Songklanakarin J. Sci. Technol.
41 (2), 397-404, Mar. – Apr. 2019

http://www.sjst.psu.ac.th

Original Article

Pharmacognostic and physico-chemical investigations of the aerial part of *Bacopa monnieri* (L.) Wettst.

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Received: 26 September 2017; Revised: 19 November 2017; Accepted: 26 November 2017

Abstract

A macroscopic, microscopic, physico-chemical, and phytochemical analysis of the aerial part of *Bacopa monnieri* (L.) Wettst. or Brahmi was undertaken for the purpose of establishing a monograph. The macroscopic and microscopic studies presented the typical characteristics of the Brahmi plant and its powder. The standard values of physico-chemical parameters were established at not more than 10.0 %w/w for loss on drying and 13.0 %w/w for total ash as well as not less than 17.0 %w/w for water soluble extractives and 10.0 %w/w for ethanol soluble extractives. Phytochemical analysis showed the presence of steroidal saponins, with confirmation by the TLC fingerprint. Total saponin content was not less than 1.0 %w/w measured by HPLC-UV method. The overall study provides pharmacognostical, physico-chemical, phytochemical details, typical TLC and HPLC fingerprints of the Brahmi, which can be used to establish a Pharmacopoeia monograph for the identification and standardization of Brahmi material.

Keywords: *Bacopa monnieri* (L.) Wettst, Brahmi, monograph, pharmacognostic study, HPLC fingerprint

1. Introduction

Bacopa monnieri (L.) Wettst. (commonly known as Brahmi) is a perennial herb belonging to the family Plantaginaceae. It has been used in Ayurvedic medicine as a brain tonic and memory enhancer for hundreds of years. The chemical constituents of Brahmi include various classes of chemical groups such as sterols (Bhandari, Kumar, Singh, & Kaul, 2006), flavonoids (Bhandari, Kumar, Gupta, Singh, & Kaul, 2007) and saponins (Nuengchamnong, Sookying, & Ingkaninan, 2016; Sivaramakrishna, Rao, Trimurtulu, Vanisree, & Subbaraju, 2005). The major compounds of this plant responsible for memory and cognition enhancement are steroidal saponin glycosides e.g. bacoside A_3, bacopasaponin C, and bacopasides I, II, and X (Deepak, Sangli, & Amit, 2005; Ganzera, Gampenrieder, Pawar, Khan, & Stuppner, 2004; Le *et al.*, 2015; Singh, Rastogi, Srimal, & Dhawan, 1988; Zhou, Peng, Zhang, & Kong, 2009). Some trials on the standardized extract of Brahmi have indicated that the extract also improved cognitive functioning (Kongkeaw, Dilokthornsakul, Thanarangsarit, Limpeanchob, & Norman Scholfield, 2014; Peth-Nui *et al.*, 2012; Stough *et al.*, 2001). Brahmi also has been shown to have anti-oxidant (Kapoor, Srivastava, & Kakkar, 2009; Simpson, Pase, & Stough, 2015), neuroprotective (Hosamani & Muralidhara, 2009; Limpeanchob, Jaipan, Rattanakaruna, Phrompittayarat, & Ingkaninan, 2008; Uabundit, Wattanathorn, Mucimapura, & Ingkaninan, 2010) and anti-inflammatory effects (Nemetchek, Stierle, Stierle, & Lurie, 2017). Supported by ethnopharmacological data and scientific studies, several Brahmi products have been developed as food supplements which are marketed around

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the world. To ensure the quality of the plant raw material for industrial uses, standardization and quality control procedures of Brahmi are needed. Up until now, only one report on a pharmacognostic study of the stem of Brahmi (Mishra, Mishra, PrakashTiwari, & Jha, 2015) and three monographs i.e. the Ayurvedic Pharmacopoeia (AP) (Government of India, 2001), the British Pharmacopoeia (BP) (British Pharmacopoeia Commission, 2014) and the United States Pharmacopeia (USP) (The United States Pharmacopeial Convention, 2017) have been published. The report of Mishra and coworkers (Mishra *et al.*, 2015) did not include the aerial part (stem and leaves) of Brahmi which is the part most used in Brahmi products. Also, the AP (Government of India, 2001) did not describe the specific method for either qualitative analysis (such as TLC) or quantitative analysis of the active compounds in Brahmi. Besides, given that Brahmi is a plant that is widely distributed in many parts of the world including Australia, North America, Africa, and Asia, the evaluation of the quality of Brahmi from various geographical origins is necessary.

The present study fills the need to establish a new standard method which covers Brahmi from different geographical areas. Thirteen samples were collected from different regions in Thailand as well as from Australia, India, and Japan (but originated from India). Macroscopic, microscopic, physico-chemical and phytochemical analyses of Brahmi were undertaken to identify and establish the necessary parameters for identification and standardization of Brahmi material.

2. Materials and Methods

2.1. Plant materials and chemicals

Samples from 13 Brahmi plants were collected from different regions of Thailand, and from Australia, India, and Japan (Table 1). The samples were identified by the third author. Voucher specimens (Saesong001-013) were identified by the third author and kept at the PNU Herbarium, Naresuan University, Phitsanulok, Thailand. All of the samples were planted at Faculty of Pharmaceutical Sciences, Naresuan University, before harvesting. Aerial parts consisting of leaves and stems cut at 10 cm intervals beneath the shoots (suggested by (Phrompittayarat *et al.*, 2011)) were used in the study. The shoots of each sample were collected, cleaned and dried at 50ºC for 24 hr in a hot air oven. The dried plants were powdered, sieved (60 mesh) and kept in plastic containers at -20°C until use.

All chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). Both AR and HPLC grade solvents were bought from RCI Labscan (Pathumwan, Bangkok, Thailand). Five saponin standards; bacoside A_3, bacopasaponin C, bacopasides I, II and X were purchased from Natural Remedies, Bangalore, India (purity > 96% by HPLC).

2.2. Methodologies

Some methods such as physico-chemical evaluations (total ash and water-soluble and ethanol-soluble extractives), and preliminary phytochemical screening (steroidal saponin tests) in this study were modified from World Health Organization (WHO) guidelines (World Health Organization, 2011). They are briefly described in the following sections.

Table 1. The details of collection numbers and locations of Brahmi in this study

Code	Collection No.	Collecting location
BM1	Saesong001	Perth, Australia
BM2	Saesong002	Bangkok, Thailand
BM3	Saesong003	Nakhon Pathom, Thailand
BM4	Saesong004	Phitsanulok, Thailand
BM5	Saesong005	Bangkok, Thailand
BM6	Saesong006	Nakhon Nayok, Thailand
BM7	Saesong007	Bangkok, Thailand
BM8	Saesong008	Ayutthaya, Thailand
BM9	Saesong009	Fukuoka, Japan (originated from India)
BM10	Saesong010	Bangkok, Thailand
BM11	Saesong011	Bangkok, Thailand
BM12	Saesong012	Phetchabun, Thailand (originated from India)
BM13	Saesong013	Phitsanulok, Thailand

BM is *Bacopa monnieri*

2.2.1. Macroscopic and microscopic analysis

The macroscopic characteristics of the Brahmi plants were examined visually and recorded. For the microscopic study, the fresh leaves and stems of each Brahmi plant were cross-sectioned with a razor blade at right angles to the longitudinal axis of the sample and then stained in 0.1 %w/v of safranin O solution for 1 min before inspection under a microscope (Olympus BX43, Spacemed Co., Ltd.) with suitable magnification. Cross-sections of each structure and component were photographed. The powder of the aerial part of Brahmi was put on a microscope slide with 1-2 drops of safranin O solution added. The characteristics of the dried powders were inspected and photographed under a microscope with suitable magnification.

2.2.2. Physico-chemical evaluations

1) Determination of loss on drying

Loss on drying of the Brahmi powder was calculated as percentage with a moisture analyzer (Sartorius MA40, Scientific Promotion Co., Ltd), in which 100 mg of powdered sample was heated to 105ºC for 30 sec. The percentage of weight loss was reported by this instrument at the end of the process.

2) Determination of total ash

The 2 g samples of the dried Brahmi powder were accurately weighed and then each was spread in an even layer in a porcelain crucible, and placed in a furnace whose temperature was increased at 15 min/ºC to 450ºC (over 12 hr) until the powder was white, indicating the absence of carbon. The sample was then cooled in a desiccator and weighed. The total ash (mg/100 mg of dried Brahmi powder) was calculated.

3) Determination of water-soluble and ethanol-soluble extractives

The 5 g samples of the dried Brahmi powder were macerated with 100 ml of water in a closed conical flask on a shaker for 6 hr and then left standing at room temperature for 18 hr. The extract solution was filtered and 20 ml of the filtrate was evaporated in an oven at 105ºC. The weight of the dried extract was recorded when constant weight was achieved. Also, the same procedure was used for the determination of ethanol-soluble extractive using absolute ethanol and the extracted matter was similarly calculated.

2.2.3. Preliminary phytochemical screening

1) Liebermann Burchard test

The 500 mg samples of the dried Brahmi powder were placed in flasks and 10 ml ethanol was added. The samples were warmed in a water bath at 50ºC for 5 min, the solutions were filtered, and 2 ml of each filtrate was placed on an evaporating disk, and dried in a water bath. The extracts were dissolved in 4-5 drops of acetic anhydride, and 3-4 drops of sulfuric acid were gradually added. Samples containing steroidal structures turned green.

2) Froth test

Samples of dried Brahmi powders (100 mg) were mixed with 5 ml of water in test tubes and shaken for 30 sec. The formation of honeycomb-like froth indicated that saponins were present.

2.2.4. Thin-layer chromatographic (TLC) identification

Dried Brahmi powders (100 mg) were extracted with 1 ml MeOH and sonication for 15 min. Each extract (1 µl) was then applied to a TLC plate coated with silica gel 60 F_{254} (Merck, Darmstadt, Germany). As standards, 0.5 µl of 1 mg/ml bacoside A_3, bacopasaponin C and bacopasides I, II, and X, were also applied to the plates. The TLC was developed in a chamber saturated with ethyl acetate, MeOH and water (7.5:1.5:1) and the developing distance was 8 cm. After developing, the TLC plates were observed under UV at 254 nm and 366 nm and then sprayed with anisaldehyde reagent and heated at 100ºC for 5 min, and the colored spots on TLC plate were recorded.

2.2.5. Determination of saponin glycosides by HPLC

One hundred mg of each powdered Brahmi was extracted with 5 ml of 70% MeOH by sonication for 15 min and the extracts were then filtered through 0.45 µm nylon filters before submitting to HPLC. The HPLC condition was modified and optimized from a prior report (Phrompittayarat *et al.*, 2011). The HPLC system (Shimadzu, Japan) consisted of SPD-20A UV/Vis detector, LC-20AT Pump, SIL-20A Auto sampler and CTO-10AS column oven. Analytical separations were carried out on a Phenomenex Luna C18 column (150x4.6 mm, 5 µm particle size) connected to a Phenomenex Luna C18 (10x4.6 mm, 5 µm) guard column, using an isocratic system of 0.2% phosphoric acid and acetonitrile (65:35 v/v), pH 3 (the pH was adjusted with 5 M NaOH) as the mobile phase. The injection volume was 20 µl and the flow rate was 1.0 ml/min, with a 205 nm detection wavelength. The temperature in the column was set at 30ºC. The separation time was 30 min. The peak heights of five saponins; bacoside A_3, bacopasaponin C, and bacopasides I, II and X at 6.25, 12.5, 25, 50, 100, and 200 µg/ml were used to make standard curves.

2.2.6. Establishment of lower limit and upper limit values

The upper limits of the loss on drying and total ash were set to the means of these values obtained from 13 Brahmi samples plus their standard deviation (SD). In the same fashion, the lower limit values of water-soluble, ethanol-soluble extractives and total saponin contents were set to the mean of these values obtained from Brahmi samples minus SD (Chonlasit, Julsrigival, & Janjom, 2015).

3. Results and Discussion

3.1. Macroscopic and microscopic studies

Brahmi is an aquatic plant often found in marshy areas. It is a small creeping herb with numerous branches, small oblong leaves, and white-purple flowers. The leaves of Brahmi are bright green, succulent, oblong, margin entire, 0.4-1.0 cm wide and 0.5-2.5 cm long and are arranged oppositely on the stem, with no petiole. The stems are a lighter green, succulent, with numerous branches, glabrous, soft, 10-40 cm long, 1-3 mm thick, and often showing sprouting rootlets. The flowers are small with a purplish hue, axillary and solitary on pedicels, 1.0-1.5 cm long. The corolla consists of 5 petals and 3 sepals is 8-10 mm long, with a dark purple didynamous stamen and bright green capitate stigma. The fruits of Brahmi are capsules up to 5 mm long, ovoid and glabrous (Figure 1a-f).

Figure 1. Brahmi whole plant and stem (a), leaf (b), flower (c, d), fruit (e), and root (f)

The transverse section of each Brahmi leaf comprises three main components consisting of the epidermis, mesophyll, and vascular tissue (Figure 2a). The epidermis is divided into two single layers, the upper epidermis and the lower epidermis, and is covered with cuticle. The underside of the upper epidermis has numerous palisade parenchyma cells attached together with spongy parenchyma cells. Both types

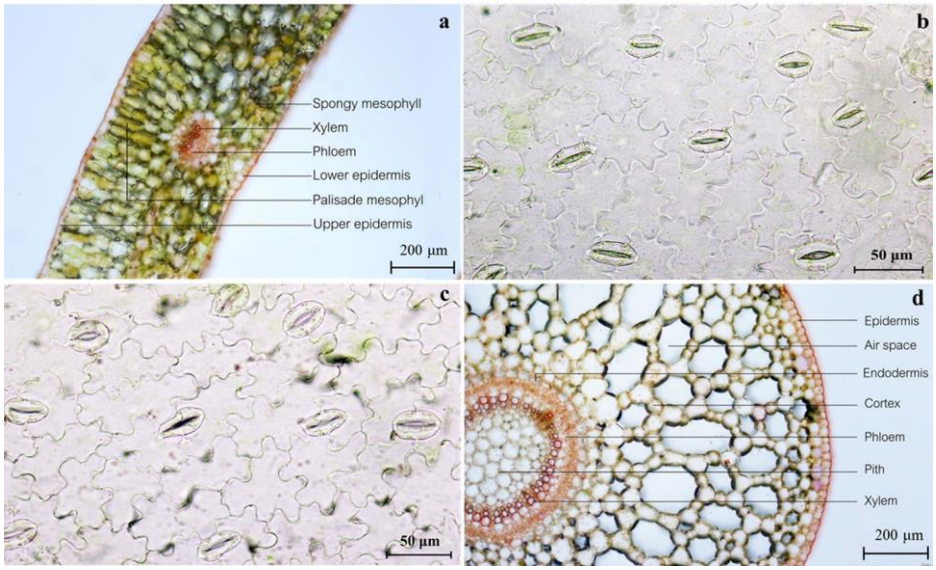

Figure 2. Representative transverse sections of leaf (a), stomata on upper epidermis (b), stomata on lower epidermis (c) and stem of Brahmi (d) using safranin O staining

of cells are filled with chlorophyll pigments. The vascular tissue found in the midrib portion of the leaf consists of xylem and phloem. The lower epidermis is very similar to the upper epidermis. The stomata are of the anomocytic type (Figure 2b, 2c) and guard cell on stomata is 30-45 µm long and 18-25 µm wide.

The transverse section of the Brahmi stem is composed of the epidermis, cortex, and stele (Figure 2d). The epidermis is the outermost thin-wall, layered with the cuticle and cortex which are below this layer. The cortex layer comprises parenchyma tissue with big airspaces (lacunae). In the stele layer there is an endodermis, vascular tissue, and pith which is located in the center of the stem and this layer area is less than half the thickness of the cortex layer. A single layered endodermis connects with the cortex but is separate from the vascular tissue, forming a ring. This tissue consists of phloem. The pith is situated above the xylem and innermost stem, and contains compactly arranged parenchyma cells.

The microscopic study of the powdered Brahmi identified the presence of several fragments of multicellular tissues from different Brahmi samples. The six main components usually found in each sample were epidermis, parenchyma with starch grains, anomocytic stomata, lignified fibers, sieve tubes, and scalariform vessels (Figure 3a-f). The shape and size of the guard cells of the stoma on the powdered Brahmi were similar to those in a fresh leaf. All these components can be used for identification and authentication of 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.

3.2. Physico-chemical parameters

The physico-chemical parameters of Brahmi samples, i.e., loss on drying, total ash, and water-soluble and ethanol-soluble extractives, are summarized in Table 2. The upper limits of loss on drying and total ash, and the lower limits of water-soluble and ethanol-soluble extractives were established. All these values agreed with those stated in the BP, except for the ethanol-soluble extractive that is not mentioned in the BP. It is noted that the values given in AP and USP differ from those in BP and in this current study. This perhaps reflects different geographic origins of Brahmi samples and its varieties.

3.3. Preliminary phytochemical screening

The Liebermann-Burchard test, which is specific for triterpenoid/steroid structures, was used in the preliminary phytochemical screening of the Brahmi samples. The ethanolic extracts of Brahmi were dissolved in acetic anhydride, and turned green when mixed with sulfuric acid, indicating the presence of steroidal saponins. The presence of saponins was confirmed by the formation of long lasting honeycomb-like foam when the Brahmi powder was shaken with water.

3.4. TLC (Thin-Layer chromatographic) fingerprint

TLC is commonly used for chemical characterization of herbal materials. TLC fingerprints of the 13 Brahmi methanolic extracts are shown in Figure 4 and the Rf values of the components in the samples are summarized in Table 3. The extracts provided fourteen spots with different Rf values and different colors after being sprayed with anisaldehyde reagent. Not all of the spots could be observed under UV at 254 nm and 366 nm. The light pink spot at Rf 0.19 corresponded to bacopaside I and the spot at Rf 0.30 represented a mixture of four saponin glycosides (bacoside A_3, bacopaside II, bacopaside X, and bacopasaponin C) by comparison with the saponin standards. In the BP and USP monographs, slightly more polar mobile phases are used, resulting in higher Rf values of bacopaside I (0.3 for BP and 0.4 for USP) and other saponins (0.4 for BP and 0.6 for USP). However, the Rf values of other spots are not fully described.

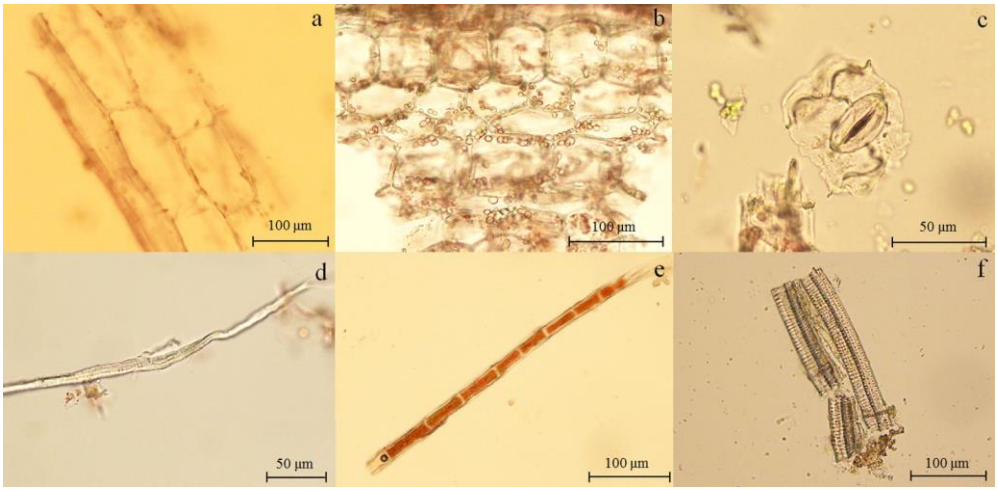

Figure 3. Photomicrographs of dry powdered aerial parts of Brahmi; epidermis (a), parenchyma with starch grains (b), anomocytic stoma (c), lignified fibers (d), sieve tube (e), and scalariform vessel (f) using safranin O Staining

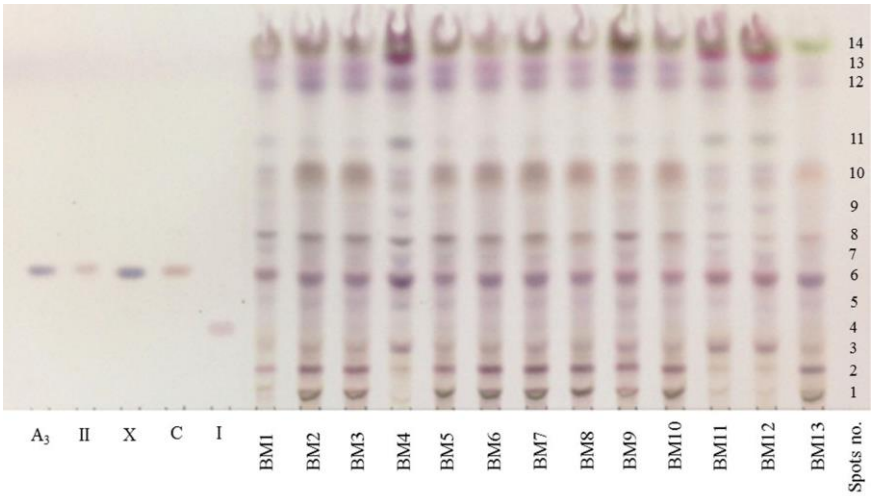

Figure 4. TLC fingerprints of the Brahmi samples (BM1-BM13, extracted as described in section 2.2.4) and of the five saponin reference standards bacoside A_3 (A_3), bacopaside II (II), bacopaside X (X), bacopasaponin C (C), and bacopaside I (I)

Table 2. Physico-chemical characteristics of the aerial parts of Brahmi (n=13) and comparison of limits set by this study (calculated by means plus or minus SD) with the limits in Brahmi monographs in the Ayurvedic Pharmacopoeia (AP), the British Pharmacopoeia (BP) and the United States Pharmacopeia (USP)

Physicochemical parameters	Mean ± SD (%w/w)	Limit values in monographs			
		This study (%w/w)	The AP (%w/w)	The BP (%w/w)	The USP (%w/w)
Loss on drying	9.07 ± 0.83	Not > 10.0	NR	Not > 11.0	Not > 12.0
Total ash	11.05 ± 1.58	Not >13.0	Not > 18.0	Not > 13.0	Not > 18.0
Water-soluble extractive	18.93 ± 2.31	Not < 17.0	Not < 15.0	Not < 15.0	NR
Ethanol-soluble extractive	10.89 ± 0.91	Not < 10.0	Not < 6.0	NR	Not < 5.0
Total saponin	1.37 ± 0.24	Not < 1.0	NR	Not < 1.0	Not < 2.5

NR, not reported

Table 3. R_f values of spots on TLC of Brahmi samples visualized after spraying with anisaldehyde reagent and heating for 5 min

Spot	Rf	Color
1	0.04 ± 0.02	Dark green
2	0.10 ± 0.02	Deep violet
3	0.14 ± 0.01	Brown violet
4*	0.19 ± 0.03	Light pink
5	0.25 ± 0.02	Violet
6**	0.30 ± 0.01	Deep Violet
7	0.34 ± 0.02	Violet
8	0.38 ± 0.01	Dark brown
9	0.45 ± 0.03	Violet
10	0.51 ± 0.01	Brown
11	0.60 ± 0.02	Dark blue
12	0.71 ± 0.02	Deep Violet
13	0.78 ±0.02	Deep violet
14	0.83 ±0.03	Violet green

* is bacopaside I
** is mixture of bacoside A_3, bacopasides II, X and bacopasaponin C

Thus, our TLC fingerprint offers better discrimination for quality assessment and identification of Brahmi materials.

3.5. HPLC for quantitative analysis of saponins

Saponin glycosides in Brahmi can be classified into 2 groups i.e. jujubogenin glycosides (bacoside A_3 and bacopaside X) and pseudojujubogenin glycosides (bacopasides I, II and bacopasaponin C). The HPLC-UV method for evaluating these saponin glycosides was modified from a prior report (Phrompittayarat *et al.*, 2011). The total saponins in Brahmi were calculated as sum total of five individual saponins. In contrast, the BP only uses bacopaside II as a standard and the amounts of other saponin glycosides are expressed as bacopaside II equivalents, while the USP uses bacoside A_3 as reference standard for quantification of other saponin glycosides. In addition, the BP and USP use a different extraction technique (reflux) from this current study (ultrasonication). However, the lower limit of total saponins found in our study and the BP are the same (> 1.0 %w/w, Table 2) while that in the USP is higher (2.5 %w/w). This probably reflects the differences in methods of calculation, extraction technique, or geographical origins of the source materials. The total saponin contents of Brahmi samples are shown in Figure 5. We found that about 80% of total saponins were pseudojujubogenin glycosides of which bacopaside I was the majority (~ 50%). A typical HPLC chromatogram of the Brahmi extract and the mixture of five saponin standards, which are useful for quantification and identification, is exhibited in Figure 6.

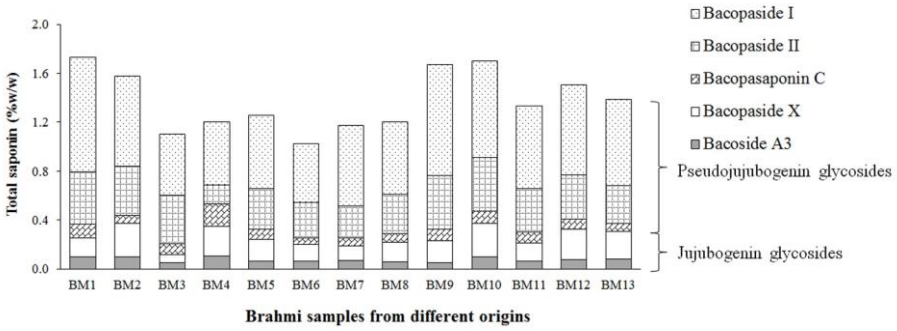

Figure 5. Saponin glycoside contents in Brahmi samples (BM1-BM13) classified into pseudojujubogenin glycosides (bacopaside I, bacopaside II, and bacopasaponin C) and jujubogenin glycosides (bacopaside X and bacoside A_3)

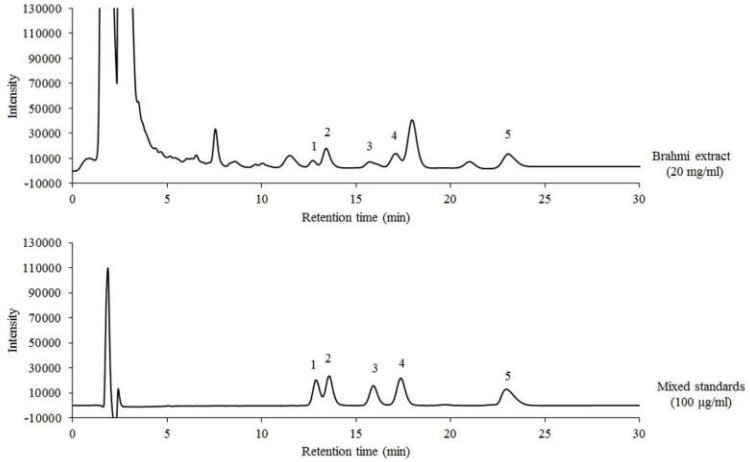

Figure 6. Representative HPLC chromatograms of Brahmi methanolic extract (upper) and a mixture of five saponin glycoside standards (lower) showing bacoside A_3 (1), bacopaside II (2), bacopaside X (3), bacopasaponin C (4), and bacopaside I (5)

4. Conclusions

The pharmacognostical and physico-chemical characteristics of Brahmi were identified and characterized. These methods are superior to those used by AP, BP, and USP, and are well suited to standardization and quality control of Brahmi and its products. Application of these characteristics will improve the reliability of Brahmi-derived pharmaceuticals and improve the reproducibility of Brahmi research studies.

Acknowledgements

The authors would like to acknowledge the financial support through grants from the Royal Golden Jubilee PhD Program (PHD/0039/2557 to Tongchai Saesong and Kornkanok Ingkaninan), Center of Excellence for Innovation in Chemistry, the Office of the Higher Education Commission. Many thanks to Mr. Roy Morien of the Naresuan University Language Centre and Dr. Norman Scholfield for their English editing of the manuscript. In additional, we would like to thank Mr. Thadecha Punthachak, Miss Kawinthip Tangtong, Miss Pennapa Aungsupachot and Miss Pattinan Amnuaychaiwat for their help on sample preparation and phytochemical tests.

References

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.200700066

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.

British Pharmacopoeia Commission. (2014). *British Pharmacopoeia Volume-IV* (Seventh Edition ed. Vol. 6). London: The Stationery Office.

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.

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.

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

Government of India. (2001). *The Ayurvedic Pharmacopoeia of India (Part-I, Volumn-II)* Vol. 2.

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

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

Kongkeaw, C., Dilokthornsakul, P., Thanarangsarit, P., Limpeanchob, 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

Le, X. T., Nguyet Pham, H. T., Van Nguyen, T., Minh Nguyen, K., Tanaka, K., Fujiwara, H., & Matsumoto, 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

Limpeanchob, N., Jaipan, S., Rattanakaruna, S., Phrompittayarat, 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

Mishra, A., Mishra, A. K., PrakashTiwari, 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

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

Nuengchamnong, N., Sookying, S., & Ingkaninan, K. (2016). LC-ESI-QTOF-MS based screening and identification of isomeric jujubogenin and pseudojujubogenin aglycones in *Bacopa monnieri* extract. *Journal of Pharmaceutical and Biomedical Analysis, 129*, 121-134. doi:10.1016/j.jpba.2016.06.052

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

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.

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

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.

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

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.

The United States Pharmacopeial Convention. (2017). *Pharmacopeial forum: Volume 40(5)*. North Bethesda, MD: The United States Pharmacopeia–National Formulary (USP–NF).

Uabundit, N., Wattanathorn, J., Mucimapura, S., & Ingkaninan, K. (2010). Cognitive enhancement and neuroprotective effects of *Bacopa monnieri* in Alzheimer's disease model. *Journal of Ethnopharmacology, 127*(1), 26-31. doi:10.1016/j.jep.2009.09.056

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

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