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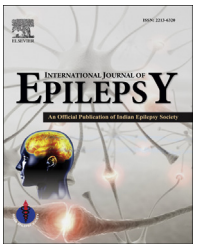

Original Article

Pharmacognostic, physicochemical and phytochemical investigation of *Bacopa monnieri* L. stem and its anticonvulsant potential in laboratory animals

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ABSTRACT

Aim: The aim of present work was to study the pharmacognostic standardization, physicochemical, phytochemical and pharmacological evaluation of the stem of *Bacopa monnieri* L.
Method: Standard method was followed for pharmacognostic, physicochemical, and phytochemical study. For quantitative analysis, HPTLC technique was employed. Maximal electroconvulsive shock (MES) and pentylenetetrazole (PTZ) method was used for anticonvulsant evaluation.
Result: Microscopic study of the stem showed presence of epidermis and cortex having large intercellular spaces. The oval shaped starch grains were present in cells of cortex and endodermis. Total ash (5.98%), water-soluble ash (4.91%), acid-insoluble ash (3.96%), water soluble extractive (15.31%) and ethanol soluble extractive (6.74%) values were evaluated for physicochemical evaluations. Quantitative estimation of Bacoside A (marker compound) by HPTLC technique was performed. The linearity (100–600 ng), the % recovery (97.81% w/w) were estimated. The Bacoside A in the alcoholic extract was found to be higher than that of the other extracts. The proposed HPTLC method was found to be simple and accurate. The anticonvulsant activities of all the extracts were evaluated by MES and PTZ models in mice.
Conclusion: The least anticonvulsant potential was recorded for acetone extract in dose of 100 mg/kg and maximum anticonvulsant action was recorded for ethanolic extract in dose of 300 mg/kg.

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1. Introduction

Bacopa monnieri L. (family Scrophulariaceae), commonly referred as *Brahmi*, is creeping plant used in Ayurveda for memory enhancement and for treatment of anxiety.[1] Due to its neuroprotective properties on the nervous system, Brahmi is known as *Medhya Rasayana*.[2] The chief traditional usages of *Bacopa monnieri* includes in anxiety, depression, learning and various neuropharmacological disorders whereas it has also been used to treat inflammation, pyrexia, constipation, cough, poisoning and blood disorders.[3] It has traditionally been given to children as brain booster.[4] As per traditional treatise of Ayurveda, Brahmi is claimed for various ailments but the literature review suggests that systematic standardization of this very plant is still lacking and scientifically chemical constituents responsible for its pharmacological activity especially anticonvulsant activity is to be proved.

In the present study, *Bacopa monnieri* Linn plant was collected, authenticated and standardized. The plant was dried, separately extracted in different solvents and extracts were concentrated under vacuum. These extracts were subjected to TLC and a solvent system was developed for the separation of Bacoside A. Bacoside A was estimated quantitatively in all three extracts by HPTLC. Keeping in view the traditional claim of Brahmi as remedy for epilepsy (Aakshepa),[5] focus is to be laid on investigation of the anticonvulsant potential of standardized extract of *Bacopa monnieri* which requires it's proving through scientific models. For this, the acetone, aqueous and ethanolic extracts of the stem of *Bacopa monnieri* were subjected to anticonvulsant evaluation by MES and PTZ test using Swiss albino mice.

2. Materials and methods

2.1. Pharmacognostical studies

2.1.1. Plant material

The plant specimens for the study were collected from the botanical garden of Jamia Hamdard, New Delhi (India). The sample was positively identified by local flora and authenticated by the Scientist-in-charge, National Botanical Research Institute (NBRI), Lucknow (India). The voucher specimen (NBRI/06/11), Reference No-Drug Authentication/2011/6/14-2 is kept preserved in laboratory for future references. Care was taken to select healthy fully grown plants with normal organs. The samples of the stems were cut suitably, removed from the plant, thoroughly washed with water to remove the adherent impurities, and dried under shade.

2.1.2. Macroscopical characterization

Visual observation was performed for macroscopical characterization by naked eyes. The shape, color, odor, and taste of stems were carried out and findings were reported.

2.1.3. Microscopical characterization

Sectioning. The fresh stem samples, embedded in paraffine were sectioned with the help of Rotary Microtome. The sections were dewaxed, transferred on slides, cleared with warming chloral hydrate, and mounted in glycerin. The thickness of the sections was kept between 12 and 15 μm. Serial dilution with ethanol viz. 30, 50, 75, 90% v/v and absolute ethanol was taken in 5 petriplates and numbered 1–5. The sections were placed for 10 min in 1st petriplate and then these were dehydrated by serially passing through petriplates 2–5 (5 min each) in reverse direction. The dehydrated sections were stained with saffranin and washed with distilled water three times. Finally, the stained sections were mounted in Canda balsam dissolved in Xylol. The microscopical study was performed by the use of trinocular microscope. Photomicrographs were taken with the help of inverted microscope (Letz Co, Japan) using different magnifications for photo documentation. The powdered drug was separately treated with phloroglucinol and hydrochloric acid (1:1) solution to determine the presence of annular vessels and parenchyma with starch grains. For normal observations, a bright field was used. For the study of powder microscopic features, polarized light was employed.[6]

2.1.4. Physicochemical evaluations

Physicochemical parameters of *Bacopa monnieri* L., stem powder were determined with special reference to total ash, water-soluble ash, acid-insoluble ash, alcohol and water soluble extractive values. In order to determine water and alcohol soluble components, alcohol and water-soluble extractive values were determined. The % moisture content and pH were also determined.[7]

2.1.5. Extraction

The stem powder of *B. monnieri* (250 g) was extracted by Soxhlet extraction method in acetone and ethanol separately.[8] For aqueous extract, cold maceration was adopted. All the extracts were concentrated on vacuum dryer separately and labeled as AE (aqueous extract), ACE (acetone extract) and EE (ethanolic extract).

2.1.6. Hemolytic evaluation

A serial dilution of ethanolic extract of Brahmi (0.5 mg/ml) was prepared with Phosphate buffer pH 7.4 and blood suspension (2% in sodium citrate 36.5 g/l) in 10 test tubes (Tables 1 and 2). Mixed and observed for 24 h. The dilution at which total hemolysis occurred, indicated by a clear, red solution without any deposit of erythrocytes was examined and recorded. The concentration of the dilution was converted into gram (g).[9]

Parallelly, a serial dilution of Saponin R(0.01 mg/ml) was also prepared and observed for total hemolysis. The total hemolytic concentration was also converted into gram (g).

The hemolytic activity was calculated by using the following formula[10]:

$$1000 \times a/b$$

where 1000 = the defined hemolytic activity of saponin R in relation to ox blood;a = quantity of saponin R that produces total hemolysis (g);b = quantity of herbal material that produces total hemolysis (g).

The process was performed in triplicate with different dilutions and result was presented in mean ± SEM.

Table 1 – Serial dilution of etanolic extract of Brahmi for hemolytic index.

Test tube	1	2	3	4	5	6	7	8	9	10
Ethanolic extract of Brahmi (0.5 mg/ml) in ml	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
Phosphate buffer pH 7.4 in ml	**0.9**	**0.8**	**0.7**	**0.6**	**0.5**	**0.4**	**0.3**	**0.2**	**0.1**	-
Blood suspension (2%) in ml	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

Table 2 – Serial dilution of Saponin R for hemolytic index.

Test tube	1	2	3	4	5	6	7	8	9	10
Saponin R (0.01 mg/ml) in ml	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1**
Phosphate buffer pH 7.4 in ml	**0.9**	**0.8**	**0.7**	**0.6**	**0.5**	**0.4**	**0.3**	**0.2**	**0.1**	-
Blood suspension (2%) in ml	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**

2.2. *Phytochemical analysis*

2.2.1. *Preliminary phytochemical screening*

All the three concentrated extracts were subjected to various phytochemical tests to detect the presence of different phytoconstituents.[11,12]

2.2.2. *Preparation of standard solution and samples for HPTLC*

A reference standard solution of Bacoside A (10 μg/ml) was prepared by dissolving 1 mg of accurately weighed Bacoside A in methanol by sonication in ultrasonic water bath to dissolve and making up the volume to 100 ml with methanol. The stock solution was further diluted with methanol to give a standard solution of Bacoside (100 ng/μl). 250 mg of extracts were sonicated, filtered through Whatman filter paper No1 and the final volumes were made up to 25 ml with methanol.

2.2.3. *Chromatographic conditions (for bacoside A)*

Stationary phase: Pre-coated silica gel 60F254 TLC plate (10 × 10 cms, 0.2 mm thickness). Mobile phase: Butanol: Glacial acetic acid: water (4:1:5v/v), saturation time: 15 min, Wavelength (λ): 225 nm, Radiation source: Deuterium.

2.2.4. *Calibration curve*

The standard solutions (100–600 ng per respective spot) were applied in triplicate on TLC plate. The plate was developed and scanned as per the chromatographic conditions mentioned above. The peak areas were recorded. Calibration curve of Bacoside A was prepared by plotting peak areas vs. concentrations of Bacoside A applied.

2.2.5. *Analytical procedure*

Extracts and standard solution of Bacoside A were spotted on a 10 × 10 cm precoated TLC plates as 6 mm wide band and 8 mm from the bottom by using Linomat V. The plates were developed in a twin trough chamber, under the chromatographic conditions given above, by ascending mode to a distance of 8 cm under chamber saturation conditions. After development the plates were dried in air and scanned at 225 nm for bacoside A by using CAMAG Scanner III. The contents of Bacoside A extracts were calculated from the respective calibration curve.

2.3. *Pharmacological screening*

2.3.1. *Animals*

Swiss albino mice (25–30 g) of either sex were obtained from Institutional Animal House and same place was used throughout the study for keeping the animals. The animals were maintained at constant room temperature (22–25 ± 2 °C) and 12 h light/12 h dark cycle was maintained. The animals were provided *ad libitum* as food and free access to water. The experimental protocols for the activity were approved by the Institutional Animal Ethics Committee (IAEC) constituted under Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA No.837/ac/04/CPCSEA) with resolution number 2011/837ac/PhD/06.

2.3.2. *Determination of acute toxicity*

The acute toxicity studies of all the three extracts were determined in swiss albino mice. The animals were fasted overnight prior to the start of experiment and fixed dose in compliance with OECD guideline No. 420 (Annexure 2d) method of CPCSEA was adopted to perform the acute toxicity studies.[13,14] The dried extracts were administered in doses of 25, 150, 500 and 1000 mg/kg p.o. to different groups of mice each containing six animals (n = 6) and mortality were observed after 24 h. The acetone, aquous and ethanolic extract of *B. monnieri* stem exhibited absence of mortality of mice at dose of 1000 mg/kg when dose was administered by p.o. route and therefore LD50 value in the present case was >1000 mg/kg. So far as 1/10th dose of LD50 is to be selected for screening the activity, 100 mg/kg body weight was used anticonvulsant evaluation.

2.3.3. *Maximum electroshock induced seizures (MES)*

In order to induce and evaluate convulsions, the maximal electroshock (MES) method was performed.[15] Mice deprived of food and water (*ad libitum*) for overnight, were randomly distributed in to eleven groups (n = 6). Group I served as control (vehicle treated), Group II served as standard (treated with Phenytoin sodium 25 mg/kg body weight); Group III, IV and V were treated with acetone extract (ACE) as 100, 200 and 300 mg/kg body weight respectively and Group VI, VII and group VIII were treated with aqueous extract (AE) dose 100, 200 and 300 mg/kg body weight respectively. Group IX, X and

group XI were treated with ethanolic extract (EE) dose 100, 200, and 300 mg/kg body weight respectively. The test extract was administered orally in 2%v/v Tween 80 solution, 1 h before inducing the convulsion. Standard drug (Phenytoin sodium 25 mg/kg) was administered i.p. 30 min before the delivery of shock. Electro convulsive shock (50 mA for 0.2 s) was delivered through corneal electrode to induce convulsions to all eleven groups of mice (n = 6). The various phases of convulsions including Flexion, Extension, Clonus and Stupor were observed. Prior to delivery, current output was checked by multimeter. After the electric stimulation, the duration of phases was noted and HLTE (Hind limb tonic extension) phase was compared with control group. Decrease in duration of HLTE was counted as protective effect.[16]

2.3.4. *Pentylenetetrazole (PTZ) induced seizures*

For this, the mice were divided in to eleven groups (n = 6). Group I served as control (vehicle treated i.e. Tween 80, 2%), Group II served as standard and received Diazepam 4 mg/kg body weight (i.p.), Group III, IV and V were treated with ACE as 100, 200 and 300 mg/kg body weight respectively and Group VI, VII and Group VIII were treated with AE dose 100, 200 and 300 mg/kg body weight respectively. Group IX, X and group XI were treated with EE dose 100, 200 and 300 mg/kg body weight respectively. 30 min after i.p. injection of Diazpam and 60 min after oral administration of extracts, 60 mg/kg PTZ was injected subcutaneously. The anticonvulsant properties of different extracts were accessed by its ability to delay the onset of myoclonic spasms and clonic convulsions.[17,18]

2.4. *Statistical analysis*

The results expressed as mean ± SEM and statistical analysis was carried out using one way ANOVA followed by Dunnett multiple test, where a difference of $P < 0.01$was considered significant in all cases.

3. Results

3.1. *Pharmacognostical studies*

3.1.1. *Macroscopic features*

The fresh plant of *B. monnieri* is succulent but in later conditions, it gets shriveled on drying, slightly bitter in taste, without any characteristic odor. The stems of this creeping herb were observed as, wiry, small, green, or purplish green (Fig. 1). The stem was little spongy, breakable 4–6 inch in length and about 1–2 mm thick. The stems were having soft nodes and internodes. Leaves were sessile, oblong and fleshy succulent.

3.1.2. *Microscopical study*

Outer cortex along with dark circular periphery was observed in TS of stem (Fig. 2a, b). In the centre of multilayered cortex of stem, calcium oxalate crystals were found (Fig. 2c). Vascular ring was continuous, composed of a narrow zone of phloem towards periphery. The centre occupied by a small pith with distinct intercellular spaces and the simple starch grains with round to oval shape were present in a few cells of cortex and endodermis.

3.1.3. *Powder microscopy*

The microscopical study of powder exhibited presence of annular vessels, compound starch grains with parenchyma cells. The powder also showed presence of prismatic crystals of calcium oxalate, lignified fibers, tannin contents, pitted vessels, simple and compound starch grains and parenchyma cells filled with starch grains (Fig. 3).

3.1.4. *Physicochemical parameters*

B. monnieri powder exhibited the presence of total ash 5.98% w/w, acid insoluble ash 3.96% w/w, water soluble ash 4.21% w/w, water soluble extractive 15.31%w/w, ethanol soluble extractive 6.74% w/w, moisture content 4.10% and pH 6.71. The stomatal index 27.99, palisade ratio 3.66, foaming index 115.74 and hemolytic index was found to be 17.50 (Table 3).

3.2. *Phytochemical analysis*

3.2.1. *Preliminary phytochemical studies*

The phytochemical analysis showed the presence of alkaloid and saponin in all three extracts. The acetone and ethanolic extract also showed presence of glycosides and sugars. Test for steroid and tannin was negative for each extract of *B. monnieri*. The test for resin showed negative result. Amino acid test was positive only in ethanolic extract sample of *B. monnieri* (Table 4).

3.2.2. *HPTLC studies*[19]

In HPTLC, Bacoside A showed single peak at 0.43 Rf, which was observed in the chromatogram of each samples. Calibration curves was prepared by plotting concentrations versus average area of the peak (Fig. 4).The linear regression analysis of calibration plots of Bacoside A exhibited linear relationship in the range of 100–600 ng with the correlation coefficient(r^2)

Fig. 1 – External morphological appearance of *B. monnieri*.

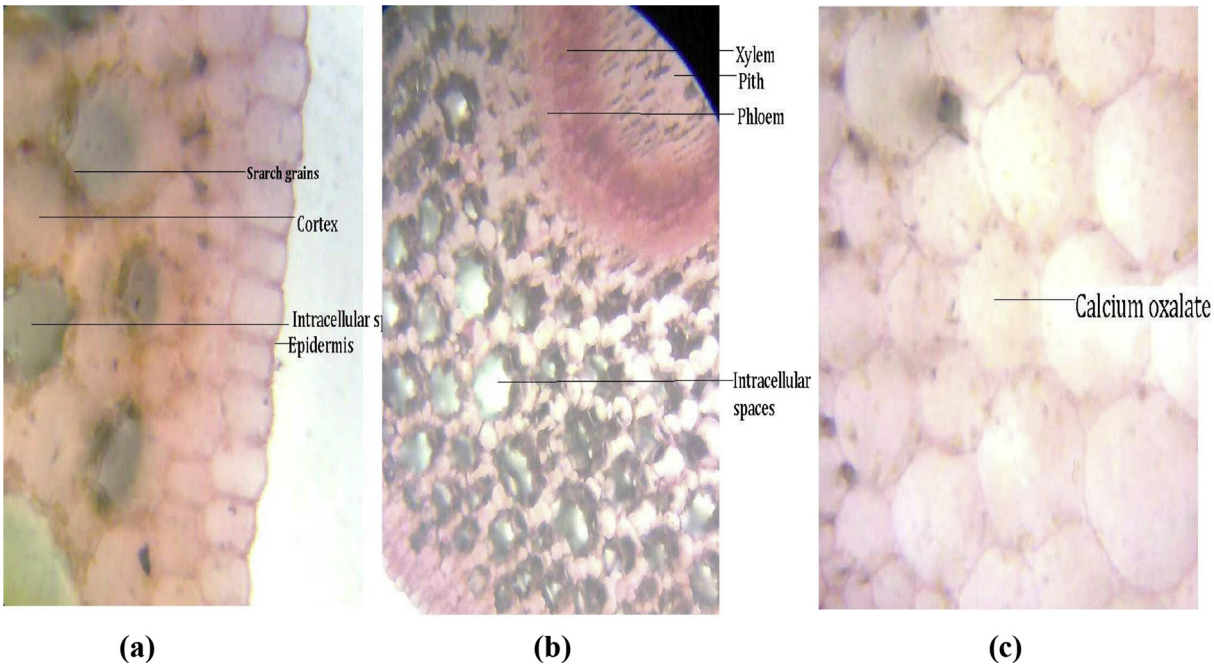

Fig. 2 – (a) Microscopical view of the T. S. of the *B. monnieri* stem-cortex and epidermis., (b) Microscopical view of the T. S. of the *B. monnieri* stem-vascular bundle and pith., (c) Microscopical view of the T. S. of the *B. monnieri* stem- Calcium oxalate.

0.991 and thus exhibits good linearity between concentration and area. Good correlation was also obtained between standard and different extract overlay HPTLC spectra of Bacoside A. The colored lines represent the absorption maxima for samples and standard Bacoside A (Fig. 5). Recovery study was carried out by spiking the pre-analyzed samples with pure Bacoside A (Table 5). The percentage recovery of Bacoside A was found to be 97.81%. The limit of detection (LOD) and limit of quantification (LOQ) were determined by signal to noise ratio method and found to be 30.6 ng/spot and 92.4 ng/spot. The precision of the method was evaluated in terms of interday and intraday precision and presented as % relative standard deviation (%RSD). %RSD for intraday (0.891) and interday (1.112) measurement was less than 2 indicating that proposed method is precise (Table 6). All the three different extracts were analyzed by the proposed method. The data from Table 7 revealed that the Bacoside A contents in ethanolic extract, acetone extract and aqueous extract were found as 1.14 ± 0.02%, 0.69 ± 0.03% and 0.83 ± 0.05%.

3.3. *Pharmacological screening*

The MES and the PTZ induced seizures may be the inhibited by drugs which causes voltage-dependent blockade of membrane Na+channels responsible for the action potential.[20] Phenytoin, Valproate, Felbamate and Lamotrigine inhibits the voltage dependant Na+channels. Prolongation of this inactive state with prolongation of the refractory period is considered as underlying mechanism of anticonvulsant drugs. For tonic seizures, same can be inhibited either by drugs that act as positive modulator of$GABA_A$ receptors and blocks N-methyl-D-aspartate (NMDA).[21,22] The different extracts of *B. monnieri* may follow any one of the above stated mechanism. The findings of anticonvulsant effect of *B. monnieri* against MES and PTZ induced convulsions are shown in Table 8 and Table 9 respectively.

The one way ANOVA analysis of the results observed indicated that the anticonvulsant potential of ACE at each dose tested was insignificant when compared with control. The aqueous and alcoholic showed significant anti-seizure effect against MES and PTZ induced convulsions when comparison was made with control group.

In MES model, Phenytoin was used as standard drug whereas Diazepam was used as standard drug in PTZ model. The time recorded for HLTE in standard group was zero sec in MES induced convulsion model. The onset time for Diazepam treated standard group was recorded as zero sec for jerks, clonus and extensor. Control group animals exhibited HLTE of 12.21 ± 0.06sec after the delivery of an electro convulsive shock. The AE and EE at the dose of 100 mg/kg body weight shown very insignificant effect on total duration of HLTE in comparison to control group, while the dose of 200 mg/kg body weight of aqueous and ethanolic extract, reduced the duration of HLTE to 7.52 ± 0.06 and 5.28 ± 0.09 s respectively. The dose of 300 mg/kg body weight of aqueous and ethanolic extract, reduced the duration of HLTE to 5.25 ± 0.04 and 3.29 ± 0.54 s respectively which indicates good anticonvulsant potential of AE and EE in 200 mg/kg body weight.

In PTZ induced seizures, the delayed onset for clonus recorded for ACE-100 mg/kg. However ACE 200 and 300 mg/kg increased the onset of action for clonus i.e. 78.58 ± 0.03 and 79.25 ± 0.01 s. The EE dose 200 mg/kg and 300 mg/kg showed the onset time for clonus as 88.68 ± 0.08 and 94.29 ± 0.54 s respectively which indicates significant anticonvulsant action in comparision to control group animals in both the models.

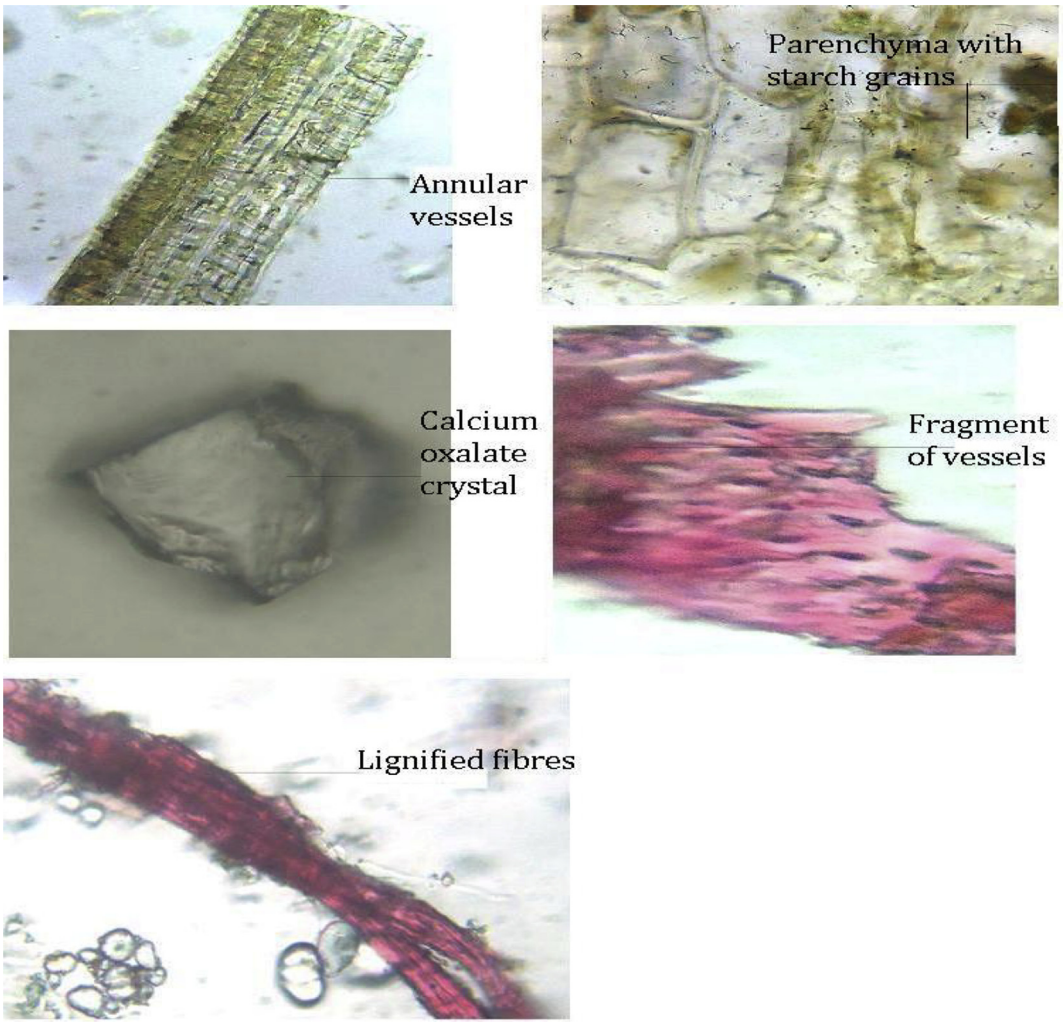

Fig. 3 – Powder microscopy of the *B. monnieri* stem (Annular vessel, Parenchyma with starch grains, calcium oxalate crystal, fragment of pitted vessels and lignified fibers).

In a study conducted on *Bacopa monniera* leaves, mechanism elucidated for anticonvulsant action was GABA agonist action.[23]

In comparative terms, it may be concluded that anticonvulsant activity of three different extracts were found in dose dependent manner. The least activity was recorded for acetone extract whereas ethanolic extract exhibited maximum anticonvulsive potential.

Brahmi is very common and important plant and is key ingredient of various formulations which includes Brahmi

Table 3 – Physicochemical analysis of stem of *B. monnieri* L.

Physicochemical parameters	Value
Total ash	5.98 ± 0.41%
Acid insoluble ash	3.96 ± 0.28%
Water soluble ash	4.21 ± 0.38%
Water soluble extract	15.31 ± 0.245
Ethanol soluble extract	6.74 ± 0.49%
Moisture content	4.10 ± 0.83%
pH	6.71 ± 0.09
Stomatal index	27.99 ± 0.50
Palisade ratio	3.66 ± 0.25
Foaming index	115.74 ± 14.10
Hemolytic index	17.50 ± 0.80

Table 4 – Phytochemical analysis of *B. monnieri* Linn. stem.

Phytoconstituents	Acetone extract	Ethanol extract	Aqueous extract
Alkaloid	+	+	+
Glycoside	+	+	+
Terpene	+	–	+
Saponin	+	+	+
Tannin	–	–	–
Amino acid	–	+	–
Sugar	+	+	+
Resin	–	–	–
Steroid	–	–	–

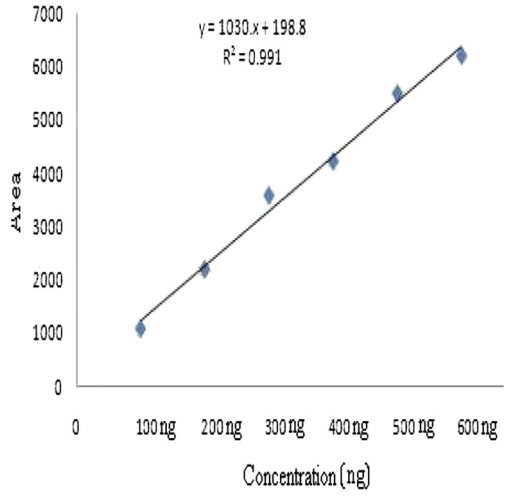

Fig. 4 – Calibration curve of Bacoside A.

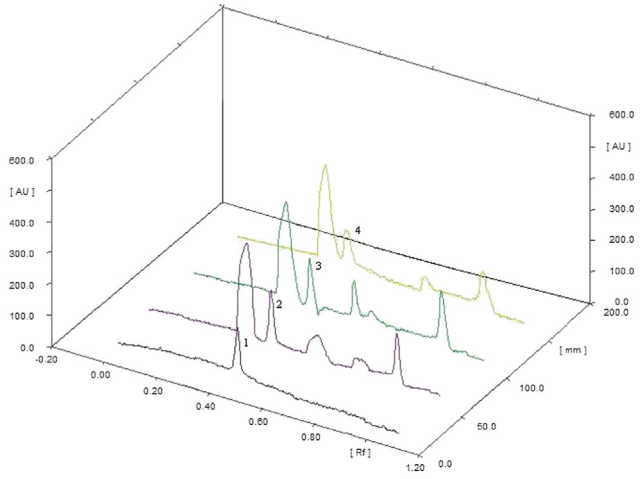

Fig. 5 – Chromatogram of Bacoside A(1), EE(2),AE(3) and ACE(4).

ghrita, Brahmi vati etc. The patients are now more concious about quality and standardization parameters. In a recent work carried on anticonvulsant potential of commonly practiced formulations of Brahmi (*Bacopa monnieri* Linn.) in Wistar rats, it was concluded that Brahmi Ghrita (BG) and Saraswatarishta (SW) are effective in promoting restorative and neuroprotective action in convulsions.[24]

Table 7 – Bacoside A content in different extracts.

Sample	% Bacoside A (mean ± SEM)
EE	1.14 ± 0.02
AE	0.83 ± 0.05
ACE	0.69 ± 0.03

Table 8 – Effect of extracts of *B. monnieri* against MES induced convulsions.

Groups	Onset time in sec (mean ± SEM)		
	Jerks	Clonus	Extensor
Gr-I (Control)	49.81 ± 0.15	75.61 ± 0.04	275.15 ± 0.21
Gr- II (Phenytoin)	0.00	0.00	0.00
Gr-III (ACE-100)	47.18 ± 0.14	76.25 ± 0.21	278.36 ± 0.11
Gr-IV (ACE-200)	50.35 ± 0.32	78.58 ± 0.03	281.59 ± 0.16
Gr-V (ACE-300)	51.22 ± 0.10	79.25 ± 0.01	285.32 ± 0.41
Gr-VI (AE-100)	48.29 ± 0.08	76.50 ± 0.23	278.26 ± 0.24
Gr-VII (AE-200)	52.19 ± 0.05*	81.52 ± 0.06*	293.54 ± 0.16*
Gr-VIII (AE-300)	54.91 ± 0.18*	83.25 ± 0.04*	305.19 ± 0.36*
Gr-IX (EE-100)	49.59 ± 0.10	78.21 ± 0.05	280.53 ± 0.31
Gr-X (EE-200)	56.67 ± 0.21*	88.68 ± 0.08*	298.21 ± 0.46*
Gr-XI (EE-300)	61.71 ± 0.06*	94.29 ± 0.54*	315.51 ± 0.11*

(* = $p < 0.01$), ACE-Acetone extract, AE-Aqueous extract, EE-Etahanolic extract, 100, 200 and 300-indicates dose of extract was 100 mg/kg bw, 200 mg/kg and 300 mg/kg body weight respectively. Values are presented in mean ± SEM., n = 6.

4. Discussion

WHO has recognized epilepsy to be a major public health problem all over world. In Ayurvedic text, Apasmara (epilepsy) has been defined as sudden abhorrent body activities accompanied by memory blackout or loss of consciousness owing to disturbance in mental functions like memory, retention and intelligence.

Bacopa monnieri is a nervine tonic used for raising the mental performance in Ayurvedic therapies. Bacoside A, the

Table 5 – Results of recovery study.

Initial concentration of bacoside A (A)	Amount of bacoside A added (B)	Theoretical concentration of bacoside A (A + B = C)	Detected amount of bacoside A (D)	% Recovery D/C × 100
200 ng	100 ng	300 ng	291.75 ± 5.8 ng	97.25%
200 ng	200 ng	400 ng	389.40 ± 8.7 ng	97.35%
200 ng	300 ng	500 ng	494.29 ± 4.3 ng	98.85%
			Average recovery	97.81%

Table 6 – Results of validation method.

Analytical method	Linearity	r^2	Accuracy (% recovery)	Precision (%RSD)		LOD (Ng/spot)	LOQ (Ng/spot)
				Intra day (n = 3)	Inter day (n = 3)		
HPTLC	100–600 ng	0.991	97.81%	0.891	1.102	30.6	92.4

Table 9 – Effect of extracts of *B. monnieri* against PTZ induced convulsions.

Groups	Time (s) in various phases of convulsions (mean ± SEM)				
	Flexion	Extension (HLTE)	Clonus	Stupor	Recovery
Gr-I (Control)	4.18 ± 0.05	12.21 ± 0.06	14.05 ± 0.51	99.51 ± 0.60	125.15 ± 0.526
Gr- II (Diazepam)	1.42 ± 0.24	0.00	06.54 ± 0.05	45.21 ± 0.21	031.22 ± 0.215
Gr-III (ACE-100)	3.84 ± 0.04	11.25 ± 0.21	13.56 ± 0.21	95.21 ± 0.16	118.18 ± 0.540
Gr-IV (ACE-200)	3.15 ± 0.02	9.58 ± 0.03	12.59 ± 0.16	90.82 ± 0.51	095.21 ± 0.220
Gr-V (ACE-300)	2.82 ± 0.11	8.25 ± 0.01	11.45 ± 0.31	88.25 ± 0.61	093.11 ± 0.156
Gr-VI (AE-100)	4.10 ± 0.21	11.50 ± 0.23	12.56 ± 0.51	90.21 ± 0.53	116.14 ± 0.410
Gr-VII (AE-200)	3.51 ± 0.10	7.52 ± 0.06*	11.54 ± 0.36	81.54 ± 0.45	108.21 ± 0.221
Gr-VIII (AE-300)	2.71 ± 0.20	5.25 ± 0.04*	8.51 ± 0.46*	50.25 ± 0.51*	095.25 ± 0.151
Gr-IX (EE-100)	3.52 ± 0.20	11.21 ± 0.05	12.45 ± 0.31	89.25 ± 0.53	111.25 ± 0.413
Gr-X (EE-200)	2.55 ± 0.11	5.28 ± 0.09*	11.52 ± 0.51	69.46 ± 0.19*	106.21 ± 0.213
Gr-XI (EE-300)	1.51 ± 0.09	3.29 ± 0.54*	8.21 ± 0.21*	48.51 ± 0.43*	096.52 ± 0.115

*$p < 0.01$ when compared with control; n = 6

putative bioactive component of the Indian medicinal plant *Bacopa monnieri*, was found to be a mixture of saponins with Bacoside A_3, Bacopaside II, Jujubogenin isomer of Bacopasaponin C as major constituents and their concentration ranges of samples of *B. monnieri* collected from different regions of India were found to be 0.14–0.85% (w/w), 0.12–0.69%, 0.05–0.72% and 0.05–0.44% respectively.[25] Bacoside A has been reported to have vasodilating and hepatoprotective activities.[26,27] It also protects the brain from the toxic effects of cigarette smoking, has free radical scavenging activity and improves mental retention capacity.[25] In the present study Bacoside A content was estimated and extracts were subjected to evaluation of anticonvulsant activity and found that anticonvulsant potential was increasing with the concentration of Bacoside A and the extract containing highest amount of Bacoside A was found to have maximum activity.

5. Conclusion

By the above results we can conclude that Bacoside A content of *B. monnieri* may be responsible for its anticonvulsant activity.

Conflicts of interest

All authors have none to declare.

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