RESEARCH ARTICLE

Anatomical markers and Phytochemical study of different plant parts of *Bacopa monnieri* (L.) Wettst.

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ABSTRACT

Bacopa monnieri (L.) Wettst. (Scrophulariaceae) commonly known as "brahmi", has been used in the traditional systems of medicines as "Medhya Rasayana" as it increases mental clarity and brain stimulating action. It is also being used as anti-inflammatory, analgesic, antipyretic, sedative, antioxidant and anti-cancerous agent. The present study was carried out to provide characteristic anatomical markers for root, stem and leaf, along with this study preliminary photochemical analysis was also carried out for the presence of major secondary metabolites which can act as the indicator of bioactivity of the different plant parts of Bacopa monnieri (L.) Wettst. The distinguishing features for root and stem are simple starch grains, xylem vessel with spiral thickenings, pitted tracheids and for leaf are subepidermal foliar idioblast, sessile, multicellular trichome and prismatic calcium oxalate crystals. Presence of two types of stomata i.e. anomocytic and diacyitc in the same field of vision under 45X magnification, are seen in the surface preparation as well as in the powder microscopy of Bacopa monnieri leaf. This is the major observation of the study. All these distinguishing features can be used as anatomical markers for correct indentification of root, stem and leaf of the plant. phytochemical analysis revealed the presence of alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins and anthroquinones in different plant parts of Bacopa monnieri while quantitative estimation showed that alkaloids (mg/g dry weight) in root, stem and leaf were found to be 1.67 \pm 0.577, 47.00 \pm 0.81 and 53.07 \pm 2.08 respectively; saponins (mg /g dry weight) in root, stem and leaf were 7.50 ± 0.71 , 23.33 ± 1.53 and 57.00 ± 2.65 respectively and flavonoids (mg/g dry weight) in stem and leaf were 25.33 ± 1.53 and 27.67 ± 2.08 respectively. Thus the findings of the present study will provide referential pharmaco - botanical information for correct identification and authentication of the crude drug and its plant parts.

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Keywords: Bacopa monnieri (L.) Wettst., foliar idioblast, anomocytic, diacytic types of stomata, prismatic calcium oxalate crystal and phytoconstituents.

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INTRODUCTION

In the last few decades there is an exponential growth in the field of herbal medicine. It is getting popularize in developing countries owing to its natural origin and lesser side effects. Nowadays, herbal medicines are being manufactured on a large scale in mechanical units, where manufacturers are facing many problems such as availability of good quality of raw material, authentication of raw material, availability of standards, proper standardization methodology of drugs and formulations, quality control parameters etc (Patil et al., 2011). For pharmacological or pharmaceutical use, scrutiny of a crude drug for its botanical identity is required (Poornima et al., 2009). Generally herbal formulations use fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained (Modi et al., 2010).

Anatomical structure is very important for studying biological specimens for the purpose of classification, pathological changes and the physiological significance of certain cell organelles or structures in relation to the habitat (Coopoosamy and Naidoo, 2011). Microscopy allows the identification of herbal drugs and the detection of individual components of a mixture. It is important to ensure quality and purity of herbal medicines in order to maximize the efficacy and minimize adverse side effects. Adulteration and misidentification of herbal drug can cause serious health problems to consumers (Serrano *et al.*, 2010).

Several medicinal plants have been used as a source of many potent and powerful drugs. Medicinal property of a plant is due the presence of chemical entities (phytochemical constituents) which are extracted from the plant or the plant parts (Mahesh and Satish, 2008). Medicinally important plants contain diverse group of phytochemicals with great variations in solubility and stability (Mallikharjuna et al., 2007). Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals (Banso, 2009). Aim for the phytochemical analysis is the characterization of an active principle responsible for some toxic or beneficial effect shown by a crude plant extract when tested against a living system. Quantification of the different phytoconstituents

present in the plants is of equal importance (Harborne, 2007). Phytochemicals are divided into two groups (according to their functions in plant metabolism), these are primary and secondary metabolites. Primary metabolites are compounds like carbohydrates, proteins, lipids which are essential for the plant metabolism. Secondary metabolites are compounds which are not essential for the plant metabolism as such but are formed as byproducts in the biochemical pathways. These include very interesting and useful classes of compounds like alkaloids, terpenoids, anthocyanins, saponins phenolic compounds like flavonoids, tannins, etc. These secondary metabolites can be utilized for the identification of the plant material (Krishnaiah *et al.*, 2009).

Bacopa monnieri (L.) Wettst., an important medicinal plant belongs to family Scrophulariaceae is used in the traditional systems of medicines as memory booster (Gohil and Patel, 2010). It is also referred to as, Herpestis monniera L. Kunth. Bacopa monnieri (L.) Wettst. locally known as water hyssop, brahmi or Jalanimba in India. The name brahmi is derived from the word 'Brahma', the mythical 'creator' in the Hindu pantheon. As the brain is the center for creative activity, any compound that improves the brain health is called brahmi, which also means 'bringing knowledge of the supreme reality (Prasad et al., 2008). Brahmi is a small, smooth, hairless, somewhat fleshy and creeping herb. The plant is a short duration annual herb, frequent in moist habitat and water edges throughout tropical and subtropical India. It grows best near flowing water and wet lands in plain and foothills and is particularly abundant in monsoon. Brahmi can grow in a variety of soil types if the habitat provides wet and semi shade conditions. Near- neutral, clayey loam to clayey soils are best suited for the growth of Bacopa monnieri. In North India, it can grow in a wide range of temperatures 15° C - 40° C and soil pH 5.0- 7.5. However, it can even grow well in soils with pH 7.5 or even more. It becomes dormant during the winter months except when grown near running water. Flowers and fruits appear in summer (Agro tech, 2008).

The pharmacological effects of *Bacopa monnieri* are attributed to the presence of a number of biologically active compounds including alkaloids (nicotine and herpestine), saponins and sterols (Monograph, 2004), d-mannitol, hersaponin, monnierin, betulic acid, stigmastarol, beta-sitosterol, as well as numerous

bacosides and bacopasaponins (Agro tech, 2008). The compounds responsible for the memory enhancing effects of *Bacopa monnieri* are triterpenoid saponins called "Bacosides" (Srinivasa *et al.*, 2011). The major chemical shown to be responsible for neuropharmacological effects and the nootropic action or antiamnestic effect of *Bacopa monnieri* was bacoside A (Gohil and Patel., 2010).

The whole plant of Bacopa monnieri is used as brain tonic in indigenous system of medicines. Brahmi is considered among one of the "Celestial drugs" (Divya ausadhi) when consumed with milk for 6 months. The herb is generally confused with another herb -Centella asiatica that is morphologically very different from that of Brahmi. The juice of the boiled whole plant is given to children for relief in bronchitis and diarrhoea. The paste of the leaves is used as a remedy for rheumatism. Ghee fried leaves are given in hoarseness complaints. The leaves and tender stalks are reported to be eaten in the west Bengal. Its juice along with ginger juice, sugar and bark ext. of Moringa oleifera is given to children in stomach disorders. Decoction of leaves is given in cough. Brahmi Ghrita - a medicated ghee of brahmi when given in a dose of 1-2 tolas along with milk twice a day remove insanity problems. Brahmi Rasayana imporves memory power when taken in a dose of 1-3 mashas along with honey and ghee (Krishnamurthy). Paste or juice of fleshy leaf and stem of brahmi were mixed with sugar, jaggery or honey to make it more palatable due to its bitter taste. Some of the known preparations with brahmi are Sarasvatarishta (a decoction used as a brain tonic), Brahmi Rasayana (a rejuvenating formulations with other herbs), Brahmi Taila (medicated oil) and Brahmi Sarbat (a cooling drink) (Prasad et al., 2008).

Brahmi has been used in Ayurvedic formulations for conditions ranging from catarrhal complaints, gastrointestinal disturbances due to excessive tobacco use, habitual abortions and high blood sugars due to anxiety disorders. In certain parts of India, brahmi is believed to be an aphrodisiac; in Sri Lanka, under the name of Loonooweella, brahmi is prescribed for fevers; in the Philippines, it is used as a diuretic (Prasad *et al.*, 2008; Russo and Borrelli, 2005).

The present study included examination of morphological and microscopical characters, powder

characters and preliminary phytochemical analysis of different plant parts (root, stem and leaf) of *Bacopa monnieri* (L.) Wettst.

MATERIALS AND METHODS

Herbarium of *Bacopa monnieri* (L.) Wettst. was prepared and authenticated from Blatter Herbarium, St. Xavier's College, Mumbai. *Bacopa monnieri* (L.) Wettst. plants were collected from Keshavsrushti, Uttan, Bhayander, Mumbai. Root, stem and leaves were separated, washed under running tap water and blotted dry. All the separated plant parts were dried in preset oven at $40 \pm 2^{\circ}$ C for about a week, grounded into powder and used for further analysis.

Anatomical Study

Fresh plant parts were used for morphological and microscopical studies. Surface preparations were used to study type and structure of stomata and trichomes and stomatal index was also calculated. The fine sections were stained by safranine and mounted in glycerine, were photographed under light microscope using 45X magnifications.

Phytochemical Analysis

Preliminary phytochemical screening of different plant parts (root, stem and leaf) of *Bacopa monnieri* (L.) Wettst. was carried out by using standard methods. Aqueous and ethanolic extracts of root, stem and leaf were used for qualitative analysis. Quantitaive estimation of secondary metabolites such as alkaloids, saponins and flavonoids in different plant parts were also carried out by using standard methods (Harborne, 1973; Khan, 2001; Boham and Kocipai method, 1994 repectively).

RESULTS AND DISCUSSION

Anatomical Study

Morphological Study

The morphological characters can be diagnostic parameters for the plant.

Roots: Creamish yellow in colour, thin tapering, wiry, small, branched and arising from the nodal region of the stem.



Figure 1: (A) Morphology of whole plant; (B) Stem with node and internode; (C) Leaves; (D) T.S. of root; (E) T.S. of stem; (F) to (G) T.S. of leaf; (H) to (J) Enlarged surface view of leaf, Keywords: E= Epidermis, Co= Cortex, Ar = Aerenchyma, Ed= Endodermis, Sg = Starch grains, Gl tr = Glandular trichome, Id= Idioblast, Caox cr = Calcium oxalate crystal, VB = Vascular bundle, Xy = Xylem, Ph = Phloem, Msl = Mesophyll, Dcy st = Diacytic stomata and Anmcy st = Anomocytic stomata.

Stem: Green or purplish-green in colour, prostrate, thick and fleshy, herbaceous, soft, with prominent nodes and internodes Figure 1 A& B].

Leaves: Simple, sessile, glabrous, opposite and decussate, obovate -oblong to spathulate in shape, apex is obtuse and margins are completely entire, 1-3 nerved or penninerved, punctate, faint green in colour Figure 1 A, B & C].

Flowers: White with violet and green bands and spangled with shining dots while fresh, short lived and colour lightens gradually, actinomorphic, solitary, axillary, bracteate, bracteoles are shorter than pedicel, pedicel is slender in shape [Figure 1 A, B & C].

Microscopical Study

Transverse section (T.S.) of root showed single layered epidermis with wide cortical arenchymatous region. Endodermis was distinct and single layered while pericycle was not differentiable. Central region was occupied by stele consisted of 1-5 layers of peripheral phloem and centrally located xylem vessels [Figure 1D].

Transverse section (T.S.) of stem showed single layer of epidermis, cortex with chlorenchymatous aerenchyma or air spaces, cortical cells with starch grains, single layered endodermis, 1-2 layered pericycle, continuous vascular ring composed of a narrow zone of phloem towards periphery and a wide ring of xylem towards centre, centrally located parenchymatous pith with simple, round to oval starch grains [Figure1 E].

Surface preparation of leaf showed the presence of two types of stomata i.e. anomocytic and diacytic [Figure1J], 8- celled sessile glandular trichomes [Figure1 H & I]. The anomocytic types of stomata were more in number in comparison to diacytic type.

Transverse section (T.S.) of leaf showed distinct upper and lower epidermis, cells of upper epidermis

Table 1: Quantitative leaf microscopy of *Bacopa monnieri* (L.) Wettst.

Parameter	Value
Stomatal index	10.21%
Stomata number	34
Number of anomocytic stomata	23
Number of diacytic stomata	11

were comparatively larger than the cells of lower epidermis and covered with striated cuticle. Presence of sub - epidermal foliar idioblasts (found in the form of empty cavity) and a centrally located conjoint, collateral vascular bundle encircled by a parenchymatous sheath were observed [Figure 1 F]. Few crystals of calcium oxalate were seen embedded in the undifferentiated mesophyll tissue [Figure 1G].

Powder characteristics

Whole plant powder showed presence of simple, round to oval shaped starch grains, anomocytic and diacytic types of stomata, prismatic calcium oxalate crystals, xylem vessels with spiral thickenings and pitted tracheids [Figure 2 A₁, A₂, A₃ and A₄].

Root powder is brown in colour. The various diagnostic characteristics of root powder are shown in Figure 2 B_1 , B_2 , B_3 and these are simple starch grains, xylem vessels with spiral thickenings and pitted xylem tracheids.

Stem powder is light brown in colour and bitter in taste. The various diagnostic characteristics of stem powder observed are xylem vessels with spiral thickenings, pitted xylem tracheids, simple, round and oval starch grains. Separate spiral rings were also observed [Figure 2 C₁, C₂, C₃ and C₄].

Leaf powder is green in colour and bitter in taste. The various diagnostic characteristics of leaf powder are the presence of anomocytic and diacytic types of stomata, prismatic calcium oxalate crystals in mesophyll tissue and simple starch grains [Figure 2 D_1 , D_2 , D_3 and D_4]. Quantitative determination of leaf content included number of both types of stomata and stomatal index was done with the help of standard methods and results are tabulated in Table 1.

Phytochemical analysis

Preliminary phytochemical analysis

The results of preliminary phytochemical analysis are tabulated in Table 2. The phytochemical analysis revealed the presence of different chemical compounds such as carbohydrates, alkaloids, saponins, flavonoids, tannins and anthroquinones. Among the different plant parts of *Bacopa monnieri* (L.) Wettst., leaves were found to contain higher concentrations of phytoconstituents (alkaloids, saponins and flavonoids)

followed by stem and root. The amount of alkaloids, saponins and flavonoids in different plant parts are described in Table 3.. The amount of alkaloids (mg/g dry weight) were found to be 53.07 ± 2.08 in leaves, 47.00 ± 0.81 in stem and 1.67 ± 0.577 in root. The

amount of saponins (mg /g dry weight) were found to be 57.00 ± 2.65 in leaves, 23.33 ± 1.53 in stem and 7.50 ± 0.71 in root while flavonoids (mg /g dry weight) were found to be 27.67 ± 2.08 in leaves and 25.33 ± 1.53 in stem.

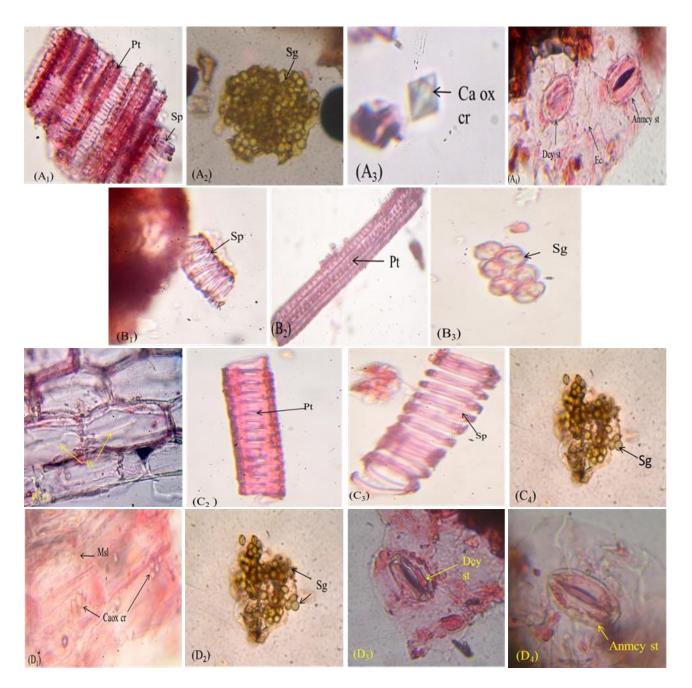


Figure 2: Powder characteristics of *Bacopa monnieri* (L) Wettst.; A₁ to A₄ whole plant; B₁ to B₃ root; C₁ to C₄ stem; D₁ to D₄ leaf

Keywords: Sg= Starch grains; Ec= Epidermal cell; Pc= Parenchymatous cells; Msl=Mesophyll; Pt = Pitted tracheid; Sp = Spiral thickening; Caox cr = Calcium oxalate crystal; Dcy st =Diacytic stomata; Anmcy st = Anomocytic stomata.

Table 2: Phytochemical constituents in different plant parts (root, stem & leaf) of Bacopa monnieri (L.) Wettst.

Tests	Name of the	Root		Stem		Leaf	
	Method /test	WE	EE	WE	EE	WE	EE
Carbohydrates	Anthrone test	-	+	+	+	+	+
Proteins	Folin – Lowry test	-	+	-	+	-	+
Alkaloids	Harborne's method	-	+	-	+	-	+
Saponins	Harborne's Froth and	+	+	+	+	+	+
	Emulsion tests	т	т	т	т.	т	т.
Flavonoids	Harborne's method	-	+	+	+	+	+
Tannins	Harborne's method	-	+	+	+	+	+
Terpenes and steroids	Harborne's method	-	-	-	-	-	-
Glycosides	Harborne's method	-	-	-	-	-	-
Anthroquinone	Harborne's method	-	+	+	+	+	+

Keys: WE = Water extract, EE = Ethanolic extract, + = Present, - = Absent

Table 3: Quantitative analysis of Bacopa monnieri (L.) Wettst. different plant parts

Phytoconstituents	Different plant parts				
(mg / g dry wet.)	Root	Stem	Leaf		
Alkaloids	1.67 ± 0.577	47.00 ± 0.81	53.67 ± 2.08		
Saponins	7.50 ± 0.71	23.33 ± 1.53	57.0 ± 2.65		
Flavonoids	0.0	25.33 ± 1.53	27.67 ± 2.08		

All values are mean of three determinants and expressed as Mean ± S.D.

CONCLUSION

Bacopa monnieri (L.) Wettst. is a popular medicinal plant, which is being used for various medicinal properties. Microscopic identification of medicinal plant is essential to distinguish any adulterants or other similar species in the crude herbal mixtures. Microscopic characteristic findings of different plant parts of Bacopa monnieri may be used as anatomical markers to identify the plant and its plant parts. The anatomical markers for root are presence of simple starch grains, xylem vessels with spiral thickenings and pitted tracheids; for stem are presence of xylem vessels with spiral thickenings, pitted xylem tracheids, simple, round and oval starch grains and for leaf are presence of anomocytic and diacytic types of stomata, upper and lower sub - epidermal idioblasts, sessile, multicellular glandular trichome, prismatic calcium oxalate crystals, simple and round starch grains. All the above mentioned anatomical markers were also observed in whole plant powder of Bacopa monnieri (L.) Wettst.

In the present study, two types of stomata i.e. anomocytic and diacytic in a same field of vision have been observed in *Bacopa monnieri* (L.) Wettst. leaf,

under 45 X magnification of light microscope and this is the major finding. Anomocytic type of stomata were observed more in number in comparison to diacytic stomata. The stomatal index and number of anomocytic and diacytic types of stomata were found to be 10.21%, 23 and 11 respectively. Presence of two types of stomata in a single plant may be the result of evolutionary changes or environmental changes.

Another distinguishing feature of the Bacopa leaf was the presence of sub- epidermal foliar idioblasts. These were in the form of air spaces. Idioblasts are isolated plant cells which differ from neighbouring cells or tissues and contain non-living substances such as oil, latex, gum, resin, tannin or pigments etc. Some can contain mineral crystals such as calcium oxalate or carbonate or silica. They have various functions such as storage of reserves, excretory materials, pigments and minerals. Karrfalt and Tomb (1983) described idioblast as "air spaces" which were irregular in shape and lacked an epithelium. Presence of foliar idioblasts in the form of air spaces were also reported in the other members of family Scrophulariaceae, such as Scrophularia and Verbascum genera (Lersten and Curtis, 1997).

These anatomical markers can be used authentication and for quality assurance of Bacopa monnieri for Herbal medicine manufacturing phytochemical industries. Results obtained from analysis could make the plant useful for treating different diseases and having a potential of providing useful drugs for human use. All these findings can also serve as an important source of information to ascertain the identity and to determine the presence of adulterants from the raw material of *Bacopa monnieri* (L.) Wettst. as well as plant parts used in it.

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