopic characterization and chemical profiling of botanical material turn out to be a pilot step in drug standardization.[10]

Hence the detailed macro-microscopic records, along with phytochemical documentation of these two plants were planned under this paper.

Materials and Methods

Fresh plant materials were collected during flowering season from Udupi District of Karnataka, authenticated referring to regional floras.[11,12] Photograph from natural habitat were taken to record habitat features. The dried ones were obtained by drying them under shade, powdered and kept for phytochemical analysis. Fresh plant material was studied for pharmacognostical characters as differences in microscopic features are not evident in the dried form. Voucher specimen and number 558.15013101-02 is deposited in the Pharmacognosy department of the SDM Centre for Research in Ayurveda and Allied Sciences, Udupi.

Representative parts from leaves were cut and preserved immediately after collection. Detailed macroscopic and microscopic studies were carried out as per standardized methodologies.[12,13]

The materials were left in a fixative solution Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol 90ml (FAA) for more than 48 hours.

Macroscopic study

Macroscopic characters of whole plant parts were recorded systematically as prescribed in text book of Pharmacognosy.[14]

Microscopic study

The preserved specimens were dehydrated with a graded series of tertiary-butyl alcohol as per the schedule. After dehydration, paraffin infiltration was carried out till super saturation of tertiary butyl alcohol was achieved. Following super saturation, materials were transferred to pure paraffin wax for two times and the materials were cast into paraffin blocks.[14]

Dermal features

Small pieces of leaves were mildly heated with Nitric acid and the droplets of cutin formed were made to dissolve using benzene. Such peelings stained with safranin were used to study the nature of cells of the epidermis, stomata etc.[14]

Histology

The paraffin wax embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10 to 12 µm. The sections were stained with toluidine blue as per the method introduced by O'Brein *et al.*, 1964.[15] Toluidine blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies. Wherever necessary, sections were stained with safranin and fast green.

Quantitative microscopy

The cleared materials were washed thoroughly, stained with safranin for quantitative microscopic studies. The tests were performed as per the procedure given by Wallis TE, 1967.[13] Few of the leaf constants such as stomatal number, epidermal cells per sq. mm, stomatal index etc. were done using micrometers.[13,14]

Stomatal number

It is the average number of stomata per square mm of the epidermis of the leaf. A minimum of ten readings were taken from different locations of the leaflet and the average value was calculated.[14]

Palisade ratio

It is the average number of palisade cells beneath one epidermal cells of the lamina. It is determined by counting the palisade cells beneath four continuous epidermal cells and dividing it by four.[13]

Photomicrography and description

In order to supplement the descriptive part, photomicrographs in different magnifications of all necessary cells and tissues were taken in Zeiss AxioLab trinocular microscope and Zeiss Stemi stereo microscope. For normal histological purposes, sections were photographed under bright field light. Magnifications of the figures are indicated by the scale-bars.

Phytochemical study

Known quantity two dry powdered drugs were extracted with petroleum ether, chloroform, ethanol and cold water. These extracts were tested for different constituents.[10]

Results

Bacopa monnieri (Linn.) is a juicy, succulent, glabrous herb rooting at nodes with numerous ascending branches. Leaves simple, opposite, sessile, entire, fleshy, obscurely veined. Flowers whitish, axillary, solitary. *Centella asiatica* is a small creeping herb with slender stem, rooting at nodes. Leaves simple with elongated petiole and sheathing leaf base, reniform, crenate, toothed. (Figure 1)

TS of leaf of *B. monnieri* passing through midrib (Fig. 2.1, 3.1) is nearly isobilateral in histology, midrib is not differentiated and both side flat without any elevation (dorsiventral in histology and midrib is differentiated, shows a broad elevation at the lower side in *C. asiatica*, see Fig. 2.2). The leaf is covered by the upper and lower epidermis, few of the lower epidermal cells being papillate, shows plenty of stomata on either side (epidermal cells of the midrib region are thick-walled shows collenchymatous hypodermis in *C. asiatica*, see Fig. 2.2, and 3.2 a, c). A vascular bundle comprising of xylem elements, which are not differentiated into protoxylem and metaxylem, and indistinct patches of phloem surrounding it is placed at the centre (oval shaped bundle with distinct patches of phloem at the lower side, vessels differentiated into protoxylem towards the upper side and metaxylem towards lower side in *C. asiatica*, see Fig. 3.2b). Beneath the upper epidermis plenty of air cavities are embedded with stomatal openings (just above the vascular bundle secretary cavity is placed in *C. asiatica*, see Fig. 3.2c).

TS of leaf passing through the lamina in *B. monneri* (Fig. 4.1) is flat and without any elevations and is covered by the upper and lower epidermis, a thick normal cuticle covers the epidermii and stomata are embedded throughout the upper and lower epidermis (cuticle striated in *C. asiatica*, see Fig. 4.2). The mesophyll region is not differentiated into palisade and spongy parenchyma but few cells beneath the upper epidermal cells are elongated and embed numerous air cavities (single layer of palisade without much air space in *C. asiatica*, see Fig. 4.2). The spongy parenchyma is many layered with a few trace bundles (few layered in *C. asiatica*, see Fig. 4.2)

Stomatal number on upper and lower epidermii of *B. monneri* was calculated to be 118 and 130 respectively, while the upper epidermis of *C. asiatica* has only 58 stomata per sq. mm. There are no glandular trichomes on the lower epidermis of *C. asiatica*. Palisade ratio is 0 on both the epidermii of *B. monneri* and lower epidermis of *C. asiatica* (Table 1, Figure 5).

Phytochemical analysis of these two drugs has revealed the presence of alkaloids, saponins, glycosides and tannins. In addition to this *C. asiatica* has shown the presence of flavanoids (Table 2 & 3).

Photograph of natural habitat Fresh leaves

Fig. 1.1: *Bacopa monneiri* Linn.

Fig. 1.2: *Centella asiatica* (Linn.) Urban

Figure 1: Habitat of the plants

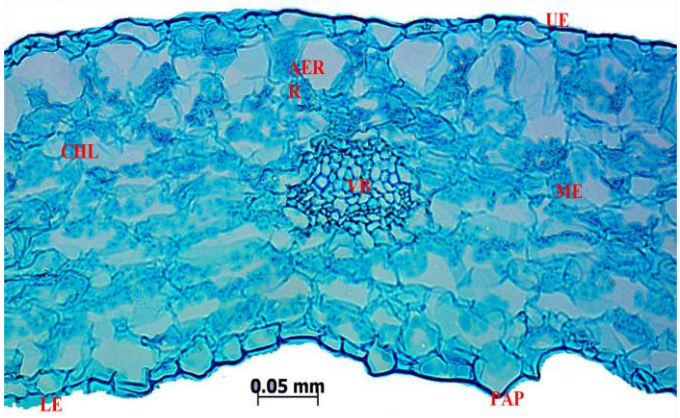

Fig. 2.1: *B. monnieri*

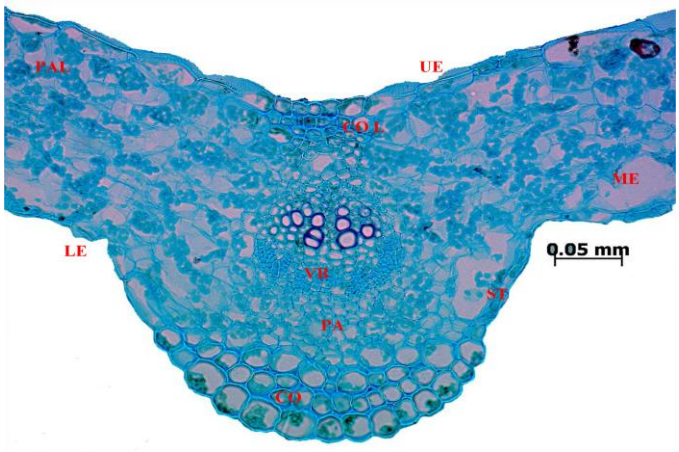

Fig. 2.2: *C. asiatica*

AER, aerenchyma; **CHL**, chlorenchyam; **COL**, collenchyma; **LE**, lower epidermis; **ME**, mesophyll; **PA**, parenchyma; **PAL**, palisade; **PAP**, papilla; **ST**, stomata; **T**, trichome; **UE**, upper epidermis; **VB**, vascular bundle.

Figure 2: TS of the leaf passing through the midrib

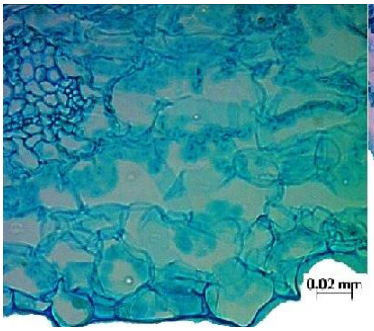

Fig. 3.1a: Lower region of midrib showing papillate epidermis

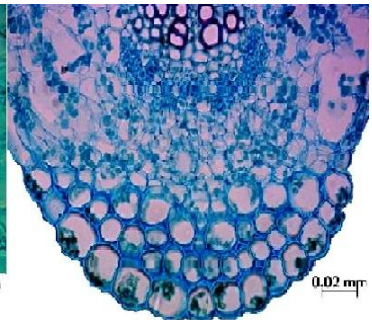

Fig. 3.2a: Lower portion of midrib showing broad elevation and normal epidermis epidermis

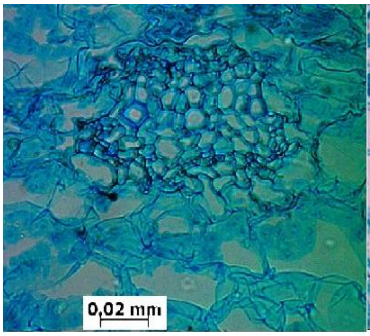

Fig. 3.1b: A vascular bundle enlarged, vessels not differentiated

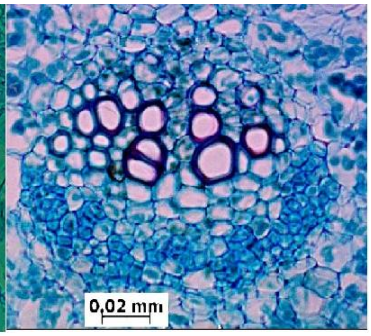

Fig. 3.2b: A VB enlarged, vessels differentiated to proto- and metaxylem

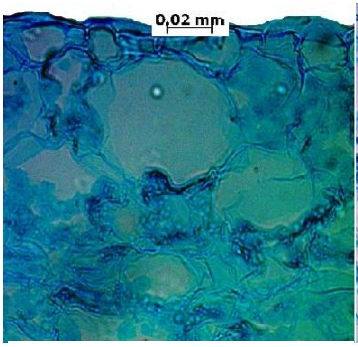

Fig. 3.1c: Upper portion of midrib showing abundant aerenchyma

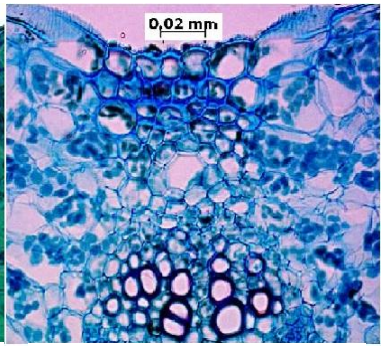

Fig. 3.2c: Upper portion of midrib showing collenchymas hypodermis and secretary cavity

Figure 3: Microscopic features of leaf **3.1.** *B. monnieri*; **3.2.** *C. asiatica*

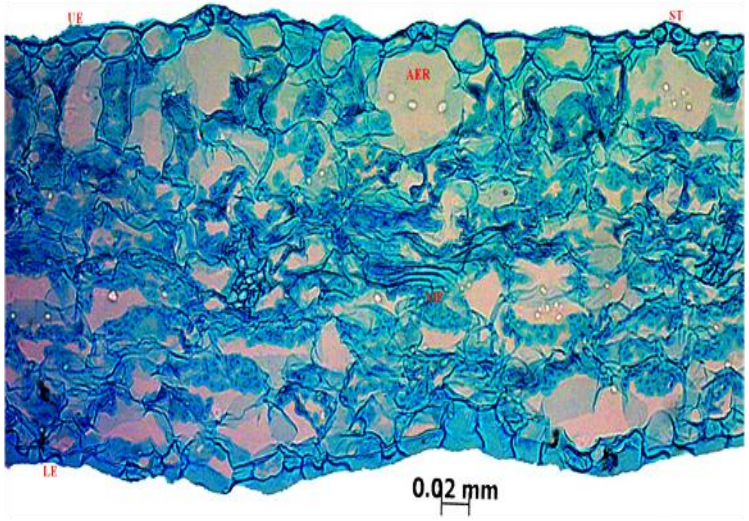

Fig. 4.1: *B. monnieri*

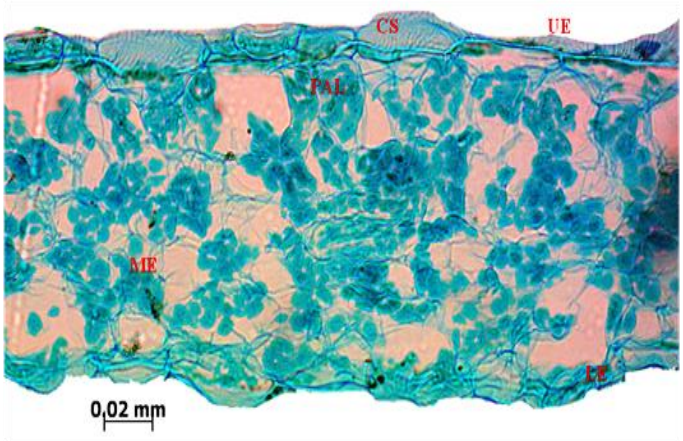

Fig. 4.2*: C. asiatica*

AER, aerenchyma; **CS**, cuticular striations; **LE**, lower epidermis; **ME**, mesophyll; **PAL**, palisade; **ST**, stomata; **UE**, upper epidermis.

Figure 4: TS of leaf passing through lamina

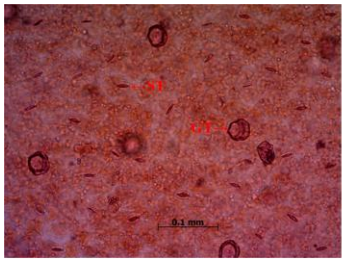

Fig. 5.1: Epidermal features upper epidermis of *B. monnieri*

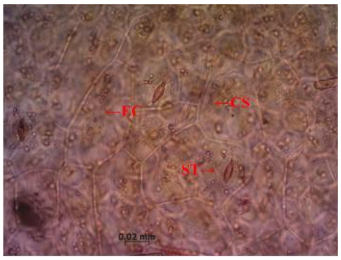

Fig. 5.2: Epidermal features of Upper epidermis of *C. asiatica*

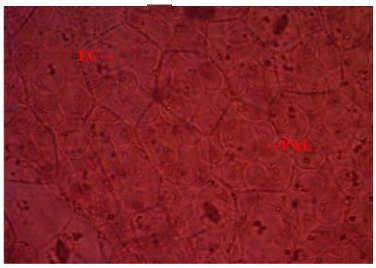

Fig. 5.3: Palisade ratio of *C. asiatica*

CS, cuticular striations; **EC**, epidermal cell; **GT**, glandular trichome; **PAL**, palisade; **ST**, stomata

Figure 5: Epidermal features of the Plants

Table 1: Quantitative microscopic values of *Centella asiatica* and *Bacopa monnieri* leaf

Per sq. mm.	***Bacopa monnieri***		***Centella asiatica***	
	UE	**LE**	**UE**	**LE**
Stomatal number	118	130	58	140
Trichome (glandular) number	25	13	16	0
Palisade ratio	0	0	6.5	0

UE – upper epidermis; LE – lower epidermis

Table 2: Phytochemical study of *Bacopa monnieri* (Dry form)

S. No	**Tests**	**Pet. ether**	**Chloroform**	**Ethanol**	**Cold infusion**
1.	**Proteins**				
a.	Biuret test	–ve	–ve	–ve	–ve
b.	Ninhydrin test	–ve	–ve	–ve	–ve
c.	Xanthoproteic test	–ve	–ve	–ve	–ve
d.	Hopkins-cole test	–ve	–ve	–ve	–ve
e.	Sulphur test	–ve	–ve	–ve	–ve
2.	**Carbohydrate test for starch**				
a.	Molisch's test	–ve	–ve	+ve	–ve
b.	Iodine test	–ve	+ve	+ve	-ve
c.	Fehling's test	+ve	-ve	+ve	+ve
d.	Benedict's test	+ve	+ve	+ve	-ve
3.	**Tannins**				
a.	Gelatin test	-ve	–ve	–ve	+ve
4.	**Anthrocyanins**				
a.	Aqueous NaOH test	–ve	–ve	–ve	–ve
b.	Conc. H_2SO_4 test	–ve	–ve	–ve	–ve
5.	**Glycosides**				
a.	Molisch's test	+ve	-ve	+ve	+ve
b.	Conc. H_2SO_4 test	-ve	–ve	–ve	+ve
c.	Keller Kiliani test	-ve	+ve	+ve	+ve
6.	**Saponin**				
a.	Foam test	+ve	-ve	+ve	–ve
7.	**Flavanoids**				
a.	Flavanoid test	–ve	–ve	–ve	–ve
b.	Pew's test for Dihydroflavanols	–ve	–ve	–ve	–ve
c.	Shinoda test	–ve	–ve	–ve	–ve
d.	Aqueous NaOH test	–ve	–ve	–ve	–ve
e.	Conc. H_2SO_4 test	–ve	+ve	–ve	-ve
8.	**Phenols**				
a.	Phenol test	-ve	–ve	+ve	–ve
9.	**Steroids**				
a.	Salkowski's test	–ve	–ve	–ve	–ve
10	**Alkaloids**				
a.	Mayer's test	–ve	–ve	+ve	–ve
b.	Dragendroff's test	–ve	–ve	–ve	–ve

Tale 3: Phytochemical study of *Centella asiatica* (Dry form)

S. No	**Tests**	**Pet. Ether**	**Chloroform**	**Ethanol**	**Cold infusion**
1.	**Proteins**				
a.	Biuret test	–ve	–ve	–ve	–ve
b.	Ninhydrin test	–ve	–ve	–ve	–ve
c.	Xanthoproteic test	–ve	–ve	–ve	–ve
d.	Hopkins-cole test	–ve	–ve	–ve	–ve
e.	Sulphur test	–ve	–ve	–ve	–ve
2.	**Carbohydrate test for starch**				
a.	Molisch's test	–ve	–ve	+ve	–ve
b.	Iodine test	–ve	+ve	+ve	-ve
c.	Fehling's test	+ve	-ve	+ve	+ve
d.	Benedict's test	+ve	+ve	+ve	-ve
3.	**Tannins**				
a.	Gelatin test	-ve	–ve	–ve	+ve
4.	**Anthrocyanins**				
a.	Aqueous NaOH test	–ve	–ve	–ve	–ve
b.	Conc. H_2SO_4 test	–ve	–ve	–ve	–ve
5.	**Glycosides**				
a.	Molisch's test	+ve	-ve	+ve	+ve
b.	Conc. H_2SO_4 test	-ve	–ve	–ve	+ve
c.	Keller Kiliani test	-ve	+ve	–ve	+ve
6.	**Saponin**				
a.	Foam test	+ve	-ve	+ve	–ve
7.	**Flavanoids**				
a.	Flavanoid test	–ve	–ve	–ve	–ve
b.	Pew's test for Dihydroflavanols	–ve	–ve	–ve	–ve
c.	Shinoda test	–ve	–ve	–ve	–ve
d.	Aqueous NaOH test	–ve	–ve	–ve	–ve

e.	Conc. H_2SO_4 test	+ve	+ve	–ve	-ve
8.	**Phenols**				
a.	Phenol test	-ve	–ve	+ve	–ve
9.	**Steroids**				
a.	Salkowski's test	–ve	–ve	–ve	–ve
10	**Alkaloids**				
a.	Mayer's test	–ve	–ve	+ve	–ve
b.	Dragendroff's test	–ve	–ve	–ve	–ve

Discussion

Lack of standards is a major problem associated with herbal medicine, while macro-microscopic scientific records are essential steps in quality assurance of a drug. Common pharmaco-therapeutic property and fewer morphological descriptions available in the texts of Ayurveda, made *Brahmi* and *Mandukaparni* as controversial drugs[3]. But their specific inclusion under particular formulations and single drug usage clarifies these as two separate drugs attributed with a specific mode of action. *Bacopa monnieri* and *Centella asiatica* are accepted source of *Brahmi* and *Mandukaparni* respectively.[5]

Transverse section of *B. monnieri* passing through midrib is found to be nearly isobilateral in histology, midrib is not differentiated and both sides are flat without any elevation. *C. asiatica* shows dorsiventral histology and the midrib is differentiated with broad elevation at the lower side. Lamina of *B. monnieri* is flat and without any elevations and is covered by upper and lower epidermii, a thick normal cuticle covers the epidermis and stomata are embedded throughout the upper and lower epidermis. However cuticle layer of *Centella asiatica* is striated. Spongy parenchyma is many layered in *Bacopa monnieri* while it is few layered in *Centella asiatica*. Presence of plenty air cavity embedded with stomatal opening beneath the upper epidermis evocative of aquatic habitat of *Bacopa monnieri*. Quantitative microscopic characters further help in identification of genuine drug.

Preliminary phytochemical studies are essential to understand the basic chemical nature of the plant. Presence of flavanoids is found to be a marked difference of *C. asiatica* though alkaloids, saponins, glycosides and tannins occur in both.

Conclusion

The macro-microscoipc profile along with their preliminary phytochemical data delineated here would be helpful to differentiate each other and other admixture.

References

1. Brain aging and Ayurveda; New Delhi, Central Council for Research in Ayurevda & Siddha, Dept of Ayush, Min of Health& family welfare, Govt of India, 2008, P. 14.

2. Singh HK, Dhawan BN, Neuropsychopharmacological effects of of Ayurvedic nootropic *Bacopa monnieri* Linn.(Brahmi). Indian J Pharmacol 1997; p 29.

3. Charaka, Charaka samhita, Chikitsa sthana, Varanasi; Choukamba Sanskrit series, 2002, P. 385.

4. VV Sivaranjan and Indira Balachandran, Ayurvedic drugs and their plant sources, New Delhi; Published by Oxford & IBH Publishing Co. Pvt. Ltd, 1994, P. 289.

5. Indian Medicinal Plants, Volume 1, Published Orient Longman Private Ltd, 1994, p.235.

6. Priyavat Sharma, Dravya guna Vijnana, Varanasi, Chaukamba Bharati Academy; Volume 2, 1998, p. 6.

7. Khare CP, Indian Medicinal Plants an Illustrated Dictionary, New Delhi, Springer; 2007, p.77.

8. Charaka, Charaka samhita, Chikitsa sthana, Varanasi; Choukamba Sanskrit series, 2009, P. 34.

9. Sunil Kumar KN, Pharmacognostical evaluation of *Cinnamomum tamala* (Buch.- Ham) Nees and Eberm (*Tamala patra*) and few of its allied species. Msc. Thesis, Institute of Ayurvedic Medical Sciences, Gujarat Ayurveda University, Jamnagar, Gujarat, 2006.

10. Quality Control Methods for Medicinal Plant Materials, WHO, Geneva, 1998, p.16- 27.

11. Nadakarni AK. Dr. KM Nadakarni's, Indian Materia Medica, Popular Prakashan, Bomaby, 1976. p579.

12. Gamble JS. Flora of the presidency of Madras. Botanical survey of India, Calcutta, 1925. Vol.II, p.556.

13. Wallis TE. Textbook of Pharmacognosy. Delhi; CBS Publishers and Distributors; 1985, p572.

14. Evans WC. Trease and Evans Pharmacognosy, London: Bailliere Tindall; 1989, p530.

15. O'Brien TP, Feder N, Mc Cull ME. Polychromatic staining of plant cell walls by toluidine blue-O. Protoplasma 1964; 59: 364-373.