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Indonesian Journal of Pharmaceutical and Clinical Research
Journal homepage: https://idjpcr.usu.ac.id

Comparative Evaluation of Phenolics, Flavonoids, Antioxidant and Antibacterial Activities of *Bacopa monnieri* (L.) Pennell Leaves from Different Geographical Regions of India

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ARTICLE INFO

Article history:
Received 05 September 2025
Revised 28 November 2025
Accepted 25 December 2025
Available online 31 December 2025

E-ISSN: 2620-3731
P-ISSN: 2615-6199

How to cite:
Ahmed, A., Dhir, N., Singh, D., Sharma, A., Parmar, B. S., & Kusheshtra, R. (2025). Comparative evaluation of phenolics, flavonoids, antioxidant and antibacterial activities of *Bacopa monnieri* (L.) Pennell leaves from different geographical regions of India. Indonesian Journal of Pharmaceutical and Clinical Research, 8(2), 48–58.

ABSTRACT

Bacopa monnieri (L.) Pennell is a medicinal herb widely used in traditional medicine for its antioxidant and antimicrobial properties. Variations in phytochemical composition and bioactivity due to geographical distribution may influence its therapeutic potential. This study aimed to comparatively evaluate the phenolic and flavonoid contents, in vitro antioxidant activity, and antibacterial potential of *B. monnieri* leaf extracts collected from different geographical regions of India. Leaf samples were collected from Delhi, Kerala, Bangalore, and Jammu. Qualitative phytochemical screening was performed, followed by quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC). Antioxidant activity was assessed using DPPH radical scavenging, hydrogen peroxide scavenging, and ferric reducing power assays. Antibacterial activity was evaluated by agar well diffusion and minimum inhibitory concentration (MIC) methods against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa*. Data were analyzed using one-way ANOVA followed by post-hoc comparison at $p < 0.05$. Leaf samples from Jammu showed the highest TPC (206.0 ± 1.01 µg/mL) and TFC (119.4 ± 0.81 µg/mL). The same samples exhibited superior antioxidant activity with DPPH radical scavenging (40.63 ± 0.35%) and the lowest IC_{50} value (313 µg/mL). The highest zones of inhibition were recorded for *Staphylococcus aureus* with ethanolic extracts from Jammu and Kerala (18.0 ± 0.34 and 18.0 ± 0.42 mm, respectively). Against *Pseudomonas aeruginosa*, ethanolic extracts from Jammu showed a maximum inhibition zone of 15.0 ± 0.50 mm. Kerala ethanolic extracts exhibited notable activity against *Escherichia coli* (11.0 ± 0.43 mm). Chloroform extracts showed moderate antibacterial activity, while aqueous extracts were inactive. The findings demonstrate that geographical origin significantly influences the phenolic and flavonoid content as well as antioxidant and antibacterial activities of *Bacopa monnieri* leaves. Samples from the Jammu region exhibited superior free radical scavenging potential, while Jammu and Kerala samples showed enhanced antibacterial activity, highlighting the importance of geographical selection for optimizing the therapeutic potential of this medicinal plant.

Keywords: *Bacopa monnieri*; Phenolic content; Flavonoids; Antioxidant activity; Antibacterial activity; Geographical variation

ABSTRAK

Karagenan merupakan polisakarida tersulfatasi yang diekstrak dari rumput laut merah (*Kappaphycus alvarezii*) dan dikenal memiliki kapasitas menahan air yang tinggi, sehingga berpotensi digunakan sebagai agen pelembap kulit. Penelitian ini bertujuan untuk merumuskan karagenan hasil isolasi ke dalam sediaan krim serta mengevaluasi karakteristik

http://doi.org/10.32734/idjpcr.v8i02.22695

fisikokimia dan efektivitas pelembapannya. Proses isolasi dilakukan melalui tahapan perendaman, dekantasi, ekstraksi, isolasi, pengeringan, dan penggilingan. Karagenan yang diperoleh dikarakterisasi berdasarkan kelarutan, viskositas, kehilangan pengeringan, dan analisis Fourier transform infrared (FTIR). Formulasi krim dibuat dengan variasi konsentrasi karagenan sebesar 2,5%, 5%, 7,5%, dan 10% (b/b), kemudian dibandingkan dengan krim gliserin 2% dan kontrol kosong. Evaluasi mutu fisik meliputi uji homogenitas, pH, jenis emulsi, stabilitas selama 12 minggu, uji iritasi kulit, serta pengujian kemampuan melembapkan secara *in vivo* pada 18 relawan manusia. Karagenan hasil isolasi memenuhi persyaratan USP XXX. Seluruh sediaan krim menunjukkan homogenitas yang baik, pH yang sesuai dengan kulit, serta stabilitas fisik yang memadai selama penyimpanan. Tidak ditemukan indikasi iritasi kulit. Efek pelembapan meningkat seiring dengan kenaikan konsentrasi karagenan, di mana krim karagenan 2,5% menunjukkan efektivitas yang hampir setara dengan krim gliserin 2%. Hasil penelitian ini mendukung potensi karagenan dari *Kappaphycus alvarezii* sebagai bahan pelembap alami dalam formulasi krim topikal.
Kata kunci: *Kappaphycus alvarezii*; karagenan; isolasi; sediaan krim; pelembap

1. Introduction

Bacopa monnieri L., habitually recognized as Brahmi, is a perennial aquatic herb including 146 species worldwide that root at nodes and have branching stems, leaves, and flowers. It has a bitter-sweet taste and is famous to contain cooling features. The Central Drug Research Institute, Lucknow, of the Indian government prepared a unique extract of *Bacopa monnieri* in 1996 under the name CDRI08 [1]. Brahmi acts as a free radical scavenging agent. It is utilized to cure back pain, mental disorders, convulsions, irritation in the bowel and joint pain [2]. It has been used for more than 3,000 years in the Ayurvedic medical system to treat various mental diseases like anxiety, etc. Due to over-exploitation, this plant, which is only used for medicine, is considered to be the most endangered [3]. The price and side effects of herbal plant items are lower than those of manufactured medications.

Secondary metabolites derived from many plant species are important for the plant's defence mechanism as well as being very beneficial to us in the treatment of various diseases. They have been found to have a range of pharmacological properties that can be accounted for by their chemical makeup and functional groups. Important secondary metabolites include glycosides, alkaloids, phenolics, terpenoids, and flavonoids. Active plant metabolites frequently have potent antioxidant activities that can be used as a source of natural antioxidants in nutraceuticals. Many secondary metabolites have a variety of therapeutic effects because they work specifically with the signalling receptors, nucleic acids, and cell membranes that are already present in the body, several secondary metabolites contain a large variety of therapeutic actions [4].

Since the dawn of time, humans have created medications to treat illnesses. They used organic materials like plants, fungus, animals, microbes, and sea species to make these remedies [5], [6]. Plants frequently produce non-nutritious substances known as active constituents when they are under stress, but these constituents are very important for medical purposes. It was found that these plant chemicals had a variety of biological effects, including those of antioxidants and antimicrobials [7], [8]. Antioxidants play a critical function in the defence against oxidative stress and free radical damage brought on by conditions including diabetes, heart disease, and cancer [9], [10]. Thus, the development of new natural antioxidant sources is crucial for the treatment of many diseases.

Even with the recent advances in technology, it was still impossible to control the spread of contagious diseases, which was vital. The issue of bacterial resistance to synthetic antibiotics spread to other countries. Thus, researchers assessed how much natural resources, including medicinal plants, were used in the production of new antibiotic medications [11]. Finding novel antimicrobial sources is crucial in the battle against harmful bacterial strains.

Geographical distribution has a significant impact on the quantity and quality of secondary metabolites produced by medicinal plants. Due to the presence of iridoid glycosides, *Picrorhiza kurroa* is typically found in the Himalayan region (3000–5000 m). It has great therapeutic properties [12]. Hyperforin, hypericin, and pseudohypericin are only a few of the secondary metabolites that are associated with the plant *Hypericum perforatum*, which may be found in the temperate western Himalayas (2000–3000 m). It also functions as an antioxidant, anti-bacterial, and neuroprotective agent in addition to being used to treat cancer, depression, and anxiety [13]. This study compared the total phenolic content, flavonoid content, antioxidant activity, and antibacterial activity of leaves from *Bacopa monnieri* (L.) Pennell collected from several Indian locations.

2. Materials and Methods

2.1 Plant material collection and authentication

Fresh leaves of *Bacopa monnieri* (L.) Pennell were collected in January 2019 from four geographically distinct regions of India: New Delhi, Kerala, Bangalore, and Jammu (Table 1). Plant specimens were

authenticated by Dr. Rizwan Ahmad by comparison with authenticated herbarium specimens deposited in the Department of Botany, Mewar University, Rajasthan, India. Voucher specimens were deposited under accession numbers BM-21 (Delhi), BM-22 (Kerala), BM-23 (Bangalore), and BM-24 (Jammu). Collected leaves were washed thoroughly with distilled water, shade-dried at room temperature (25 ± 2 °C) for 10–12 days, and pulverized using a mechanical grinder. The powdered samples were stored in airtight containers until further analysis.

Table 1: Samples of *Bacopa monnieri* (L.) Pennell leaves from various geographical locations.

Botanical name	Plant family	Time of collection	Location of collected samples	Geographic coordinates
Bacopa monnieri (L.) Pennell	Schropulariaceae	January 2019	Herbal garden of Jamia Hamdard, New Delhi, India	28.7041° N,77.1025°E
Bacopa monnieri (L.) Pennell	Schropulariaceae	January 2019	Kerala University,Kerala, Kerala	10.8505°N,76.2711E
Bacopa monnieri (L.) Pennell	Schropulariaceae	January 2019	University of Agriculture Science, Bangalore,India	12.9716°N, 77.5946°E
Bacopa monnieri (L.) Pennell	Schropulariaceae	January 2019	Regional Research Laboratory, Jammu,India	32.7329° N,74.4642°E

2.2 Chemicals and reagents

Gallic acid and rutin reference standards were procured from Normal Cures Pvt. Ltd., Bangalore, India. Folin–Ciocalteu reagent, sodium carbonate, aluminium chloride, sodium acetate, DPPH, potassium ferricyanide, ferric chloride, trichloroacetic acid, methanol, ethanol, acetone, chloroform, and DMSO were purchased from Merck India Ltd. All chemicals were of analytical grade.

2.3 Preparation of methanolic extracts for phenolic and flavonoid assays

One gram of dried leaf powder was refluxed with 50 mL methanol for 2 h. The extract was filtered through Whatman No.1 filter paper and concentrated under reduced pressure using a rotary evaporator (Buchi R-210, Switzerland). The dried extract was reconstituted in methanol to obtain a stock solution of 10 mg/mL.

2.4 Determination of total phenolic content (TPC) [14]

2.4.1 Assay procedure

To 0.5 mL of extract (10 mg/mL), 5 mL of 10% Folin–Ciocalteu reagent and 4 mL of 1 M sodium carbonate were added. The mixture was incubated for 15 min at room temperature, and absorbance was measured at 765 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan).

2.4.2 Calibration curve

Gallic acid solutions (50–150 µg/mL) were used to generate a standard curve. Results were expressed as µg gallic acid equivalents (GAE)/mL extract.

2.5 Determination of total flavonoid content (TFC) [14]

2.5.1 Assay procedure

To 0.5 mL of extract (10 mg/mL), 1.5 mL methanol, 0.1 mL of 0.1 M aluminium chloride, 0.1 mL of 1 M sodium acetate, and 2.8 mL distilled water were added. After incubation for 30 min at room temperature, absorbance was measured at 415 nm.

2.5.2 Calibration curve

Rutin (10–100 µg/mL) was used as standard, and results were expressed as µg rutin equivalents (RE)/mL extract.

2.6 Antioxidant activity assays

2.6.1 DPPH radical scavenging assay

A reaction mixture containing 0.5 mL extract, 3 mL methanol, and 0.3 mL of 0.5 mM DPPH solution was incubated in the dark for 45 min. Absorbance was recorded at 517 nm. Radical scavenging activity was calculated as:

% inhibition = [(Acontrol − Asample)/Acontrol] × 100

Acontrol = Absorbance of negative control at the moment of solution preparation

Asample = Absorbance of sample after 45 min

IC_{50} values were calculated using linear regression analysis.

2.6.2 Ferric reducing antioxidant power (FRAP)

Extracts (500 µg/mL) were mixed with phosphate buffer (200 mM, pH 6.6) and 1% potassium ferricyanide, incubated at 50 °C for 20 min, followed by addition of trichloroacetic acid. After centrifugation, ferric chloride (0.1%) was added, and absorbance was measured at 700 nm. Results were expressed as µg ascorbic acid equivalents/g extract [15].

2.6.3 Hydrogen peroxide scavenging assay

Hydrogen peroxide solution (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). Extracts (50 µg/mL) were added, and absorbance was measured at 230 nm. IC_{50} values were expressed in µg/mL [16].

2.7 Antibacterial activity techniques

2.7.1 Microorganisms

Reference bacterial strains Bacillus subtilis (MTCC 441), Staphylococcus aureus (MTCC 96), Escherichia coli (MTCC 443), and Pseudomonas aeruginosa (MTCC 424) were obtained from the Department of Biotechnology, Mewar University, India.The reference strains of bacteria should be prepared in supplement broth (Hi-media) at 37°C, subcultured regularly (every 30 days), and stored in supplement agar incline plates at 4°C and at -20°C by preparing suspensions in 20% glycerol.

2.7.2 Stock culture preparation and maintenance

The bacterial strains were cultivated on nutrient agar plates and incubated at 37 °C for 24 h. Actively growing cultures were stored at 4 °C on nutrient agar slants. Fresh subcultures were prepared at intervals of three to four weeks to maintain viability and purity of the strains.

2.8 Preparation of aqueous extract for antimicrobial activity

Twenty-five grams of dried leaf powder were suspended in hot distilled water and boiled for 30 min, followed by maceration at room temperature for 24 h. The extract was filtered using sterile Whatman No. 1 filter paper and collected in a clean conical flask. The filtrate was concentrated under reduced pressure using a rotary vacuum evaporator at 60 °C. The dried crude extract was reconstituted in 20% (v/v) DMSO and stored at 4 °C in amber-colored bottles until further use.

2.9 Preparation of extract solution for antibacterial activity

Dried and powdered leaf material (25 g) was extracted separately with chloroform, acetone, and 95% (v/v) ethanol using a Soxhlet apparatus. Each extraction was carried out with 250 mL of solvent at 40 °C until the siphon tube showed colorless solvent, indicating complete extraction. The extracts were concentrated under reduced pressure and stored at 4 °C for antibacterial assays.

2.10 Preparation of bacterial inoculum

Bacterial inocula were prepared by transferring well-isolated colonies from 24-hour-old cultures into 5 mL of sterile nutrient broth. The broth cultures were incubated at 37 °C for 4 h to obtain actively growing bacterial suspensions, which were used for antibacterial assays.

2.11 Agar well-diffusion assay [17]

Antibacterial activity was evaluated using the agar well-diffusion method. Individual bacterial strains were grown in nutrient broth for 8 h and uniformly spread on nutrient agar (NA) plates using a sterile cotton swab. Wells of 6 mm diameter were aseptically punched into the agar using a sterile cork borer. Plant extracts prepared in ethanol, chloroform, acetone, and distilled water were dissolved to obtain a stock concentration of 1 mg/mL. A measured volume of each extract was carefully introduced into the wells using a sterile syringe. The plates were allowed to stand at room temperature for 2 h to facilitate diffusion of the extracts into the agar medium.

Plates were incubated at 37 °C for 18–24 h for bacterial strains. Gentamycin served as the positive control, while the respective extraction solvent served as the negative control. After incubation, antibacterial activity was assessed by measuring the diameter of the zone of inhibition (mm) around each well. All experiments were performed in triplicate (n = 3), and results were expressed as mean ± standard deviation (SD).

2.12 Determination of Minimum Inhibitory Concentration (MIC) [18]

The minimum inhibitory concentration (MIC) of the plant extracts was determined using the broth tube dilution method. Two-fold serial dilutions of each extract were prepared aseptically in nutrient broth to

obtain concentrations ranging from 30 mg/mL to 0.23 mg/mL. Each tube was inoculated with 0.1 mL of standardized bacterial suspension (approximately 10^8 CFU/mL).

The inoculated tubes were incubated at 37 °C for 24 h, and bacterial growth was assessed by visual inspection for turbidity. The MIC was defined as the lowest concentration of the extract that showed no visible bacterial growth. Standard antibiotics Ampiclox and Zosyn were used as positive controls at concentrations ranging from 0.5 mg/mL to 0.0004 mg/mL. A positive growth control containing inoculated broth without plant extract and a negative control containing extract without bacterial inoculum were included in each experiment.

2.13 Statistical analysis

All experiments were conducted using a completely randomized design (CRD). Each assay was performed in triplicate (n = 3), and results are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using COSTAT software (Version 6.4; Cohort Software, USA). Differences among means were evaluated using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) post-hoc test. A probability level of $p < 0.05$ was considered statistically significant. In addition, regression analysis was performed to determine correlation coefficients (R^2) between total phenolic content, total flavonoid content, and antioxidant activity.

3. Results

3.1 Screening for phytochemicals

Table 2. Phytochemical screening of methanol extracts of four leaf samples from *Bacopa monnieri* in various geographical areas.

S.No.	Constituents	Delhi	Bangalore	Kerala	Jammu
1	Alkaloids	+	+	+	+
2	Carbohydrates	+	+	+	+
3	Glycosides (anth)	_	_	_	_
4	Phenolic compounds and tannins	+	+	+	+
5	Flavonoids	+	+	+	+
6	Proteins and free amino acids	+	+	+	+
7	Saponins	+	+	+	+
8	Mucilage	_	_	_	_
9	Steroids	+	+	+	+
10	Terpenoids	+	+	+	+

According to Table 2, all of the samples' leaf extracts in methanol showed the presence of alkaloids, carbohydrates, phenolics and tannins, flavonoids, proteins and amino acids, saponins, steroids, and terpenoids, but none of the samples contained mucilage or anthraquinone glycosides.

3.2 Determination of extractive value of various solvent extracts

According to Table 3, the solvent extracts of the Jammu leaf sample had the maximum extractive value, while the Bangalore leaf sample had the lowest extractive value.

Table 3. Extractive Values (% w/w) of Different Solvent Extracts from Plant Samples Collected from Jammu, Kerala, Delhi, and Bangalore (Mean ± SD, n = 3)

S. No	Name of Extract	Jammu (% w/w)	Kerala (% w/w)	Delhi (% w/w)	Bangalore (% w/w)
1	Methanol extract	3.787^a	3.345^b	3.088^c	2.876^d
2	Ethanol extract	2.654^a	2.457^b	2.234^c	2.145^d
3	Acetone extract	2.098^a	1.897^b	1.765^c	1.543^d
4	Aqueous extract	1.076^a	1.023^b	0.987^c	0.768^d

n = 3, mean ± SD, Values are expressed as ***Mean ± SD (n = 3)***. ***Different superscript letters (a–d)*** within the same column indicate ***significant differences (p < 0.05)***. Statistical significance was assessed using ***one-way ANOVA followed by Tukey's HSD post hoc test***.

3.3 The total phenolic and flavonoid content of Bacopa monnieri (L.) Pennell leaf samples collected from four distinct geographic areas

According to Table 4 and Figure 1, the leaf sample from Jammu had the highest concentration of total phenolics, whereas the leaf sample from Bangalore had the lowest concentration.

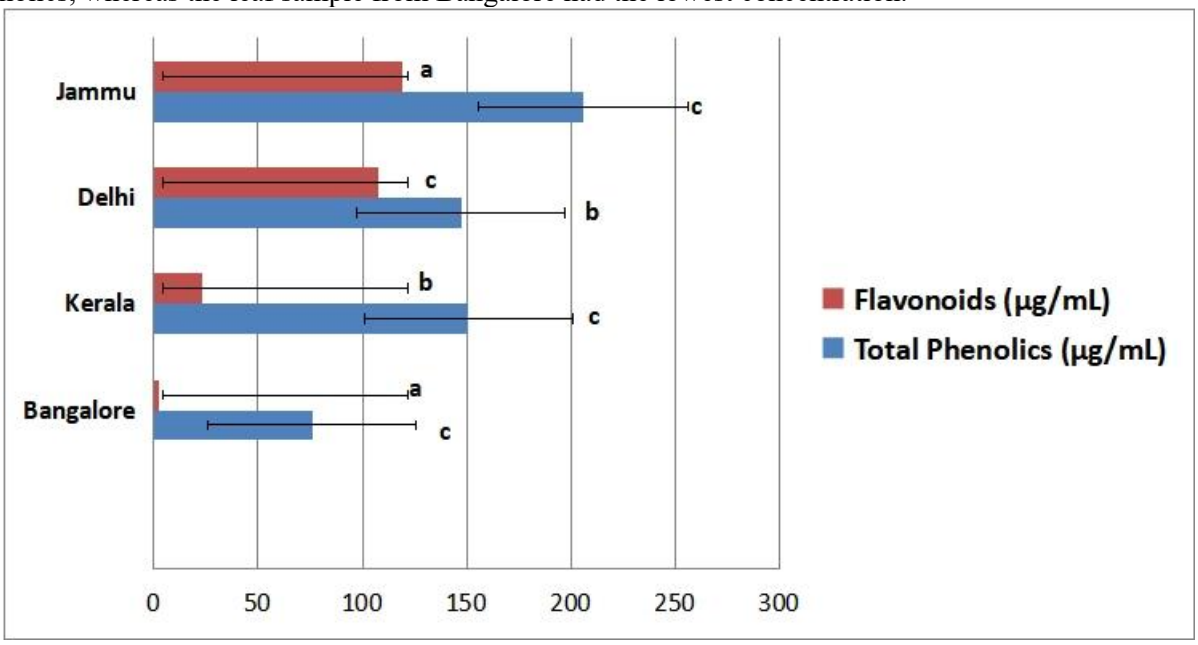

Figure 1. Concentration of total phenolics and flavonoids in samples of *Bacopa monnieri* (L.) Pennell leaves from various geographic locations.

Table 4. Total Phenolic Content of Samples from Different Geographical Regions

Sample Name	Absorbance (Mean ± SD)	Total Phenolics (µg/mL) (Mean ± SD)	% w/w
Bangalore	0.249 ± 0.0015^{a}	76.00 ± 1.65^{a}	0.09
Kerala	0.623 ± 0.002^{b}	150.8 ± 1.50^{b}	1.88
Delhi	0.605 ± 0.0017^{b}	147.2 ± 0.87^{b}	1.84
Jammu	0.899 ± 0.0027^{c}	206.0 ± 1.01^{c}	2.57

Values are expressed as ***Mean ± SD (n = 3)***.

Different superscript letters (a–d) within the same column indicate ***significant differences ($p < 0.05$)***. Statistical significance was assessed using ***one-way ANOVA followed by Tukey's HSD post hoc test***.

According to Table 5 and Figure 1, the leaf sample from Jammu had the highest quantity of flavonoids, whereas the leaf sample from Delhi had the lowest quantity of flavonoids.

Table 5: Total Flavonoid Content of Samples from Different Geographical Regions

Sample Name	Absorbance (Mean ± SD)	Flavonoids (µg/mL) (Mean ± SD)	% w/w
Delhi	0.352 ± 0.0019^{a}	2.60 ± 0.17^{a}	0.06
Bangalore	0.456 ± 0.0018^{b}	23.40 ± 0.31^{b}	0.59
Kerala	0.876 ± 0.0016^{c}	107.40 ± 1.21^{c}	2.67
Jammu	0.936 ± 0.0015^{d}	119.40 ± 0.81^{d}	2.98

Values are expressed as ***Mean ± SD (n = 3)***.

Different superscript letters (a–d) within the same column indicate ***significant differences ($p < 0.05$)***. Statistical significance was assessed using ***one-way ANOVA followed by Tukey's HSD post hoc test***.

3.4 Comparative in vitro antioxidant activity of Bacopa monnieri (L.) Pennell leaf samples from various geographical areas.

3.4.1 Activity to scavenge free radicals in DPPH

Using the DPPH method, the antioxidant capacities of *Bacopa monnieri* (L.) Pennell leaf samples from various geographical locations and the reference antioxidant BHT were assessed. In comparison to other leaf samples, the leaf sample from Jammu had a considerably higher radical scavenging activity (40.63%) and an IC50 value (291 µg /mL), as shown in Table 6. Compared to other leaf samples, the scavenging activity of BHT as a standard showed a greater activity of 45.63% with an IC50 value of 213 µg/mL.

Table 6. Comparative in vitro antioxidant activity of *Bacopa monnieri* (L.) Pennell leaf samples from different geographical regions

Sample extract	DPPH scavenging activity (%)	IC_{50} of DPPH (µg/mL)	Ferric reducing power (µg/g, dw)	H_2O_2 scavenging activity (%)	IC_{50} of H_2O_2 (µg/mL)
Jammu	40.63 ± 0.35^{b}	291.0 ± 5.49^{b}	25.4 ± 0.98^{b}	68.63 ± 0.31^{b}	51.65 ± 0.77^{b}
Kerala	37.87 ± 0.29^{c}	361.0 ± 8.07^{c}	22.2 ± 0.36^{c}	65.98 ± 0.11^{c}	68.87 ± 0.21^{c}
Delhi	35.31 ± 0.67^{d}	391.0 ± 9.83^{d}	18.8 ± 0.69^{d}	61.34 ± 0.46^{d}	63.43 ± 0.31^{d}
Bangalore	34.31 ± 0.57^{e}	393.0 ± 5.04^{e}	20.5 ± 0.68^{e}	60.54 ± 0.31^{e}	62.43 ± 0.40^{e}
Control (BHT)	45.63 ± 0.51^{a}	213.0 ± 6.49^{a}	30.4 ± 0.54^{a}	78.63 ± 0.41^{a}	41.65 ± 0.67^{a}

Values are expressed as mean ± SD (n = 3). Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$) as determined by one-way ANOVA followed by Tukey's HSD post hoc test.

3.3.2 Calculation of the ferric reducing power

As a result of the presence of antioxidant phytochemical substances in the sample mixture, the Fe3+ form in the complex changed to the ferrous form. The amount of ascorbic acid equivalent per gramme of sample on the basis of dry weight was used to communicate the transformation of iron (III) to iron (II)-reducing activity in the leaf samples of methanol extracts. Table 6 shows that a leaf sample from Jammu had the highest lowering power capacity (25.4 µg /g d.w.) when compared to the activity of the control (30.4 g/g d.w.).

3.3.3 Scavenging activity for hydrogen peroxide

Inside the cell, hydrogen peroxide can slowly oxidize a variety of substances. Depleting superoxide anion and hydrogen peroxide is therefore crucial for maintaining food systems. The leaf sample from Jammu had the highest H2O2 scavenging activity (68.63%) and the highest IC50 value (51.65 g/mL), according to Table 6.

3.4 Assessment of the antibacterial properties of several solvent extracts from Bacopa monnieri (L.) Pennell leaf samples collected from various geographical locations.

Table 7: Zone of Inhibition (mm) of Different Solvent Extracts of Plant Samples

Sample	Solvent Extract	BS (mm)	SA (mm)	EC (mm)	PA (mm)
Delhi	Water Extract	No inhibition	No inhibition	No inhibition	No inhibition
	Chloroform Extract	11 ± 0.6453	15 ± 0.2314	11 ± 0.6578	12 ± 0.4325
	Acetone Extract	9 ± 0.6754	12 ± 0.5429	No inhibition	No inhibition
	Ethanol Extract	12 ± 0.8765	16 ± 0.9812	11 ± 0.2367	13 ± 0.3478
	Gentamycin	20 ± 0.8098	25 ± 0.5432	21 ± 0.9043	20 ± 0.3409
Bangalore	Water Extract	No inhibition	No inhibition	No inhibition	No inhibition
	Chloroform Extract	13 ± 0.2309	16 ± 0.5612	10 ± 0.4310	11 ± 0.3208
	Acetone Extract	10 ± 0.7429	13 ± 0.4208	No inhibition	No inhibition
	Ethanol Extract	13 ± 0.9643	17 ± 0.6509	11 ± 0.5430	13 ± 0.4320
Kerala	Water Extract	No inhibition	No inhibition	No inhibition	No inhibition
	Chloroform Extract	14 ± 0.6320	17 ± 0.3108	12 ± 0.6501	14 ± 0.5430
	Acetone Extract	11 ± 0.8709	14 ± 0.6506	No inhibition	No inhibition
	Ethanol Extract	13 ± 0.4308	18 ± 0.4208	11 ± 0.4308	14 ± 0.3208
Jammu	Water Extract	No inhibition	No inhibition	No inhibition	No inhibition
	Chloroform Extract	15 ± 0.3098	17 ± 0.6403	12 ± 0.7605	15 ± 0.4508
	Acetone Extract	11 ± 0.4309	15 ± 0.5409	No inhibition	No inhibition
	Ethanol Extract	18 ± 0.8706	14 ± 0.3408	14 ± 0.2306	15 ± 0.5043

Values are expressed as mean ± SD (n = 3). Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$) as determined by one-way ANOVA followed by Tukey's HSD post hoc test. The antibacterial activity of solvent extracts from Delhi, Bangalore, Kerala, and Jammu samples was assessed against *Bacillus subtilis* (BS), *Staphylococcus aureus* (SA), *Escherichia coli* (EC), and *Pseudomonas aeruginosa* (PA) using the agar well diffusion method.

Water extracts from all regions showed no zone of inhibition (0 mm) against any of the tested organisms, indicating the absence or very low concentration of water-soluble antibacterial constituents. Chloroform extracts exhibited notable antibacterial activity across all regions. The zone of inhibition against BS ranged from 11 ± 0.65 mm (Delhi) to 15 ± 0.31 mm (Jammu), while activity against SA ranged from 15 ± 0.23 mm (Delhi) to 17 ± 0.64 mm (Jammu). Moderate inhibition was observed against Gram-negative bacteria, with EC showing zones between 10 ± 0.43 mm (Bangalore) and 12 ± 0.76 mm (Jammu), and PA between 11 ± 0.32 mm (Bangalore) and 15 ± 0.45 mm (Jammu).

Acetone extracts showed selective activity only against Gram-positive bacteria. Zones of inhibition against BS ranged from 9 ± 0.68 mm (Delhi) to 11 ± 0.87 mm (Kerala and Jammu), while SA inhibition ranged from 12 ± 0.54 mm (Delhi) to 15 ± 0.54 mm (Jammu). No inhibition was observed against EC and PA (0 mm) for any acetone extract. Table 7 and figure 2, 3 shows ethanol extracts demonstrated the highest antibacterial activity among all solvents. Against BS, zones ranged from 12 ± 0.88 mm (Delhi) to 18 ± 0.87 mm (Jammu). The strongest overall inhibition was recorded against *S. aureus*, with zones of 16 ± 0.98 mm (Delhi), 17 ± 0.65 mm (Bangalore), and 18 ± 0.42 mm (Kerala). Ethanol extracts also showed appreciable activity against Gram-negative bacteria, with EC inhibition up to 14 ± 0.23 mm (Jammu) and PA up to 15 ± 0.50 mm (Jammu).

The standard antibiotic gentamycin produced significantly higher zones of inhibition, ranging from 20–25 mm against all tested bacteria, confirming the validity of the assay. Overall, the antibacterial potency followed the order: Ethanol extract > Chloroform extract > Acetone extract >> Water extract, with Jammu and Kerala samples showing comparatively higher antibacterial activity, particularly against *Staphylococcus aureus*.

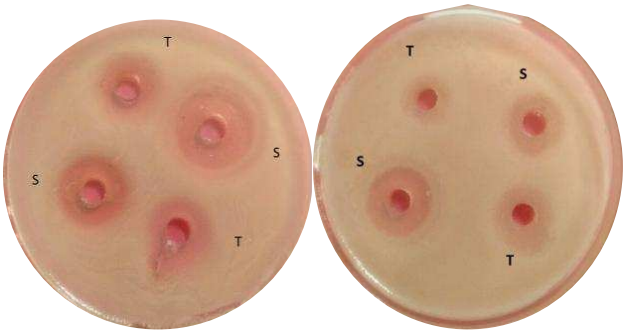

Figure 2: A. Ethanol extract (1000µg/mL) of leaves sample of Jammu against *B. subtilis*; B. Ethanol extract (1000µg/mL) of leaves sample of Jammu against *S. aureus*.

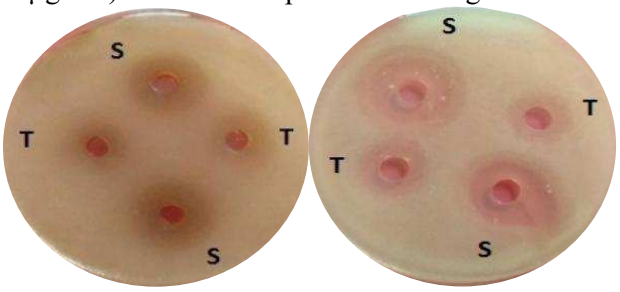

Figure 3: A. Ethanol extract (500µg/mL) of leaves sample of Jammu against *B. subtilis*; B. Ethanol extract (500µg/mL) of leaves sample of Jammu against *S. aureus*.

4. Discussion

The biochemical characteristics of specific plant and medicine species and even external elements like the environment, geographical dispersion, season, soil properties, and growth circumstances can sometimes change the potency of crude pharmaceuticals [19]. According to research, plants differ in the concentration of their active ingredients. It has been noted that the active ingredients in several plants with anticancer activity fluctuate quantitatively with the season [20], [21].

The variety in the active ingredients and pharmacological effects of the same plant parts from the same plant harvested at various geographic locations is more astounding. New active components may be produced by bioassays of extracts made from various plant parts, or from plant parts collected at various periods or from various locations. A difference in the activity may also result from post-collection treatment, such as storage and preparation [22], [23]. As a result, it's possible that the same medicinal plants grown in various locations don't always generate the same active ingredients in the same amounts.

Reactive oxygen species (ROS) are naturally produced by-products of oxygen's regular metabolism and play a significant role in cell signalling. But, at times of stress, ROS levels can soar, causing serious harm

to cell components. Antioxidants are therefore used to neutralise the surplus ROS. When reacting with the appropriate reducing chemicals, DPPH behaves as a steady free radical in methanol that readily takes an electron or hydride radical and transforms into a stable diamagnetic molecule. DPPH radicals create the equivalent hydrazine [24]. Ascorbic acid's IC50 and *Bacopa monnieri*'s hydroethanolic extract's (HEBM) IC50 were around 28 g/ml and 270 g/ml, respectively, with regression coefficients (r2) of 0.93 and 0.89. According to the values of the regression coefficients, HEBM has high antioxidant properties on par with ascorbic acid. The phenolic, triterpene, and flavonoid content of the studied plant may be responsible for its antioxidant action. Other researchers' in vitro research revealed somewhat related results [25], [26]. Jammu leaf samples in this investigation displayed more pronounced DPPH scavenging activity than other leaf samples.

By using an electron, the *Bacopa monnieri* leaf extract's sufficient antioxidant concentration led to the reduction of Fe3+ and Fe2+. By observing the Perl's blue colour, it is possible to determine the number of Fe3+ and Fe2+ ions present and gauge their amount using the absorbance at 700 nm [27]. Phenolic chemicals' relationship to reducing power as antioxidants [28]. Due to the existence of the plant's strong phenolic chemicals, reducing capacity is seen [29]. Due to the presence of the highest phenolic content and flavonoids, Jammu leaf samples had the highest ferric reduction power.

Low quantities of H2O2 may have a significant role in biological systems. Normally, naturally occurring iron complexes in the cell interact with H2O2 in vivo to form highly reactive hydroxyl radicals that could be harmful to an animal's or person's body [30]. Due to the possibility of producing a hydroxyl radical in cells, hydrogen peroxide is highly reactive and potentially harmful to cells [31]. Therefore, the removal of H2O2 is crucial for the security of our food systems. The phenolics in extracts may be responsible for scavenging H2O2 since they can provide H2O2 electrons, neutralising it in water. The leaf sample from Jammu have the highest potential for scavenging hydrogen peroxide.

The majority of the bio-active compounds, including glycosides, alkaloids, reducing sugars, phenolic compounds, steroids and terpenoids, flavonoids, tannins, and saponin glycosides, were found in Aloe vera samples collected from India's six distinct agro-climatic zones, including highland, semi-arid, arid, humid subtropical, tropical wet and dry, and tropical wet, though the amounts varied between accessions. The highest antioxidant potential was demonstrated by highland (low temperature) accessions, whereas the lowest antioxidant potential was seen in tropical zone extracts. Low temperature caused a large number of phenolics to be produced [12]. In this investigation, the samples from Kerala (south region) and Jammu (north region with high altitude) were found to have the highest and lowest percentages of bacoside A contained in plant leaves, respectively. According to the experimental findings, the variation of B. monnieri from Jammu is superior to that from other parts of India. Thus, it might be said that the Jammu region is the ideal place to cultivate B. monnieri in order to produce bacoside A [32].

The present investigation demonstrated that *Bacopa monnieri* leaf samples collected from the Jammu region possessed significantly higher total phenolic and flavonoid content, which was closely associated with enhanced antioxidant and antibacterial activities. This observation is consistent with recent reports indicating that phenolic- and flavonoid-rich extracts of B. monnieri exhibit superior biological activity due to their strong redox properties and ability to neutralize reactive oxygen species [33], [34].

Phenolic compounds are well recognized for their hydrogen- or electron-donating capacity, which plays a crucial role in scavenging free radicals and inhibiting oxidative chain reactions. Several recent studies have demonstrated a positive correlation between total phenolic content and antioxidant potential in B. monnieri extracts [35], [33]. The higher DPPH radical scavenging activity and lower IC_{50} values observed for Jammu samples in the present study can therefore be attributed to the elevated phenolic concentration, which enhances free radical neutralization efficiency.

Flavonoids are another important class of secondary metabolites contributing significantly to antioxidant and antimicrobial activities. High flavonoid content has been reported to improve ferric reducing power and hydrogen peroxide scavenging activity through metal chelation and free radical quenching mechanisms [34]. Similar trends were observed in the present study, where samples with higher flavonoid content exhibited stronger ferric reducing antioxidant power and hydrogen peroxide scavenging activity, particularly in methanolic and ethanolic extracts.

The antibacterial activity observed in this study further supports the therapeutic relevance of B. monnieri. Ethanolic extracts exhibited the strongest antibacterial activity against both Gram-positive and Gram-negative bacterial strains. This finding is in agreement with recent studies demonstrating that ethanol efficiently extracts bioactive phytoconstituents such as phenolics, flavonoids, and saponins, which disrupt bacterial cell membranes and interfere with essential metabolic processes [36], [35]. The greater susceptibility of Staphylococcus aureus compared to Gram-negative bacteria may be attributed to differences in cell wall

structure, as Gram-negative bacteria possess an outer lipopolysaccharide membrane that limits the penetration of antimicrobial compounds [36].

Geographical variation plays a critical role in determining phytochemical composition and bioactivity in medicinal plants. Environmental factors such as altitude, temperature, soil nutrients, and climatic stress influence secondary metabolite biosynthesis [37]. Plants growing in regions with higher altitude and temperature fluctuations, such as Jammu, are often exposed to environmental stress that stimulates the accumulation of phenolic and flavonoid compounds as protective mechanisms [37], [34]. This may explain the superior antioxidant and antibacterial performance of Jammu samples compared to those from other regions.

Overall, the findings of the present study are in strong agreement with recent literature emphasizing that *Bacopa monnieri* is a valuable source of natural antioxidants and antimicrobial agents, and that its bioactivity is significantly influenced by geographical origin [33], [35]. These results support the potential use of geographically optimized B. monnieri extracts in the development of antioxidant and antibacterial formulations.

The current finding demonstrated that leaves from B. monnieri in Jammu exhibit the highest levels of antibacterial activity when ethanol and chloroform extracts are used (Table 6). This shows that using this plant to treat many pathogenic conditions may have clinical advantages. These extracts' bioactive components may be employed to create brand-new antibacterial medications. Since antibiotic-resistant pathogenic microorganisms are common, finding new antibiotics is a never-ending endeavour.

5. Conclusion

Based on our research, it was shown that methanol extracts of Jammu-grown *Bacopa monnieri* (L.) Pennell leaves included greater levels of total phenolics and flavonoids. They significantly increase the hydroxyl radical's, superoxide radicals, and DPPH radical's antioxidant activity. The amount of flavonoids present is directly correlated with the sample molecules' antioxidant activity. As an antioxidant herb for adjuvant therapy, Jammu leaf extract from *Bacopa monnieri* (L.) Pennell could be utilised. Because of the adverse effects on humans of the synthetic antioxidant Butyl Hydroxy Toluene, the creation of natural antioxidants was significant and promising.

More research into *B. monnieri* crude extracts, particularly ethanol and chloroform extracts may be necessary to create a novel antibiotic that could help treat a variety of bacterial diseases in tropical areas. In summary, *B. monnieri* may be useful for treating a variety of pathogenic conditions. Our research could help with the creation of antibacterial medications from *B. monnieri* (L.) Pennell.

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