ORIGINAL ARTICLE

A study to evaluate bacoside A in *Brahmi Ghrita* by HPTLC method

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Context: *Brahmi (Bacopa monnieri* Linn. Pennel), the main ingredient of *Brahmi Ghrita* is a popular *Medhya* drug referred in Ayurvedic classics. Recent advances have established the action of bacoside, one of the constituents of Brahmi in various psycho-neurological disorders. **Aim:** Present study was planned to detect bacoside A and to do quantitative and qualitative analysis of bacoside A in *Brahmi Ghrita* using standard bacoside A sample through HPTLC method. **Materials and Methods:** Stationary phase was precoated silica gel GF 254 and mobile phase was dichloromethane: methanol: water (4.5:1.0:0.1 v/v/v). The plate was scanned and quantified at 254 nm for bacoside A. **Result:** The correlation co-efficient of bacoside A was found to be 0.9989 or 20% in 5 µl *Brahmi Ghrita* sample. **Conclusion:** The study confirms the presence of bacoside A in *Brahmi Ghrita*. The correlation coefficient of bacoside A was found to be 0.9989 or 20% in 5 µl *Brahmi Ghrita* sample. The simple, accurate and cost-effective HPTLC method can be utilized for the routine analysis of quantitative determination of bacoside A in *Brahmi Ghrita*.

Key words: Bacoside A, *Brahmi Ghrita*, HPTLC

INTRODUCTION

Brahmi Ghrita is recommended for the management of *Unmada* (insanity), *Alakshmi* (inauspicious), *Apasmara* (epilepsy) and *Papavikaras* (diseases due to sinful acts)[1] and *Graha Rogas* (diseases afflicted by evil spirits).[2] Bacoside, *Brahmi's* active principle is responsible for improving memory related functions, attributed to the capability to enhance the efficiency of transmission of nerve impulses, there by strengthening memory and cognition. The constituents responsible for *Bacopa's* cognitive effects are bacoside A and B.[3-7] In the present day context everyone would like to know on what basis drug is acting? Most of the researches have proven that the bacoside plays a role in repair of damaged neurons by enhancing kinase activity, neuronal synthesis, and restoration of synaptic activity, and ultimately nerve impulse transmission.[8]

HPTLC is a planar chromatography where separation of sample components can be achieved on high performance layers with detection and data acquisition using an advance workshop. The advantage of HPTLC in the analytical testing of herbal products is that it provides positive identification as well as visualization of the separated fractions of the sample component and helps in quantitative, qualitative and preparative analysis with the same system if possible.[9] The aims and objectives of the present study include, detection of bacoside A and to do quantitative and qualitative analysis of bacoside A in *Brahmi Ghrita* using standard bacoside A sample through HPTLC method.

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MATERIALS AND METHODS

Collection of the Sample

Whole plant of *Brahmi* (*Bacopa monnieri* (L.) Pennel) was collected from Foundation for Revitalization of Local Health Traditions (FRLHT), Bangalore in the month of December, 2011 and other ingredients of *Brahmi Ghrita* i.e., *Vacha* (*Acorus calamus* Linn.), *Kushtha* (*Saussurea lappa* C. B. Clarke), *Shankhapushpi* (*Convolvulus pluricaulis* Choosy) were collected in powder form, from Pharmacy, Gujarat Ayurved University, Jamnagar. All these were identified and authenticated in Pharmacognosy Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda (IPGT and RA), Jamnagar. *Ghrita* (Cow's ghee) was purchased from Khadi Gramodyoga Bhandar and *Brahmi Ghrita* was prepared in Pharmacy of University. Reference standard of bacoside A was procured from M/s Natural remedies Pvt. Ltd. Bangalore.

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Preparation of Sample Solution

The *Ghrita* sample was adsorbed on Silica gel. This mixture was extracted with hexane and hexane fraction was discarded. The residue material was extracted with methanol and process was repeated for three times. The methanol layers were collected, filtered and evaporated off. The dried material was again dissolved in methanol and used for TLC identification.

Preparation of Standard Solution

The reference standard stock solution (1 mg/ml) of bacoside A was prepared in methanol. From stock solution, further dilutions were done to procure the lower concentrations.

Chromatographic Conditions

To quantify the bacoside A, stationary phase: silica gel GF 254 (E. Merck) precoated TLC plates, mobile phase: Dichloromethane: Methanol: Water (4.5:1.0:0.1 v/v/v), sample volume: 5 µl, sample for HPTLC: Methanol extracts of *Brahmi Ghrita*, standard bacoside A solution, spray reagent: Vanillin-sulfuric acid.

Instrumental Conditions

Application mode: Camag Linomat V, development chamber: Camag twin trough chamber, plate: Precoated Silica Gel GF254 plate, chamber saturation: 30 min, development time: 30 min, development distance: 7 cm, scanner: Camag scanner III, detection: Deuterium lamp and tungsten lamp, data System: Win CATS software.

Procedure

The TLC plate was washed with methanol and standard as well as sample solutions were applied to the plate as sharp bands by means of Camag Linomat V sample applicator. This plate was dried in a current of air. Later the mobile phase (20 ml) was poured into a twin trough glass chamber and whole assembly was left to equilibrate for 30 min then the plate was placed in the chamber. The plate was developed until the solvent front had travelled at a distance of 80 mm above the base of plate. It was then removed from chamber and dried in a current of air. Later detection and quantification of bacoside A was done with Camag TLC scanner III at wavelength 254 nm.[10]

Assay

Standard and sample solutions were spotted on HPTLC plate (E. Merck). The percentage of bacoside A present in *Brahmi Ghrita* extract was calculated by comparing the areas measured for standard solution.

Linearity

Linearity was done by applying standard solution at different concentrations ranging from 2.5 to 7.5 µg/µl spot on 10×10 cm HPTLC plate, precoated with silica gel GF 254 (E. Merck) in the form of sharp 6 mm bands, the distance between two adjacent bands being 9.5 mm. The plate was developed in a solvent system of dichloromethane: Methanol: Water (4.5:1.0:0.1 v/v/v), up to a distance of 80 mm, at room temperature. The plate was dried in air. The detector response for bacoside A was measured for each band at wavelength of 254 nm, using Camag TLC scanner and win CATS software. The peak areas of bacoside A was obtained by plotting a graph of peak Vs applied concentration of bacoside A (5 µl) [Figures 1 and 2].

RESULTS AND DISCUSSION

The method applied in the present study utilizes silica gel GF 254 HPTLC plate as stationary phase and dichloromethane: Methanol: Water (4.5:1.0:0.1 v/v/v as mobile phase gave separation of bacoside A (Rf=0.78) standard, though the *Ghrita* sample was found loaded with number of components [Table 1]. The identified band of bacoside A in the sample extract was confirmed by overlapping the UV absorption spectra of sample with that of reference standard at 254 nm [Figure 3a-c]. The calibration curve was linear in the range of 2.5-7.5 µg/spot and the correlation coefficient

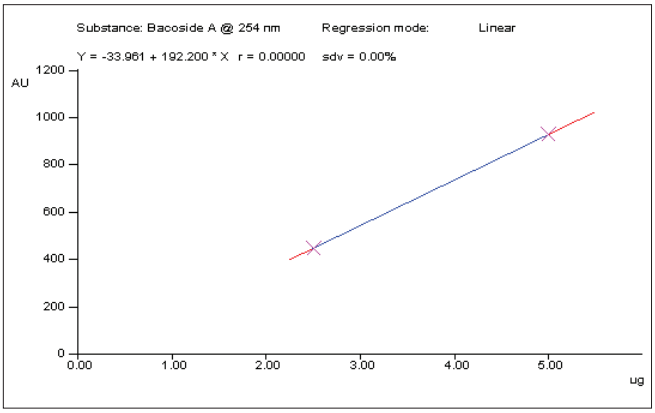

Figure 1: Linearity graph bacoside A (Range 2.5 µg-7.5 µg)

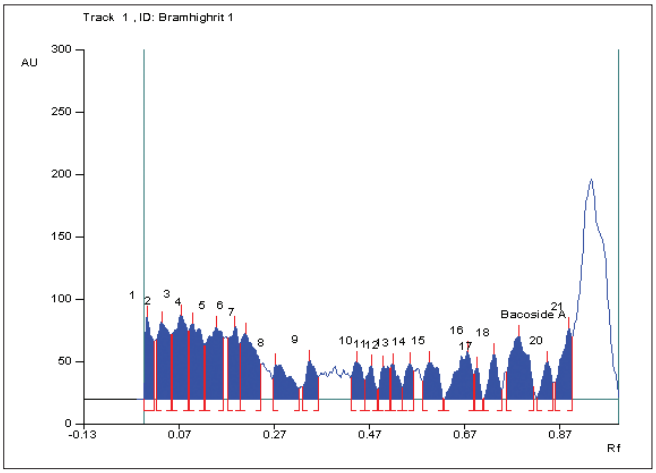

Figure 2: Peak display *Brahmi Ghrita* (densitogram)

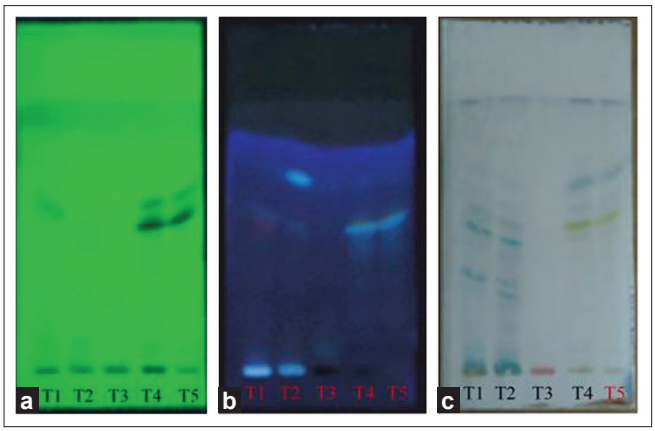

Figure 3: (a) At 254nm; (b) At 366nm; (c) At after spray. T1 - *Brahmi Ghrita* sample, T2 - *Brahmi Ghrita*, sample T3 - std (2.5), T4 - std (5), T5 - std (7.5)

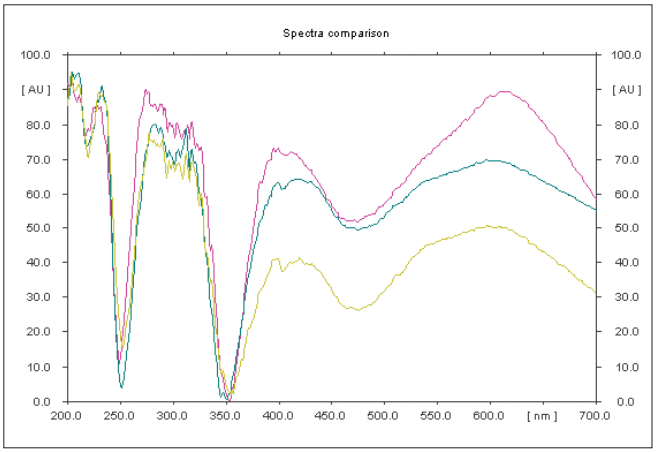

Figure 4: *In situ* spectral comparision at Rf value 0.78

Table 1: Chromatographic separation of *Brahmi Ghrita* on Silica gel GF 254

Track	No. of spot	Rf value
Brahmi Ghrita	9	0.03, 0.06, 0.13, 0.20, 0.78, 0.83, 0.86, 0.49, 0.63
Standard 1	2	0.70, 0.77
Standard 2	2	0.70, 0.77
Standard 3	2	0.70, 0.77

was determined. The correlation coefficient of bacoside A was found to be 0.9989 or 20% in 5 µl *Brahmi Ghrita* sample [Figure 4]. After derivatisation with vanillin sulfuric acid the *Brahmi Ghrita* sample shows similar colour reaction i.e pink colour spots as that of standard bacoside A at Rf value 0.78.

CONCLUSION

The study confirms the presence of bacoside A in *Brahmi Ghrita*. The correlation coefficient of bacoside A was found to be 0.9989 or 20% in 5 µl *Brahmi Ghrita* sample. The simple, accurate and cost-effective HPTLC method can be utilized for the routine analysis of quantitative determination of bacoside A in *Brahmi Ghrita*.

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