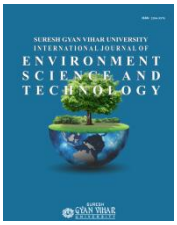

SGVU International Journal of Environment, Science and Technology

E-ISSN: 2394-9570

Vol.8 Issue 2 Page No 1-12

Research article

Pharmacognostical and Phytochemical Investigation on *Bacopa Monnieri* leaves

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Keywords

Bacopa Monnieri
Solvent,
Methanol,
Dichloromethane**.**

Abstract

A microscopic, macroscopic, physico-chemical, and phytochemical analysis was carried of the aerial part of *Bacopa Monnieri (L.) or Brahmi* to establish a monograph. In current research study the leaves of *Bacopa Monnieri* were collected and powdered. Methanolic and dichloromethane extracts were prepared by using successive cold maceration process and subsequently fractioned with different solvents for detailed chemical analysis. Fluorescence characters of different successive extracts and powder were noted under ultraviolet and under normal ordinary light, which signifies their characteristics. The total ash value, acid insoluble value, water soluble value and sulphated ash value were 9.5, 5.0, 4.5 and 8.0% respectively. The percentage yield of extractive values for water soluble, alcohol soluble and petroleum ether were 9.0, 2.0 and 2.0% respectively. Reaction behaviour of leaves with various reagents were also noted, which revealed characteristic development of particular color. Preliminary qualitative chemical tests of powered drug was found positive for phytosterol, flavonoids, carbohydrate, Proteins and amino acid, tannin and phenolic compounds, In pharmacognostical study of this plant, various parameters are evaluated like microscopical evaluation in which the observed histological characters are straight walled epidermis, Spiral vessels, Reticulate vessels, Vein-lets, Palisade cells, Unicellular covering trichome, Phloem fibers, Anmocytic stomata, Prismatic calcium oxalate crystals

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Introduction

In the last few decades there has been an exponential growth in the field of herbal medicine. (Kritikar & Basu, 1995) It is getting popularize in developing and developed countries owing to its natural origin and lesser side effects. In older times, vaidyas used to treat patients on individual basis, and prepared drugs according to the requirement of the patients (Ali, 1994). But the scene has been changed now; herbal medicines are being manufactured on a large scale in mechanical units, where manufactures are facing many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of drugs and formulations, quality control parameters and etc.

Bacopa monnieri (L.) Wettst. (Commonly known as *Brahmi*) belonging to the family Plantaginaceae is a perennial herb and has been used as Ayurvedic medicine for treatment of a brain tonic and memory enhancer for hundreds of years. Its chemical constituents include various classes of chemical groups such as flavonoids (Bhandari, et al., 2007), sterols (Bhandari, et al., 2006), and saponins (Nuengchamnong, et al., 2016; Sivaramakrishna, et al., 2005). The steroidal saponin glycosides are major compounds of this plant responsible for memory and cognition enhancement as reported in literature; e.g. bacoside A3, bacopasaponin C, and bacopasides I, II, and X (Deepak, et al., 2005; Le et al., 2015; Ganzera, et al., 2004; Singh, et al., 1988; Zhou, et al., 2009). Several trials are carried out on the standardized extract of Brahmi which indicates that the extract also improve the cognitive functioning (Peth-Nui et al., Kongkeaw, et al., 2014; 2012; Stough et al., 2001). Brahmi is also used as neuroprotective (Hosamani & Muralidhara, 2009; Limpeanchob, et al., 2008; Uabundit, et al., 2010), anti-oxidant (Kapoor, et al., 2009; Simpson, et al., 2015), and anti-inflammatory effects (Nemetchek, et al., 2017).

As per the ethnopharmacological data and scientific studies, several Brahmi products have been developed and marketed as food supplements, around the world. To ensure the plant raw material quality for their uses in industry standardization and quality control procedures of Brahmi are needed. Certain reports on a pharmacognostic study of Brahmi stem (Mishra, et al., 2015) and monographs i.e. the British Pharmacopoeia (BP) (British Pharmacopoeia Commission, 2014), the Ayurvedic Pharmacopoeia (AP) (Government of India, 2001) and the United States Pharmacopeia (USP) (The

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United States Pharmacopeial Convention, 2017) have been published. Mishra et al. (2015) did not include the most used aerial part (stem and leaves) of Brahmi in Brahmi products. The specific method did not described by the AP (Government of India, 2001) for either qualitative analysis (such as TLC) or quantitative analysis of the active compounds in Brahmi. Besides that, Brahmi is a plant and widely distributed in many parts of the world including North America, Africa, Australia, and Asia, the quality evaluation of Brahmi from various geographical origins is necessary.

The present study is helpful in filling the needs to establish a new standard method which covers Brahmi from different geographical areas. Various samples have been collected from different regions of Rajasthan, India. For identification and standardization of Brahmi material certain analysis like microscopic, macroscopic, physico-chemical and phytochemical of Brahmi were carried out and established the necessary parameters.

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MATERIALS AND METHODS

Plant material collection and authentication:

The mature green leaves of *Bacopa Monnieri* were collected locally from Jaipur District, Rajasthan, India in the month of July 2019. The plant was identified and authenticated by Central drug research institute (CDRI) Lucknow U.P. and deposited in the Rajasthan University India.

Drying and size reduction of leaves:

The leaves of *Bacopa Monnieri* were subjected to shed drying and further crushed to powder, and then the powder is passed through the mesh 22 and stored in air tight container for further use (As suggested by Phrompittayarat et al., 2011).

Pharmacognostical studies:

Macroscopic: Morphological studies of *Bacopa Monnieri* were done. The shape, apex, base, margin, taste, and odour were determined. (Table 1) (As suggested by Phrompittayarat et al., 2011). Botanical evaluation is illustrated in table 2.

Microscopic:

Microscopic studies were done by preparing a thin hand section of midrib and lamina region of *Bacopa Monnieri* leaf. The section was cleared with chloral hydrate solution, stained with phloroglucinol and hydrochloric acid, and mounted with glycerin. A separate section was prepared and stained with iodine solution for the identification of starch grains. Powder of the dried leaves was used for the observation of powder microscopical characters. The powered drug was separately treated with

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phloroglucinol and HCL solution, glycerin and iodine to determine the presence of lignified cells, calcium oxalate crystals, trichomes and starch grains (As suggested by Phrompittayarat et al., 2011). Results are depicted in table 3 and 4.

Determination of physicochemical parameters:

The leaves of *Bacopa Monnieri* were subjected to following physicochemical parameters.

Loss on drying, Total Ash value, acid insoluble, water soluble, sulphated ash value, Ethanol soluble, water soluble and Petroleum ether soluble extractives and PH determination. The results of physicochemical parameters are reported in tables below (As suggested by Phrompittayarat et al., 2011). Result is depicted in table 5.

Extraction of *Bacopa Monnieri* leaves: (WHO) guidelines (World Health Organization, 2011). The shade dried and 22 mesh powdered leaves of *Bacopa Monnieri* were subjected to successive cold extraction with Dichloromethane, methanol so as to get respective 1.98%w/w and 2.5%w/w extracts after due filtration and evaporation to dryness and stored in a desiccators over anhydrous Ca hydroxide for further use. Results are depicted in table 6.

Preliminary phytochemical screening of powdered leaves: (WHO) guidelines (World Health Organization, 2011).

Test for carbohydrates, gums and mucilage, proteins and amino acids, alkaloids, glycosides, phytosterol, flavonoids, tannin and phenolic compounds, saponins and volatile oil were carried out, results are displaced in table 7.

Treatment of the powder drug with different chemical reagents: (WHO) guidelines (World Health Organization, 2011).

The powdered drug was subjected to various chemical reagents and noted under ultraviolet and under normal ordinary light, which signifies their characteristics. Behaviour of leaves of *Bacopa Monnieri* with different chemical reagent was tabulated in table 8.

Fluorescence analysis of powder drugs: (WHO) guidelines (World Health Organization, 2011).

Fluorescence characters of powdered drug were noted under ultraviolet and under normal ordinary light, which signifies their characteristics.

RESULT AND ANALYSIS

This studied were followed evaluation of drugs by colour, odour, taste, size, shape and special features like touch, texture etc. In macroscopical studies fresh *Bacopa Monnieri* was selected for

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evaluation with the help of human eye and scale. In case of microscopic studies of *Bacopa Monnieri*, a fresh part was taken and cut it after that inner gel was carefully separated from plant part outer layer by using sharp knife. Now within 1 hour of separation all parameters of macroscopical studies were examined. Table 1 consist of complete description of macroscopic properties for *Bacopa Monnieri.*

Table 1.Morphological features of *Bacopa Monnieri* Leaf

Sr. No.	Features	Observation
1	Upper surface	Dark green color
2	Lower surface	Light green color
3	Odour	Characteristic
4	Taste	Characteristic
5	Shape	Dorsiventral
6	Size	5.1-7.6x2.5-4.5cm

Several fragments of multicellular tissues from different Brahmi samples were identified by the microscopic study of the powdered Brahmi.

In each sample, six main components usually found are parenchyma with starch grains, anomocytic stomata, epidermis, lignified fibers, scalariform vessels and sieve tubes. Both, powdered Brahmi and its fresh leaf were have the similar shape and size of the guard cells of the stoma. All these components can be utilised to identify and authenticate the Brahmi material in combination with other parameters. It is noted that no trichome were found, which conflicts with the monograph of Brahmi in the BP.

For this examination powders of leaves of leaves of *Bacopa Monnieri* were taken on watch glass, wet it with water. Very small quantity of that powders were put and spread on glass slide with the help of brush, at last mounted with glycerin, covered with cover slip and gone for examination process under compound microscopy 10x and 45x. For the examination of lignified characters, starch grains, calcium oxalate crystals and other specific characters, different chemical reagents were used. Table 2 contents detail description of microscopic features shows microscopic features of leaves of *Bacopa Monnieri* respectively.

Table 2.Botanical evaluation of *Bacopa Monnieri* leaf

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Sr. No.	Leaf Portion	Observation
1	Apex	Acuminate
2	Margin	Crenate-Serrate
3	Shape	Oblong or Ovate
4	Lamina Surface	Entire
5	Dorsal surface	Glabrous
6	Ventral surface	Smooth Shining
7	Petiole	
8	Size	2.5-5mm
9	Shape	long minutely pubescent
10	Colour	Light green colour
11	Leaf Base	Symmetrical

Qualitative Microscopy

Examination by Transverse Section (TS)

For this examination fresh, mature and washed leaves of *Bacopa Monnieri* was taken and very thin transverse sections were cut with the help of sharp blade and put in the clean glass plate filled with water. After that a very thin section was selected, put on glass slide, mounted with glycerin, covered with cover slip and at last gone for examination process under compound microscopy at 10x. For the examination of lignified characters, the section was treated with few drops of phloroglucinol solution (1:1 of HCL: Phloroglucinol) (Nuengchamnong, et al., 2016; Sivaramakrishna, et al., 2005).

After application of same procedure for microscopy by transverse section (TS), a clear microscopic image of TS view of leaves of *Bacopa Monnieri* have found. Table 3 contents detail description of microscopic features shows T.S of leaves of *Bacopa Monnieri* leaves respectively.

Table 3. Transverse section of leaf

Sr. No.	Features	Observation
1	Trichomes	Unicellular covering trichome
2	Upper epidermis	Covered with cuticle and multicellular hairs
3	Midrib	Hypodermis is made up of Collenchyma
4	Lamina	It is differentiated into upper Palisade and lower spongy tissue
5	Vascular bundles	Xylem towards upper epidermis.
6	Collenchyma cells	Present
7	Parenchyma	Spongy

For this examination powders of leaves of leaves of *Bacopa Monnieri* were taken on watch glass, wet it with water.

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Very small quantity of that powders were put and spread on glass slide with the help of brush, at last mounted with glycerin, covered with cover slip and gone for examination process under compound microscopy 10x and 45x. For the examination of lignified characters, starch grains, calcium oxalate crystals and other specific characters, different chemical reagents were used. Table 4 contents detail description of microscopic features shows microscopic features of leaves of *Bacopa Monnieri* respectively (Bhandari, et al., 2007).

Table 4. Powder Microscopy

Sr. No.	Features	Observation
1	Nature	Coarse powder
2	Colour	Dark green
3	Odour	Slightly characteristic
4	Taste	Slightly characteristic
Microscopic		
5	Straight walled epidermis	Present
6	Spiral vessels	Present
7	Reticulate vessels	Present
8	Vein-lets	Present
9	Palisade cells	Present
10	Unicellular covering trichomes	Present
11	Phloem fiber	Present
12	Anomocytic stomata	Present
13	Prismatic Ca oxalate crystal	Present

The moisture content of drug should be determined and should be controlled in order to prevent decomposition of crud drug either due to chemical or microbial contamination.

According to procedure for determination of moisture content by %LOD, accurately weighted 5 mg of powdered crud drug was put in plate and dry in the oven at 100 Ċ or 105Ċ for duration of 15 min. after that plate was take out from oven and kept into desiccators for cooling without any air or moisture contacts to the drug followed by weighting of that. The same procedure was repeated until two same values of drug weight were not come. At last percentage of loss of drying was calculated (Table 5).

Table 5. PH Determination of *Bacopa Monnieri* leaves

Ph	1% Solution	10% solution
	7.14	7.26

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Preliminary phytochemical screening includes alcohol soluble, water soluble extractive values and phytochemical characterization. Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values (as shown in table 6) indicate the nature of the constituents present in a crude drug. The detection of active principles in medicinal plants plays an important role in phytochemical investigation of crude plant extracts and is very important in regards to their potential pharmacological affects.

Table 6. A. Extractive values, B. Moisture content, C. Ash values of *Bacopa Monnieri* leaves

Sr. No.	Parameters	Determined value % w/w
A 1	**Water soluble extractive**	**9.0**
2	Alcohol soluble extractive	2.0
3	Petroleum ether extractive	2.0
B	**Moisture content**	**15**
C 1	**Total ash**	**9.5**
2	Acid insoluble ash	5.0
3	Water soluble ash	4.5
4	Sulphated ash	8.0

All the above values are agreed with those stated in the BP, except for the ethanol-soluble extract that is not listed in the BP. The values given in AP and USP are differ from those listed in BP and are observed in current study. This presumably reflects different geographic origins of Brahmi samples and its varieties (Bhandari, et al., 2007).

Chemical Analysis

All drugs show their own colour reaction when treated with different chemicals and this resulting color reaction will be utilized in identification of drugs. Table 7 shows response of powder of leaves of *Bacopa Monnieri* on chemical analysis.

Table 7. Phytochemical analysis of *Bacopa Monnieri* leaves

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Sr. No.	Reagents	Constituent Treatment	
		Under ordinary light	under ultra violet
1	Powder	Green	Dark green
2	Powder + conc. HCl	Dark green	Dark brown
3	Powder + conc.HNO_3	Greenish brown	Reddish brown
4	Powder + conc H_2SO_4	Greenish brown	Blackish green
5	Powder + Glacial acetic acid	Grayish green	Blackish green
6	Powder + 5%NaOH	Greenish brown	Greenish brown
7	Powder + 5%KOH	Greenish brown	Greenish brown
8	Powder + 5% ferric chloride	Blackish green	Blackish green
9	Powder + picric acid (saturated solute)	Yellowish green	Yellowish green
10	Powder + Ammonia	Dark green	Dark green

Sr. No.	Constituent	Under ordinary light	under ultra violet
1	Carbohydrate	-	-
2	Gums and mucilage	-	-
3	Proteins and amino acids	+	+
4	Alkaloids	-	-
5	Glycosides	-	-
6	Phytosterol	+	+
7	Flavonoids	+	+
8	Tannin and phenolic compounds	+	+
9	Saponins	-	-
10	Volatile oil	-	-

Table 8. Treatment of the powder drug with different chemical reagents:

Both primary and secondary metabolites of plants are known as phytochemical constituents. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites. So, for determination and identification of any drug its required does complete phyto chemical study. Table 8 consists of the observations of phytochemical screening tests of leaves of *Bacopa Monnieri*. The physico-chemical and pharmacognostical characteristics of Brahmi have been identified and characterized. Present methods are superior to those used by AP, BP, and USP, and are well suited in terms of standardization and quality control of Brahmi and its products. Application of these characteristics of Brahmi and its products will improve the reliability of Brahmi-based pharmaceuticals and also improve the reproducibility of Brahmi research studies.

CONCLUSION

Present work was undertaken to lay down microscopic and physicochemical standards of *Bacopa Monnieri* leaves, which can be used to identify the genuine drug. Microscopical and phytochemical characters are discussed as distinguishing parameters to identify and decide the authentication of this herbal drug .They can be included as microscopic standards in Indian Pharmacopoeia. The plant, *Bacopa Monnieri* leaves are worth for further chemical isolation and pharmacological investigation

Funding:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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REFERENCES

1. Bhandari, P., Kumar, N., Gupta, A. P., Singh, B., & Kaul, V. K. (2007). A rapid RP-HPTLC densitometry method for simultaneous determination of major flavonoids in important medicinal plants. *Journal of Separation Science*, 30(13), 2092-2096. doi: 10.1002/jssc.2007 00066.
2. Bhandari, P., Kumar, N., Singh, B., & Kaul, V. K. (2006). Bacosterol glycoside, a new 13,14-seco-steroid glycoside from *Bacopa monnieri*. *Chemical and Pharmaceutical Bulletin*, 54(2), 240-241.
3. British Pharmacopoeia Commission. (2014). *British Pharmacopoeia* Volume-IV (Seventh Edition ed. Vol. 6). London: The Stationery Office.
4. Chonlasit, K., Julsrigival, S., & Janjom, S. (2015). Chemical and physical specifications of Kot Namtao. *Bulletin of the Department of Medical Sciences*, 57(2), 352-362.
5. CK Kokate (1994). Practical Pharmacognosy, 4th Edition (Vol. 22), V*allabh prakasan*, Delhi.
6. Deepak, M., Sangli, G. K., & Amit, A. (2005). Quantitative determination of the major saponin mixture bacoside A in *Bacopa monnieri* by HPLC. *Phytochemical Analysis*, 16, 24-29.
7. Ganzera, M., Gampenrieder, J., Pawar, R. S., Khan, I. A., & Stuppner, H. (2004). Separation of the major triterpenoid saponins in *Bacopa monnieri* by high-performance liquid chromatography. *Analytica Chimica Acta*, 516(1–2), 149-154. doi:10.1016/j. aca.2004.04.002
8. Government of India. (2001). *The Ayurvedic Pharmacopoeia of India* (Part-I, Volumn-II) Vol. 2.
9. Hosamani, R., & Muralidhara. (2009). Neuroprotective efficacy of *Bacopa monnieri* against rotenone induced oxidative stress and neurotoxicity in Drosophila melanogaster. *Neurotoxicology*, 30(6), 977-985. doi:10.1016/j.neuro.2009.08.012
10. Indian Pharmacopoiea (1995).Vol.II, 3rdEdition, Ministry of health and family welfare, *Govt. of India, Controller of publication*, New Delhi ,44.
11. Kirtikar, K.R., & Basu, B.D. (1995). Indian Medicinal Plant. 4thEdition Vol.I *International Distributors*, Dehradun, 218-223.

*Corresponding author's e-mail addresses: hariom.nagar@mygyanviahr.com, hariomnagariitr@gmail.com

12. Kapoor, R., Srivastava, S., & Kakkar, P. (2009). *Bacopa monnieri* modulates antioxidant responses in brain and kidney of diabetic rats. *Environmental Toxicology and Pharmacology*, 27(1), 62-69.doi:10.1016/ j.etap.2008.08.007
13. Kongkeaw, C., Dilokthornsakul, P., Thanarangsarit, P., Lim-peanchob, N., & Norman Scholfield, C. (2014). Meta-analysis of randomized controlled trials on cognitive effects of *Bacopa monnieri* extract. *Journal of Ethnopharmacology*, 151(1), 528-535. doi:10.1016/j.jep.2013.11.008
14. Le, X. T., Nguyet Pham, H. T., Van Nguyen, T., Minh Nguyen, K., Tanaka, K., Fujiwara, H., & Matsu-moto, K. (2015). Protective effects of *Bacopa monnieri* on ischemia-induced cognitive deficits in mice: The possible contribution of bacopaside I and underlying mechanism. *Journal of Ethnopharmacology*, 164, 37-45. doi:10.1016/j.jep.2015.01.041
15. Limpeanchob, N., Jaipan, S., Rattanakaruna, S., Phrompitta-yarat, W., & Ingkaninan, K. (2008). Neuroprotective effect of *Bacopa monnieri* on beta-amyloid-induced cell death in primary cortical culture. *Journal of Ethnopharmacology*, 120(1), 112-117. doi:10.1016/ j.jep.2008.07.039
16. Ali, M.(1994). Text book of Pharmacognosy. Ist Edition, *CBS publishers and Distributers*, Delhi, 112,411.
17. Mishra, A., Mishra, A. K., Prakash Tiwari, O., & Jha, S. (2015). Pharmacognostic, physicochemical and phytochemical investigation of *Bacopa monnieri* L. stem and its anticonvulsant potential in laboratory animals. *International Journal of Epilepsy*, 2(1), 19-27. doi:10.1016/j.ijep.2015.02.004
18. Nemetchek, M. D., Stierle, A. A., Stierle, D. B., & Lurie, D. I. (2017). The Ayurvedic plant *Bacopa monnieri* inhibits inflammatory pathways in the brain. *Journal of Ethnopharmacology*, 197, 92-100. doi: 10.1016/j.jep.2016.07.073
19. Nuengchamnong, N., Sookying, S., & Ingkaninan, K. (2016). LC-ESI-QTOF-MS based screening and identification of isomeric jujubogenin and pseudojujubo-geninaglycones in *Bacopa monnieri* extract. *Journal of Pharmaceutical and Biomedical*

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Analysis, 129, 121-134. doi:10.1016/j.jpba.2016.06. 052

20. Peth-Nui, T., Wattanathorn, J., Muchimapura, S., Tong-Un, T., Piyavhatkul, N., Rangseekajee, P., & Vittaya-areekul, S., (2012). Effects of 12-week *Bacopa monnieri* consumption on attention, cognitive processing, working memory, and functions of both cholinergic and monoaminergic systems in healthy elderly volunteers. *Evidence-Based Complementary and Alternative Medicine*, 2012, 606424.doi:10. 1155/2012/606424
21. Phrompittayarat, W., Jetiyanon, K., Wittaya-Areekul, S., Putalun, W., Tanaka, H., Khan, I., & Ingkaninan, K. (2011). Influence of seasons, different plant parts, and plant growth stages on saponin quantity and distribution in *Bacopa monnieri*. Songklanakarin *Journal of Science and Technology*, 33(2), 193-199.
22. Simpson, T., Pase, M., & Stough, C. (2015). *Bacopa monnieri* as an antioxidant therapy to reduce oxidative stress in the aging brain. *Evidence-Based Complementary and Alternative Medicine*, 2015, 615384.doi:10. 1155/2015/615384
23. Singh, H. K., Rastogi, R. P., Srimal, R. C., & Dhawan, B. N. (1988). Effect of bacosides A and B on avoidance responses in rats. *Phytotherapy Research*, 2(2), 70-75.
24. Sivaramakrishna, C., Rao, C. V., Trimurtulu, G., Vanisree, M., & Subbaraju, G. V. (2005). Triterpenoid glycolsides from *Bacopa monnieri*. *Phytochemistry*, 66(23), 2719-2728. doi:10.1016/j.phytochem.2005. 09.016
25. Stough, C., Lloyd, J., Clarke, J., Downey, L. A., Hutchison, C. W., Rodgers, T., & Nathan, P. J. (2001). The chronic effects of an extract of *Bacopa monniera* (Brahmi) on cognitive function in healthy human subjects. *Psychopharmacology*, 156(4), 481-484.
26. The United States Pharmacopeial Convention. (2017). Pharmacopeial forum: Volume 40(5). North Bethesda, MD: *The United States Pharmacopeia–National Formulary* (USP–NF).
27. Uabundit, N., Wattanathorn, J., Mucimapura, S., & Ingkaninan, K. (2010). Cognitive enhancement and neuroprotective effects of *Bacopa monnieri* in Alzheimer's disease

*Corresponding author's e-mail addresses: hariom.nagar@mygyanviahr.com, hariomnagariitr@gmail.com

model. *Journal of Ethnopharmacology*, 127(1), 26-31. doi:10.1016/j.jep.2009.09. 056

28. World Health Organization. (2011). *Quality control methods for herbal materials*. Geneva, Switzerland: WHO Press.

29. Zhou, Y., Peng, L., Zhang, W. D., & Kong, D. Y. (2009). Effect of triterpenoid saponins from *Bacopa monniera* on scopolamine-induced memory impairment in mice. *Planta Medica*, 75(6), 568-574. doi:10.1055/s-0029-1185339

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