

## Determination and Quantification of Bacoside A From *Bacopa monnieri* (L) By High Performance Thin Layer Chromatography

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### ABSTRACT

*Bacopa monnieri* (L) has been used in Ayurvedic medicine as nervine tonic for promoting mental health and improving brain function. The plant has been reported to contain several phytoconstituents mainly flavonoids (luteolin and apigenin), betulic acid, stigmastanol, beta sitosterol, bacopasaponins and the minor components like bacopasaponin F, bacopasaponin E, bacopaside N1, bacopaside III, IV, V. Bacoside A is a major bacopasaponin constituent of *Bacopa monnieri* (L).

The aim of the present investigation was to develop a simple, sensitive and reproducible HPTLC method for the determination of Bacoside A from the methanolic extract of *Bacopa monnieri* (L).

The HPTLC method developed for separation of Bacoside A by TLC on stationary phase i.e. Silica gel 60 F<sub>254</sub> with a solvent system Toulene:Ethylacetate:Methanol:Glacial Acetic acid (3:4:3:1v/v) and detection of Bacoside A was carried out by scanning and quantifying the peak at 540 nm by winCATS Planar Chromatography Manager.

The calibration curve was linear in the range of 0.5 - 4 µg/spot with correlation coefficient 0.9977. The coefficient of variance was found to be 1.766. The proposed HPTLC method was found precise and can be used for monitoring, detection, identification and quantification of Bacoside A from the methanolic extract of *Bacopa monnieri* (L).

**Keywords :** *Bacopa monnieri* (L), Phytoconstituents, Bacoside A, HPTLC, TLC, Quantification

### INTRODUCTION

*Bacopa monnieri* (L) belongs to the family Scrophulariaceae. It is a medicinal herb found throughout the Indian subcontinent in wet, damp and marshy area<sup>1</sup>. *Bacopa monnieri* (L) is used in the indigenous systems of medicine for the treatment of various nervous system ailments such as insomnia, anxiety, epilepsy, hysteria and in improving intellect, memory<sup>2-4</sup>. In addition to the memory boosting activity, it also claimed to be useful in the treatment of cardiac, respiratory, neuropharmacological disorder like insanity, depression, psychosis and stress<sup>5,6</sup>. It was reported that *Bacopa monnieri* (L) to possess anti inflammatory, analgesic, antipyretic, sedative, free radical scavenging, antioxidant and anti lipid peroxidative activities<sup>7,8,9</sup>.

Triterpenoid saponins, the major components in Brahmi, were reported to be responsible for the cognitive enhancing activity of Brahmi<sup>3,6</sup>. The major bioactive dammarene type triterpenoid saponin isolated from the *Bacopa monnieri* (L)<sup>10</sup>, that carries the neuropharmacological activities, is Bacoside A, which is a mixture of Bacoside A3, Bacoside II, Bacopasaponin C and an isomer of Bacopasaponin C<sup>11</sup>. Bacoside A is held in high repute as a potent nerve tonic<sup>12</sup>. Now a days standardization and quantification of medicinal plant extracts is essential for formulation<sup>13</sup>. As the number of aged people suffering from cognitive problems, the memory boosters have gained immense importance and there is an urgent need to develop sensitive and reliable quality control techniques to establish the

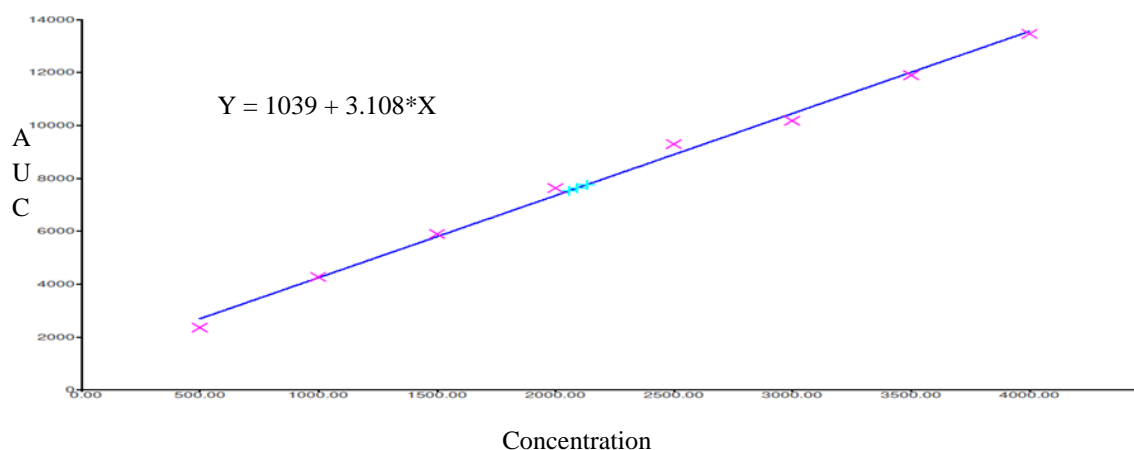
authenticity and purity of memory boosting drugs<sup>14</sup>. The use of medicinal plants in both crude and prepared forms has increased substantially. Use of chromatography for standardization of plant products was introduced by the WHO and is accepted as a strategy for identification and evaluation of the quality of plant products<sup>15-17</sup>.

Methods described in the literature for analysis of bacosides are mainly based on UV Spectroscopy<sup>4,18</sup>, Thin Layer Chromatography<sup>19</sup> and HPLC<sup>11,20,21</sup>. Therefore in the present study a suitable, sensitive and reliable quantitative High Performance Thin Layer Chromatography method has been developed for qualitative and quantitative estimation of the phytochemical marker namely Bacoside A from methanolic extract of *Bacopa monnieri* (L).

### MATERIAL AND METHOD

#### Material

The herbarium of *Bacopa monnieri* (L) was prepared and authentication has been obtained from Scientist D and HOD, Botanical survey of India, Pune, Maharashtra. The specimen (MGJ-1) was kept to herbarium department in Botanical Survey of India, Pune. The whole plant material was shade dried at room temperature and kept in oven for 40°C to remove moisture. The dried plant was then finely grinded by mechanical grinder manually. The powder obtained was then sieved and kept in air tight containers for further extraction process. The reference standard of Bacoside A (C<sub>41</sub> H<sub>68</sub> O<sub>13</sub>) was purchased from M / S



Result via area	Rf	X(average)	CV [%]	n	Regression	Remark
Bacoside A	0.31	2.094 µg	1.766	3	Linear	

Figure 1: Linearity curve for standard Bacoside A.

Natural Remedies Pvt. Ltd. Bangalore, India and used for quantification.

#### Method

##### Extraction of *Bacopa monnieri* (L)

The methanolic extract of *Bacopa monnieri* (L) was prepared by traditional maceration method for the determination and quantification of Bacoside A. *Bacopa monnieri* (L) dry plant powder was dissolved in methanol in the glass jar and it was wrapped by aluminium foil and covered well to avoid evaporation. Mixture was kept for 72 hrs at room temperature with occasional shaking. After completion of the maceration, the supernatant was decanted and the mixture was filtered through the muslin cloth. The extract was concentrated to dryness by keeping filtrate for complete evaporation of solvent. After evaporation of solvent the extract was weighed and kept in air tight glass container for further use.

##### Preparation of plant extract solution

The methanolic extract was taken and dissolved in methanol and sonicated for 15min. Then from this, stock solution was pipette out and methanol was added to make final dilution.

##### Preparation of standard solution

The reference standard stock solution of Bacoside A was prepared in methanol and sonicated for 15 min.

##### Chromatographic conditions

The following chromatographic conditions were used to quantify the Bacoside A :

Stationary phase : Silica gel 60 F<sub>254</sub> (E. Merck) precoated TLC plates

Mobile phase : Toulene:Ethylacetate:Methanol:Glacial Acetic acid (3:4:3:1 v/v)

Developing chamber – Twin trough glass chamber

Sample volume : 2µl

Mode of application : Band

Saturation time : 30min

Temperature : Ambient room temperature

Migration distance: 8mm

Detection wavelength: 540nm

##### Assay / development of chromatogram/ procedure

For determination of Bacoside A content in the methanolic extract of *Bacopa monnieri* (L), 2µl sample was used in High Performance Thin Layer Chromatography (HPTLC). Standard and sample solutions were applied to the Silica gel 60 F<sub>254</sub> (E. Merck) precoated TLC plates as sharp bands by means of CAMAG Linomat V sample applicator. The spots were dried in a current of air. Chromatography was carried out in a glass chamber (CAMAG). The mobile phase Toulene : Ethylacetate:Methanol:Glacial Acetic acid (3:4:3:1v/v) was poured into a twin trough glass chamber whole assembly was left to equilibrate and for presaturation for 30 min. The plate was then developed until the solvent front had travelled at a distance of 80mm above the base of the plate, at 20°C and 50% relative humidity. The plate was visualized by immersing it in vanillin–sulphuric acid-ethanol (1g:5ml:95ml) solution, using an automatic immersion device (CAMAG), followed by heating on CAMAG TLC plate heater for few min. For detection and quantification TLC spots corresponding to Bacoside A were scanned at 540nm using CAMAG TLC scanner. The percentage of Bacoside A present in *Bacopa monnieri* (L) extract was calculated by comparison of the areas measured for the sample and standard solution.

##### Linearity

Linearity was performed by applying solution at different concentrations ranging from 0.5 to 4 µg/spot on 20×10 cm HPTLC plates, precoated with Silica gel 60F<sub>254</sub> (E. Merck) in the form of sharp 8mm bands. The distance between two adjacent bands was 12.3mm. The plates were developed in a solvent system of Toulene:Ethylacetate:Methanol:Glacial Acetic acid (3:4:3:1v/v), upto a distance of 80mm, at room temperature. The plates were dried in air.

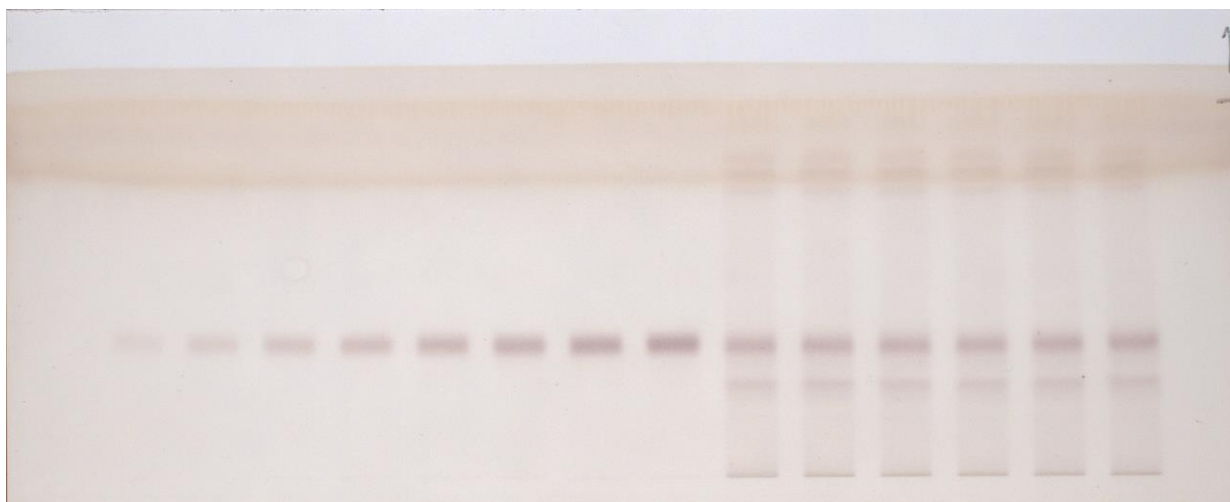


Figure 2: TLC Plate Visualized under CAMAG Visualizer : 150503 White remission showing separation of Bacoside A compound

Track 4, ID: Standard4

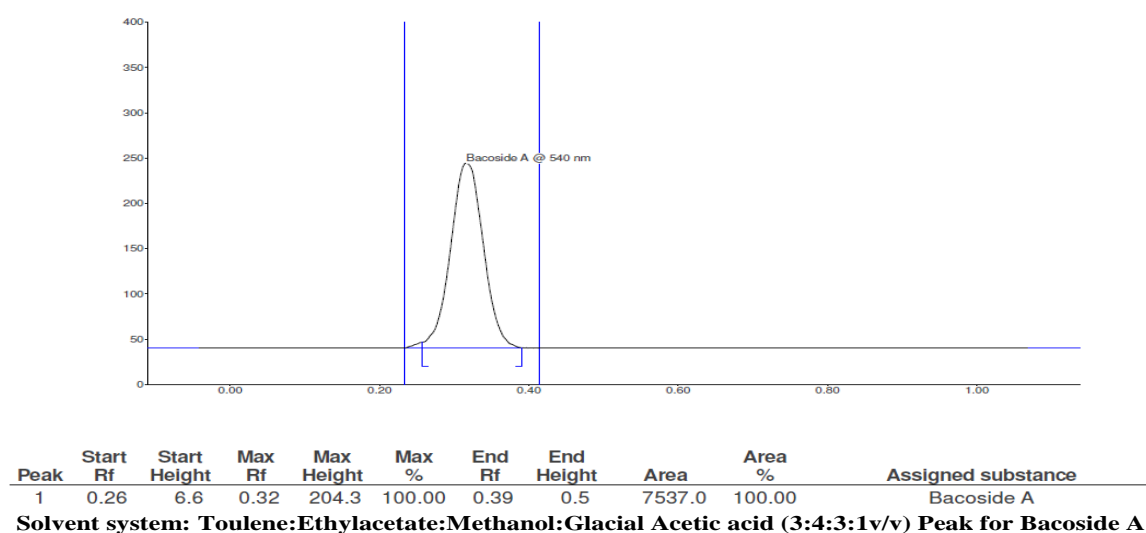


Figure 3: HPTLC Chromatogram of Standard Bacoside A

The detector response for Bacoside A was measured for each band at wavelength 540nm, using CAMAG TLC scanner and with WinCAT software. The peak areas of Bacoside A were recorded for each concentration. The linearity curve of Bacoside A was obtained by plotting a graph of peak area of Bacoside A vs applied concentration of Bacoside A.

## RESULT

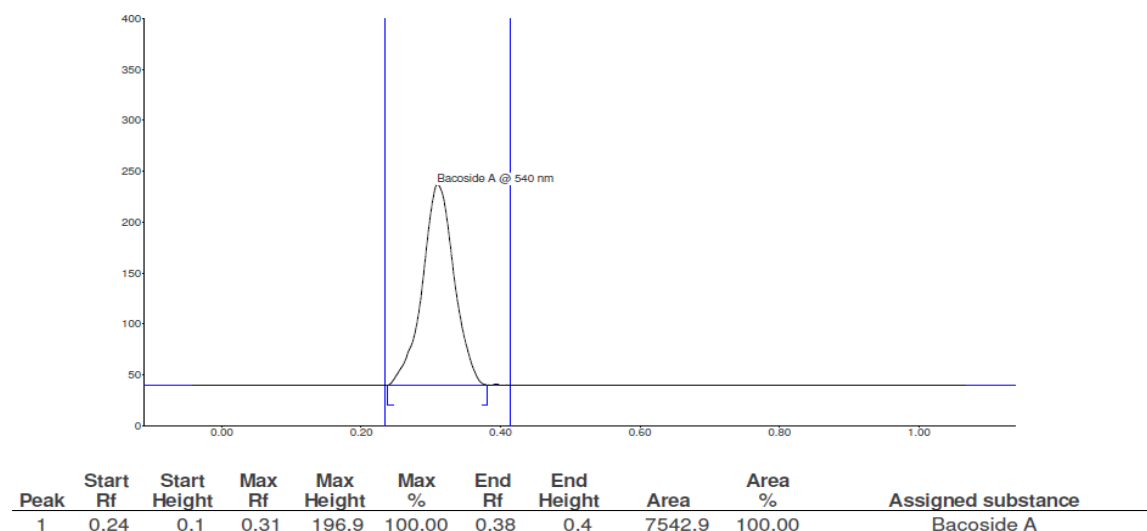
The method described utilizes Silica gel 60F<sub>254</sub> HPTLC plates as stationary phase and Toulene : Ethylacetate: Methanol: Glacial Acetic acid (3:4:3:1v/v) as mobile phase which gives good separation of Bacoside A ( $R_f = 0.31$ ). The calibration curve was linear in the range of 0.5 - 4  $\mu\text{g}/\text{spot}$  as shown in fig 1. The correlation coefficient ( $r$ ) was determined it was found to be 0.9977 indicating good linearity between concentration and peak area. Well defined bands were obtained which are shown fig 2. The percentage coefficient of variance (CV) for peak area was

found to be 1.766. The HPTLC chromatograms of standard Bacoside A and methanolic extract of *Bacopa monnieri* (L) are presented in fig. 3 and fig. 4.

## DISCUSSION

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. In the present study by using various mobile phase compositions, a better resolution of Bacoside A with symmetrical and reproducible peak was achieved with Toulene:Ethylacetate:Methanol:Glacial Acetic acid (3:4:3:1v/v). With the developed HPTLC method the  $R_f$  value of Bacoside A was found to be 0.31. Linearity range was found to be in the range of 0.5 - 4  $\mu\text{g}/\text{spot}$  with a

## Track 11, ID: Extttract



Solvent system: Toulene:Ethylacetate:Methanol:Glacial Acetic acid (3:4:3:1v/v) Peak for Bacoside A

Figure 4: HPTLC Chromatogram of *Bacopa monnieri* methanolic extract

coefficient of variance of 1.766 indicating good linearity between concentration and peak area.

Several kinds of techniques were employed for plant analysis. Amit *et al.* (2012) developed the HPTLC method for the quantification of Bacoside A and fingerprinting of the in-house mother tincture (*Bacopa monnieri*) and marketed samples of homeopathic medicines in India by using Dichloromethane: Methanol : Water as a solvent system<sup>22</sup>. Rastogi *et al.* (1994) isolated and characterized bacoside A3 from *Bacopa monnieri* (L)<sup>23</sup>. Chakravarty *et al.* (2001) isolated two saponins, bacoside I and II from *Bacopa monnieri* (L) by 2D NMR technique<sup>24</sup>. Pal *et al.* (1998) performed quantitative analysis of bacoside by HPLC<sup>25</sup>. Shrikumar *et al.* (2003) determined and analysed the Bacoside A in the *Bacopa monnieri* (L) and its commercial monoherbal capsule formulation by HPTLC<sup>26</sup>. Deepak *et al.* (2005) performed quantitative analysis of Bacoside A from *Bacopa monnieri* (L) by HPLC<sup>27</sup>. Kawai *et al.* (1978) performed acid hydrolysis of Bcoside A from *Bacopa monnieri* (L) and obtained ebelin lactone and bacogenin A<sup>28</sup>. Agrawal *et al.* (2006) carried out separation of Bacoside A<sub>3</sub> and Bacopaside II which are the major triterpenoid saponins in *Bacopa monnieri* (L) by HPTLC and Super Critical Fluid Chromatography techniques<sup>29</sup>. Watoo *et al.* (2007) determined the saponin glycosides in *Bacopa monnieri* (L) by reversed phase High Performance Thin Layer Chromatography<sup>30</sup>. Five major saponins namely bacoside A<sub>3</sub>, bacopaside II, bacopasaponin C isomer, bacopasaponin C and bacopaside I in the extracts of *Bacopa monnieri* (L) were determined by using HPLC technique<sup>31</sup>. Shinde *et al.* (2011) developed the HPTLC method for the simultaneous determination of Withanolide A and Bacoside A in spansules from *Withania somnifera* and *Bacopa monnieri* (L)<sup>32</sup>. An HPLC – UV method was

developed by Mishra *et al.* (2013) for the standardization of Brahmi Vati and simultaneous quantitative estimation of Bacoside A<sub>3</sub> and Piperine which are the major constituents of *Bacopa monnieri* (L) and *Piper longum* respectively<sup>33</sup>.

## CONCLUSION

According to Ayurvedic literature, *Bacopa monnieri* (L) is ethnically used in various diseases in humans and animals. Various separation techniques have been reported for separation and quantification of specific phytoconstituents in the medicinal plant. 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. Multiple numbers of samples can be analyzed in a single run allows one to save time and thus cost of analysis and the sample preparation requirements are often minimal because the stationary phase is disposable. From the present study it can be concluded that, the developed HPTLC method is fast, precise and reliable and may be useful for quantitative monitoring of Bacoside A in methanolic extract of *Bacopa monnieri* (L).

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