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COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION OF TWO PLANT SPECIES VALUED AS MEDHYA RASAYANAS.

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ABSTRACT : *Centella asiatica* and *Bacopa monnieri widely known as* Brahmi are considered as 'Medhya rasayanas' in Ayurveda meaning brain tonic. However, these plants are distinctly different. The name Brahmi, Jala-brahmi or water-brahmi is assigned to *Bacopa monnieri whereas* "mandukaparni", often confused with Brahmi, refers to *Centella asiatica*. A detailed investigation of whole plant and roots of both the crude drugs was carried out. The diagnostic characteristics includes presence of stomata, vessels with spiral thickening, rosette crystals of calcium oxalate in *Centella asiatica* and xylem vessels with reticulate thickening, glandular hairs, simple, round and oval starch grains, measuring 4-14 µ in diameter in *Bacopa monnieri*. Various physicochemical parameters were also established. Phytochemical screening revealed the presence of many therapeutically important classes of phytoconstituents such as glycosides, alkaloids, saponins, protiens and carbohydrates. The present work was undertaken to generate information regarding relevant pharmacognostical, phytochemical and physicochemical data needed for proper identification and authentification of two crude drugs belonging to two different families, mainly focusing and exploring the aspects leading to common effect of improving memory.

Key words: Brahmi, Mandukaparni , Medhya rasayanas, memory, tonic

INTRODUCTION

In India, *Bacopa monnieri*, known as "Brahmi", is revered in the indigenous system of medicine as a nerve tonic. In early literature, the name Brahmi was also used to refer to another plant species, *Centella asiatica* Linn., known as Indian penniwort[1]. However, these plants are distinctly different. The name Jala-brahmi or water-brahmi assigned to *Bacopa monnieri* in ancient Sanskrit writings provides the differentiation. Brahmi is found in marshy areas near streams and ponds throughout India especially in the North eastern regions. It is found at an elevations from sea level to altitudes of 4,400 feet. It is found in `Uttar Pradesh, Punjab, Haryana, Bihar, Bengal, Tamil Nadu, Kerala, Karnataka, Foot hills of Himachal Pradesh & Uttaranchal[2]. The vernacular name "mandukaparni", often confused with Brahmi, in fact refers to *Centella asiatica*. Mandukaparni is commonly found as a weed in crop fields and other waste places throughout India up to an altitude of 600 m. It is abundant in tropical and subtropical regions particularly in damp, shady places along marshy banks of rivers, streams, ponds, irrigated fields. In Himalayas, it grows wild in natural habitat all round the year[3]. The Charaka Samhita considers them both to be promoters of cognitive functions, but it suggests that Brahmi is superior to mandukaparni. Chemically both species are rich in saponins. Madecassoside and Asiaticoside are the important saponins of *Centella asiatica* whereas *Bacopa monnieri* contains bacosides A and B having biological activity[4]. Both are medhya rasayanas or 'mental rejuvenatives[5]. Brahmi is used to treat specific mental disorders such as insanity and epilepsy[6], while mandukaparni is a general rejuvenative tonic which improves mental health[7]. Brahmi promotes fertility and sustains implantation of the embryo in the uterus[8], while mandukaparni tends to reject the embryo[9]. This suggests that the plant materials have opposite effects on uterine functions.

The Sushruta Samhita defines the properties of the herbs. Brahmi belongs to tikta rasa (bitter), while mandukaparni belongs to kasaya rasa (astringent). mandukaparni is cooling, making it better for pitta, whereas Brahmi is warming, indicated in kapha/vata. mandukaparni is also indicated in skin issues and for wound-healing, whereas Brahmi has additional properties for helping throat and lung[10,11]. The information generated by this particular study will generate relevant morphological, anatomical, macroscopical, microscopical, physicochemical, phytochemical data of plants to confirm identification and authentification of plants of these particular species for the benefit of taxonomists as well as common man.

The scientific classification of the plants is mentioned in Table 1[12].

Table 1: Plant Profile

Scientific Classification	*Centella asiatica*	*Bacopa monnieri*
Kingdom	Plantae	Plantae
Division	Angiospermae	Angiospermae
Class	Dicotyledonae	Dicotyledonae
Order	Umbelliferae	Tubiflorae
Family	Apiaceae	Scrophulariaceae
Genus	*Centella*	*Bacopa*
Species	*Asiatica(Linn)*	*monnieri(Linn)*
Synonym	*Hydrocotyle asiatica(Linn)*	*Herpestis monnieria(Linn)*
Common names	*Mandukaparni, Indian pennywort,Gotu Kola*	*Brahmi, Nira-brahmi, Jalabrahmi, Thyme-Leaved Gratiola*

MATERIALS AND METHODS

The whole plants of *Centella asiatica* and *Bacopa monniera* were procured from Zandu Foundation Valsad District (Gujarat) in the month of August and September and authenticated by Botany Department, The Zandu Pharmaceutical Works Ltd. (Now Emami Limited). Vapi, Gujarat, India. A voucher specimen (14474-Mandukaparni, 14708-Brahmi) was retained for further references.
Fresh entire plant of brahmi and mandukaparni were used for pharmacognostic evaluation and some preliminary phytochemical tests. For the anatomical study, leaf, petiole, stem, root of fresh plants were collected, sectioned and stained with safranin[13]. Powder of the whole plants was used for the observation of powder microscopic characters[14].
Photomicrographs were obtained by observing free hand sections of plant parts under Digital Binocular Motic microscope (DMB series PAL System) with built in analogue camera. Computer Images were captured using Motic Image Software (VQCI038). Physicochemical constants, Phytochemical analysis, and chromatographic studies were carried out from shade-dried powder following prescribed methods[15-18].

RESULTS

MACROSCOPY

Mandukaparni herb consists leaves, stems, and very few small flowers and fruits, odour strong, faintly aromatic, taste- pungent. It is a prostrate, perennial herb with slender, glabrous, pink stem, rooting at nodes giving rise to thin, brownish-grey, roots of about 2.5 to 6.0 cm in length; leaves 1 to 3 from each node, orbicular-reniform, crenate, base cordate, petioles channelled with adnate stipules; flowers fascicled umbels each carrying 3 or 4 flowers, short stalked; fruits are oblong, dull brown, with laterally compressed seeds, the pericarp hard, thickened, woody and white. [Figure 1-A, B, C]

Figure 1: Mandukaparni A- Habitat; B- Entire Plant ; C- Leaf

Brahmi is the small creeping herb with the numerous branches. It grows to a height of 2 -3 feet and its branches are 10 -35 cm long. Roots are thin, wiry, small, branched creamish-yellow. Stem is thin, green or purplish green, about 1-2 mm thick, soft, nodes and internodes prominent, glabrous; taste, slightly bitter. Leaf is simple, opposite, decussate, green, sessile, 1-2 cm long, obovate-oblong; taste, slightly bitter. Flowers are Small, axillary and solitary, five petaled white- purple in colour, pedicels 6-30 mm long, bracteoles shorter than pedicels. Fruits are capsules upto 5 mm long, ovoid , glabrous and sharp at apex.

Figure 2: Brahmi D- Habitat; E- Entire Plant: F- Leaf

MICROSCOPY

Mandukaparni shows greyish green leaves with stomata on both surfaces, 30 by 28µm, mostly rubiaceous type. Palisade cells differentiated into two layers of cells, 45 by 25µm; spongy parenchyma of about three layers of cells with many intercellular spaces, some with crystals of calcium oxalate; Small-sized leaves usually hug the ground and have a short petiole stem; however, large leaves can have a petiole up to 20cm long. When plants are grown in the shade, they tend to have large leaves and very long petioles. This petiole stem can have a pink/purple tinge. petiole shows epidermis with thickened inner walls; collenchyma of two or three layers of cells; a broad zone of parenchyma; seven vascular bundles within parenchymatous zone, two in projecting arms and five forming the central strand; vessels 15–23µm in diameter. Some parenchymatous cells contain crystals of calcium oxalate.

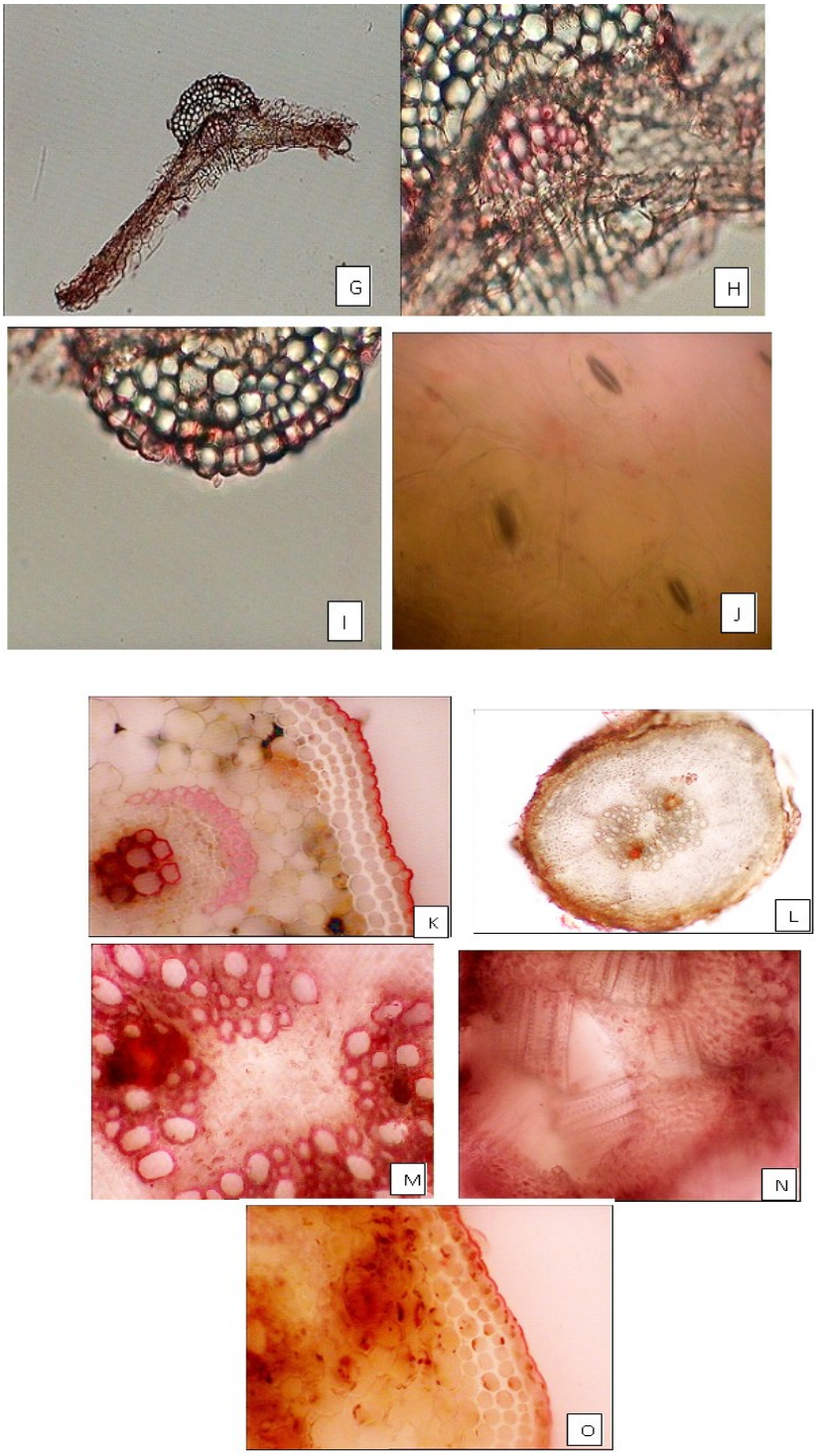

Figure 3: Mandukaparni :- G-T.S. of leaf, H- leaf vascular bundles, I- leaf collenchyma cells, J- leaf stomata, K- T.S of leaf Petiole, L- T.S of Root, M- T.S of root showing Secondary growth, N- T.S. of root showing reticulate & spiral vessels, O- T.S. of stem.

Midrib region shows two or three layers of parenchymatous cells without chloroplastids. Pink flowers 5mm across, usually set two to four, side by side, as an umbel, developing from the stem nodes. Flowers are so small and often hidden underneath leaves. Fruits, shows several ridges in outline, epidermis of polygonal cells, each ridge having a vittae and patch of sclerenchyma, trichomes similar to the leaves, sheets of elongated parquetry layer cells, bundles of narrow annular vessels, and parenchymatous cells contain single large prisms of calcium oxalate. Macerates show presence of vessels with spiral, annular and reticulate thickenings. [Figure 3- G- O]

Transverse section of Brahmi root shows a single layer of epidermis, cortex having large air cavities, endodermis single layered, pericycle not distinct; stele consists of a thin layer of phloem with a few sieve elements and isolated material from xylem shows vessels with reticulate thickenings. Stem shows single layer of epidermis followed by a wide cortex of thin-walled cells with very large intercellular spaces, endodermis single layered, pericycle consisting of 1-2 layers; vascular ring continuous, composed of a narrow zone of phloem towards periphery and a wide ring of xylem towards centre; centre occupied by a small parenchymatous pith with distinct intercellular spaces, starch grains simple, round to oval, present in a few cells of cortex and endodermis. Leaf shows upper and lower epidermii, the cells of the upper is bigger in size and covered with striated cuticle, both the epidermii shows stomata and sessile glandular trichomes with multicellular head. A narrow collenchymatous band is located below both the epidermis of the midrib, a centrally located conjoint collateral vascular bundle encircled by a parenchymatous sheath is placed in the centre. The mesophyll is spongy parenchymatous with vascular strands, prismatic crystals of calcium oxalate are embedded throughout the parenchymatous cells of the leaf. No distinct midrib present. [Figure 4- P-A1].

POWDER MICROSCOPY

The powder of *Mandukaparni* is greyish green with characteristic odour and slightly bittersweet taste. When powder was mounted with chloral hydrate, phloroglucinol and HCl, stained with safranin it shows fragments of epidermal cells polygonal in surface view with stomata, palisade cells, vessels with spiral, reticulate and annular thickening, microsphenoidal and rosette crystals of calcium oxalate; simple, oval to round starch.[Figure 5-A2, A3]

DISCUSSION

The physicochemical studies showed the average value of moisture content of mandukaparni and brahmi 6.7 % w/w and 5.8 % w/w indicating presence of high moisture content in mandukaparni. [Table 2]. Total ash and acid insoluble were found in higher amount in mandukaparni (2.1 % w/w and 1.9 % w/w) indicating presence of inorganic radicals like carbonates, phosphates, silicates, silica etc. The higher acid insoluble ash value indicates the contamination with siliceous materials like earth and sand. The water soluble extractive value of both the crude drugs were found almost similar but the alcohol soluble extractive value of brahmi (14.54% w/w)was found higher than mandukaparni (12.60 % w/w). The pHs of aqueous solutions of both the crude drugs were found to be almost neutral. Phytochemical analysis of different solvent extracts showed presence of saponins, glycosides, carbohydrates, proteins in both crude drugs. Brahmi also showed presence of alkaloids [Table 3, 4].

Figure 4: Brahmi :- P- TS of root; Q- TS of root showing cork cells and air spaces; RTS of root showing starch grains; S- Root Vascular Bundles; T- TS of Stem showing Single layered epidermis covered with thin layer cuticle; U- TS of Stem showing Vacular Bundle ring with parenchymatous pith; V-TS of Leaf; W-TS of Leaf showing upper bigger epidermis cells; X- TS of Leaf showing lower smaller epidermis cells; Y- Vascular bundle of leaf; Z- Leaf Stomata; A1- Leaf Trichome

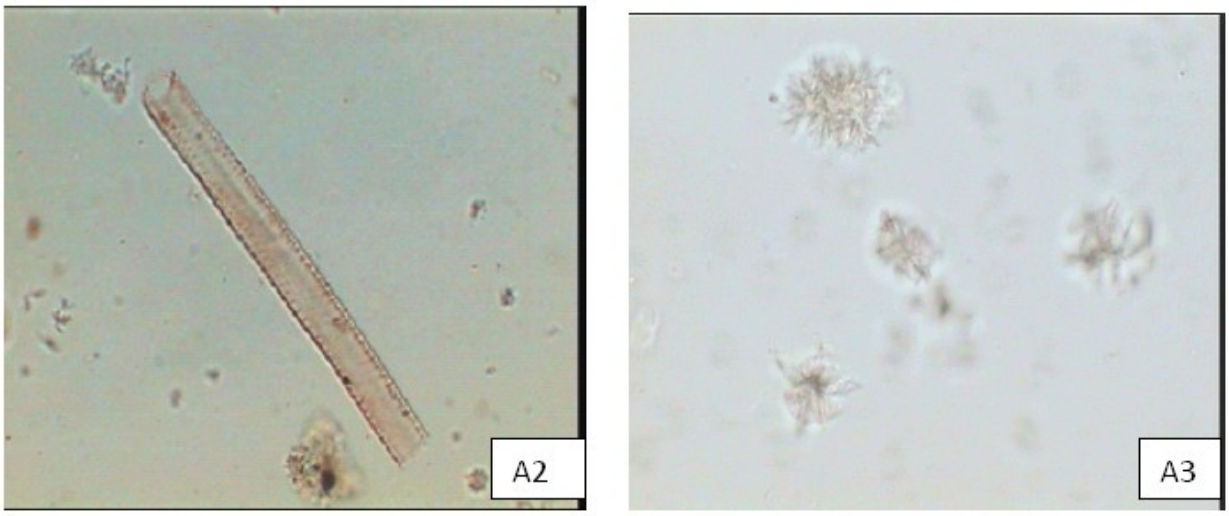

Figure 5: A2- Spiral vessels; A3- Rosette crystals of calcium oxalate

The powder of Brahmi shows xylem vessels with reticulate thickening, glandular hairs, simple, round and oval starch grains, measuring 4-14 µ in diameter. [Figure 6 -A4-A6]

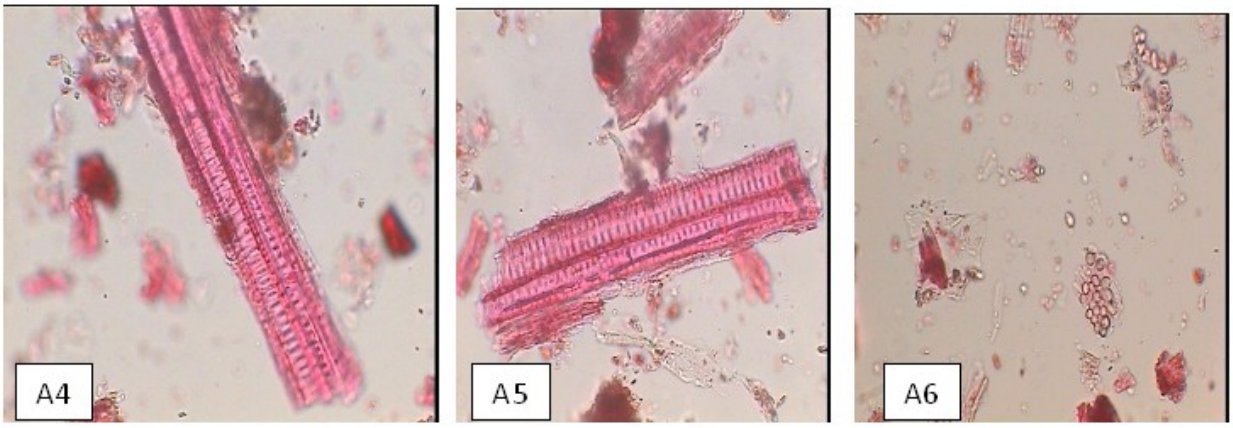

Figure 6: A4,A5 -Xylem vessels with reticulate thickening; A6- Round and oval starch grains

Table 2: Physicochemical Analysis of *Centella asiatica* and *Bacopa monnieri*

Physicochemical parameters	***Centella asiatica* value**	***Bacopa monnieri* value**
Total Ash	2.1 % w/w	1.9 % w/w
Acid insoluble ash	1.9 % w/w	1.3%w/w
Water soluble ash	1.73 % w/w	1.4%w/w
Water soluble extract	20.25 % w/w	20.95%w/w
Ethyl alcohol soluble extract	12.60 % w/w	14.54%w/w
Moisture content	6.7 % w/w	5.8% w/w
pH	6.7	6.9

Table 3. Phytochemical Analysis of *Bacopa monnieri*

Phytoconstituents	Pet.Ether	Methanol	Water	Ethyl acetate	Chloroform
Carbohydrates	+	+	+	+	+
Protiens	+	+	+	+	+
Amino acids	−	−	−	−	−
Glycosides	+	+	+	+	+
Alkaloids	+	+	+	+	+
Flavanoids	−	−	−	−	−
Tannins	−	−	−	−	−
Saponins	+	+	+	+	+
Steroids	−	−	−	−	−
Resins	−	−	−	−	−

+ = Present − = Absent

Table 4. Phytochemical Analysis of *Centella asiatica*

Phytoconstituents	Pet.Ether	Methanol	Water	Ethyl acetate	Chloroform
Carbohydrates	+	+	+	+	+
Protiens	+	+	+	+	+
Amino acids	−	−	−	−	−
Glycosides	+	+	+	+	+
Alkaloids	−	−	−	−	−
Flavanoids	−	−	−	−	−
Tannins	−	−	−	−	−
Saponins	+	+	+	+	+
Steroids	−	−	−	−	−
Resins	−	−	−	−	−

+ = Present − = Absent

CONCLUSION

Pharmacognostic, phytochemical and physicochemical evaluation *of Centella aiatica* (whole plant) and *Bacopa monnieri* (whole plant) provided specific parameters that will be useful in scientific evaluation, identification and authentification of both the crude drugs.

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