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Phytochemical Screening and Spectral Analysis of a Bioactive Compound from the Fraction of Bacopa monniera (L)

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Abstract

The present paper highlights Phytochemical study and Spectral Analysis of Bacopa monniera whole plants which was collected, identified, shade dried, authenticated, and powdered for the isolation of extract by soxhlation apparatus, then obtained extract was further purified by thin layer, column chromatography and purified fraction was sent to SAIF, CDRI Lucknow (UP) for spectral analysis (IR, UV, HNMR, CNMR and Mass). As a result of spectral analysis, a compound Bacopasaponin was isolated and confirmed in the biologically active fraction of Bacopa monniera extract. Maximum percentage yield was observed in methanol (9.54%) followed by water 3.56% in the results. In preliminary phytochemical screening, various tests were applied and aqueous and methanolic extract was found to be strong positive for saponin and sapogenin. In Column chromatography of Bacopa monniera methanolic extract, solvent systems viz. Toluene: n-hexane: Ethyl acetate (8:1:1), Toluene: Ethyl acetate: Methanol (3:4:3), Toluene: Ethyl acetate: Methanol: Glacial acetic acid (2:4:3:1), n-Butanol: Distilled water (5:5) and Methanol: distilled water (5:5) were applied and Bm-2 fraction was found to be biologically active. In Thin layer chromatography of Bacopa monniera methanolic extract solvent system viz. Toluene: Ethyl acetate: n-Hexane (8:1:1) was used and Rf value 0.68 was found to be of Bm-2 fraction of Bacopa monneira extract.

Keywords: *Secondary metabolites; Bacopa monniera; Bacopasaponin; Bioactive compound.*

1. Introduction

In the present study, Bacopa monniera was ranked outstanding in a priority list of the most important medicinal plants, evaluated on the basis of their medicinal importance, commercial value and potential for further research and development. Bacopa monniera is one of the most important plants used as brain tonic and as restorative in debilitated condition which belongs to family Scrophulariaceae and distributed on the Indian subcontinent [1] in wet, damp marshy areas. Bacopa monniera is also referred as,Water hyssop, Herpestis monniera and Brahmi. It is classified as a Medhyarasayana, a drug used to improve memory and intellect (Medhya) [2].It is used in Ayurveda for the treatment of anxiety and in improving the memory for several centuries. In addition to memory boosting activity, it is also claimed to be useful in the treatment of cardiac, respiratory and neuro-pharmacological disorders. It was reported to possess anti-inflammatory, analgesic, antipyretic, sedative, free radical scavenging and anti-lipid per-oxidative activities and the compounds responsible for the memory enhancing effects of Bacopa monniera are triterpenoid saponins called Bacosides". In the present study, the pharmacological properties of phytoconstituents of Bacopa monniera was evaluated by applying phytochemical techniques and the mast cell de-granulation activities was attributed mainly due to the presence of active phyto-constituent called Bacosides. Besides this, the pharmacological effects of Bacopa monniera are attributed to the presence of a number of biologically active compounds, including alkaloids, saponins and sterols. Later on, other alkaloids like nicotine and herpestine were also reported [3] The isolation of D-mannitol and a saponin, hersaponin and potassium salts from Bacopa

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monniera was noticed by Rajniet al. [4] as a Medhya Rasayana drug. Therefore, the present study was aimed to isolate a compound Bacopasaponin from the Bacopa monniera medicinal plants for the inhibition of mast cell de-granulation activities.

2. Materials and Methods

Plant Bacopa monniera of family Scrophulariaceae that is commonly known as "Jalneem or Brahmi" was taxonomically identified and authenticated by Prof. (Dr.) P.N. Shrivastava and procured in the Herbarium (S.No.45) at Pest Control and Ayurvedic Drug Res. Lab., S. S. L. Jain P.G. College Vidisha (M.P.) for further use. The whole plant material was collected from the marshy areas of Vidisha, where it is available plentifully. About 2 kg fresh whole plant material of Bacopa monniera was shade dried and powdered which was used for the isolation of extract in methanol by steam distillation method using Soxhlet apparatus and semisolid crude extract was evaporated on vacuum evaporator and percentage yield of the extract was calculated (Table 1).

Table 1 Percentage Yield of Curde Extract Obtained by Soxhletion

Table-1: Percentage yield of crude extract obtained by Soxhletion.

Plant Name	Solvent	Wt. of Dried Powder	Volume of Solvent	Weight of Extract	Percentage yield
Bacopa monniera	Methanol	255 gm	1500 ml	24.35 gm	09.54 %
	Water	255 gm	1000 ml	09.10 gm	03.56 %

Preliminary phytochemical screening of the Bacopa monniera extract was performed following phytochemical methods of Harborne [5] to confirm the presence of phytoconstituents such as alkaloids, phenols, terpenoids, phytosterols, saponins, flavonoids and tannins (Table 2). The purified fraction of the Bacopa monniera extract was applied in Thin Layer Chromatography also on silica coated glass plate G' 60 F2 54 of 0.2 mm thickness by using solvent system (menstruum) Toluene: EtoAc: n-Hexane (8:1:1) for the detection of saponin and Rf value were calculated by the formula of Brimley and Barrett[6]as Distance travelled by solutes/Distance travelled by solvents (Table 3).

Table 2 Preliminary Phytochemical Screening of Bacopa Monniera Extracts

Table-2: Preliminary phytochemical screening of *Bacopa monniera* extracts.

Phytoconstituents	Successive extraction	
	Methanol	Aqueous
Alkaloids	-	-
Flavonoids	++	++
Saponins and sapogenin	++++	++++
Glycosides	++	++
Tannins	-	-
Phytosterol	+	+
Triterpene	+	+

Key: Positive (+), Strong positive (++++), Negative (-)

Table 3 Thin Layer Chromatography of Bacopa Monniera Extract

Table-3: Thin layer chromatography of *Bacopa monniera* extract

Solvent System Used	Spots	Rf Value	Color characterization		
			Visual light	Uv-light 260 nm	Iodine chamber
Toluene: Ethyl acetate: n-Hexane (8:1:1)	Bm-1	0.12	Light green	Dark green	Black
	Bm-2	0.38	Light yellow	Yellow	Light brown
	Bm-3	0.68	Brown yellow	Dark yellow	Brown

The extract of Bacopa monniera was partitioned by column chromatography[7] using various solvent systems viz. Toluene: n-Hexane: EtoAc (8:1:1), Toluene: Ethyl acetate: Methanol (3:4:3),Toluene: Ethyl acetate: Methanol: Glacial acetic acid (2:4:3:1), Methanol: Distilled Water (50:50) and n Butanol: Distilled Water (50:50) and the purified fractions were obtained (Table 4). The purified fraction of

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Bacopa monniera were sent to SAIF, CDRI, Lucknow for spectral analysis viz. IR, UV, 1HNMR,13CNMR and Mass (Graph 1-5) and obtained graph were used for structural elucidation of the biologically active compound. Figure 1 shows IR Spectrum.

Table 4 Column Chromatography of Bacopa Monniera Extracts

Table-4: Column chromatography of *Bacopa monniera* extracts.

Plant extract	Solvent systems used	Fraction obtained	Wt. of fraction in gm.	Color of fraction	Fraction used
Bacopa monniera	Toluene: n-hexane: Ethyl acetate (8:1:1)	Bm-1	0.47	Light green	
	Toluene: Ethyl acetate: Methanol (3:4:3)	Bm-1	0.25	Light green	
	Toluene: Ethyl acetate: Methanol: Glacial acetic acid (2:4:3:1)	Bm-1	0.38	Green	
	n-Butanol: Distilled water (5:5)	Bm-1	1.47	Dark Brown	
	Methanol : distilled water (5:5)	Bm-1	1.34	Green	
		Bm-2	3.12	Yellow	
		Bm-3	2.05	Blackish green	BM-2
		Bm-4	1.13	Red	

In the present study, IR Vmax (KBr) (cm 1) spectrum of Bacopa monniera showed 13 peaks of different ranges and frequency 3019.89was found in the range between 3020 to 3100 cm -1 with medium intensity showed =C-H, =CH2 and C=C, frequency 1654 cm -1 showed C=C stretching and frequency 1385 cm-1 showed OH group. Characteristic bands at Vmax (KBr) at 3019 and 1654 cm-1 showed double bonds whose presence was further confirmed by positive color test with tetra nitro methane (Graph-1). Characteristic bands at Vmax (KBr) at 3019 and 1654 cm- 1 showed double bonds. Fraction BM-2 has displayed absorption band in the UV spectrum at 283 nm in MeOH which confirmed Presence of Bacopasaponin (Graph- 2). Figure 2 shows UV spectrum.

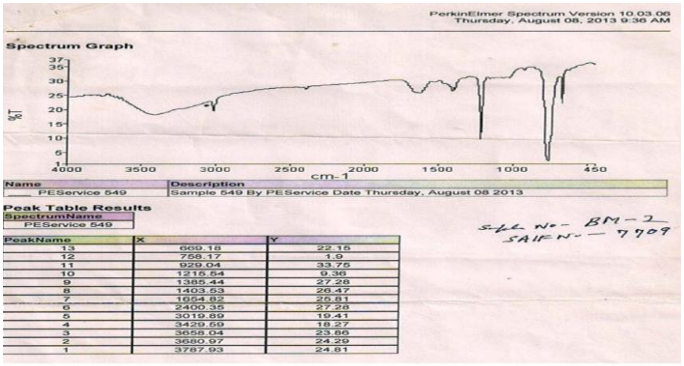

Figure 1 IR Spectrum

Graph-1: IR spectrum of Bacopa monniera; BM-2 fraction.

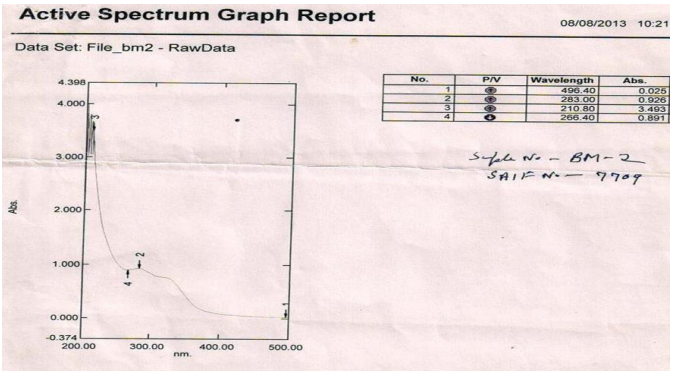

Figure 2 UV spectrum

Graph-2: UV spectrum of Bacopa monniera; BM -2 fraction.

3. Results and Discussion

In the present study, maximum percentage yield of the Bacopa monniera extract was found to be 9.54% in methanol, followed by water 3.56%. Similarly, Volluriet al. [8] have reported a very good percentage yield 15.47 % of Bacopa monniera methanolic extract. In preliminary phytochemical screening of the Bacopa monniera extracts, aqueous and methanolic extract were found to be strong positive for saponin and sapogenin, positive for flavonoids, glycosides, sterols and terpenoids and negative for tannins and alkaloids. Deepak et al. [9] have also reported phytoconstituents of Bacopa monniera especially, major saponin Bacoside A which was found to be a mixture of saponins with bacoside A3, bacoside II, jujubogenin, isomer of bacopasaponin as major constituents which was reported as major bioactive phytoconstituents in the present study for the inhibition of mast cell de granulation activity. The thin layer chromatography of Bacopa monniera

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methanolic extract was experienced using solvent system viz. Toluene: Ethyl acetate: n-Hexane (8:1:1) and Rf values viz. 0.12, 0.38, 0.68 were calculated. Saxena et al. [10] have described thin layer chromatography of Bacopa monniera extract using chloroform: methanol: water as solvent system for the separation of saponins and detection of spot was done at 630 nm. Chatterji et al. [11] have also reported the chromatography of crude saponin mixtures of Bacopa monniera and Rf value were calculated for saponin i.e. 0.09 and 0.43. Both the Rf value were found to be nearer to the present Rf values. Then, column chromatography of Bacopa monniera methanolic extract was also done in the present study by trying different solvent systems viz. Toluene: n-hexane: Ethyl acetate (8:1:1), Toluene: Ethyl acetate: Methanol (3:4:3), Toluene: Ethyl acetate: Methanol: Glacial acetic acid (2:4:3:1), n-Butanol: Distilled water (5:5) and Methanol: distilled water (5:5) and number of fractions were obtained. Chatterji et al.11 have also isolated number of fractions in column chromatography including the sample of Bacopasaponin by applying ethyl acetate: pyridine: water (4:1:1) and butanol: ethyl acetate: water (4:1:5) and butanol: acetic acid: water (4:1:5). In the present study, fraction Bm-2 was tested for the inhibition of mast cell de granulation activity. On the basis of spectral graphs the compound (BM-2) was analyzed for molecular formula C33H38O, mp.240-2420C, [α] D31-31.16°(in CH3OH) and isolated an amorphous powder from the purified fraction which gave positive froth test for Saponin, Lieberman Burchard test [12] for terpenoid and Molish test [13] for sugar. In the present study, IR Vmax (KBr) (cm-1) spectrum of Bacopa monniera showed 13 peaks of different ranges and frequency 3019.89 was found in the range between 3020 to 3100 cm-1 with medium intensity showed =C-H, =CH2 and C=C. The IR spectrum also showed bands at 3429.59 cm-1 which indicates poly hydroxyl system, 1654 cm-1showed C=C stretching and 1385 showed OH group. Characteristic bands at Vmax (KBr) at 3019 and 1654 cm-1 showed double bonds whose presence was further confirmed by positive color test with tetra nitro methane (Graph-1).14 Fraction BM-2 has displayed absorption band in the UV spectrum at 283 nm in MeOH which confirmed the presence of Bacopasaponin (Graph-2). In the present study, 1HNMR spectrum of Bacopa monniera active fraction (BM-2) at δ 3.612 showed 2H (m, -CH2-CO-), δ 1.572 showed 3H, s, =C (CH3)-CH3 and δ 0.991 showed 3H (s, CH3). The 1HNMR of BM-2 showed the presence of methyl group at signals δ 0.991 (s, 3H, CH3), 1.31 (s, H3-28), 1.572 (s, 3H, H=27), (d, J=8, 1Hz, 1H, Hβ2), 1.99 (d, J=10.5 Hz, 1 H, Hα-15), 2.06 (d, J=10.5 Hz, 1 H, Hα-15), 2.51 (m, 1H, H-13), 3.26 (m, 1H, H-3), 3.29 (dd, J=11.4, 4.2, H-3), 3.36 (dd, J=11.5, 4.3), 3.791 (d, J=7.2Hz, 1H, Hα-30), 3.72 ((d, J=7.2Hz, 1H, Hβ 30), δ 3.858 (m, 1H, Hβ-5'), 4.528 (d, J=4.5 Hz, 1H, H-2'), 4.800 (d, J=3.6 Hz, 1H, H-1') and the 1HNMR spectrum clearly exhibited 5.199 (m, H-15) and 5.270 (d, J=6.0 Hz, H-14) conforming the presence of double bond in the compound. The 1HNMR spectrum of Bacopasaponin showed anomeric proton signals at δ5.454 for 3-O-α-L-arabinopyranosyl unit. The 1HNMR of BM-2 displayed one proton doublet at δ4.247 shifted to 4.528 in its acetate assignable to C-3 on the basis of biogenetic analogy and its coupling interaction of δ4.05 and 3.52 Hz indicated the β-orientation of methine proton and α-orientation of hydroxyl group. The presence of one signal at δ4.52 (dd, J=1.5, 3.0 Hz, β-H-29) was attributed to methylene group at position-29. The 1HNMR of BM-2 also exhibited coupling between β-H-29 (4.528 ppm), α-H-29 (4.800 ppm), H-13 (2.52 ppm) and H-27 (1.572 ppm) indicating C-29→C-13→C-27 correlation (Graph-3). Figure 3 shows 1HNMR Spectrum.

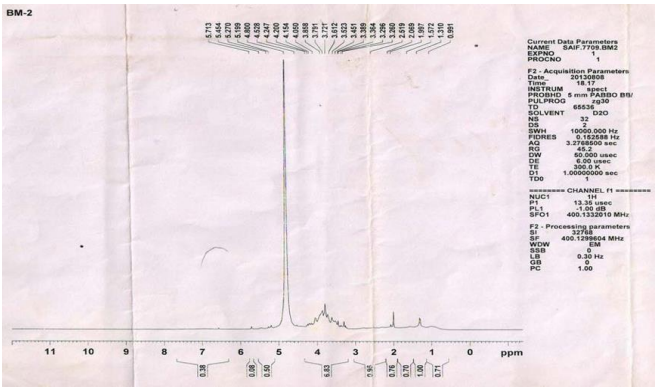

Figure 3 ^{1}HNMR Spectrum

Graph-3:^{1}HNMR spectrum of Bacopa monniera BM-2 fraction

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In the present study, 13CNMR spectrum of the fraction BM-2 of Bacopa monniera, showed the presence of a peak at δ 69.13 for pseudojujubogenin type of skeleton in the compound. The 13CNMR spectrum showed two arabinose units, one was present in pyranose and another in furanose. 13CNMR chemical shifts of the aglycone portion were well in agreement with those assigned for pseudojujubogenin, the genuine aglycone isolated from this plants[15] excepting the chemical shift of C 3 which appeared at δ77.28 in pseudojujubogenin, but at 89.69 in the saponin, due to the linkage of sugar chain at C-3 and the chemical shift values for arabinose indicated its existence in the pyranose form. Based on these observations, the structure of Bacopasaponin was assigned to be 3-O-α-L arabinopyronosyl pseudojujubogenin (Graph-4). Figure 6 shows Mass Spectrum. The MS spectrum of the purified fraction of the Bacopa monniera (m/z), relative intensity data suggested the compound to be Bacopasaponin. The data of the compound was found in full agreement with the literature [16-17]and the different species formed during fragmentations are shown in the (Graph-5). The significant fragmentation pattern observed in the FAB-MS of Bacopa monniera (BM-2) showed m+/z=476.16 (M+H-Arabinosyl)+, 459.15 (M+H Arabinose), m/z 438.15, 419.14, 245.06, 204.03, 203.12, 197.07, 186.03, 170.05 and 136.03. On the basis of above fragmentation pattern, the structure of the compound Bacopasaponin was obtained as amorphous powder and molecular composition of C33H38O as established on the basis of high-resolution mass spectrum (m+ 459.15). The appearance of the carbon resonance as a primary carbon at δ 63.93 attributable to C-22 and C-23 of the present compound indicates that the nature of aglycone i.e. pseudojujubogenin. Figure 5 shows 13CNMR spectrum. The attachment of sugar moiety in the present compound at C-3 can be inferred from carbon chemical shifts of C-3 which appeared at δ77.28 as secondary carbon in pseudojujubogenin.18-19]Hence, a new dammarane type pseudojujubogenin Bacopa saponin was isolated from the BM-2 fraction of Bacopa monniera and characterized as 3-O-(α-L arabinopyranosyl) pseudojujubogenin by spectral and phytochemical analysis. The structure of the compound (Figure-1). Similarly, Pawar and Bhutani [20] have isolated new dammarne type triterpenoidalsaponins from Bacopa monniera. Pawar et al. [21] have also isolated glycosides of 20-deoxy derivatives of jujubogenin and pseudojujubogenin from Bacopa monniera. Figure 4 shows Bioactive Compound Bacopasaponin Elucidated from Bacopa Monniera.

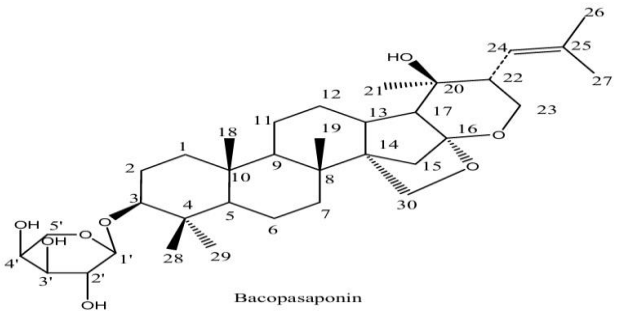

Figure-1: Bioactive compound Bacopasaponin elucidated from *Bacopa monniera.*

Figure 4 Bioactive Compound Bacopasaponin Elucidated from Bacopa Monniera

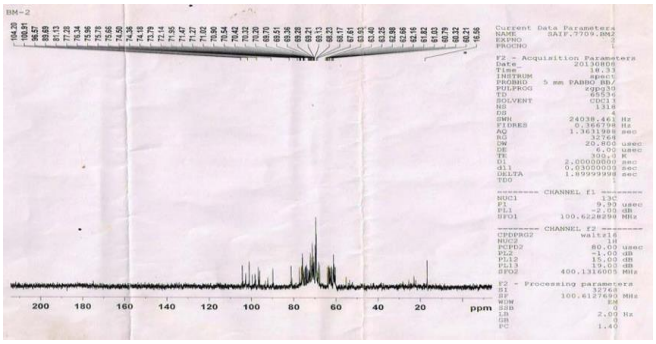

Figure 5 ^{13}CNMR spectrum

Graph-4: ^{13}CNMR spectrum of Bacopa monniera 2BM-2 fraction

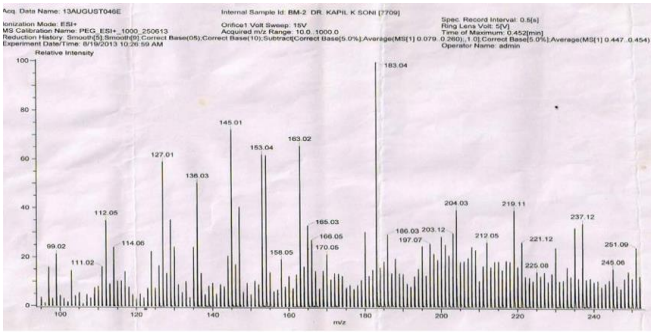

Figure 6 Mass Spectrum

Graph-5: Mass spectrum of Bacopa monniera BM-2 fraction

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Conclusion

In the present study, phytochemistry of Bacopa monniera whole plant was done by applying various phytochemical techniques and a bioactive compound Bacopasaponin was isolated from the purified fraction of Bacopa monniera extract on the basis of spectral analysis (IR, UV, HNMR, CNMR and Mass).

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