Research Paper

Qualitative and Quantitative Assessment of Four Marketed Formulations of Brahmi

NEETI SAINI, RAJANI MATHUR AND S. S. AGRAWAL*
Delhi Institute of Pharmaceutical Science and Research, Pushp Vihar, Sector-III, MB Road, New Delhi-110 017, India

This study was conducted with the aim to compare two batches each of four popular commercial formulations of *Bacopa monnieri* (Brahmi), and report, if any, inter-batch variations. The formulations were procured from local market and analyzed for label specifications, uniformity of weight of capsule, identity, purity and strength parameters (total ash content test, acid insoluble ash content, water soluble extractive, alcohol soluble extractive, loss on drying). Bacoside A, one of the pharmacologically active saponin present in *B. monnieri*, was quantified in all the formulations using UV-spectrophotometer. In addition each formulation was assessed and compared for variation in biological activity using *in vitro* test for hemolytic activity using human erythrocytes. The results of the study show that there is a wide variation in the quality and content of herbal drugs marketed by different manufacturers. More importantly this study demonstrates that there exists a bigger challenge of batch-to-batch variation in the quality and content of herbal formulations of the same manufacturer. This challenge of providing standardized formulations is being faced by not any one manufacturing house but by all, and may be attributed firstly to, lack of stringent regulations and secondly to high variability in raw material quality.

Key words: Bacoside A, *Bacopa monnieri,* hemolytic activity, quality standards, UV spectrophotometry

Herbal medicines have widespread use in both developed as well as developing countries. Although herbal medicines have been used for centuries, they have not been incorporated into mainline health care system as their use is fraught with numerous challenges such as systematic pharmacologic investigation, standardized method of formulation, dosage, proof of safety and herb-drug interactions[1,2]. In June 2000, the Government of India took steps to establish herbal drug legislation and released Good Manufacturing Practices (GMP) for pharmacies manufacturing Ayurvedic, Siddha and Unani medicines to improve the quality of drugs[3]. On similar lines, Indian Pharmacopoeia (2007), Ayurvedic Pharmacopoeia of India (1999), Indian Herbal Pharmacopoeia (2002), have been compiled, with the objective to standardize herbal medicines. However, in actual practice, these standards have not been implemented in letter and spirit and gaping loopholes remain in the manufacturing and quality control process of herbal formulations that are currently available in the market.

***Address for correspondence**
E-mail: agrawal_shyam@indiatimes.com

Bacopa monnieri (family: Scrophulariaceae) commonly known as Brahmi is an important medicinal plant that has been attributed with medicinal properties in traditional literature. The chemical constituents of whole plant of *B. monnieri* are saponins (Bacoside A, A1 and B), betulinic acid, D-mannitol, β-stigmasterol and stigmastanol[4,5]. In pharmacologic studies, the whole plant of *B. monnieri* has been attributed with various medicinal properties including memory enhancing, antiinflammatory, antioxidant, analgesia, antipyretic, sedative, cardiotonic and antiepileptic and these have been correlated to the presence of bacosides[6-8].

According to the summary report submitted to Department of Ayush, Ministry of Health and Family Welfare in August 2008, Brahmi (*Bacopa monnieri*) is one of the most popular medicinal plants due to its wide array of therapeutic properties and sought after for export development. Despite its wide acceptability and use, it is not known whether the various manufacturing houses of *B. monnieri* based formulations are adhering to the set quality standards for herbal formulations. Hence, this study was designed to address the issue and investigate

the quality standard of different batches of herbal formulations that are currently available in the market of this widely used medicinal plant.

MATERIALS AND METHODS

Four formulations of *B. monnieri* from different manufacturing houses were purchased from local market in two batches each. Immediately following purchase, the batch details were recorded and coded (Table 1).

Characterization of material:
Label data was checked in accordance with the guidelines laid down by Dietary Supplement and Health Education Act (DSHEA), 1994, a framework for FDA regulation of dietary supplement. In accordance with the method elaborated in Indian Pharmacopoeia, 1996, the intact capsules were checked for uniformity in weight[9]. As per the method detailed in Ayurvedic Pharmacopoeia of India, tests were performed on powdered content of capsules to evaluate total ash content, acid insoluble ash content, alcohol soluble extractive test and water soluble extractive[10]. Loss on drying test was performed on all the samples as per the method elaborated in Indian Pharmacopoeia, 1996. It was calculated as percent loss in weight (% w/w) resulting from water and volatile matter of any kind driven off under specified conditions[11].

Bacoside A content:
In accordance with the method elaborated by Pal and co-workers, Bacoside A, a triterpenoid glycoside, was primarily hydrolysed to release the aglycone which was then quantified by UV spectrophotometry[12]. Briefly, equal volumes of sulfuric acid and stock solution of standard Bacoside A (1 mg/ml) were mixed and refluxed on a water bath for 4 h at a temperature of 60º. The mixture was cooled and diluted with distilled water. The solution was extracted with chloroform (four times, 10 ml each). The combined extract was washed with ammonia solution (0.1%), followed by distilled water. Chloroform extract was dried over anhydrous sodium sulfate and the yield was calculated. Standard curve of Bacoside A was plotted over concentration range of 37.5 to 300 µg/ml at the detection wavelength of 278 nm using UV spectrophotometer. The samples were subjected to similar treatment and quantified using the standard curve.

Hemolytic activity of saponins:
Human peripheral whole blood (5 ml) was collected in EDTA-coated vacutainers and red blood cells (RBCs) were separated from it using standard biochemical protocol. Briefly, whole blood was centrifuged at 1000 *g* for 10 min at 4° to separate RBCs. The pellet was re-suspended in phosphate-buffered saline (PBS, pH 7.4) and re-centrifuged. The upper 1–2 mm layer of packed cells was aspirated along with liquid phase to remove white blood cells. The exercise was repeated four times and the separated RBCs were pooled. The cells were re-suspended in 10 volumes of PBS and centrifuged at 2000 *g* for 10 min[13]. Buffy coat of the pellet was removed, and RBC suspension (10%) was prepared in PBS. Aliquots of either sample (1 ml of 0.5 mg/ml), Triton-X (1 ml of 0.2%) or PBS (1 ml) were shaken with RBC suspension (5 ml of 10% v/v at 37°) to serve as test, positive control, and vehicle control, respectively. After 40 min, the supernatant was removed and the liberated hemoglobin in the supernatant was measured spectrophotometrically as absorbance at 541 nm using a double-beam spectrophotometer (U-2900 Hitachi, Japan). The experiment was done in triplicate and mean±SE. was calculated[14]. Triton-X in concentration range of 0.025-0.2% was used to plot standard curve and hemolytic index for each sample was calculated using the following formula:

$$\text{Hemolytic Index (\%)} = Abs_{(test)} - Abs_{(negative\ control)} / Abs_{(positive\ control)} - Abs_{(negative\ control)}$$

TABLE 1: BATCH DETAILS OF DIFFERENT MANUFACTURERS

Company, batch code	Batch number	Manufacturing date	Expiry date
Himalaya Drug Company, Bangalore, India, H1	F076006G	December, 2006	3 years from the date of manufacture
Himalaya Drug Company, Bangalore, India, H2	F078005G	June, 2008	3 years from the date of manufacture
Multani Pharmaceuticals Pvt. Ltd., New Delhi, India, M1	1202-E	March, 2008	February, 2011
Multani Pharmaceuticals Pvt. Ltd., New Delhi, India, M2	362-E	August, 2007	July, 2010
Surya Herbal Ltd. (Sunova), Noida,India, S1	SBC-7004	October, 2007	September, 2010
Surya Herbal Ltd. (Sunova), Noida,India, S2	SBC-7003	June, 2007	May, 2010
Suraj Bala Exports Pvt. Ltd, Delhi, India, SBE1	SBE/001801/08-09	November, 2009	November, 2010
Suraj Bala Exports Pvt. Ltd, Delhi, India, SBE2	SBE/001802/08-09	November, 2009	November, 2010

Statistical analysis:
Statistical analysis of parameters of identity, purity and strength, loss on drying, amount of bacoside A in each sample, hemolytic index of sample, are represented as mean±standard error. The difference in percentage was compared for batch to batch variation by applying student's *t*-test using Sigmastat 3.5 software.

RESULTS AND DISCUSSION

Herbal medicines have gained global importance, both medically as well as economically. In developing countries, 80% of the population depends on traditional systems of medicine as their primary source of healthcare. While in developed nations like United States, the sale of herbal products has skyrocketed from $200 million in 1988 to >$3.3 billion in 1997 and within the European Community, herbal medicines represent pharmaceutical market share, with annual sales in the range of $7 billion[14]. Despite such widespread acceptability of herbal medicines throughout the world, questions concerning their quality, safety and efficacy still eclipse their usage[14]. Although herculean efforts are being made by scientific community to establish their safety and efficacy, there is paucity of systematic protocols regarding their formulation and packaging. Consequently, the public at large is exposed to herbal drugs that are unregulated with respect to purity, safety, and efficacy[15]. Thus, the present study was undertaken to address this issue, and in consonance with the existing guidelines laid down in DSHEA, 1994 of US FDA, Indian Pharmacopoeia 2007, EMEA, Ayurvedic Pharmacopoeia of India, 1999, the qualitative and quantitative assessment of four marketed formulations of *B. monnieri* and their batch to batch consistency was carried out.

As part of quality control parameters, the labels were checked and compared with detailed criteria as per DSHEA, 1994 and Indian Pharmacopoeia 2007. The label was confirmed for presence or absence of important information including, name of the herbal supplement, net quantity of contents, any disclaimer, supplement facts panel (serving size, amount and active ingredient), other ingredients, name and address of manufacturer, packer or distributor for authenticity of product. The labels of the marketed formulations of Brahmi were not in conformation with guidelines and variations in label claim in terms of type of extract, extraction ratios and content were recorded (Table 2). Very importantly, 28 and 30 capsules were counted in bottles of SBE1 and SBE2, respectively, against a label claim of 60.

The weight of hard gelatin capsules filled with active ingredient and adjuvant is required to be uniform for a formulation, within set pharmacopoeial limits. Large variation in weight of capsules arise due to formulation practices that do not comply with good manufacturing practices and lead to variation in the content of capsule[16]. Such formulations expose the patients to pharmacodynamic and pharmacokinetic fluctuations, on consumption. Thus, in this study, the uniformity of weight of capsule content was checked according to method described in Indian Pharmacopoeia, 1996. The number of capsules deviating from average weight in different batches were- about 2 in H2 and SBE2, 1 in M2, 4 and 7 in S1 and S2, respectively. Consequently S1, S2, M2 failed the test limits as the number of capsules found to deviate were greater than permissible limits. This highlights the wide variation in content and lack of stringent quality control measures which allow such batches to be released in the market.

Identity, purity and strength of formulations were tested in accordance with the methodology detailed in Ayurved Pharmacopeia of India, 1999. The tests

TABLE 2: LABEL CLAIM OF DIFFERENT MANUFACTURERS

Criteria according to DSHEA,1964	Name of manufacturing company			
	Himalaya pure herbs	Sunova	Suraj Bala Exports	Multani purest herbs
Statement of identity	✓	✓	✓	✓
Net quantity of contents	✓	✓	✓	✓
Structure function claim	✓	✓	✓	✓
Directions	✓	✓	✓	✓
Supplement fact panel	✓	✓	✓	✓
Other ingredients	✓	✓	Not specified	Not specified
Name and place of Mfr	✓	✓	✓	✓
Alternative information	✓	Not specified	✓	Not specified
Storage	✓	✓	✓	✓
Mfg license and Batch no.	✓	✓	✓	✓
Mfg and expiry date and price	✓	✓	✓	✓

revealed that the formulations do not conform to the prescribed limits and a significant batch-to-batch variation exists. M1, M2, S1, S2 failed total ash content test. When H1 and H2, M1 and M2 formulations were incinerated an inorganic ash was obtained indicating high carbonate, phosphate, silicate and silica content (Table 3). Similarly, H2, M2, S2 and SBE2 failed acid-insoluble ash content test; H1 and H2, S1 and S2, M1 and M2, SBE1 and SBE2 showed significant batch-to-batch variation which may be due to presence of large amount of contaminants including silica, heavy metal etc. that were not degraded in presence of strong acid (Table 3). All samples except H1 and H2, were within specified limit for water-soluble extractives value (Table 3). H2, M1 and M2 failed the test for alcohol-soluble extractives value and H1 and H2, M1 and M2 showed significant batch-to-batch variation (Table 3).

Loss in weight on drying results due to loss of water and volatile matter of formulation upon drying and helps to determine the amount of moisture present in the sample. The acceptable limit for loss of weight upon drying as set by Indian Pharmacopoeia 2007 is not more than 6% of initial weight of sample taken. The weight loss of H1, M1, M2, S1, S2, SBE1, SBE2 was neither within specified limit nor consistent over the batches. M1 and M2, H1 and H2, S1 and S2, SBE1 and SBE2 showed significant batch-to-batch variation in the moisture content (Table 4).

TABLE 3: TOTAL ASH, ACID INSOLUBLE ASH, WATER SOLUBLE EXTRACTIVE AND ALCOHOL SOLUBLE EXTRACTIVE VALUES FOR DIFFERENT BATCHES of *B. monnieri* FORMULATIONS

Sample	Total Ash (%)	Acid insoluble Ash (%)	Water soluble extractive (%)	Alcohol soluble extractive (%)
H1	17.39	4.40	19.60	30.40
H2	13.90**	7.60**	48$^{\#}$	12**
M1	32.40	4.80	22.40	10.40
M2	28.00$^{\#\#}$	18.80$^{\sim}$	20	6.40$^{\#\#}$
S1	25.60	5.20	64	23.20
S2	26.00	20.80$^{\#\#}$	24*	24
SBE1	15.20	4.10	36.80	35
SBE2	15.80	9.65$^{\$\$}$	36.80	36.90

Limit of total ash content as per the Ayurvedic Pharmacopoeia of India not less than content 18%-**P<0.05 *vs* H1, $^{\#\#}P$<0.05 *vs* M1, compared with student's *t*-test. Limit of Acid Insoluble Ash Content as per the Ayurvedic Pharmacopoeia of India not less than content 18%. **P<0.05 *vs* H1, $^{\#\#}P$<0.05 *vs* S1, $^{\sim}P$<0.05 *vs* M1, $^{\$\$}P$<0.05 *vs* SBE1, compared with student's t-test. Limit As per the Ayurvedic Pharmacopoeia of India not less than 18%. $^{\#}P$<0.05 *vs* H1, *P<0.05 *vs* S1, compared with student's t-test. Limit As per the Ayurvedic Pharmacopoeia of India not less than 18%. **P<0.05 *vs* H1, $^{\#\#}P$<0.05 *vs* M1,compared with student's *t*-test.

Although literature describes spectofluorimetry, HPTLC based methods of estimation of bacoside A[17], we used UV spectroscopy based method to obtain standard curve of hydrolyzed bacoside A marker compound which was found to be linear over concentration range of 37.5-150 µg/ml with regression coefficient of 0.998. The amount of bacoside A present in each sample was calculated. The results reveal that quantity of bacoside A varies from manufacturer to manufacturer (Table 5). More importantly, there was significant difference in Bacoside A content within the two batches of M1 and M2, S1 and S2. This reveals that herbal formulations that are commercially available are not standardized with respect to quantity of bioactive molecule. Such pharmaceutically inequivalent formulations are rampantly being sold OTC and consumed.

Bacoside A and B, dammarane type triterpenoid saponins, present in *B. monnieri* possesses hemolytic activity[18,19] and this attribute was used to quantify total saponins present in the formulations. Absorbance of hemoglobin released from RBC lysis using Triton-X as the standard, was plotted to obtain a standard curve that was linear over the concentration range of 0.0125-0.1% v/v with regression coefficient of 0.986. A statistically different hemolytic activity of the two batches of the formulations H1 and H2, SBE1 and SBE2 was recorded (Table 6). This simple *in vitro* test highlights the fact that variation in content of the formulation can lead to variation in its biological activity.

Thus, a pharmaceutical formulation that is not standardized does not consistently provide the required amount of drug that is required for therapeutic benefit. In such a situation, a known

TABLE 4: PERCENTAGE OF WEIGHT LOSS ON DRYING OF DIFFERENT FORMULATIONS

Samples	Initial weight (g)	Final weight (g)	Percentage (%) loss of drying
H1	4.98	4.62	7.2
H2	5	4.74	5.2*
M1	5	4.69	6.2
M2	4.98	4.53	9$^{\sim}$
S1	4.93	4.47	9.3
S2	4.95	4.56	7.8$^{\#}$
SBE1	5.01	4.55	9.18
SBE2	5	4.56	8.8$^{\$}$

Limit for loss on drying as per Indian Pharmacopoeia-2007 not more than 6% of initial weight taken. *P<0.05 *vs* H1, $^{\sim}P$<0.05 *vs* M1, $^{\#}P$<0.05 *vs* S1, P<0.05 *vs* SBE1, compared with student's *t*-test.

TABLE 5: AMOUNT OF BACOSIDE A IN DIFFERENT FORMULATIONS

Sample	Amount of extract obtained (µg)	Amount of Bacoside A (µg)	Bacoside A (% w/w)
H1	640	34.43	5.3
H2	800	34.47	4.3
M1	150	47.63	31.75
M2	350	47.89	13.68$^{\$}$
S1	350	39.7	11.3
S2	200	35.52	17.76$^{\#}$
SBE1	140	44.73	31.95
SBE2	160	42.36	26.4$^{\sim}$

$^{\$}P<0.05$ *vs* M1, $^{\#}P<0.05$ *vs* S1,$^{\sim}P<0.05$ *vs* SBE1, compared with student's *t*-test.

TABLE 6: HEMOLYTIC INDEX (%) OF DIFFERENT FORMULATIONS

Sample	Hemolytic Index (%)
H1	17.90
H2	24.69*
M1	28.70
M2	25.66
S1	33.19
S2	34.68
SBE1	12.63
SBE2	7.59$^{\sim}$

*$P<0.05$ *vs* H1, $^{\sim}$P<0.05 *vs* SBE1; compared with student's *t*-test

therapeutically useful drug may cause more harm than benefit to the patient as it exposes him to sub- or-over therapeutic concentrations which may lead to either failure-of-therapy or unwanted adverse effects, respectively. In conclusion, qualitative and quantitative assessment of marketed formulations of *B. monnieri* shows that they are affected by the scourge of batch-to-batch variation. A multi-pronged approach that includes greater awareness amongst both consumers and manufacturers, stricter guidelines and their stringent implementation needs to be adopted, in order to derive the complete benefit of herbal products.

REFERENCES

1. Gedif T, Hahn HJ. The use of medicinal plants in self-care in rural central Ethiopia. J Ethnopharmacol 2003;87:155-61.
2. Gogtay NJ, Bhatt HA, Dalvi SS, Kshirsagar NA. The use and safety of non-allopathic Indian medicines. Drug Saf 2002;25:1005-19.
3. Dyaneshwar W, Patwardhan B. Botanical: Quality and Regulatory Issues. J Sci Ind Res 2005;64:83-92.
4. Prasad R, Bagde US, Puspangadan P, Varma A. *Bacopa monniera* L. Pharmacological Aspects and Case Study Involving *Piriformospora indica*. Int J Integr Biol 2008;3:100-10.
5. Brahmi. In: Agrawal SS, Tamrakar BP, Paridhavi M, editors. Clinical Useful Herbal Drugs. Delhi: Ahuja Publishing House; 2005. p. 157.
6. Roodenrys S, Booth D, Bulzomi S, Phipps A, Micallef C, Smoker J. Chronic effects of Brahmi (*Bacopa monnieri*) on human memory. Neuropsychopharmacol 2002;27:279-81.
7. Singh HK, Dhawan BN. Neuropsychopharmacological effects of the Ayurvedic nootropic *Bacopa monniera* Linn. (Brahmi). Indian J Pharmacol 1997;29:359-65.
8. Kasture SB, Kasture VS, Josuha AS, Damodaran A, Amit A. Nootropic activity of Bacomind, an enriched phytochemical composition from *Bacopa monnieri*. J Nat Remedies 2007;7:166-73.
9. Uniformity of Weight: Capsules; Indian Pharmacopoeia, Vol. 2. Govt. of India, Ministry of Health and Family Welfare; 1996. p. 134-5.
10. Ayurvedic Pharmacopoeia of India (API), Vol. 2, 1st ed, Part 1, Govt. of India, Min. of Health and Family Welfare, Depts. of ISM and H, New Delhi; 1999. p. 190-1.
11. Determination of Loss on Drying; Indian Pharmacopoeia (IP), Govt. of India, Min. of Health and Family Welfare; 2007. p. 134-5.
12. Pal R, Sarin JP. Quantitative determination of Bacosides by UV spectrophotometry. Ind J Pharm Sci 1992;54:17-8.
13. Sharma P, Sharma JD. *In vitro* hemolysis of human erythrocytes-by plant extracts with atiplasmodial activity. J Ethnopharmacol 2001;74:239-43.
14. Mahady GB. Global harmonization of herbal health claim. J Nutr 2001;131:1120S-3S.
15. Lipman TO. Herbal supplements. Curr Gastroenterol Rep 2005;7:302-7.
16. Felton LA, Garcia DI, Farmer R. Weight and weight uniformity of hard gelatin capsules filled with microcrystalline cellulose and silicified microcrystalline cellulose. Drug Develop Ind Pharm 2002;28:467-72.
17. Shrikumar S, Sandeep S, Ravi TK, Umamaheshwari M. A HPTLC determination and fingerprinting of Bacoside A in *Bacopa monnieri* and its formulation. Indian J Pharm Sci 2004;66:132-5.
18. Pawar R, Gopalakrishnan C, Bhutani KK. Dammarane triterpene saponin from *Bacopa monniera* as the superoxide inhibitor in polymorphonuclear cells. Planta Med 2001;67:752-4.
19. Dou DQ, Chen YJ, Liang LH, Pang FG, Shimizu N, Takeda T. Six new dammarane-type triterpene saponins from the leaves of *Panax ginseng*. Chem Pharm Bull (Tokyo) 2001;49:442-6.

Accepted 02 January 2012
Revised 27 December 2011
Received 07 February 2011
Indian J. Pharm. Sci., 2012, 74 (1): 24-28