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Research Article

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Fingerprinting Profile of Saponin Glycosides in *Bacopa Monnieri* (L.) Wettst

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Abstract: As a medhya rasayana in Ayurveda formulations, *B. monnieri* has undergone extensive research into its bioactive chemicals in the last few years. To date, no research has been done on the chemical profiles of different extract components, such as the leaf and stem compounds that are crucial for pre-formulation purposes. Routine *B. monnieri* testing requires the use of well-defined concentrations of bacopaside II and baposide A3. As a result, the study's goal was to identify the active ingredients found in the leaves and stems of the *Bacopa monnieri* plant. We want to standardize phytoconstituents in *B. monnieri* leaves and stems using two different markers and the HPLC technique described here. The HPLC fingerprinting analysis of the extract of *B. monnieri* was carried out. Phosphoric acid: acetonitrile (65: 53 v/v) was used as the mobile phase, and the absorbance at 240 nm was measured after a 50-minute run time. Saponins found in the leaf extract included apigenin, bacopasaponin A, bacoside A3, and bacoside A1, with retention durations of 21.217, 17.843, 15.241, 14.540, 11.274, and 11.263, respectively. Stem extract, on the other hand, revealed the existence of major compounds such as bacopaside II, bacoside A3, bacopaside I, and bacosaponin C, with retention durations of 21.133, 14.186, 14.021, and 13.970, respectively. Phytoconstituents were found in greater abundance in the leaf extract of the plant than in the stem extract. The standard bacopaside II and bacoside A3 were used to match the phytoconstituents found in leaves and stems. HPLC fingerprinting was used to check the quality of plant extracts and their fractions, such as methanol.

Keywords: *Bacopa Monnieri*, Bacoside, Saponin, Nootropics, Dammarane-Type Triterpenoid

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1. INTRODUCTION

About 3000 years ago, the Ayurvedic medical community began using *Bacopa monnieri* to treat neurological disorders, and it has been proven to be effective.[1] Nootropic herb *Bacopa monnieri* can be found in the Southern United States as well as South Asia's subcontinent. Field-grown *Bacopa monnieri* showed seasonal, plant part, and growth stage variations in morphology and phytochemicals analyses, which may be related to changes in environmental conditions. [2] A low amount of genetic diversity in plant germplasm gathered from various parts of India has been found in previous studies; this may be due to the plant species' dependence on vegetative growth. [3] Bacosides A and B are said to be present in many products produced from *B. monnieri* that may be found on the international market. [4] *B. monnieri* leaves and stems contains several phytoconstituents responsible for therapeutics activity in certain diseases. [5] Neurological illnesses have taken over the world and are a major factor in slowing down any country's economic progress. [6] An estimated number of people suffer from Alzheimer's disease and other forms of dementia to account for roughly 6.3 percent of the worldwide burden of illness in 2005, and this figure is expected to rise to 12 percent by 2030, according to WHO research. [7] Existing drugs have negative side effects and do not slow down the progression of the disease over time. Many people have relied on botanicals such as herbal supplements or medicinal plants to treat neurological problems because of their proven efficacy. [8] Nootropic plant *Bacopa monnieri* (family: Scrophulariaceae) has been mentioned in ancient Ayurvedic treaties. [9] Brain-boosting benefits of *B. monnieri* include improved cognition and memory as well as revitalization of the senses. [10] Dammarane-type triterpenoid saponins were found in the alcoholic extract and characterized. It is possible to find aglycone-containing saponins in *B. monnieri* that contain jujubogenin and pseudo-jujubogenin, including bacosides A1–A3 [11] and the bacopasaponins A–G and the bacopasides I–V. [12-13] It is the nootropic effects of bacoside A, a component of this extract, that are to be attributed to this supplement, while the saponin bacoside B is of greater significance. [14-15] Thus, the protocol of the study was designed to fingerprint an extract of valuable *B. monnieri* leaves and stems using the HPLC technique in order to identify the phytoconstituent present in it.

1.1 About phytoconstituents identification

Bacosides are key triterpenoid saponins. They appear to boost nerve impulse transmission. The bacosides boost neuronal production and kinase activity to help heal damaged neurons. Bacosides help restore synaptic activity and nerve impulse transmission. The extract of *B. monnieri* has also been shown to have anti-inflammatory, antipyretic, antidepressant, antibacterial, anticancer, and antioxidant properties. Several plant phytocompounds are responsible for these therapeutic properties, including saponins, which play an important role in neuroprotection by modulating antioxidant enzyme activity in stressed neuronal cells (like SOD and catalase). [16]

1.1.1 Bacoside A3

The triterpenoid saponin, bacoside A3, is a key active ingredient in *Bacopa monnieri*. Bacoside A3 has already been shown to have neuroprotective properties.

1.1.2 Bacopaside II

The triterpenoid saponin Bacopaside II was isolated from the *Bacopa monnieri* plant, which has been shown to be an antidepressant. The ATPase activity of P-glycoprotein is inhibited by it.

1.1.3 Bacopasaponin C

Bacopasaponin C is a plant saponin present in *Bacopa monnieri.* It prevents memory deficits and retrieves memories in mice. Bacopasaponin C also reduces spleen parasite burden in a hamster model of leishmaniasis.

1.1.4 Bacopaside I

Bacopa monnieri is commonly used for anxiety and epilepsy treatment. It is an MAO-A and MAO-B inhibitor. Forcible swimming and tail suspension tests, and MDA levels in the brains of mice are reduced by Bacopaside I. This supplementation also increases GPX and SOD activities in the brain, which is consistent with previous research. It increases the time spent in the target quadrant of the Morris water maze and decreases the formation of amyloid plaques in the APP/PS1 transgenic mouse model of Alzheimer's disease. By blocking the middle cerebral artery, bacopaside I reduce the infarct volume in the rats used to study transitory focal ischemia.

1.1.5 Bacopasaponin A

Bacopasaponin A is a natural product found in *Bacopa monnieri*. It is a dammarane and protostane triterpenoid. It helps with learning and memory.

1.1.6 Bacoside A1

In the plant *Bacopa monnieri*, there is a saponin by the name of Bacoside A1. Jujubogenin is a 3-O [-l- arabinofuranosyl (1→ 3) -l-arabinopyranosyl] jujubogenin, structurally.

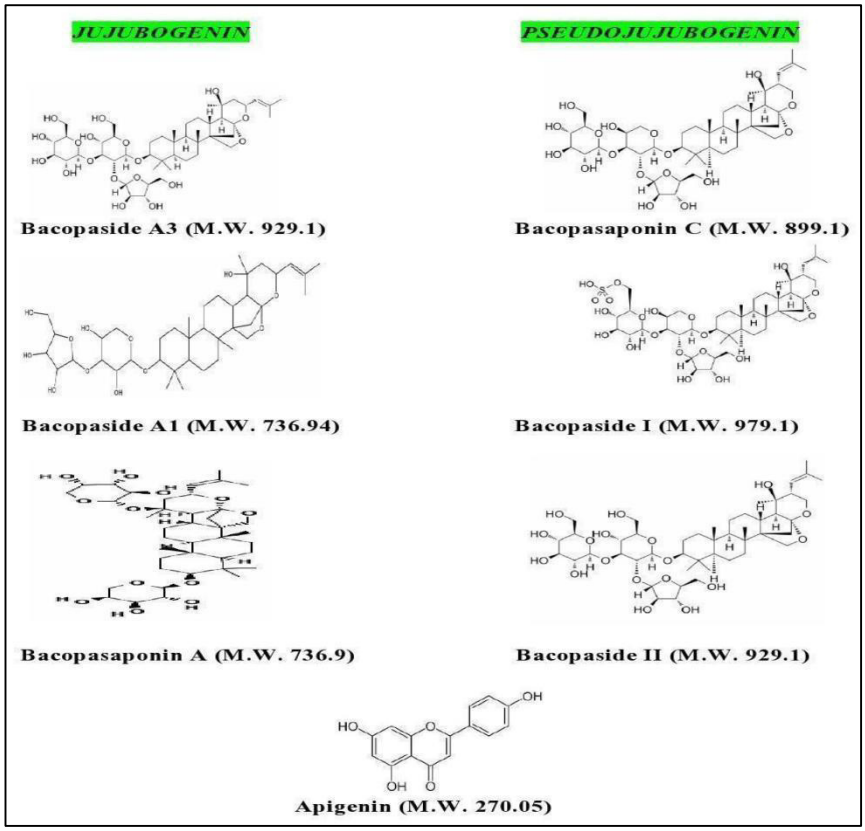

Fig. 1: Chemical structure of phytoconstituents identified through HPLC techniques

1.1.7 Apigenin

Trihydroxyflavone Apigenin is a flavone substituted at positions 4', 5', and 7" with hydroxy groups. It is proven to reduce anxiety and sedate people. It is also used as an effective anti-cancer substance. With strong selectivity for cancer cells as compared to non-cancerous cells, it offers excellent protection against a wide range of malignancies. Medicinally relevant phytoconstituents from the plant can be used to treat CNS disorders and other diseases after screening. Standardization approaches are needed to overcome differences in therapeutic potential between commercially available *Bacopa monnieri* formulations. The chemical structure of different phytoconstituents present in the plant extract is shown in Figure 1.

2. MATERIALS AND METHODS

An HPLC method and an optimized extraction procedure were used to determine the components in the extract of *B. monnieri.* A soxhlet apparatus was used to extract *B. monnieri* leaves and stems using methanol as the solvent.

2.1 Sample Collection

B. monnieri fresh plant was air dried in April 2021. All plant materials were obtained from the Ayurvedic Research Centre, Jhansi (U.P.). The Central Council for Research in Ayurvedic Sciences, Jhansi, recognised *Bacopa monnieri* (L.) Wettst's collection of leaves and stems for its value. The specimen was catalogued as voucher number 28718 in the institute's herbarium.

2.2 Chemicals and Reagents

The reagents utilized in the experiment were acetonitrile, methanol, and phosphoric acid from Rankem Pharmaceuticals. Rankem Pharmaceuticals, Gurgaon, Haryana, India, supplied both the LR and HPLC quality solvents. Sigma-Aldrich (St. Louis, MO, USA) provided the Bacoside A and Bacopaside II standards (purity > 99%).

2.3 Chromatography Protocols and Condition Details

In the bloodstream, *B. monnieri* operated as a mental chelating agent, removing any extra poisonous metals and utilizing them to remove heavy metals (chromium and cadmium). A Waters 515 HPLC pump, manufactured by Waters, the 2998 Photodiode Array Detector (PAD), and an FRC-10A fraction collector for the HPLC analysis of our sample were used. With a SunFire C18 column that measured 4.6 by 220 mm and a Waters Temperature and Pump Control Module II, the configuration was completed. There was a flow rate of 1.2 millilitres per minute and a detection wavelength of 240 nanometers. At 30 °C, this experiment used a mobile phase of 0.2 percent phosphoric acid and 53 percent acetonitrile (65: 53).

2.4 Preparation of A Standardized B. Monnieri Extract

The methanolic extract of *B. monnieri* was subjected to HPLC fingerprinting analysis. The injection volume for the leaves, stems samples and for standard was 10 µl, and the concentrations for these two samples were 20 mg/ml and 100 µg/ml for standard solution, respectively, to record the chromatogram.

2.5 Sample Preparation

In methanol, measured *Bacopa monnieri* dried extracts were dissolved. 2.8 mg of methanol extract was added to 1 ml of solvent to create the stock solutions for the samples. Plant

leaves and stem methanolic extract were ultrasonically processed at 30 °C and dried under reduced pressure. The residue from the sonicated contents of the flask was filtered through a 0.45-micron filter was used to remove any impurities and then injected into the chromatographic apparatus.

2.6 Standard Preparation

By dissolving 1.7 mg of standard with around 3 ml of solvent (A: B at a ratio of 65:35v/v), a standard stock solution was produced. The pH of the solution was adjusted to the desired 3.0 by adding 5 M NaOH. To obtain the 1-liter volume mark, use distilled water last.

2.7 Fingerprinting Analysis

In this study, HPLC fingerprint profiles of the methanol extract were developed in a 0.2% phosphoric acid: acetonitrile solvent system at a ratio of 65:35. Two solvents, acetonitrile (Solvent A) and phosphoric acid (0.2% in water) (Solvent B), were slowly eluted. The gradient programme began with 8% of A for the first 35 minutes, increased to 22% for the following 15 minutes, and then dropped once again to 8%. HPLC was used to examine the detector linearity at various concentrations of extract (from 20 mg/mL to 30 mg/mL), as well as triplicate samples (10 µl) from each concentration. The amount injected (g) was plotted against the associated peak areas (m Abs) to generate calibration curves, and regression equations were calculated. [17]

3. RESULT AND DISCUSSION

According to previous research, the amount of bacosides found in various parts of cultivated plants varies over time (an annual analysis), season, and plant growth stage. The lack of well-defined standard identification tests for diverse Ayurvedic formulations of *B. monnieri*, as well as their record in pharmacopoeias and monographs, is a major source of concern. [18] Variations in phytochemicals can also be found in the formulations used in this study. Because *B. monnieri* is standardised and assessed primarily on the concentration of bacoside A3 and bacopaside II, HPLC (with UV detection) was created for qualitative assessment of active components present in the extract. A modified HPLC-UV technique was used to obtain chromatographic fingerprints of the methanolic extracts of leaf and stem, as shown in figures 2 and 3, respectively. [19] Data on the atoms and molecules that make up a substance can be found in Tables 1 and 2 respectively. Six and four metabolites were tentatively identified in the leaf and stem extracts using the optimised HPLC conditions based on their elemental composition and patterns, respectively. [20] All detected bacoside A components showed linearity of detection at 240 nm with coefficients of determination (r2) > 0.998 in the 3–72 µg (injected amount) range. In all of the injected concentrations (n = 3), there was a 4.0 RSD in this sample's data.

3.1 Detail Analysis of the Leaf Extracts

Bacoside A3 and bacopaside X are examples of jujubogenin glycosides, while pseudojujubogenin glycosides are the major metabolites of *B. monnieri*. Both types of steroidal saponins are found in high concentrations in the leaves of *B. monnieri* (bacopasides I, II and bacopasaponin C). [21] The leaves of *B. monnieri* contain the most important components of the plant, including bacoside A3, bacopaside II, bacopasaponin C, bacopasaponin A, and apigenin (Fig 2). The retention time for these fingerprints was 11.263, 11.274, 14.540, 15.241, 17.843, and 21.217 for bacoside A1, bacoside A3, bacopasaponin C, apigenin, bacopasaponin A, and bacopaside II, respectively (Table 1). Similar compounds were reported by Saesong et al. [22] Similarly, there were five saponin glycosides, including bacoside A1 and bacopaside F, as well as seven pseudo-jujubogenin saponin glycosides, including bacopaside (I, II, and III), as well as oxybacopaside I and bacopasaponin (C and D) in the hydro-ethanolic extract of B. monnieri that were identified by the UPLC-ESI-MS analysis of Waly et al. findings. [23] Also, the study of Phrompittayarat et al. indicate the presence of saponins in *B. monnieri*. [24] It has been found that this plant extract is mainly composed of triterpenoid saponins, including pseudojujubogenin and jujubogenin glycosides. [25-26] However, it has noted that Ganzera et al. did not disclose their HPLC analysis of Brahmi showed the presence of Bacopaside I. [27]

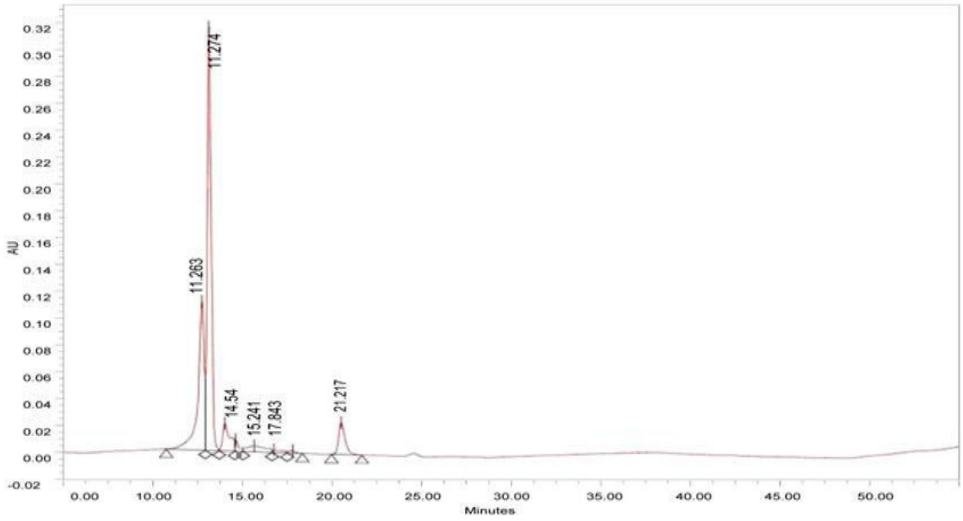

Fig. 2: HPLC chromatogram of six reference compounds extracted from B. monnieri leaves

Table no 1: Analysis of B. monnieri leaves extract by HPLC fingerprinting

S. No.	Retention Time (Min)	Area	% Area	Height	Compounds
1	11.263	1167941	26.43	100704	Bacoside A1
2	11.274	1843270	50.77	304873	Bacoside A3
3	14.540	235321	5.01	21543	Bacopasaponin C
4	15.241	249364	6.44	24633	Apigenin
5	17.843	121017	3.57	4254	Bacopasaponin A
6	21.217	49863	1.49	8336	Bacopaside II

3.2 Detail Analysis of the Stem Extracts

An active fraction including four major saponins was previously identified using HPLC-MS coupled anthelmintic assay-guided fractionation of *B. monnieri*'s methanolic extract, which is remarkable. The major constituents of *B. monnieri* in the stem, namely bacopaside II, bacopasaponin C, bacopaside I, and bacoside A1, were mentioned in figure 3 matched to the published work. [28] The retention times of bacosaponin C, bacopaside I, bacoside A3, and bacopaside II, as determined by HPLC fingerprinting of the stem extract, were 13.970, 14.021, 14.186, and 21.133 for bacosaponin C, bacopaside I, bacoside A3, and bacopaside II, respectively (Table 2). Phrompittayarat et al. observed similar results in their research. [28] Five main saponins were identified and quantified, with Bacopaside II (0.27-0.59%) or Bacopaside I (0.27-0.59%) being the most abundant (0.35-0.71 percent). The total saponin concentrations in the samples ranged from 1.11 to 2.16 percent.

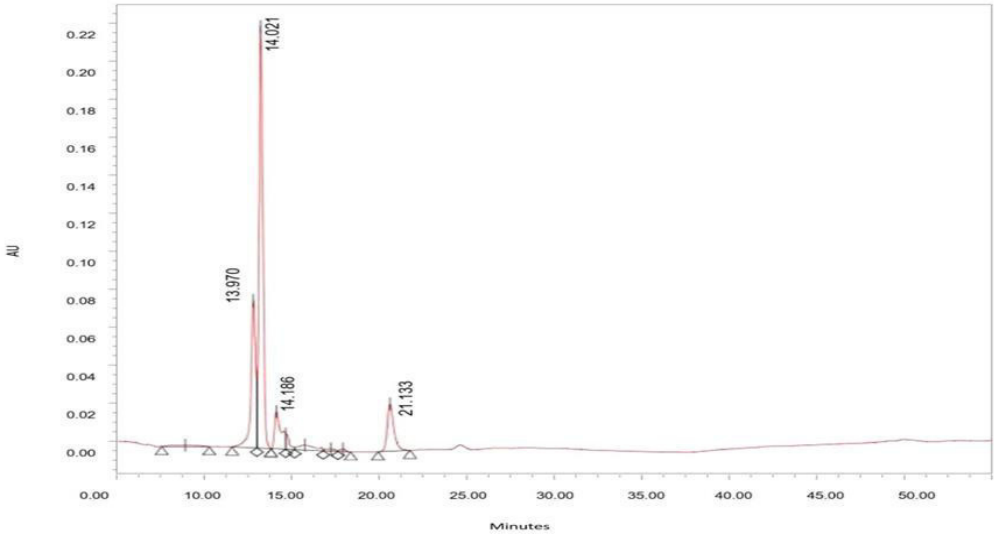

Fig. 3: HPLC chromatogram of four reference compounds extracted from B. monnieri stems

In contrast, Ganzera et al. [27] and Deepak et al. [17] did not include HPLC tests for Bacopaside I in their studies of Brahmi HPLC. However, Ganzera et al. found an unidentified saponin peak in their chromatogram that had the same pattern as Bacopaside I, which was identified in our study.

Table no 2: Analysis of B. monnieri stems extract by HPLC fingerprinting

S. No.	Retention Time (Min)	Area	% Area	Height	Compounds
1	13.970	32052	1.27	723	Bacopasaponin C
2	14.021	567117	22.31	87711	Bacopaside I
3	14.186	1311063	52.74	123234	Bacoside A3
4	21.133	193123	7.68	17629	Bacopaside II

3.3 Detail Analysis of Markers

Peaks were found by the analysis of the standard bacosides A3 and II (figure 4), and preparative HPLC was used to identify the substances that each peak belonged to Bacopaside II was discovered to have a retention time of 20.622 seconds, whereas the typical bacoside A3 retention time was determined to be 9.560 seconds (Table 3). Naik et al. accessed the Bacopaside A content in various *B. monnieri* organs and accessions. [29] Using HPLC, Murthy et al. found twelve bacopa saponins in *B. monnieri* preparations. [30] The authors discovered material that was comparable to what we saw in our study. Based on the peaks found in the standard, various chemicals found in the leaf and stem extract were interpreted. Further research on the phytoconstituents of *B. monnieri* extract is required, particularly in light of the biological activity of bacoside A3 and bacopaside II that we have observed in our studies. Other valuable plants' saponin contents can also be standardised and estimated using this method. [31]

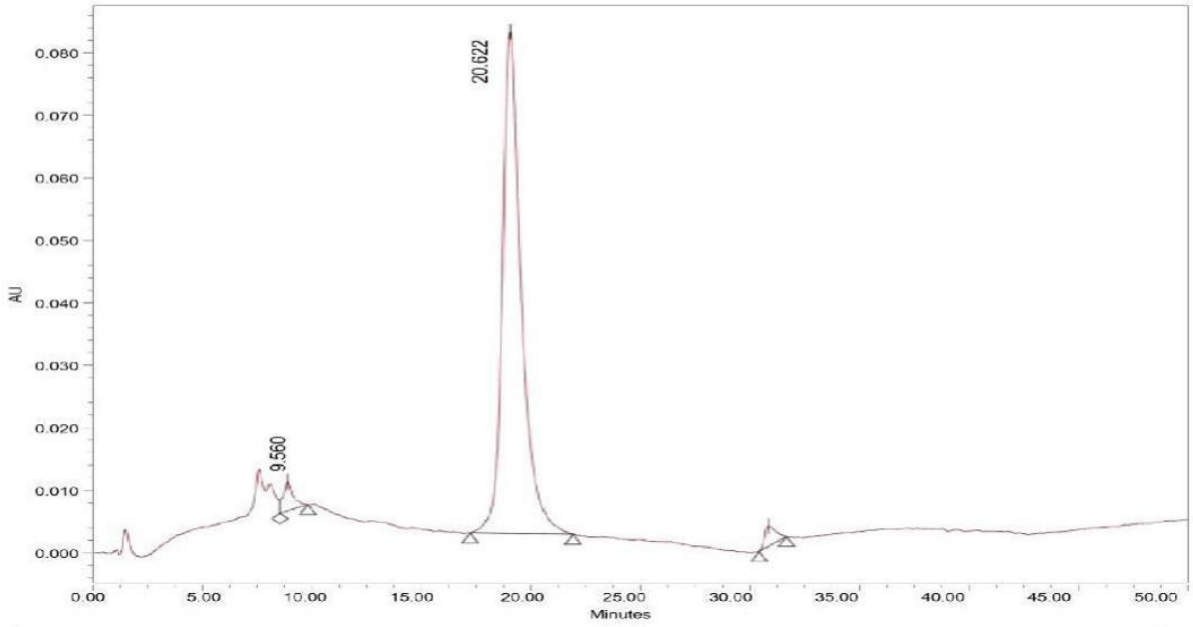

Fig. 4: HPLC chromatogram of Standards Bacoside A3 and Bacopaside II

Table 3: Analysis of 2 markers by HPLC fingerprinting

S. No.	Retention Time (Min)	Area	% Area	Height	Compounds
1	9.560	60792	2.99	4692	Bacoside A3
2	20.622	1929250	94.78	80300	Bacopaside II

4. CONCLUSION

This study looked at the saponins in *B. monnieri* extracts from various parts of the plant, such as leaves and stems. The phytoconstituents of *B. monnieri* can be qualitatively analyzed using the HPLC method described in this study. Most of these saponin glycosides are found in both parts; however, the majority are widely available in the leaf parts as the leaves of *B. monnieri* plants are mostly used as a potent medicinal booster for brain and nerve cells. After HPLC fingerprinting, saponins were found to be the most active components in *B. monnieri* extract. However, further research is needed to have a deeper understanding of these active components' therapeutic potential and the mechanisms through which they work.

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6. AUTHORS CONTRIBUTION STATEMENT

Chhaya Singh has written the original manuscript; Dr. Rishi Kumar Saxena has proofread the article; and Dr. Pawan Tripathi has guided the design of the protocol. Dr. Sonika Gupta critically reviewed the manuscript before approving it.

7. CONFLICT OF INTEREST

The authors declared no conflict of interest.

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