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Content Variation Studies of Marketed Monoherbal Formulations Containing *Bacopa monnieri* By HPLC Technique

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ABSTRACT

The standardization of herbal formulations has become very essential as there is increase in the demand and frequent usage of the herbal formulations by the people. Monoherbal formulations containing *Bacopa monnieri* extract were collected from the commercial market and analysed for their phytoconstituents viz: Bacopaside-I, Bacoside A_3, Bacopaside-II, Jujubogenin isomer of Bacopasaponin C and Bacopasaponin C contents by High Performance Liquid Chromatographic Technique (HPLC). The Capsule and Powder forms of different brands of marketed herbal formulations of *Bacopa monnieri* were selected and analysed by HPLC Technique. The total peak area of standards and the corresponding peak area of samples were compared and the amount present in them was calculated. The results reveal that there are lots of variations between marketed Bacopa products and the content of phytochemicals are not uniform. The present study indicates the necessity of development of analytical procedures for all herbal formulations available in the market to ensure the quality and efficacy of the products.

Keywords: *Bacopa monnieri, HPLC Analysis, Analytical markers, Content variation*

INTRODUCTION

Standardization of herbal formulations are essential in order to assess the quality of drugs based on the concentration of their active principles, physical, chemical, phytochemical, standardization and *invitro-invivo* parameters. The quality assessments of herbal formulations are of paramount importance in order to justify their acceptability in modern system of medicines. One of the major problems faced by the herbal industry is the unavailability of rigid quality control profile for herbal materials and their formulations. The subject of herbal drug standardization is massively wide and deep[1]. There is so much to know and so many seemingly contradictory theories on the subject of herbal medicines and their relationship with human physiology and mental functions[2]. India needs to explore the medicinally important plants. This can be achieved only if the herbal products are evaluated and analysed using sophisticated modern techniques of standardization.

The aim of the present study is to determine the content variations of five analytical markers viz: Bacopaside-I, Bacoside A_3, Bacopaside-II, Jujubogenin isomer of Bacopasaponin C and Bacopasaponin C in the monoherbal formulations containing *Bacopa monneri* available in the commercial market. The Capsule and Powder forms of different brands of marketed monoherbal formulations of Brahmi were selected and analysed for their content by HPLC Technique.

MATERIALS AND METHODS

Sample collection

The capsules and powder forms of different brands of six monoherbal formulations containing *Bacopa monneri* were

collected from various community pharmacies and given number from1 to 6 and used for the study.

Standard preparation

Bacoside A

Accurately weighed 20mg of Bacoside A which contains known amount of Bacoside A_3, Bacopaside-II, Jujubogenin isomer of Bacopasaponin C, and Bacopasaponins C to a 50 ml volumetric flask. Added 25 ml of methanol to the above, gently heated, sonicated and cooled then make upto 50 ml with methanol.

Bacopaside-I

Weighed accurately 5mg of Bacopaside-I reference standard to a 50 ml volumetric flask, dissolved in 25 ml of methanol, gently heated, sonicated and cooled then make upto 50ml with methanol.

Sample Preparation

Extract

Accurately weighed quantity of samples equivalent to standard concentration to 100ml volumetric flask, added 50ml of methanol, sonicated for 6 minutes, boiled on water bath for5 minutes, Cooled to room temperature then made up the volume to 100ml with methanol. This solution was mixed well and filtered through 0.2µ membrane filter paper and used for analysis.

Chromatographic conditions

Solvent A

Dissolved 0.136gm of anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) in 900ml of HPLC grade water and added 0.5ml of Ortho-phosphoric acid. The water is added to the above to make up the volume upto 1000ml. The above solution was filtered through 0.45µ membrane and degasses it in a sonicated for 3 minutes[3-5].

Solvent B - Acetonitrile solution

Column	:	Pinnacle DB C18 column, 250mm, 4.6mm / 5 micron (Restek)
Detector	:	Photo diode array detector or UV Detector
Wave length	:	205nm
Flow rate	:	1.5ml/min
Injection volume	:	20µl

Table No: 1 Gradient conditions

Time (min)	Buffer concentration. (Solvent A)	Acetonitrile concentration (Solvent B)
0.01	70	30
25	60	40
30	40	65
35	15	85
40	10	90
45	10	90
50	70	30
55	70	30

RESULT AND DISCUSSION

The HPLC analysis of different monoherbal marketed formulations were carried out for the quantitative estimation of possible phytoconstituents listed in the table including Bacoside -A, active principle present in *Bacopa monnieri*. The results are tabulated in Table No:3 and Fig No : 1-8.

The mono herbal formulations containing *Bacopa monnieri,* the label claim of 250mg, 250mg, 30mg, 500mg, 500mg and 500mg were taken for HPLC analysis. The retention time of the five standards were found to be 11.509, 15.529, 16.162, 17.678& 18.679 for Bacopaside I, Bacoside A_3, Bacopaside II, Jujubogenin isomer of Bacopasponin C and Bacopasaponin C respectively. The retention time of six various herbal formulations containing *Bacopa monnieri* were matching with each other and confirmed the presence of all five standards in the formulations except sample 4 and 6 (Table No.2).

Table : 2. Results of HPLC analysis of standards and samples with Retention Time

AnalyticalMarkers	Retention Time of Standard	Retention Time of Formulations					
		Product 1	Product 2	Product 3	Product 4	Product 5	Product 6
Bacopaside I (%w/w)	11.509	11.460	11.711	11.307	NIL	11.557	NIL
Bacoside A_3 (%w/w)	15.529	15.542	15.668	15.676	NIL	15.622	NIL
Bacopaside II (%w/w)	16.162	16.172	16.295	16.309	NIL	16.259	NIL
Jujubogenin isomer of Bacopasaponin C (%w/w)	17.678	17.698	17.825	17.834	NIL	17.791	NIL
Bacopasaponin C	18.679	18.699	18.823	18.840	NIL	18.781	NIL

The content of Bacopasaponin C (5.73%W/W) and Bacoside II (4.70%W/W) is more in sample 1 followed by sample 5 (Bacopasaponin C : 2.46%W/W & Bacopaside II (3.51%W/W), sample 3 (Bacopasaponin C : 2.46%W/W & Bacopaside II (3.51%W/W), sample 2 (Bacopasaponin C: 0.98%W/W & Bacopaside II (1.17%W/W) and remaining standards were also present in the same manner (Table No.3). The two products namely sample 4 and 6 does not produce any peaks for all five standards listed in the table (Table No. 2 & 3).

Table : 3. Results of Percentage Content of Various Herbal Formulations by HPLC Analysis.

Samples	Bacopaside I (%w/w)	Bacoside A3 (%w/w)	Bacopaside II (%w/w)	Jujubogenin isomer of Bacopasaponin C (%w/w)	Bacopasaponin C (%w/w)
Sample 1	3.74	2.83	4.70	4.35	5.73
Sample 2	0.76	0.95	1.17	0.96	0.98
Sample 3	1.09	1.10	1.32	1.15	1.41
Sample 4	ND	ND	ND	ND	ND
Sample 5	2.92	1.93	3.51	2.63	2.46
Sample 6	ND	ND	ND	ND	ND

*ND-Not Detected

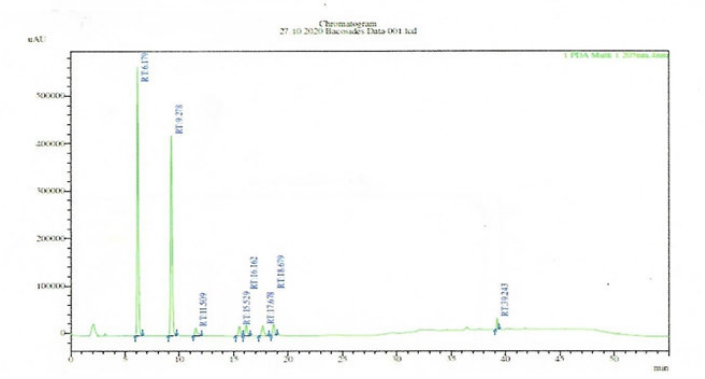

Fig.No.1. HPLC Chromatogram of Standards

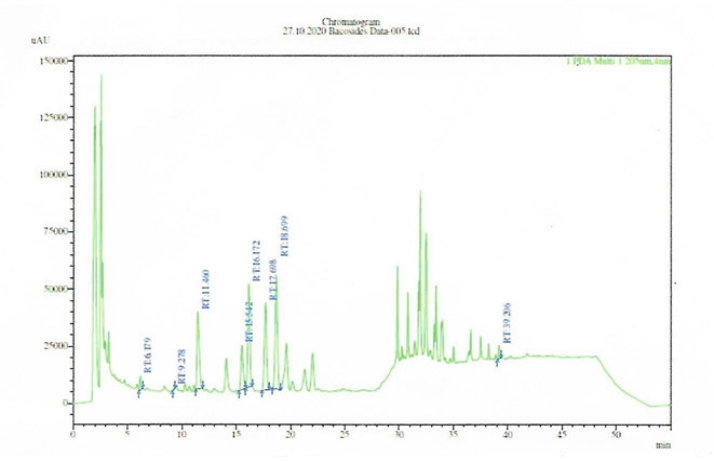

Fig.No.2. HPLC Chromatogram of SAMPLE 1 - Monoherbal Formulation Containing Brahmi

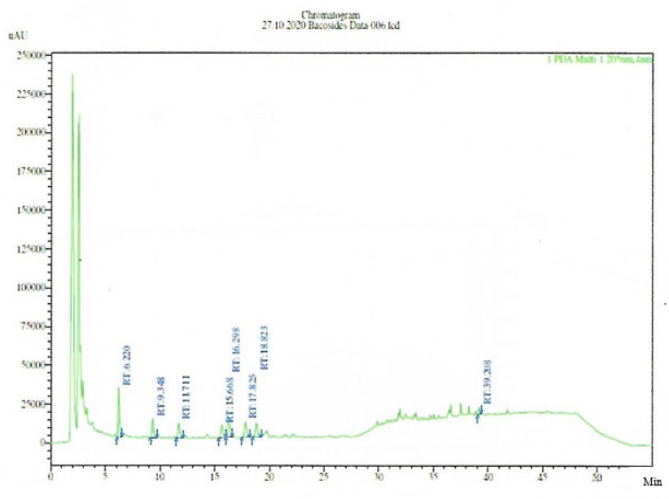

Fig.No.3. HPLC Chromatogram of SAMPLE 2 - Monoherbal Formulation Containing Brahmi.

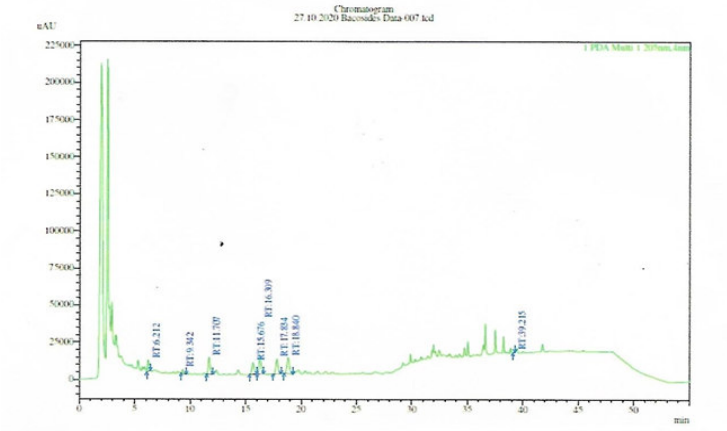

Fig.No.4. HPLC Chromatogram of SAMPLE 3 - Monoherbal Formulation Containing Brahmi.

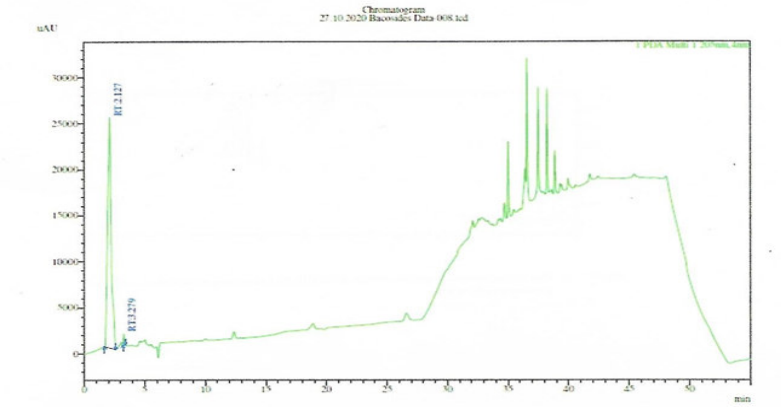

Fig.No.5. HPLC Chromatogram of SAMPLE 4 - Monoherbal Formulation Containing Brahmi.

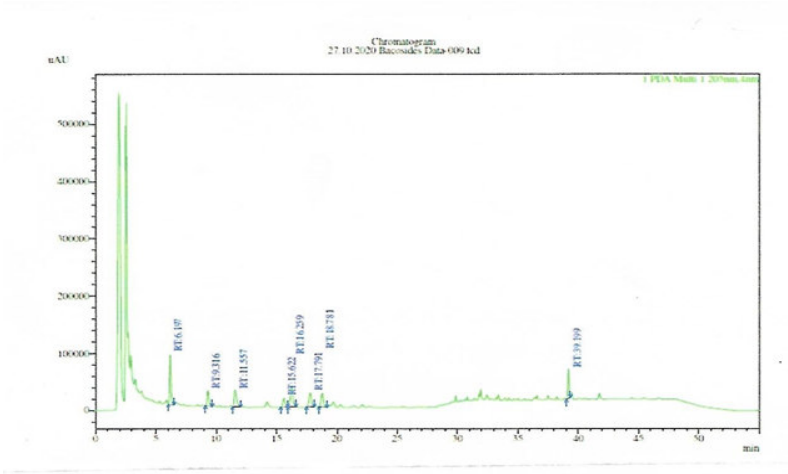

Fig.No.6. HPLC Chromatogram of SAMPLE 5 - Monoherbal Formulation Containing Brahmi

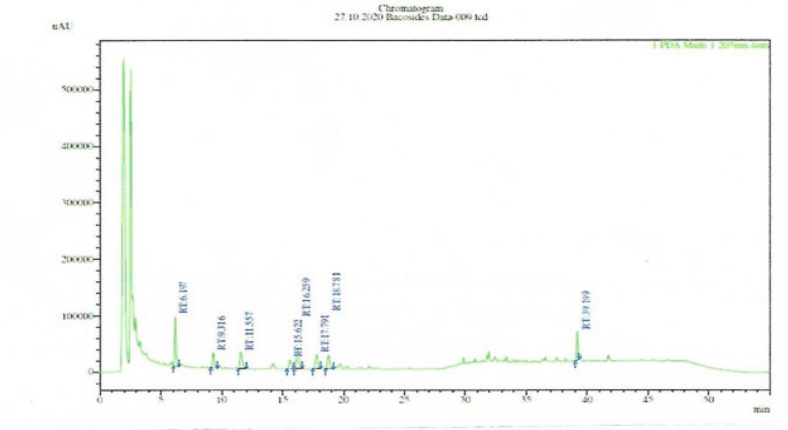

Fig.No.7. HPLC Chromatogram of SAMPLE 6 - Monoherbal Formulation Containing Brahmi

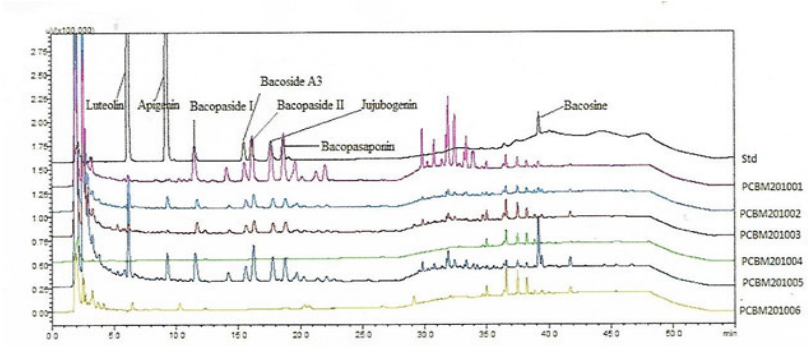

Fig.No.8. Overlay chromatogram of samples with standards

SUMMARY AND CONCLUSION

Herbal medicine is very important in the developed countries for primary health care. The less side effects of herbal medicines have increased their popularity in humans. Such herbal medicines also have a large safety profile that showing fewer side effects and better absorptions. Hence standardization of herbal drugs is of great importance. Hence the quality of herbal medicines should be question. HPLC analysis of Bacosides and other standards present in *Bacopa monnieri* were carried out and the content of same are compared with marker compounds. The six various brands containing *Bacopa monnieri* were collected from various parts of India and analysed by using HPLC.

From the HPLC analysis the variation of Bacosides and other contents from marketed formulations were studied and this study reveals that lot of variation in the content and it may affect the potency of formulations. Standardization of herbal medicines should be well established as that of the allopathy medicines to maintain the standard of all herbal medicines. In the commercial market there are many herbal formulations and herbal based Ayurveda, Siddha, Homeopathy, Unani and Neutraceutical formulations available and people are consuming regularly. But people do not know about the quality and efficacy of the product and based on the advertising and publicity they are purchasing for their personal use. Based on the present study it was reveal that there are lot of variations in the content and quality of the herbal formulations, depends upon the content present in the formulation the pharmacological activity will be varied. The content of the individual herb will vary due to many reasons like climate, soil, temperature, humidity, storage and package etc., If individual herb content varies

then the content of finished product also will vary and this will affect the quality and efficacy of the finished product. So it was concluded that similar to Allopathic medicines there should be an analytical procedure for testing of herbal raw materials as well as finished formulations to maintain the uniform quality and efficacy of all herbal based formulations available in the commercial market.

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