

Short Communication

ESTIMATION OF BACOSIDE-A IN *BACOPA MONNIERI* AERIAL PARTS USING TLC DENSITOMETRY

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ABSTRACT

Objective: To estimate bacoside A in *Bacopa monnieri* aerial parts using a validated TLC densitometric method.

Methods: The content of bacoside A was estimated using standardized procedures. A standard curve was prepared by applying different concentrations of the marker. The estimation of bacoside A was achieved on pre-coated TLC plate using solvent system, i.e., toluene: ethyl acetate: methanol: formic acid (3:3.5:2.5:1) followed by scanning at 225 nm. The developed method was validated for the parameters described in the ICH guidelines.

Results: The content of bacoside A was determined to be 1.13±0.019% w/w in *B. monnieri* aerial parts. The instrumental precision, repeatability, linearity range, correlation coefficient, intra-day precision, inter-day precision, limit of detection (LOD), limit of quantification (LOQ) and accuracy were found to be 0.61% CV, 0.87% CV, 150-900 ng, 0.998, 1.43% CV, 1.79% CV, 32 ng/spot, 92 ng/spot and 97.19±0.39% respectively.

Conclusion: The developed method was found to be simple, economic, accurate, precise, and can be utilized for the quantitative as well as qualitative estimation of bacoside A in *Bacopa monnieri*.

Keywords: Bacopa, Nootropic, Saponin, TLC densitometry.

Pharmaceutical industries are investing enormous resources in identifying potentially beneficial agents i.e. phytoconstituents which can be used to treat various ailments. This is the reason why several plants have been selected based on their traditional uses. *Bacopa monnieri* (Brahmi; family-Scrophulariaceae) is one of such plants used traditionally in the treatment of cognitive disorders. Brahmi is commonly grown in marshy areas of India [1].

Brahmine, nicotine, bacoside A and steroids have been isolated from *B. monnieri* [2]. Bacoside A, a dose-dependent inhibitor of acetylcholinesterase, is mainly used in brain disorder and memory-related disorder. Acetylcholinesterase is an enzyme which metabolizes the neurotransmitter acetylcholine. Bacoside A also aid in the repair of damaged neurons by enhancing kinase activity, neuronal synthesis and restoration of synaptic activity and ultimately nerve impulse transmission [3, 4]. *B. monnieri* has also reduced β -amyloid levels in the brain suggesting mechanisms of action relevant to Alzheimer's disease [5]; antioxidant activity in the hippocampus, frontal cortex and striatum [6]; modulate the expression of certain enzymes involved in generation and scavenging of reactive oxygen species in the brain [7]; protective effect against DNA damage in astrocytes and human fibroblasts [8]; affect the oxidative stress cascade by scavenging reactive oxygen species, inhibiting lipo oxygenase activity and reducing divalent metals [9]. The results of clinical trials also showed improvement of various components of cognitive functions in geriatric patients suffering from Alzheimer's disease who consumed *Bacopa monnieri* for six months [10].

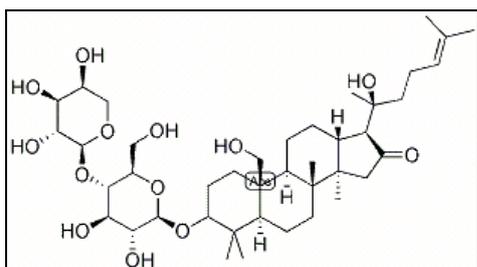


Fig. 1: Bacoside A

A survey of literature revealed that no work has been carried out to standardize *B. monnieri* aerial parts on the basis of main chemical constituent(s) using TLC-densitometry. Thus, the main objective of the present investigations is to develop validated TLC densitometric method for the estimation of bacoside A in *B. monnieri* aerial parts.

B. monnieri aerial parts were procured from Una (Himachal Pradesh) and authenticated from the Central Council for Research in Ayurveda & Siddha, Tamil Naidu. The bacoside A was a generous gift from Prof. Ikhlas A. Khan, School of Pharmacy, University of Mississippi.

Methanol (E Merck, Delhi, India), of LR grade, was used for the preparation of crude extract of *B. monnieri* aerial parts. Methanol, toluene, ethyl acetate, formic acid (E Merck, Delhi, India) of AR grade, were used for thin layer chromatographic studies.

The stock solution of bacoside A (5 mg/ml) was diluted with methanol to get dilutions of different concentrations (15, 30, 45, 60, 75 and 90 μ g/ml). A volume of 10 μ l from each dilution was applied in triplicate on pre-coated TLC plate (E Merck, Mumbai, India; 0.2 mm; aluminum base) using CAMAG LINOMAT 5.

The plate was developed in a solvent system toluene: ethyl acetate: methanol: formic acid (3:3.5:2.5:1) in a chamber saturated for 10 min, to a distance of 8 cm. The developed plate was dried in a current of hot air and then scanned in TLC scanner at 225 nm. The area under the curve (AUC) of the peak corresponding to marker compound was noted in each track.

Coarsely powered aerial parts of the plant (10 gm) were exhaustively extracted with methanol in a Soxhlet apparatus [11]. The methanol extract was filtered, concentrated under reduced pressure and the volume was adjusted to 10 ml with methanol.

Test solution (10 μ l) of methanol extract was applied in triplicate on pre-coated TLC plate (5×10 cm). The plate was developed and scanned following the same procedure as used for the preparation of standard plot. The average AUC of the peak corresponding to marker compound was noted at 225 nm in the test sample. The content of the marker compound was calculated from the regression equation of the standard plot.

The developed methods were validated for the parameters described in ICH guidelines such as linearity, range, limit of detection, limit of quantification, inter-day precision, intra-day precision, accuracy, repeatability and specificity [11]. Comparative TLC fingerprint studies confirmed the presence of bacoside A in methanol extract. Thus, bacoside A was taken as a marker to standardize *B. monnieri* aerial parts using validated TLC densitometric method. The best resolution of bacoside A in methanol extract was achieved in toluene: ethyl acetate: methanol: formic acid (3:3.5:2.5:1). Fig. 2 shows comparative fingerprint profile of bacoside A against methanol extract. Standard plots were prepared between different concentrations of bacoside A versus their peak areas after scanning at 225 nm (fig. 3). The estimation of bacoside A was done from the regression equation of their

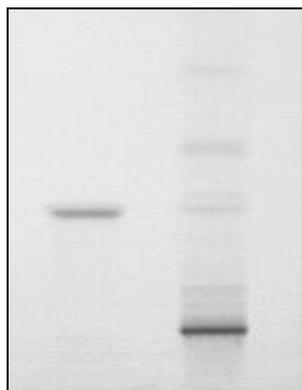


Fig. 2: Comparative TLC fingerprint profile of bacoside A and methanol extract of *B. monnieri* aerial parts under UV chamber at 254 nm

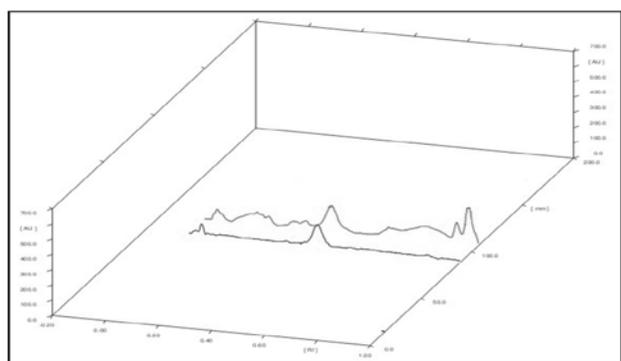


Fig. 4: TLC densitometric chromatogram of bacoside A and methanol extract of *B. monnieri* aerial parts

HPTLC is a sophisticated instrumental technique with merits of easy method development and validation, automation, scanning, full optimization, selective detection principle, minimum sample preparation, etc., enable it to be a powerful analytical tool for quantitative determination of particular compound(s) in complex mixtures of inorganic, organic and biomolecules [12,13]. Percentage coefficient of variance observed in validation parameters of developed method complied within the prescribed limit. Recovery of bacoside A in accuracy studies was more than 98%. Further, no deviation was observed in ultraviolet spectra and thin layer chromatograms of sample and standard. These observations infer that the developed method for estimation of bacoside A in *B. monnieri* aerial parts is precise, accurate, reproducible and specific. It is well documented that bacoside A is bioactive triterpenoidal saponin of *B. monnieri* aerial parts and is actually responsible for its pharmacological activity useful in Alzheimer's disease. Therefore, it was selected as a marker to standardize *B. monnieri*.

Finally, it can be concluded that validated TLC densitometric method for estimation of bacoside A in *B. monnieri* aerial parts is specific,

respective standard plot. The estimation of marker compound and their accuracy (recovery) studies were done in triplicate, and the values were expressed in percent w/w (mean±S. D.). The content of bacoside A in *B. monnieri* aerial parts was found to be 1.13±0.019% w/w. TLC densitometric method was validated as per ICH guidelines. The instrumental precision, repeatability, linearity range, correlation coefficient, intra-day precision, inter-day precision, LOD, LOQ and accuracy were found to be 0.61% CV, 0.87% CV, 150-900 ng, 0.998, 1.43% CV, 1.79% CV, 32 ng/spot, 92 ng/spot and 97.19±0.39% respectively. It is clearly evident from ultraviolet spectra and thin layer chromatogram overlay that there is no interference in quantitative analysis (fig. 4 and 5), thus, confirming the specificity of the developed TLC densitometric method for each standard marker.

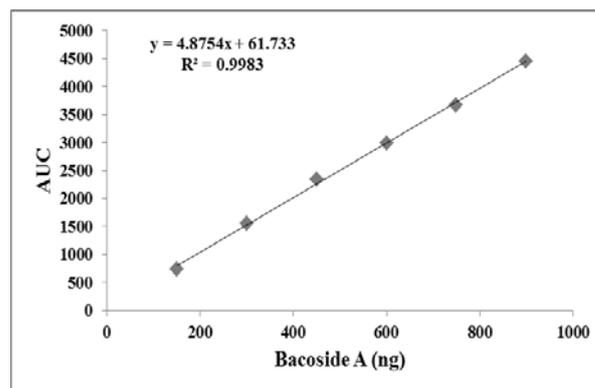


Fig. 3: Standard plot of bacoside A in TLC densitometric analysis.

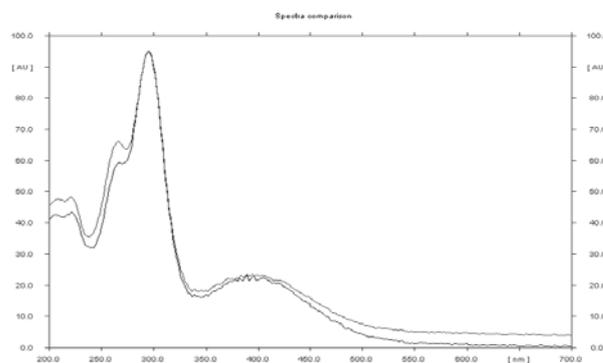


Fig. 5: Spectra overlay of bacoside A with corresponding peak in methanol extract of *B. monnieri* aerial parts

accurate, precise and acceptable for the estimation of bacoside A in commercially available herbal medicines.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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