wjpmr, 2025, 11(2), 182-190

SJIF Impact Factor: 6.842

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

ELEMENTAL MAPPING OF *BACOPA MONNIERI* VIA SCANNING MICROSCOPY WITH CHARACTERIZATION OF ITS EXTRACT AND VALIDATION OF ITS ANTIOXIDATIVE POTENTIAL

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Article Received on 23/11/2024 **Article Revised on 13/12/2024** **Article Accepted on 02/01/2025**

ABSTRACT

The present study is focused on the elemental mapping and validation of extraction and characterization of *Bacopa monnieri* (Brahmi), the only used herb in Ayurveda*;* it shows the scavenging activities towards the free radicals, which are induced due to the exogenous chemical sources. We have mainly focused on the *Bacopa monnieri* plant herbal extraction of crude and characterization of its bioactive constituents like alkaloids, flavonoids, and bacosides. The BM herbal plant was collected from the botanical garden of Dr. Harisingh Gour Vishwavidyalaya Sagar and then identified by the Botany department via the herbarium file. Then, the dried plant was used for extraction by the Soxhlet method. The extract was dried in the oven, and the powder extract was used for the phytochemical screening and biological activity like Free radical scavenging activity or antioxidant activity, and H_2O_2 scavenging activity. We also perform SEM elemental mapping, FTIR, and NMR analysis. The results revealed that the extract possesses alkaloids, carbohydrates, terpenoids, Bacoside A, and Bacoside B as principal bioactive constituents. This study highlights the chemical constituents and biological properties of *Bacopa monnieri.* The study further validates its properties to promote it as a promising therapeutic effect.

KEYWORDS: *Bacopa monnieri*, Elemental mapping, SEM, Characterization, Herbal plant, Extract.

INTRODUCTION

Bacopa monnieri is a medicinal plant belonging to the Scrophulariaceae family. It has a history of use in traditional medicine to treat various neurological disorders and enhance memory and cognitive function. Neer Brahmi is the indigenous term for this therapeutic botanical species **(Lal & Baraik, 2019).** *Bacopa monnieri***,** a medicinal plant from the Scrophulariaceae family, is a perennial herbaceous plant with a creeping growth habit. It is commonly found in wetland areas and muddy shorelines, with its fleshy, dense, oblong leaves arranged in pairs on the stem. Brahmi is particularly fascinating in humid, boggy, and wet regions throughout India, and its presence in Nepal, Sri Lanka, China, Taiwan, Vietnam, and select southern states of the United States **(Promsuban et al., 2017)** Brahmi extract contains bacosides, which are triterpenoid saponins, explicitly A and B. Bacosides are a set of 12 analogues that have been identified. Alkaloids, saponins, and sterols are the chemical compounds accountable for Bacopa's pharmacological effects. The substance possesses various pharmacological properties, including antioxidant, antidepressant, endocrine stimulant, hepatoprotective, and anticancerous effects **(Kar, et al., 2009; Chourasiya & Mahobiya, 2023).** Despite all these facts, its elemental mapping has not been done so far. We have now carried over not only the elemental mapping of *B. monnieri,* but also validated its bioactive compounds and the antioxidant potential. Plant with the bioactive compound shown in **(Fig. 1).**

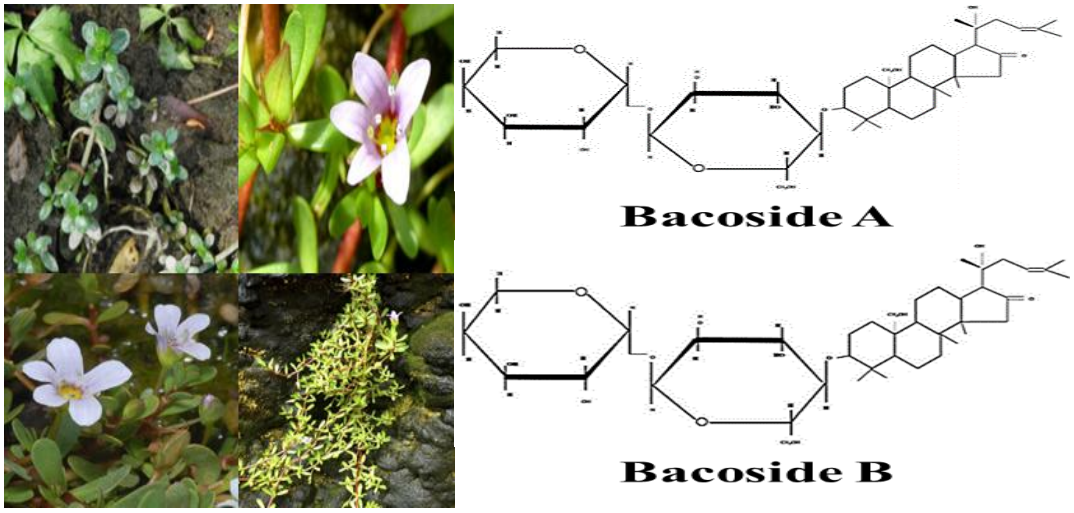

Fig. 1: Herbal plant *Bacopa monnieri* (Neer Brahmi) and Structure of Bioactive compound Bacoside A & Bacoside B.

MATERIAL METHOD

Chemicals

Brahmi Extract, Ethanol, H_2O_2, bismuth subnitrate, potassium iodide, Dragendorff's reagent, α-naphthol solution, Benedict's reagent, sulphuric acid, sodium hydroxide, copper sulphate, NaOH, ferric chloride solution, m-dinitrobenzene, chloroform, Fehling solution, DPPH, methanol, ascorbic acid, phosphate buffer, TBA, DMSO are the following chemical used in this study were procured from the CDH, Himedia.

Preparation of Plant Extract and identification

The plant, BM, was locally collected, and the specimen samples were deposited in the herbarium of the Department of Botany, Dr Harisingh Gour Vishwavidyalaya, Sagar, M.P., which received a herbarium No. Bot/H/05/13/548. Plant extracts were prepared using the methods previously described by others. For the extraction of *B. monnieri* leaf extract, the process of Shobana et al., 2012 was followed. Fresh plant was collected, shade-dried, and powdered. The leaf powder was extracted with 50% ethanol employing the Soxhlet extraction method, which was repeated twice. The resulting extracts were concentrated and evaporated to dryness in the oven. **(Shobana et al., 2012)**. The extraction method is listed below in **(Fig. 2).**

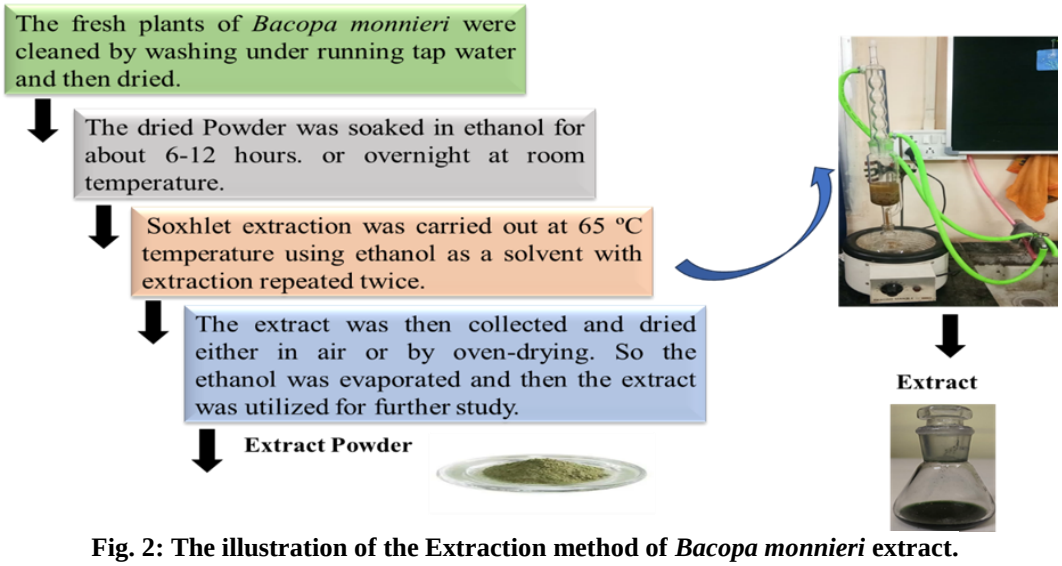

Fig. 2: The illustration of the Extraction method of *Bacopa monnieri* extract.

Characterizations of Prepared Plant Extract

Elemental Analysis of *Bacopa monnieri* Extract Via SEM

Electron microscopic images were used to examine morphological analysis. The scanning electron microscope (SEM) images of *Bacopa monnieri* extract show a clear and consistent pattern of elements clustering together uniformly. Dry powder is extracted and used for elemental mapping via SEM, which shows the presence of elements present in the extract **(Ahmed et al., 2023).**

Preliminary Analysis of Extract

Phytochemical analysis was conducted on the ethanolic

extract to identify the presence or absence of primary and secondary metabolites such as alkaloids, sterols, phenolics, flavonoids, glycosides, and terpenoids via qualitative methods.

Test for Alkaloids

Dragendorff's Test: The reagent was prepared by combining a solution of bismuth subnitrate (5 g) in distilled water (100 mL) with a solution of potassium iodide (60 g) in distilled water (20 mL). In order to determine the presence of alkaloids, a small amount of the sample solution (typically alkaloidal extracts) was added to a test tube. Next, a small amount of Dragendorff's reagent was added to the test tube. A solution containing alkaloids was added, resulting in the formation of an orange-red to brown precipitate **(Sen et al. 2013)**.

Test for Flavonoids

Alkaline Reagent Test: A 2 ml solution containing 2.0% NaOH was combined with an aqueous plant crude extract. This resulted in the formation of a concentrated yellow colour, which turned colourless upon adding 2 drops of diluted acid to the mixture. Flavonoids were detected in this result **(Gul et al., 2017).**

Test for Tannins

Ferric chloride test: A freshly prepared ferric chloride solution was added to the 1 mL extract. The formation of a dark blue or greenish-black colour indicates the presence of tannins **(Gul et al., 2017).**

Test for Glycosides

Raymond's test: Combine the drug with a small amount of 50% ethanol and a very small amount (0.1 ml) of a 1% solution of m-dinitrobenzene in ethanol. Add two or three drops of a 20% sodium hydroxide solution to the current solution. The presence of the active methylene group causes the emergence of violet colours **(Sen et al. 2013)**.

Test for Terpenoids

Salkowski test: The extract was tested with chloroform treatment, followed by the addition of an equal volume of concentrated sulphuric acid. The presence of terpenoidal components in the extract is indicated by the development of a bluish-red to cherry colour in the chloroform layer and a green fluorescence in the acid layer **(Sen et al., 2013)**.

Test for Sterols

Chloroform test: The 5 ml aqueous plant crude extract was combined with 2 ml of chloroform and concentrated H_2SO_4. The chloroform layer became red, indicating the presence of sterols **(Gul et al., 2017).**

Test for Phenolics

Folin-Ciocalteu Reagent test: The alcoholic or aqueous extract was combined with a small amount of Fehling solution. The presence of phenolic chemicals was indicated by the green or blue colour **(Sen et al. 2013)**.

Biological Antioxidant assays

The biological antioxidant assays, viz., DPPH free radical scavenging assay, H_2O_2 Scavenging assay, and Hydroxyl radical scavenging assays, were carried out.

DPPH Scavenging Activity

The DPPH radical scavenging activity is used to measure the ability of the prepared plant extract to remove free radicals, specifically ascorbic acid. The kit consists of a pre-prepared solution containing 1mg/ml of ascorbic acid dissolved in methanol, along with 0.1Mm concentration of DPPH, which was prepared in the dark. Test samples were acquired and mixed with methanol, with a maximum volume of 1 ml and 0.1–1 ml of ascorbic acid. For each sample, 1 ml of 0.1Mm methanolic DPPH was mixed with 1 ml of methanol in the blank. The reaction mixture was incubated at 37°C in the dark for a duration of thirty minutes. The absorbance at a wavelength of 517 nm was measured relative to the blank. The formula was utilized to determine the radical scavenging activity, which was expressed as the percentage of DPPH inhibition **(Sen et al., 2013).**

The calculation for DPPH assay

DPPH inhibition % = [(A control – A sample) / A control × 100] (Sen et al., 2013).

H_2O_2 Radical Scavenging Activity

The H_2O_2 scavenging activity assay is a widely used technique for assessing the capacity of a material to counteract or diminish hydrogen peroxide, which is a kind of reactive oxygen species. A sequence of dilutions was made using phosphate buffer and distilled water to get various concentrations of flavonoids and their complexes. The concentrations generally varied from 10 to 200 µg/ml. The hydrogen peroxide solution and sample solutions were combined in separate test tubes, with equal amounts in each tube. The quantities of hydrogen peroxide at the end are the same in every tube. A control without the sample was created by using the same amount of hydrogen peroxide and buffer. This served as a standard solution. The reaction mixtures were incubated at an optimal temperature, often at room temperature, for a predetermined time of around 10-30 minutes. Following the incubation period, the reaction was terminated by applying heat, as dictated by the test procedure. The residual hydrogen peroxide in each tube was quantified. The process can be accomplished by employing a spectrophotometer set to a wavelength of around 240 nm, at which hydrogen peroxide exhibits light absorption. The absorbance values for every sample and the blank control were recorded **(Sinha et al., 1972).** The hydrogen peroxide scavenging activity was quantified by calculating the percentage using the following **formula.**

% H_2O_2 Scavenging Activity = [(A blank – A sample) / A blank] × 100 (Sinha et al., 1972).

Where: The absorbance of the blank control, devoid of the sample, is called a blank.

The absorbance of the sample following the reaction is called a sample. A higher percentage of scavenging activity indicates a greater ability of the test sample to scavenge hydrogen peroxide.

Hydroxyl Radical Scavenging Activity Assay

The extractives' ability to scavenge hydroxyl radicals was assessed using the method developed by Halliwell et al. in 1989. The Fe3+-ascorbate-EDTA-H_2O_2 system (Fenton reaction) produced the hydroxyl radical. The assay relies on measuring the amount of the degradation product of 2-deoxy-D-ribose, which produces a pink chromogen when heated with TBA under acidic conditions. The assay was conducted following the method described by Rahman et al. in 2015. The spectrophotometer was used to measure the absorbance of the solution at a wavelength of 532 nm. The capacity to scavenge hydroxyl radicals was assessed by measuring the inhibition percentage of 2-deoxy-D-ribose oxidation (Halliwell et al., 1989; Rahman et al., 2015). The hydroxyl radical scavenging activity percentage was determined using the following formula.

% Hydroxyl Radical Scavenging activity = [A0−(A1−A2] × 100/A0] (Rahman et al., 2015).

Fourier Transformed Infrared Resonance (FTIR)

The Bruker FTIR was used to conduct the FTIR analysis. Apply one drop or a tiny (15–20 mg) extract powder with a micro spatula. Press the sample with two fingers and measure the IR spectra of the sample (500-3500cm^{-1}) (Jawarkar et al., 2019).

Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is employed to ascertain the phytoconstituents present in the crude plant extract that has been prepared. The 1H NMR spectroscopy of the crude extract of Neer Brahmi (*Bacopa monnieri*) was conducted using a 500MHz Variant FT-NMR Spectrometer at room temperature. The sample was prepared by dissolving 5 mg and 25 mg of Brahmi in 0.7 ml of DMSO for 1H NMR analysis. The samples were thoroughly mixed and subsequently filtered and de-gassed. The specimens were relocated to a 50 mm NMR tube, and the spectra were taken (Trivedi et al., 2017).

Statistical Analysis

The results of the experiment were presented as Mean ±SEM. A two-way ANOVA was used to analyze the data, followed by the Bonferroni post hoc test. Significance was considered at $p<0.05$ or less confidence level using Graph-Pad Prism version 9.0 software.

RESULTS

Elemental Analysis of *Bacopa monnieri* Extract Via SEM

The elemental analysis findings demonstrate the presence of different elements using different colors, and SEM results show the different colors used for the identification of different elements like carbon (C), oxygen (O), potassium (K), sodium (Na), magnesium (Mg), and calcium (Ca) through powder extract surface scanning. The scanning electron microscope (SEM) images of *Bacopa monnieri* extract showed a clear and consistent pattern of elements clustering together uniformly. The merged image was also taken to show the exact localization of the element after the extracted powder surface scanning via SEM. The elemental mapping is shown in **(Fig. 3.)** and **(Table 1.)** shows the element composition.

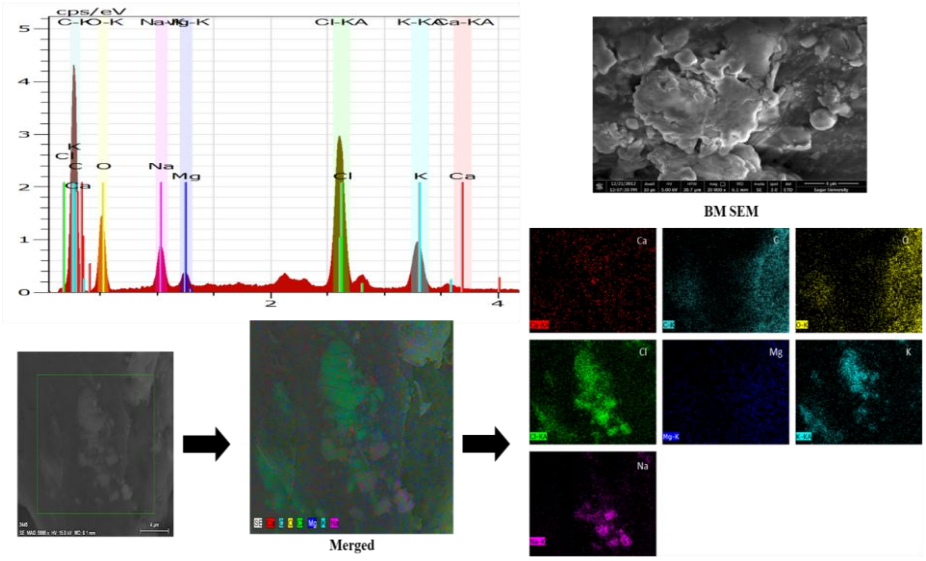

Fig. 3: Elemental mapping of *Bacopa monnieri* extract through SEM mapping in this graph red colour indicate the Ca (Calcium), light blue colour indicate the C (Carbon) yellow colour indicate the O (Oxygen), green colour indicate the Cl (Chlorine), Dark blue colour indicate the Mg (Magnesium), sea green colour indicate the K (Potassium), purple colour indicate the Na (Sodium) content respectively.

Table 1: This table shows the composition of elements present in *Bacopa monnieri*.

Element (color used)	[wt. %]	[at. %]
C light blue	33.89	62.63
O Yellow	17.22	23.92
Cl Green	10.92	6.85
K Sea green	4.87	2.77
Na Purple	3.07	2.97
Mg Dark Blue	0.74	0.68
Ca Red	0.16	0.29

Preliminary Phytochemical Analysis

The phytochemical screening of the ethanolic extract of *Bacopa monnieri* revealed the presence of alkaloids, flavonoids, phenols, tannins, sterols, terpenoids, and glycosides through various confirmatory tests. The findings of the phytochemical analysis are presented in **(Table 2.)** below.

Table 2: The preliminary Screening of all the phytochemicals contents present in the crude extract of *Bacopa monnieri.* (- Absent, +Present, ++ Moderately Present, +++ Strongly Present showed respectively).

S.No.	Group	Test	Presence
1.	Alkaloids	Dragendorff's test	+++
2.	Flavonoids	Alkaline reagent test	++
3.	Tannins	Ferric Chloride test	+
4.	Glycosides	Raymond's test	++
5.	Terpenoids	Salkowski test	+
6.	Sterol	Chloroform test	+++
7.	Phenol	Folin-Ciocalteu method	+++

Biological Antioxidant Assay

DPPH Scavenging Activity

The findings of the DPPH assay demonstrated a positive correlation between the concentration of the ethanolic extract of *Bacopa monnieri* and its ability to scavenge free radicals, indicating an increasing antioxidant scavenging activity. The radical scavenging activity at a lower dose of 20 µg/ml is approximately 25%, and it increases to 85% at a dose of 100 µg/ml, compared to the radical scavenging activity of the bioactive compound ascorbic acid **(Fig. 4).**

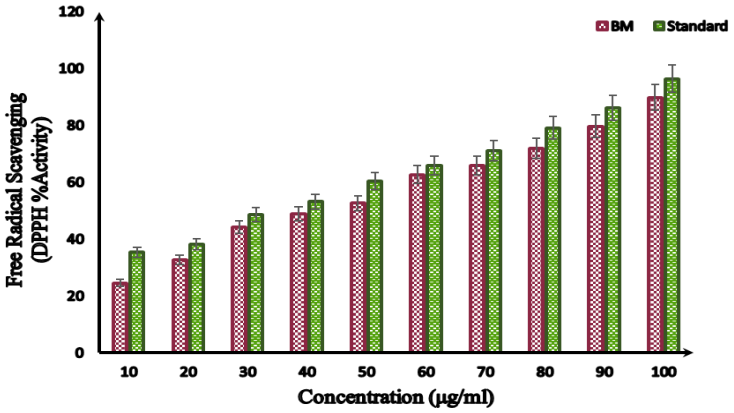

Fig. 4: The percentage of free radical scavenging activity (%RSA) of a preprepared ethanolic extract of *Bacopa monnieri* (Neer Brahmi) was determined using the DPPH assay.

H_2O_2 Radical Scavenging Activity

The results of the H_2O_2 assay revealed a positive correlation between the concentration of the ethanolic extract of *Bacopa monnieri* and its capacity to scavenge hydrogen peroxide radicals, indicating an enhanced H_2O_2 scavenging activity. The H_2O_2 radical scavenging activity at a lower dosage of 200 µg/ml is approximately 25%, increasing to 85% at a dosage of 1000 µg/ml, in comparison to the H_2O_2 radical scavenging activity of the standard ascorbic acid **(Fig. 5).**

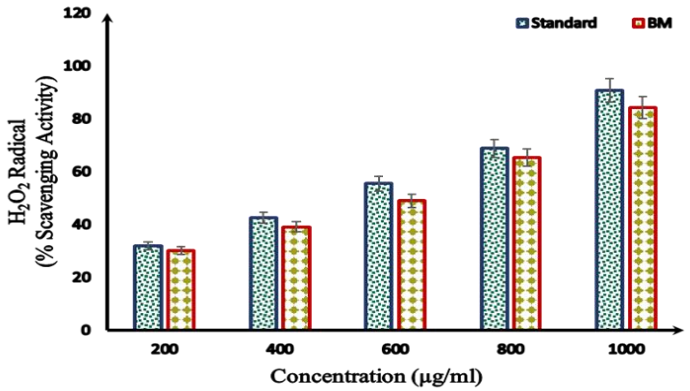

Fig. 5: The percentage of H_2O_2 radical scavenging activity (%SA) of a preprepared ethanolic extract of *Bacopa monnieri* (Neer Brahmi) was determined using the H_2O_2 radical scavenging assay.

Hydroxyl Radical Scavenging Activity

The results of the hydroxyl assay revealed a positive correlation between the concentration of the ethanolic extract of *Bacopa monnieri* and its capacity to scavenge OH radicals, indicating an enhanced hydroxyl radical scavenging activity. The OH radical scavenging activity at a lower dosage of 200 µg/ml is approximately 25%, increasing to 85% at a dosage of 1000 µg/ml, in comparison to the OH radical scavenging activity of the standard ascorbic acid **(Fig. 6).**

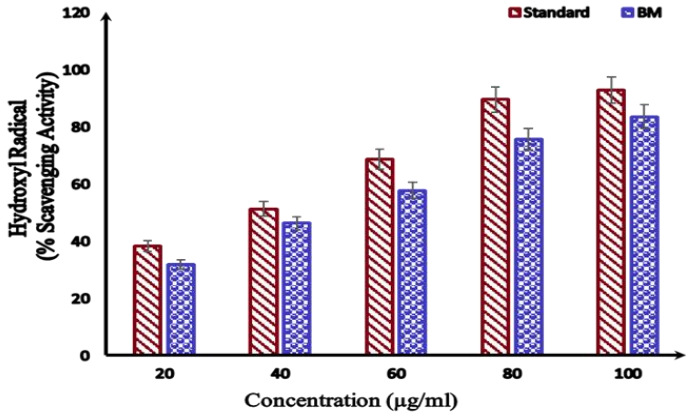

Fig. 6: The percentage of Hydroxyl (OH) radical scavenging activity (%SA) of a preprepared ethanolic extract of *Bacopa monnieri* (Neer Brahmi) was determined using the Hydroxyl radical scavenging assay.

Fourier Transformed Infrared Resonance (FTIR) Analysis

The outcomes of FTIR analysis of a *Bacopa monnieri* extract. The FTIR spectrum displays significant bands at 3830.67 cm-1, 2915.84 cm-1, 2847.91 cm-1, 1564.60 cm-1, 1363.46 cm-1, 1237.90 cm-1, 1025.66 cm-1, 633.30 cm-1, and 590 cm-1.93 cm^{-1}, 535.71 cm^{-1} shown in **(Fig. 7)**. The various functional groups and their spectra are presented in the table below **(Table 3).**

Table 3: This demonstrated that the crude extract of *Bacopa monnieri* included various functional groups and their spectra.

S.NO.	Spectrum	Functional Group (Compounds)
1.	3830.67 (H-bonded)	O-H (Alcohol)
2.	2912.88 – 2847.91 cm-1	O-H (Carboxylic Acid)
3.	1564.60 cm-1	C-O Stretching (aromatic compound)
4.	1363.46 cm-1	S-O Stretching (sulfonate)
5.	1237.90 cm-1	C-O Stretching (alkyl aryl ether)
6.	1025.66 cm-1	S-O Stretching (Sulfoxide)
7.	633.30 cm-1	C-C bending
8.	590.93- 535.71	C-H bending

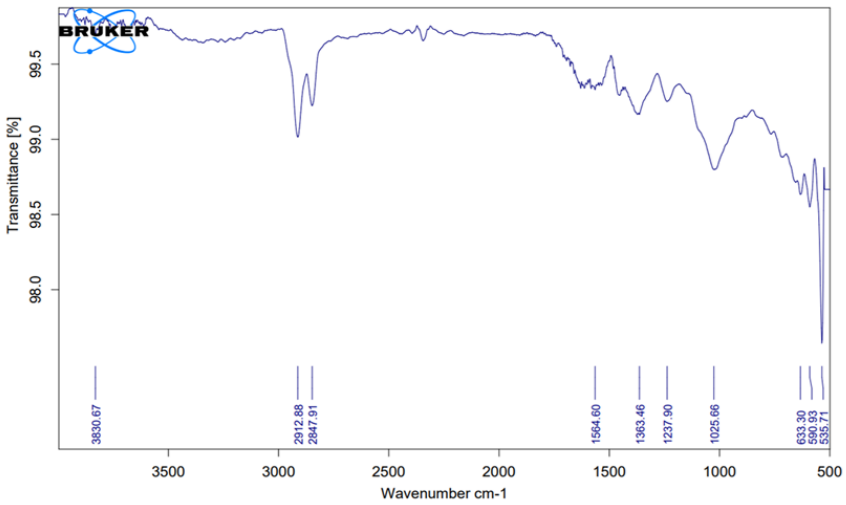

Fig. 7: FTIR graph shows the different Spectra present in the *Bacopa monnieri* Extract.

Nuclear Magnetic Resonance (NMR)

The NMR spectroscopy results revealed the phytochemical constituents found in the ethanolic extract of *Bacopa monnieri* **(Fig. 8).**

The ^{1}H NMR spectra of the prepared extract displayed two prominent peaks, corresponding to Bacoside A and B, as indicated by their chemical shift (δ) values. **Jwarkar et al. (2019)** determined that our Extract contains Bacosides A and B, which are responsible for its antioxidant activity.

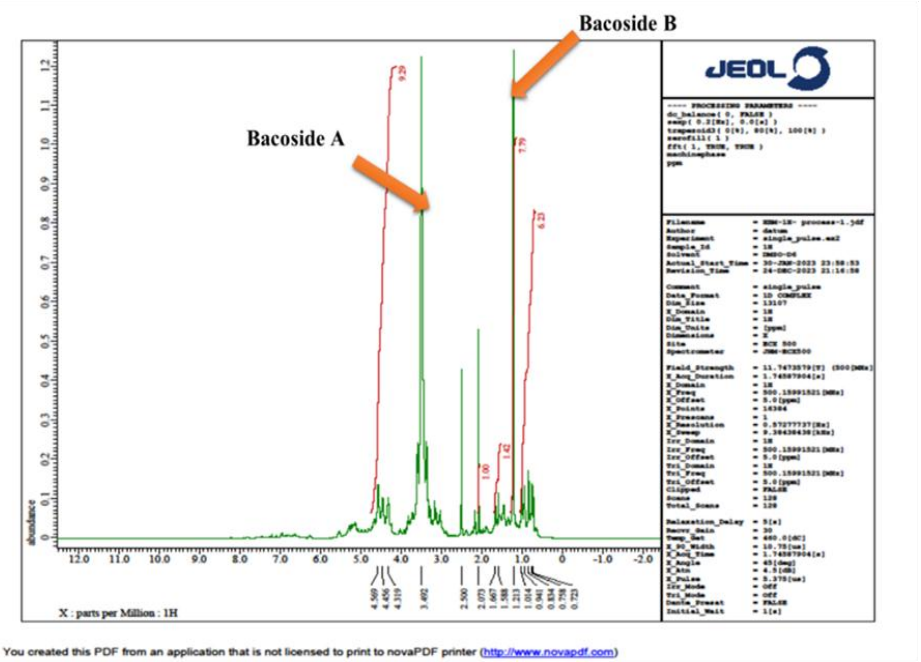

Fig. 8: ^{1}H NMR graph of ethanolic extract of *Bacopa monnieri.* The illustrated two peaks shows the presence of bacoside A and Bacoside B.

DISCUSSION

The results of phytochemical screening of the ethanolic extract of the whole *B. monnieri* plant used in this study **(Table 1)** showed that while steroids were absent, phenolic compounds (tannins and flavonoids) and non-phenolic compounds (alkaloids, sterol, terpenoids, and glycosides) were present among the phytocompounds examined. The phytochemical components of *B. monnieri* may be in charge of its therapeutic role and other pharmacological characteristics; the plant components described here and found during the experiments inspire us to explore its potential therapeutic applications **(Praveen et al., 2016).**

The results of the elemental composition of the extract revealed carbon (C), oxygen (O), chlorine (Cl), potassium (K), magnesium (Mg), sodium (Na), and calcium (Ca) were determined by elemental mapping of

the *bacopa monnieri* extract. Consistent with previous research, the extract has similar components found in the SEM Edx in ethanolic extract **(Dashteh et al., 2020).** Similarly, in line with our results, Ansari Iqubal et al. also studied SEM-Edx elemental analysis of many plants **(Ansari et al., 2020).**

A substance can only be classified as an antioxidant if it can neutralize and combat free radicals. As concentration is raised, the prepared BM extract has the ability to scavenge radicals rises. The current study demonstrated the bioactive compound's ability to scavenge radicals in this crude ethanolic *Bacopa monnieri extract*. With the increasing concentration, the bioactive components of crude extracts showed increasing antioxidant activity **(Fig. 4),** hydroxyl radical scavenging activity **(Fig. 6)**, and H_2O_2 scavenging activity **(Fig. 5)** compared with the standards. In line with our research, *Bacopa monnieri's* root and leaf extract have radical-scavenging properties that shield against oxidative damage and stop apoptosis **(Pal et al., 2015).** In line with our results, kar et al. show the Antioxidant and thyroid-stimulant properties of *Bacopa monnieri* with a dose of 200mg/kg BW in mice **(Kar et al., 2002).**

The active phytoconstituents are characterized using a comprehensive range of methods, ensuring the robustness and reliability of our findings. The elements of the crude extract used in the current study, as determined by FTIR **(Fig. 7)** and NMR **(Fig. 8),** matched the reported FTIR and NMR data. This alignment of our analysis with reported data provides strong evidence for the presence of Bacoside A and Bacoside B as active ingredients in the extract **(Jwarkar et al., 2019)**. The present study's findings indicate that *Bacopa monnieri* possesses active ingredients called Bacoside A and B, which are accountable for the plant's anti-inflammatory, hepatoprotective, thyroid stimulant, neuroprotective, and antioxidant properties **(Jeyasri et al., 2020).** One of our in-vitro studies also revealed that bacopa monnieri extract mitigates cypermethrin-induced hypothyroidism and oxidative stress in female mice **(Chourasiya et al., 2024).**

CONCLUSION

The study concludes that we are the first one to demonstrate the results of elemental mapping of *Bacopa monnieri* extract via Scanning microscopy and shows the presence of the following elements: carbon (C), oxygen (O), chlorine (Cl), potassium (K), magnesium (Mg), sodium (Na), and calcium (Ca). Our extract shows the revalidation of the presence of preliminary phytochemicals like flavonoids, tannins, alkaloids, proteins, alkaloids, sterols, terpenoids, and glycosides. The FTIR and NMR results show the presence of bacoside A and bacoside B. The biological activity like DPPH activity, H_2O_2 radical scavenging activity, and hydroxyl radical scavenging activity also revalidate the strong antioxidative properties, suggesting its use as a therapeutic agent in many diseases.

Conflict Interest

The authors assert that there are no conflicts of interest in this article.

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ACKNOWLEDGEMENTS

Ms. Surbhi Chourasiya acknowledges the fellowship received from the Indian Council of Medical Research (ICMR) for this study. A project from the ICMR in New Delhi, India (3/1/3(12)/Endo-fellowship/22-NCD-III) provided funding for this study. Additionally, the authors express deep gratitude to DST-FIST Central instrumental facility, Department of Zoology, Dr. H.S Gour (Central) University, Sagar, M.P., for providing the necessary instruments and infrastructure required for this study.

REFERENCES

1. Ahmed RR, Waqar A, Maria A, Zahid AC, Ahmed F, and Parmar V. The role of green innovation on environmental and organizational performance: Moderation of human resource practices and management commitment. Heliyon, 2023; 9: 1.
2. Ansari, I, Sundararajan M, Kumar R, Sharma S, & Arora C. SEM-EDX: a potential tool for studies medicinal plants. Essential Techniques for Medical and Life Scientists, 2020; 124-41.
3. Chourasiya, S, Kar A, & Sharma V. Bacopa monnieri extract mitigates Cypermethrin-induced hypothyroidism and oxidative stress in female mice, 2024. https://doi.org/10.21203/rs.3.rs-4216353/v1
4. Chourasiya S and Mahobiya P. A systematic review on the scavenging and elucidating efficacy of Bacopa monnieri as a thyroid stimulant. int j pharm sci & res, 2023; 14(6): 2645-50. doi: 10.13040/IJPSR.0975-8232.14(6).2645-50.
5. Dashteh M, Mohammad AZ, Khazaei A, Baghery S, Yarie M, Makhdoomi S, and Safaiee M. Synthesis of cobalt tetra-2, 3-pyridiniumporphyrazinato with sulfonic acid tags as an efficient catalyst and its application for the synthesis of bicyclic ortho-aminocarbonitriles, cyclohexa-1, 3-dienamines and 2-amino-3-cyanopyridines. RSC advances, 2020; 10(46): 27824-27834.
6. Gul R, Jan SU, Faridullah S, Samiullah S, and Jahan N. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan. The Scientific World Journal, 2017; 1,3: 587-648.
7. Halliwell B. Oxidants and the central nervous system: some fundamental questions. Is oxidant damage relevant to Parkinson's disease, Alzheimer's disease, traumatic injury or stroke? Acta Neurologica Scandinavica, 1989; 80: 23-33.

8. Jawarkar SV, Shete SD, and Kane SR. Green synthesis of silver nanoparticles using bacopa monnieri leaf extract and assessment of their dpph activity. Pharmaceutical Research, 2019; 9(02).
9. Kar A., Panda S., & Bharti S. Relative efficacy of three medicinal plant extracts in the alteration of thyroid hormone concentrations in male mice. Journal of ethnopharmacology, 2002; 81(2): 281-285.
10. Pal A, Kumar M, Saharan V, Bhushan B, & Hau H. Anti-oxidant and free radical scavenging activity of Bacopa monnieri leaves. Journal of Global Bioscience, 2015; 4(1): 1127-1137.
11. Parveen R, Shamsi T.N, Kumar H, & Fatima S. Phytochemical analysis and in vitro biological characterization of aqueous and methanolic extract of Bacopa monnieri. int j pharm sci & res, 2016; 8(12): 90-6.
12. Promsuban C, Limsuvan S, Akarasereenont P, Tilokskulchai K, Tapechum S, & Pakaprot N. Bacopa monnieri extract enhances learning-dependent hippocampal long-term synaptic potentiation. Neuroreport, 2017; 28(16): 1031-1035.
13. Rahman MM, Das R, Hoque MM, & Zaman W. Effect of freeze drying on antioxidant activity and phenolic contents of Mango (Mangifera indica). International Food Research Journal, 2015; 22(2): 613.
14. Sen S, De B, Devanna N, & Chakraborty R. Total phenolic, total flavonoid content, and antioxidant capacity of the leaves of Meyna spinosa Roxb., an Indian medicinal plant. Chinese journal of natural medicines, 2013; 11(2): 149-157.
15. Shobana C, Kumar R, & Sumathi T. Alcoholic extract of Bacopa monniera Linn. protects against 6-hydroxydopamine-induced changes in behavioral and biochemical aspects: a pilot study. Cellular and molecular neurobiology, 2012; 32: 1099-1112.
16. Sinha, AK. Colorimetric assay of catalase. Analytical biochemistry, 1972; 47(2): 389-394. https://doi.org/10.1016/0003-2697(72)90132-7
17. Lal S, & Baraik B. Phytochemical and pharmacological profile of Bacopa monnieri-an ethnomedicinal plant. int j pharm sci & res, 2019; 10(3): 1001-13.
18. Trivedi MK, Panda P, Sethi KK, & Jana S. Metabolite profiling in Withania somnifera roots hydroalcoholic extract using LC/MS, GC/MS, and NMR spectroscopy. Chemistry & Biodiversity, 14(3): e1600280.