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Analytical Method Development of Bioactive Compounds in Bacopa Monnieri Leaf Extract By RP-HPLC

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ABSTRACT
A novel very rapid, sensitive, reverse phase High performance liquid chromatography (RP-HPLC) technique was developed for the quantitative estimation of bioactive compounds in the medicinal plant Bacopa monnieri. Five bioactive compounds namely Bacopasaponin A, Bacoposide I, Bacopaside II, Bacoside A3, Jujobogenin of Bacopasaponin C was resolved by using a mobile phase of phosphate buffer, acetonitrile in the ratio of 60 : 40 % v/v at a flow rate of 1.5ml/min using UV-visible detector at the wavelength of 205nm for quantification. Efficient separation was achieved for Bacopa monnieri on Hypersil BDS C18 (4.6mm X250 mm, 5.0 micron) Column at 30C.With an isocratic elution the separation was obtained in a total run time of 30 mins. The retention time of active constituents was found to be 16.4,9.3,14,13.5,15.4 minutes respectively. Based on the studies it was concluded that the method development of bio active components from Bacopa monnieri were found to be simple, sensitive and inexpensive method. Hence, the development method can be recommended for routine quality control analysis.

Keywords: Bacopa monnieri, RP – HPLC, UV Detector, Isocratic elution.

INTRODUCTION

Bacopa monnieri is an important medicinal plants which is belong to the family of Plantaginaceae. It is the perennial, creeping herb which is indigenous to the wetlands of Southern and Eastern India, Australia, Europe, Africa, Asia, and North and South America. The common name of Bacopa monnieri is also called water hyssop, water hyssop, Brahmi, thyme- leafed gratiola, and Indian pennywort. It is also used in the Ayurveda. It is a non- aromatic herb. It' s flowers is the small, white in colour, it may be present 4 to 5 petals. It has been extensively used in traditional medicine to enhance memory, learning, and cognitive function. Recent studies have validated its potential in improving cognitive performance, making it an attractive supplement for individuals seeking to enhance their mental acuity. High performance liquid chromatography (HPLC) is a process, which separates mixture containing two or more components under high pressure. In this the stationary phase is packed in a

column one end of which is attached to a source of pressurized liquid mobile phase. High performance liquid chromatography is the fastest growing analytical technique for the analysis of drugs. Its simplicity, high specificity and wide range of sensitivity makes it ideal for the analysis of many drugs in both dosage forms and biological fluids. HPLC is also known as high pressure liquid chromatography. Development procedures by using High Performance Liquid Chromatography (HPLC) and UV detector for the determination of Bacopasaponin C, Bacopaside I, Bacopaside II, Bacoside A3, Jujubogenin of Bacopasaponin C. Isocratic elution is used and more than 30 minutes run time is needed. Our study seeks to contribute to the growing body of research on this fascinating herb, shedding light on its chemical composition and potential applications in the realm of health.

MATERIALS AND METHODS

PLANT MATERIALS

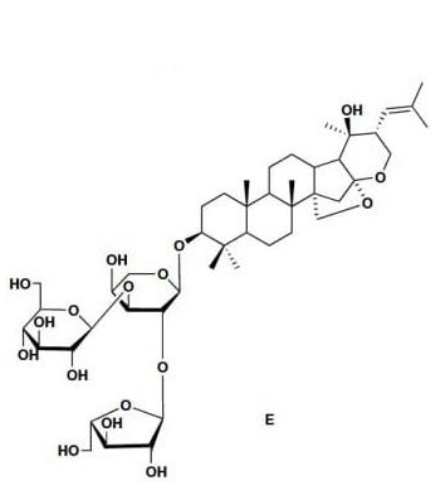
The aerial parts of Bacopa monnieri were collected from rural areas near Namakkal, Tamil Nadu. The plants were identified by Assistant professor DR.V.ARAVINDHAN, Department Of Botany, Kongunadu Arts and Science College Coimbatore. The Bacopa leaves were repeatedly washed to remove dirt and other impurities and subsequently dried in air until it attained constant moisture content. Then, Bacopa leaves were pulverized to get the particle sizes of 355µm and prepared for extraction process (Maria et al. 2008).

EXTRACTION PROCESS

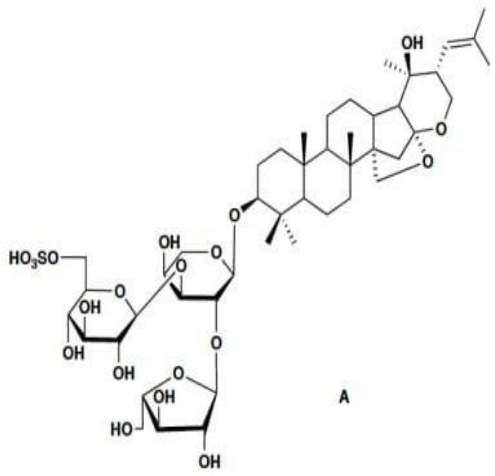
Extraction of Bacopa monnieri was carried by Soxhlet Extration Method using Ethanol used as a solvent. Take 100 g of Bacopa monnieri powder was placed into the thimble and Soxhlet chamber and placed 500 ml of ethanol were placed in a round bottom flask and assembled for Soxhlet extractor then the distillation process was done. After completed the extraction process, the solvent (Ethanol) and extractor were placed on a water bath to evaporate the solvent and after evaporate collect the sample for analysis.

CHEMICALS

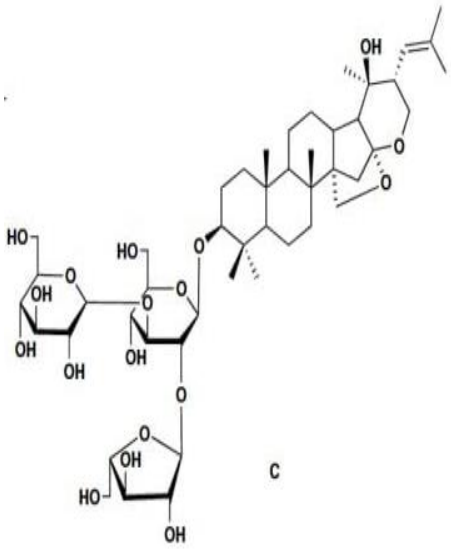
Ethanol, Acetonitrile, Methanol, Phosphate Buffer, Saponoin reference standards Bacopasaponin C, Bacopaside I, Bacopaside II, Bacoside A3, Jujubogenin of Bacopasaponin C.



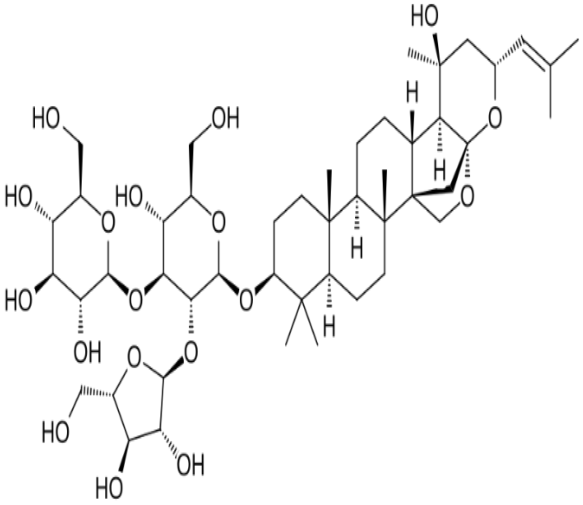
Bacopasaponin C



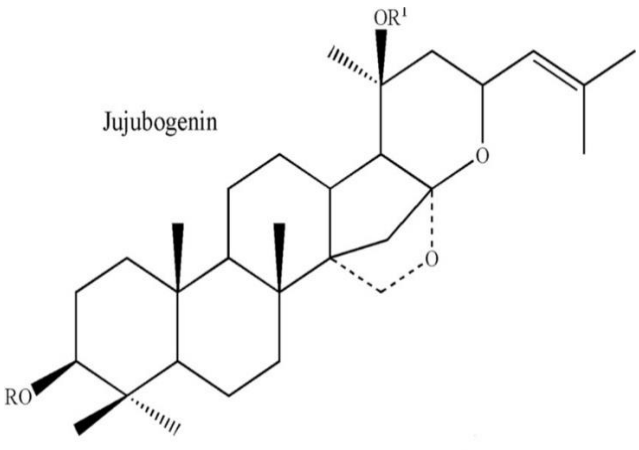
Bacopaside I



Bacopaside II



Bacoside A3



Jujubogenin of Bacopasaponin C

STANDARD SOLUTION PREPARATION

5 mg of Bacopasaponin C , Bacopaside I, Bacopaside II, Bacoside A3 and Jujubogenin of Bacopasaponin C were accurately weighed respectively and transfered into a 100 ml volumetric flask. Dissolve in 25 ml of diluent and sonicated for 15 mins and make upto the volume with diluent. Take 5 ml of above stock solution into 50 ml volumetric flask and make upto volume with diluent and filter the solution through sartorious 292 filter paper.

PREPARATION OF SAMPLE

1.0 g of Bacopa monnieri leaf extract sample were accurately weighed and transfered into a 100 ml volumetric flask. Dissolve in 15 ml of diluent and sonicated for 15 minutes. Then make up the volume with diluents and filter the solution through sartorious 292 filter paper.

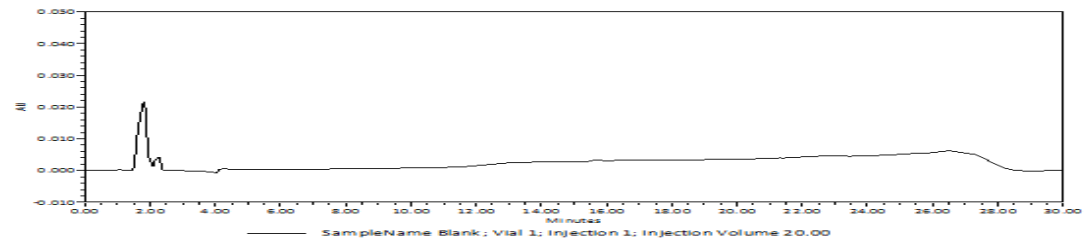
CHROMATOGRAPHIC METHOD

Instrument	:	HPLC
Mobile phase	:	0.001M of phosphate buffer : acetonitrile (60:40)
Column	:	Hypersil BDS C18 5µ, 250×4.6mm
Flow rate	:	1.5 mL/min
Injection volume	:	20 µl
Run time	:	30 min
Detector Wavelength	:	205 nm
Column temperature	:	30ºC

RESULT AND DISCUSSIONS

In the present study, new RP-HPLC method was carried out for analytical method development of Bacopasaponin C, Bacopaside I, Bacopaside II, Bacopaside A3 and Jujubogenin of Bacopasaponin C from Bacopa monnieri Extract. The results of the studies are summarized as follows.

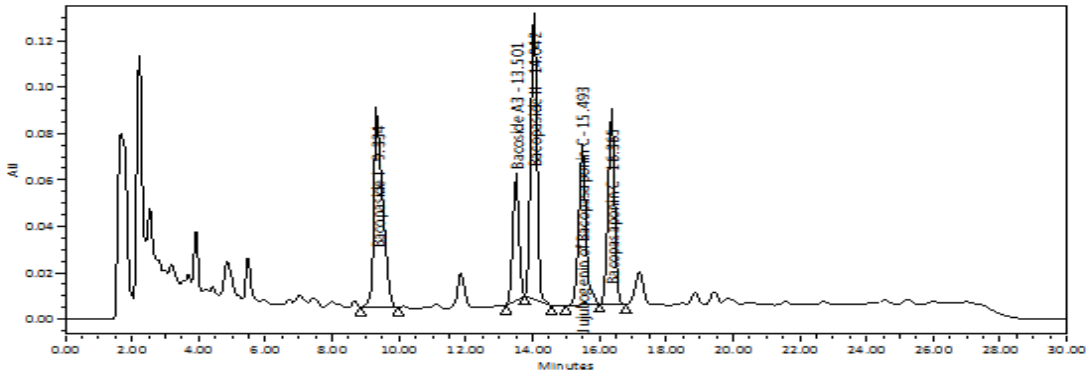
- Several trials were performed and 4th trial was optimized for the method development.
- Successful separation was achieved on a Hypersil BDS C18 5µ, 250x4.6 mm column, mobile phase composition of PH 3.0 phosphate buffer and acetonitrile (60:40) in the ratio 60:40 %v/v, Flow rate 1.5 ml/ min. UV detection was carried out at 205 nm. The retention time of was found to be Bacopasaponin C 16.365 mins, Bacopaside I – 9.334 mins, Bacopaside II – 14.042 mins, Bacoside A3 – 13.501 mins and Jujubogenin of Bacopasaponin C – 15.493 mins respectively.
- The run time was found to be 30 minutes.



System Suitability Summary Results

	Name	Vial	Inj	RT	Int Type
1	Bacopasaponin C	1	1	16.371	Missing
2	Bacopaside I	1	1	9.350	Missing
3	Bacopaside II	1	1	14.027	Missing
4	Bacoside A3	1	1	13.500	Missing
5	Jujubogenin of Bacopasaponin C	1	1	15.501	Missing

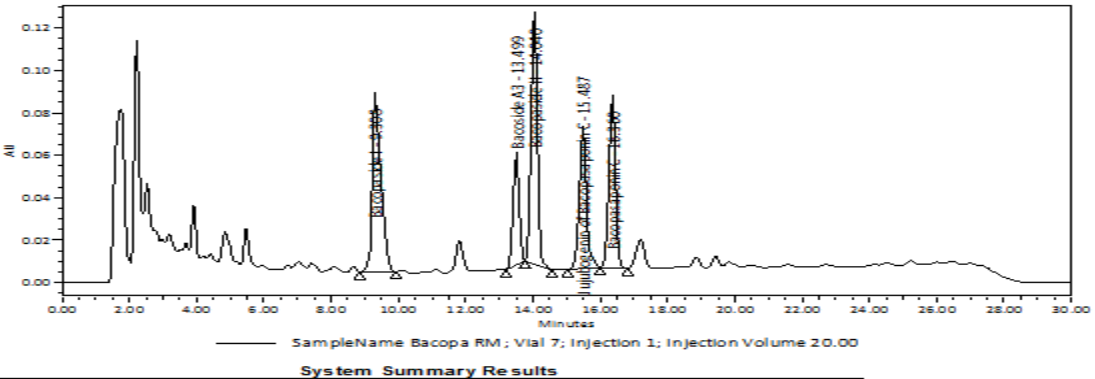
Figure 1: Chromatogram for Blank



System Summary Results

	Name	Vial	Inj	RT	Area	USP Resolution	USP Tailing	USP Plate Count	Int Type
1	Bacopasaponin C	6	1	16.365	1143873	2.2	1.0	30063	BB
2	Bacopaside I	6	1	9.334	1547662		1.4	4985	BB
3	Bacopaside II	6	1	14.042	1584875	1.5	1.0	25660	BB
4	Bacoside A3	6	1	13.501	680989	9.3	1.0	23014	BB
5	Jujubogenin of Bacopasaponin C	6	1	15.493	1022965	3.9	1.1	25861	BB

Figure 2: Chromatogram for Standard



System Summary Results

	Name	Vial	Inj	RT	Area	USP Resolution	USP Tailing	USP Plate Count	Int Type
1	Bacopasaponin C	7	1	16.360	1097378	2.3	1.0	30839	BB
2	Bacopaside I	7	1	9.308	1488801		1.4	5380	BB
3	Bacopaside II	7	1	14.040	1523669	1.5	1.0	25751	BB
4	Bacoside A3	7	1	13.499	657790	9.5	1.0	23232	BB
5	Jujubogenin of Bacopasaponin C	7	1	15.487	973638	3.9	1.1	26098	BB

Figure 3: Chromatogram for Sample

CONCLUSION

Based on the studies it was concluded that the method development of Bacopasaponin C, Bacopaside I, Bacopaside II, Bacopaside A3 and Jujubogenin of Bacopasaponin C from Bacopa monnieri extract were found to be simple, sensitive and inexpensive method. Hence, the developed method can be recommended for routine quality control analysis.

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