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Research Article

Standardization of *Bacopa Monnieri* and its Formulations with reference to Bacoside A, by High Performance Thin Layer Chromatography

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ABSTRACT
A simple and reproducible High Performance Thin Layer Chromatographic method for the determination of bacoside A in *Bacopa monnieri* and its prepared formulations was developed and is described. Phytochemical screening of methanolic extract of *Bacopa monnieri* is performed and a Thin Layer Chromatographic method was developed using n-Butanol: Acetic acid: Water (36: 6: 8 v/v). The High Performance Thin Layer Chromatographic method involved separation of components by TLC on stationary phase i.e. precoated silica gel GF_{254} with a solvent system of n-butanol: Acetic acid: Water (36:6:8) and detection was carried out by scanning and quantifying the peak at 580 nm for bacoside A. The method was validated in terms of linearity, accuracy and precision. The sensitivity of High Performance Thin Layer Chromatographic method was found to be 46 ng and the linearity was observed in the range of 200 ng to 1200 ng. The proposed method was found precise and sensitive and can be used for detection, monitoring and quantification of bacoside A in *B. monnieri* and its formulations.

Keywords: *Bacopa monnieri*, bacoside A, formulations, HPTLC, standardization.

INTRODUCTION

Standardization of Ayurvedic drugs, herbal formulations and plant materials is the need of the day. Many of them do not have standard identification tests or analytical procedures to maintain their consistent quality. Several Pharmacopoeias and books containing monographs on plant materials describe only the physico-chemical parameters and are lacking in identification and quantification of active compounds. Hence modern methods describing the identification and quantification of biomarkers in plants may be useful for proper standardization of herbs and their formulations. *B. monnieri*, Scrofulariaceae (sometimes also called as *Bacopa monniera*, *Herpestis monniera*) is a medicinal plant which has proven brain tonic properties or capable of improving mental ability and memory.[1-3] *B. monnieri* is widely used in indigenous system of medicine. It was found to contain different types of saponins, out of them the steroidal tetracyclic triterpenoid saponin bacoside A was found to be the major active principle having biological activity. [4, 5, 6] Quality control of synthetic drugs offers no problem with very well defined parameters of analysis as compared to herbal formulations of plant origin, which are prone to deterioration and variation. [7] The quality control of herbal medicines is therefore highly desired to ensure their authenticity, stability and consistency. HPTLC is becoming a routine analytical technique because of its advantages of low operating cost, high sample throughput, simplicity and speed, the need for minimum sample clean up, reproducibility, accuracy, reliability and robustness[8]. In the present study a suitable, sensitive and reliable quantitative HPTLC method has been developed for quality control determination of Bacoside A from *B.monnieri* plant and its formulations.

MATERIALS AND METHODS

Fresh aerial parts of *B. monnieri* were collected from local (Dadar) Market Mumbai. The crude drug materials were authenticated at Agharkar Research Institute, Pune as Bacopa monnieri. Standard bacoside A was purchased from Natural Remedies Pvt. Ltd., Bangalore.

Extraction

Around 50 g of air dried whole plant sample was ground to pass through No.40 mesh sieve and extracted with 80% methanol by soxhlet extraction method. The extract was filtered and concentrated. The final solution for spotting was prepared by reconstituting the extract in methanol to give a final concentration of 1 mg/ml. This solution was used further for HPTLC analysis as per the procedure mentioned below.

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Table 1: Results of accuracy by recovery for methanolic extract of *b. Monnieri* and its developed formulations.

Sample	Amount of sample taken (mg) **(a)**	Amount of Bacoside A present (mg) **(b)**	Amount of Bacoside A added (mg) **(c)**	Amount of Bacoside A taken (mg) **(d = b + c)**	Total Bacoside A found (E) mg	% recovery E/D x 100
B. monnieri	1000	31	10	41	40	97.96
B.monnieri	1000	31	12.5	43.5	40.2	92.42
B. monnieri	1000	31	15	46	42.1	91.92
Capsule	1000	12.9	6	18.9	18.7	98.94
Capsule	1000	12.9	3.5	16.4	16.04	97.80
Capsule	1000	12.9	1.2	14.1	13.9	98.58
Tablet	1000	12.4	6	18.4	17.93	97.45
Tablet	1000	12.4	3.5	15.9	15.38	96.73
Tablet	1000	12.4	1.2	13.6	13.07	96.11

Table 2: Percentage of Bacoside a in *B.Monnieri* extract and its formulations.

Sample	% Bacoside A* ± SD
B. monnieri extract (80 % methanolic)	3.1± 0.169
Capsule formulation	1.30 ± 0.03443
Tablet formulation	1.26 ± 0.05859

* The values are mean of three determinations, observation ± standard deviation.

For *B. monnieri* formulations (developed tablet and capsule formulations) 10 tablets as well as capsules were ground to a fine powder. A weight equivalent to 10 mg of bacoside A was transferred to a conical flask and extracted with methanol. The extracts were filtered through whatman filter paper and the residue was washed with 10 ml of methanol. Both extract and washings were transferred to a 100 ml of volumetric flask and volume was made up to 100 ml with methanol.

Qualitative Phytochemical Evaluation

The qualitative chemical test were performed on the methanolic extract obtained by the solvent extraction of *Bacopa monnieri* to detect the presence of phytochemicals such as alkaloids, glycosides, tannins, saponins, phenols etc. These phytoconstituents were tested using standard procedures [9, 10]

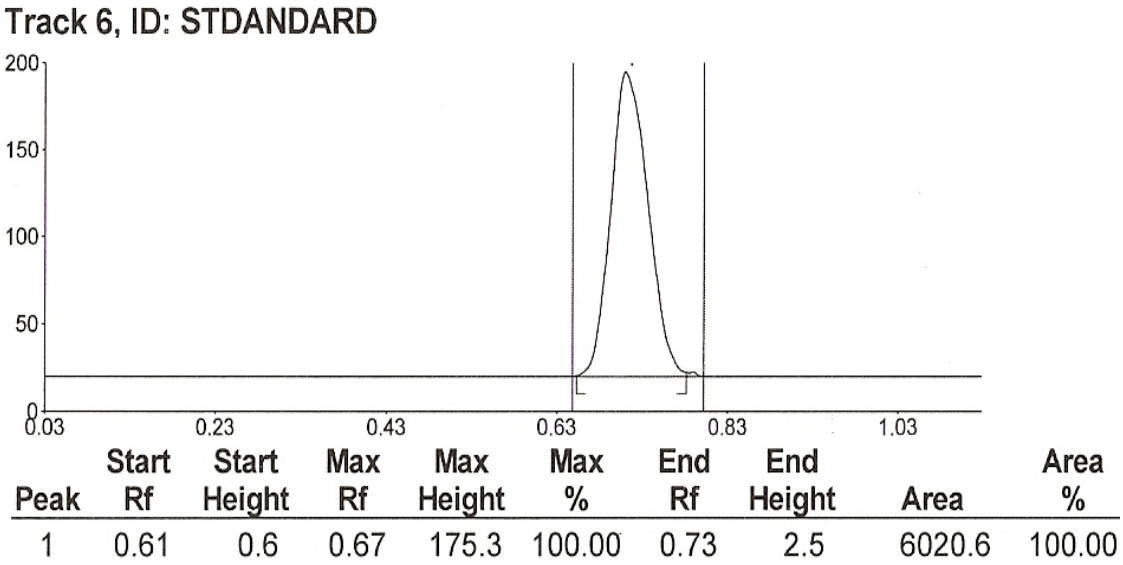
Thin layer chromatography analysis

Since Bacoside A has been reported as a major phytoconstituent in the *Bacopa monnieri*, the standard bacoside A was used as a marker for TLC evaluation[11,12,13]. In this evaluation, concentrated methanolic extract was used to spot on the precoated Silica gel 60 GF_{254} E. Merck plates. The plate was developed using n- Butanol: Acetic acid: water (36:6:8 v/v) in mobile phase and 20 % sulphuric acid in methanol was used as the spraying reagent for visualization.

High Performance Thin Layer Chromatography:

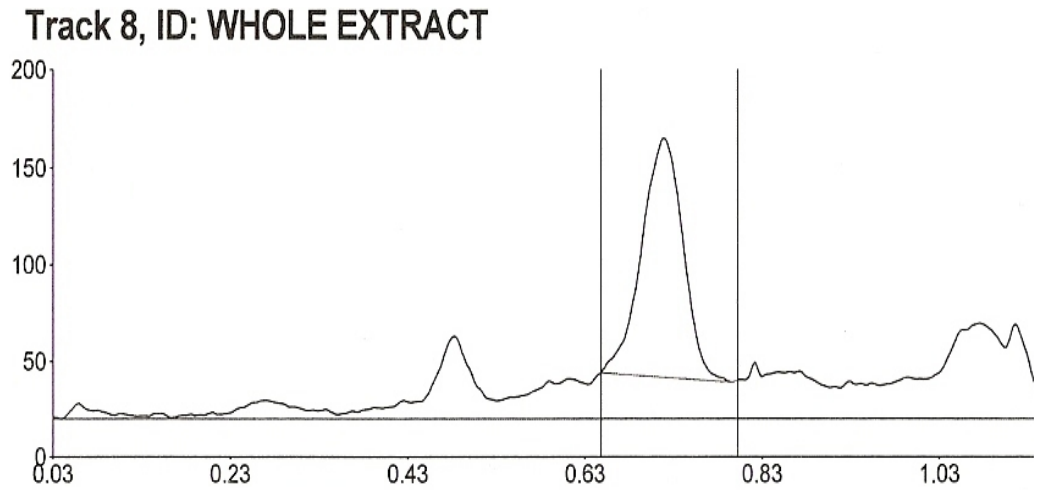
Chromatographic conditions:

Instrument: A Camag HPTLC system equipped with a sample applicator Linomat IV, Twin through plate



Solvent system: n-Butanol: acetic acid: water (36: 6: 8 v/v). Peak for is Bacoside A (R_f = 0.67)

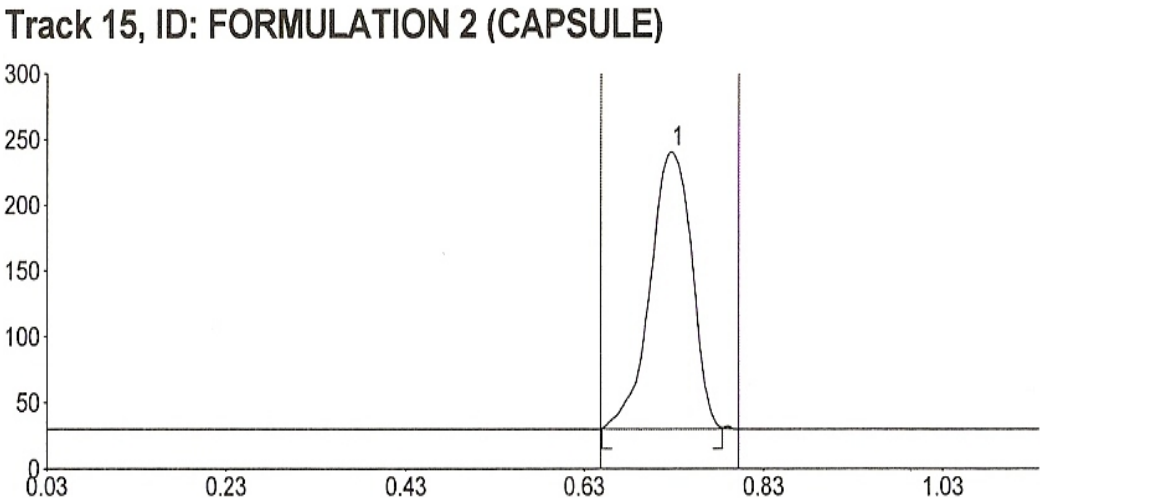
Fig. 1: HPTLC Chromatogram of Standard Bacoside A.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.61	0.9	0.68	123.4	100.00	0.74	0.1	4526.0	100.00

Solvent system: n-Butanol: acetic acid: water (36: 6: 8 v/v). Peak for is Bacoside A (R_f = 0.68)

Fig. 2: HPTLC Chromatogram of *Bacopa monnieri* methanolic extract.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.61	0.5	0.68	211.0	100.00	0.74	1.3	7686.6	100.00

Solvent system: n-Butanol: acetic acid: water (36: 6: 8 v/v). Peak for is Bacoside A (R_f = 0.68)

Fig. 3: HPTLC Chromatogram of *B. monnieri* capsule formulation.

development chamber, TLC Scanner III and an Integration software CATS.

Adsorbent: TLC aluminum plate Precoated with silica gel GF_{254} (E. Merck)

Solvent system: n-Butanol: Acetic acid: Water (36:6:8)

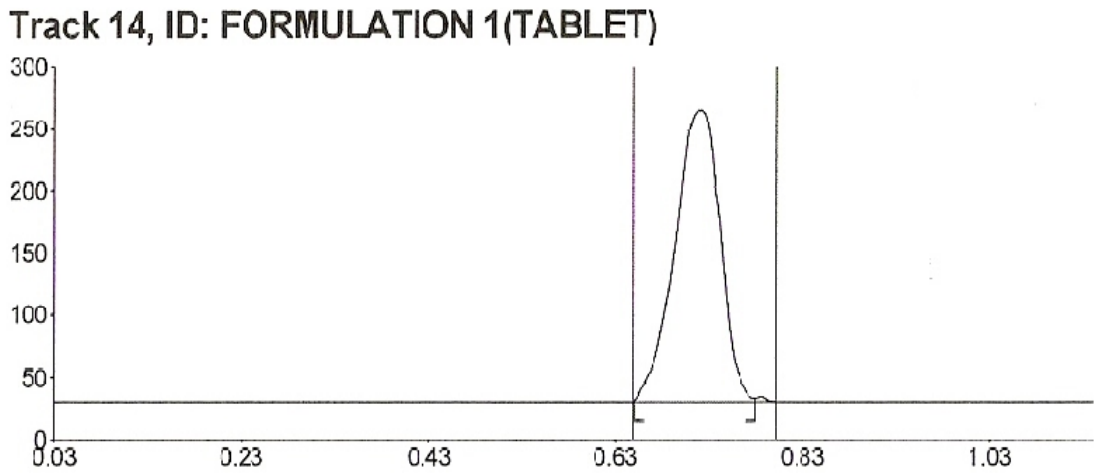
Solvent run up to: 80 mm.

Scanning wavelength: 580 nm.

Standard preparation: A 0.1 mg/ml solution of Bacoside A reference standard was prepared in methanol.

Procedure

Standard solution of pure bacoside A (1 mg/ml) in methanol was prepared to yield a stock solution of 100 µg/ml. From this various volumes of 1, 2, 4, 6, 8, and 10 µl of standard bacoside A solution were applied on precoated TLC silica gel $G60F_{254}$ plates, using a camag linomat IV automatic sample applicator from about 1 cm edge of TLC plate using a band width of 6 mm. The chromatogram was developed upto 80 mm under chamber saturation condition (saturation for 30 mins), with n-butanol: acetic acid: water (36:6:8) in a twin



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.61	1.3	0.68	213.9	100.00	0.73	3.1	8765.0	100.00

Solvent system: n-Butanol: acetic acid: water (36: 6: 8 v/v). Peak for is Bacoside A (R_f = 0.68)

Fig. 4: HPTLC Chromatogram of *B. monnieri* tablet formulation.

through chamber. The plate was air dried for 15 min, derivatised with vaniline in sulfuric acid and heated at 120^{0}C for 15 min in hot air oven. The plate was then scanned and quantified at 580 nm using camag TLC scanner III. Linearity curve for standard bacoside A in the range of 0.2 µg to 1.2 µg was developed by plotting the peak area against concentration of bacoside A. The amount of bacoside A was determined using the standard calibration curve. The linearity curve shows a correlation coefficient of 0.998.

Validation and recovery studies

The developed HPTLC method was validated for LOD, LOQ, accuracy, precision and reproducibility. A known amount of bacoside A was added to about 1.0 gm of powdered test samples in which the content of bacoside A was estimated previously by proposed method. The samples were extracted and analyzed separately as per the procedure mentioned above. The contents of bacoside A were quantified using proposed method and recovery was calculated. The results are depicted in Table 1. Precision of the HPTLC instrument was checked by scanning the same spot (900 ng) of bacoside A five times. Reproducibility of the method was checked by analyzing a standard solution of bacoside A (300

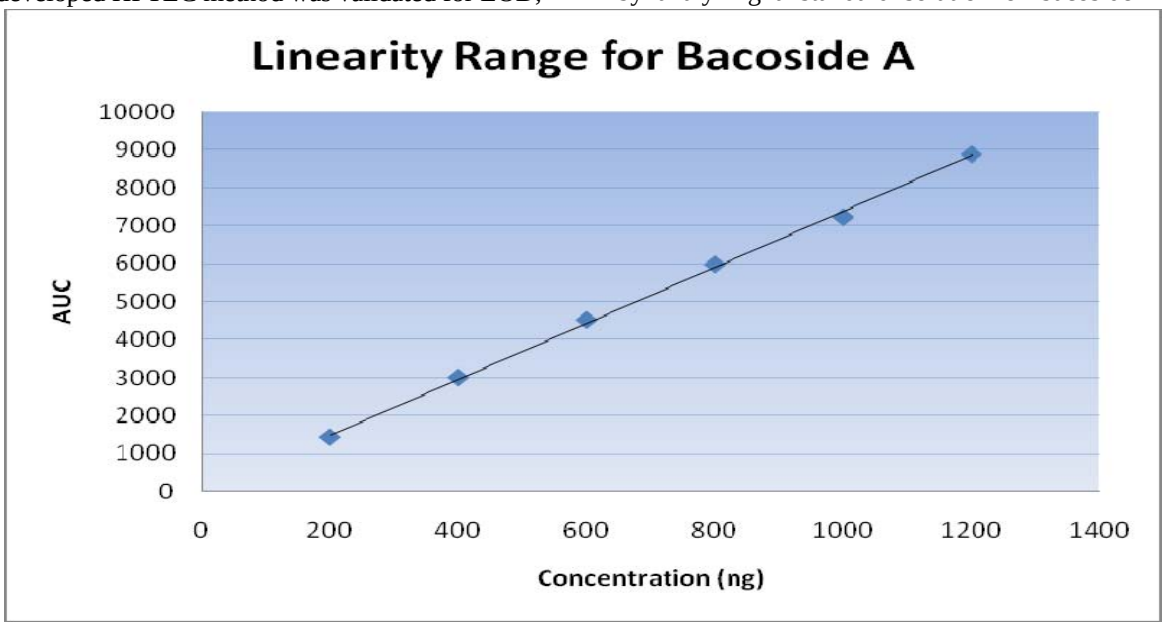


Fig. 5: Linearity curve for standard Bacoside A.

Average percentage recovery of *B. monnieri* extract and its capsule and tablet formulation are 94.10 ± 1.93, 98.44 ± 0.335 and 96.76 ± 0.39 respectively.

ng/spot).

RESULTS AND DISCUSSIONS

Phytochemical screening of methanolic extract of *Bacopa monnieri* showed the presence of alkaloids, carbohydrates, saponins, flavonoids, steroids and terpenes, and tannins and phenolic compounds. The results are depicted in table 3.

The Thin Layer Chromatographic analysis of *B. monnieri* methanolic extract showed the presence of two distinct spots using n-Butanol: Acetic acid: Water (36: 6: 8 v/v) as a solvent system. The spots with Rf value 0.45 and 0.68 were observed out of which the spot with Rf 0.68 was matched with standard bacoside A spot.

Sample preparation and development of suitable mobile phase or solvent system are two important stages in development of the analytical procedures, which becomes more significant for herbal drugs because of their complexity of the chemical compounds and their affinity towards different solvent systems. By using various mobile phase compositions a better resolution of bacoside A with symmetrical and reproducible peaks was achieved with n butanol: acetic acid: water (36:6:8). With the developed HPTLC method the R_f value of bacoside A was found to be 0.67 (fig. 1).

Linearity range was found to be in the range of 200 to 1200 ng with a correlation coefficient (r) of 0.998 indicating good linearity between concentration and peak area. The limit of detection and limit of quantification was found to be 46 ng and 139.4 ng respectively. The content of bacoside A in the extract of *B. monnieri* extracts and formulations was depicted in Table.2. Developed method is found to be specific for bacoside A (R_f = 0.67) for the extract of *B. monnieri* (fig. 2) and even in the presence of other excepients in case of formulations (fig. 3 and fig. 4). Precision of the HPTLC instrument was checked by scanning the same spot (900 ng) of bacoside A five times. Reproducibility of the method was checked by analyzing a standard solution of bacoside A (300 ng) after application (5 µl) on a TLC plate (n=5) and the % Coefficient of Variation (CV) for peak area was found to be 4.96. Hence the developed HPTLC method is reliable for quantitative monitoring of bacoside A in the raw materials as well as in its formulations. Accuracy of the method was evaluated by carrying out recovery study which showed that the average % recovery for whole methanolic extract and developed formulation was sufficient to quantify the extract and formulations in terms of bacoside A. The results of accuracy study are compiled in Table 1.

The HPTLC chromatograms of standard Bacoside A, *B. monnieri* extract and its formulations are given in fig. 1- fig 4.

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