

Phytochemical analysis, antioxidant and antimicrobial activity in leaves samples of *Bacopa monnieri* (L.) Pennell from different geographical regions in India

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Research Article

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Abstract

Background: To determine quantitative analysis of total phenolic compound and flavonoids content and evaluate antioxidant and antibacterial activity in leaves samples of *Bacopa monnieri* (L.) from four different geographical regions in India.

Result: Phytochemical analysis was performed on all four leaves samples and estimation of Total phenolic and flavonoid content were carried out. Evaluation of antioxidant activities such as DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity, hydrogen peroxide (H_2O_2) scavenging activity, ferric reducing power and antibacterial activity in different solvent extracts of leaves samples from four different geographical regions in India were performed.

The highest content of total phenolics and flavonoids were observed in leaves sample of Jammu region. Highest DPPH radical scavenging activity, H_2O_2 scavenging activity and ferric reducing power were observed in leaves samples of Jammu. Maximum zone of inhibition against bacteria *Staphylococcus aureus* was reported with ethanol extract of leaves sample of Jammu. Maximum zone of inhibition against bacteria *Bacillus subtilis* was reported with chloroform extract of leaves sample of Kerala. Maximum zone of inhibition against bacteria *Pseudomonas aeruginosa* was reported with ethanol extract of leaves sample of Jammu. Maximum zone of inhibition against bacteria *Escherichia coli* was reported with ethanol extract of leaves sample of Kerala.

Conclusion: Excellent antioxidant activity was given by extract of leaves samples of Jammu. So they can be used as natural antioxidants after isolation and purification. Excellent antibacterial activity was reported in leaves samples of Jammu and Kerala.

1. Background

Free radicals are chemical moieties occurs as independent existence that possess one or more unpaired electrons that make them highly reactive (Dinis et al. 1993), and must be converted to non-toxic form to avoid causing damage to the cell. They have been implicated in many human diseases due to their increased formation, which accompany tissue injury (Bridges et al. 1993). Antioxidants, on the other hand, are substances that counteract the harmful effects of these free radicals and are effective because they are willing to give up their own electrons to free radicals. When a free radical obtains the electron from antioxidants it will be neutral for cell damage and the chain reaction of oxidation is broken (Dekkers et al. 1996). Naturally there is a dynamic balance between the amount of free radicals produced in the body and the antioxidants to scavenge or quench them to protect the body against deleterious effects. However, the quantity of antioxidant exit under normal physiological conditions may be not enough to neutralize free radicals produced under pathological conditions (Amaeze et al. 2011). Therefore, it is important to enrich animal diet with antioxidants to protect against harmful pathogen.

Plant chemical have the potency to inhibit oxidative damage caused by free radicals, oxygen anions, and interfere with the oxidation process by reacting with the free radicals, chelating catalytic metals, and also

by acting as oxygen scavengers (Shahidi and Wanasundara 1992). The potentially reactive derivatives of oxygen attributed as reactive oxygen species (ROS) such as superoxide anion, hydroxyl radicals, hydrogen peroxide and singlet oxygen are continually generated from normal metabolic activity inside animal body, which are detoxified by antioxidant present in the body such as superoxide dismutase, catalase, glutathione peroxidase, etc. However, over production of ROS especially when exposed to unfavourable condition e.g temperature and salinity, and or inadequate antioxidant defense can easily affect and persuade oxidative damage to various biomolecules including cellular proteins, membrane lipids, lipoproteins, disruption of cellular functions and breaking of DNA strands (Farber 1994, Patra et al. 2008).

However, several studies have indicated that medicinal plants contain compounds like peptides, unsaturated long chain fatty acids, aldehydes, flavonoid, alkaloids, essential oils, phenols and water or ethanol soluble compounds. These metabolites are important in therapeutic use against micro organisms (Khan et al. 2003, Pavrez et al. 2005). These secondary metabolites produced by plants are organic chemicals of high structural diversity, which play different functions including chemotherapeutic, bacteriostatic, bacteriocidal and antimicrobial functions (Purohit and Mathur 1999), and are used in nutraceutical industry.

In this research paper, comparative research study was carried out on total phenolics, flavonoid content, antioxidant and antibacterial activity in leaves samples of *Bacopa monnieri* (L.) Pennell from different geographical regions in India.

2. Methods

2.1 Collection of drugs

The fresh leaves of *Bacopa monnieri* were collected from the Herbal Garden Jamia Hamdard and three other different places of India (Kerala, Bangalore and Jammu) as given in Table 1. It was authenticated by comparing with the specimen available at Deptt. of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi. The leaves were dried at room temperature and powdered.

2.2 Preparation of Reagents [Harborne 1998; Khandelwal 2004]

2.2.1 Dragendorff's reagent

It was arranged by blending two arrangements. Arrangement A (17 gm of bismuth subnitrate + 200 gm tartaric corrosive + 800 ml refined water) and arrangement B (160 gm potassium iodide + 4 ml refined water) were blended in 1:1 v/v extent. From this arrangement working standard was arranged by taking 50 ml of this arrangement and including 100 gm of tartaric corrosive and making up to 500 ml with refined water.

2.2.2 Mayer's reagent

A mixture was created with Mercuric chloride (1.36 g) and 60 ml of refined water. An arrangement of 5 g of Potassium iodide (KI) in 20 ml of refined water was included to deliver to 100 ml and kept in a holder to utilize.

2.2.3 Wagner's reagent

A mixture was created with iodine (1.27 g) and potassium iodide (2 g) with 5 ml of water and the arrangement was weakened to 100 ml with water.

2.2.4 Liebermann-Buchard's reagent

Sulphuric acid (2.5 ml) was blended with acidic anhydride (2.5 ml), to it 45 ml of liquor was included, shaken well and put away for use.

2.2.5 Fehling's arrangement A (Copper solution) Carefully chosen little precious stones of cupric sulfate (34.66 g) were dissolved appearing no follow of foam or following dampness in adequate water to create 500 ml, and put away in well closed container.

2.2.6 Fehling's solution B (Alkaline tartarate solution)

Sodium potassium tartarate (176 g) and Sodium hydroxide (77 g) were broken down in adequate water to deliver 500 ml and put away in well stoppered container. These two arrangements (Fehling's arrangement A and B) were blended in break even with volumes promptly some time recently use.

2.2.7 Ninhydrin's reagent

Ninhydrin (30 g) was blended in 10 ml of n- butanol taken after by 0.3 ml of 98% v/v in acidic acid.

2.2.8 Millon's reagent (Mercuric nitrate solution) It can be prepared by dissolving 3 g of mercury in 27 ml of raging nitric acid and cooled.

2.3 Qualitative chemical tests for active compounds

2.3.1 Tests for Alkaloids

Each of the extract buildups was taken independently in five ml of 1.5 % hydrochloric acid and sifted. The filtrate was at that point tried with taking after reagents:

2.3.1.1 Dragendorff's Test

Small quantity of Dragendorff's reagent were integrated in each of the extract and watched for arrangement of orange yellow precipitate.

2.3.1.2 Hager's Test

A small number of drops of Hager's reagent were integrated in each of the extract and watched for arrangement of yellow precipitate.

2.3.1.3 Wagner's Test

A small number of drops of Wagner's reagent were included in each of the extract and watched for arrangement of precipitate.

2.3.1.4 Mayer's Test

Few drops of Mayer's reagent were included in each of the extract and watched for arrangement of white or cream colored precipitate.

2.3.2 Tests for Carbohydrates

2.3.2.1 Molisch Test: Small amounts of alcoholic and watery extricates were broken up independently in five ml of refined water and sifted. To this arrangement, 2-3 drops of α -naphthol were incorporated. To this, approximately one ml of concentrated sulphuric corrosive was included along the sides of slanted test tube so as to create two layers and watched for arrangement of violet colored layer at the border.

2.3.2.2 Fehling's Test (Location of lessening sugar) : Few drops of Fehling's arrangements A and B in break even with volume were included in weaken extricates and warmed for 30 minutes and watched for the arrangement of brick ruddy coloured precipitate.

2.3.3 Tests for Glycosides

About two ml alcoholic extracts were utilized for the glycoside test.

2.3.3.1 Keller-Killiani Test: Dried extract was included with acetic acid containing ferric chloride and one ml of strong sulphuric acid and watched for the arrangement of ruddy brown colour at the intersection of two layers and the upper layer turns somewhat blue green within the nearness of glycosides.

2.3.3.2 Borntrager's Test: Small amount of benzene and weak smelling salts arrangement were incorporated to the ethanolic extract and watched for the arrangement of ruddy pink colour.

2.3.3.3 Legal Test: concentrated ethanolic extracts were made antacid with few drops of 10% sodium hydroxide arrangement and after that naturally arranged sodium nitrosopruside arrangement was included to the arrangement and watched for arrangement of blue colour.

2.3.3.4 Baljet test: To the concentrated ethanolic extracts sodium picrate reagent was included and watched for arrangement of orange or yellow colour.

2.3.4 Tests for phenolics compounds

2.3.4.1 Test of Ferric chloride: The extracts were blended in water and heated. To this, two ml of ferric chloride arrangement was included and watched for arrangement of green or blue colour.

2.3.4.2 Lead Acetic acid test: To the extracts (2 ml) leads acetic acid arrangement was included and watched for arrangement of accelerate.

2.3.4.3 Gelatin solution: A few ml of gelatin arrangement was included to the watery extract and watched for arrangement of accelerate or turbidity.

2.3.5 Chemical tests for Flavonoids

2.3.5.1 Smelling salts test: Filter paper strips were plunged in alcoholic arrangement of the extracts, ammoniated and watched for colour alters from white to yellow.

2.3.5.2 Shinoda / seat test: A little amount of buildup was broken up in five ml of ethanol and treated with few of drops concentrated hydrochloric corrosive and 0.5 g of magnesium turnings and watched for arrangement of pink colour.

2.3.6 Tests for Proteins and Amino-acids

2.3.6.1 Millon's test: To small amount of ethanol extract, five ml refined water was included and sifted. To two ml of this filtrate 5-6 drops of Millon's reagent (arrangement of mercuric nitrate and nitrous acid) were included and watched for arrangement of ruddy precipitate.

2.3.6.2 Xanthoprotein test: Small amount of extract was integrated with nitric acid and watched for arrangement of yellow colour.

2.3.6.3 Biuret Test: Soluble filtrate of the dried extracts was included with small amount of 0.02 % copper sulfate arrangement were included and watched for arrangement of ruddy or violet colour.

2.3.6.4 Ninhydrin Test: To the extract, lead acetate arrangement was included to accelerate tannins. The accelerate was spotted on a paper chromatogram, splashed with ninhydrin reagent and warmed at 110°C for 5 minutes and watched for arrangement of ruddy or violet colour.

2.3.7 Tests for Saponins

2.3.7.1 Froth Test: Few mg of residue was taken in a test tube with little sum of water and shaken enthusiastically for one minute and watched for arrangement of wealthy foam which was steady for above ten minutes. To the alcoholic extracts, few drops of sodium bicarbonate arrangement were included, shaken well, and watched for the arrangement of honeycomb like frothing.

2.3.8 Tests for Acidic compounds: To the alcoholic extract sodium bicarbonate arrangement was included and watched for the generation of foam (Harborne, 1998).

2.3.9 Litmus paper test: A little sum of alcoholic extracts were taken in warm water and sifted. The filtrate was at that point tried by litmus paper and methyl orange and watched for the visibility of blue colour.

2.3.10 Tests for Mucilages: The extricate was treated with ruthenium red solution in lead acetate and watched for the arrangement of pink colour.

2.3.11 Tests for Resins: Distilled water was included to the extracts and watched for turbidity. A blend of extract in acetone (3 ml) and HCl (3 ml) was warmed on a water shower for half an hour and checked for a pink colour.

2.4 Assay for total phenolics and flavonoid content

2.4.1 Material and Reagent

Reference standard, for flavonoid Rutin and for phenol compound gallic acid were received from M/s Normal cures Pvt. Ltd. Bangalore, India. Each chemicals and reagents utilized were of explanatory rating and acquired from Merck Chemicals, India.

2.4.2 Preparation of methanol extracts of all samples: one gm of leaf and callus sample were dried and powdered then refluxed in 50 ml of methanol for two hours. Collected filtrate was filtered and heated till residue formation .The weight of residue was measured and reconstituted in methanol.

2.4.3 Reagents

- 10% Folin Ciocalteu reagent solution in distilled water
- 1M Na_2CO_3 (sodium carbonate) solution in distilled water
- Standard (Gallic acid) 1 mg/ml solution in methanol
- Dilution of standard Gallic acid: 50 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$ and 150 $\mu\text{g/ml}$ in methanol

2.4.4 Samples preparation: 10 mg / ml methanol leaf extract and 8 mg/methanol calli extracts were made. 0.5 ml sample was poured to mixture of 5 ml F.C compound and 4 ml volume of Na_2CO_3 then took absorbance on 765 nm after 15 minutes.

2.4.5 Preparation of Standard: 0.5 ml of each standard dilution was mixed to mixture of 5 ml volume F.C. reagent and 4 ml Na_2CO_3 solution, then took absorbance at 765 nm after 15 minutes.

2.4.6 Blank solution: Methanol 5 ml solvent and 5 ml F.C. reagent were taken and added to 4 ml Na_2CO_3 solution.

2.5 Evaluation of entire flavonoid content [Pourmorad et al. 2006]

2.5.1 Reagents

- 0.1 mg/ml AlCl_3 (aluminium chloride) solution in distilled water
- 1M CH_3COONa (sodium acetate) solution in distilled water
- Standard (Rutin) 1 mg/ml solution in methanol
- Dilution of standard Rutin: 10 $\mu\text{g}/\text{ml}$ to 100 $\mu\text{g}/\text{ml}$ in methanol

2.5.2 Samples preparation: 100 mg /ml solution for leaves extract and calli extract were included in methanol. Half ml of sample was added to 1.5 ml methanol. Combination of 0.1 ml volume of AlCl_3 and 1/10 ml volume of CH_3COONa reagents were poured to above solution. After that 2.8 ml volume distilled water mixed to solution and kept for half hour after that absorbance was maintained at 415 nm.

2.5.3 Preparation of Standard: 0.5 ml volume of standard dilution were taken and added to 1.5 ml methanol. After that 0.1 ml volume of AlCl_3 and 0.1 ml of CH_3COONa reagents were poured to above solution and then added 2.8 ml Distilled water and kept for half hour after that absorbance was taken at 415 nm.

2.5.4 Blank solutions: two ml methanol was added to mixture of 0.1 ml volume of aluminium chloride and 0.1 ml volume of CH_3COONa and then added to 2.8 ml volume purified water.

2.6 Methods for antioxidant activity

2.6.1 DPPH liberated radical rummaging activity

0.1 mM DPPH was mixed in methanol and 1 ml mixture was included to 3 ml alcohol extract at one concentration (500 $\mu\text{g}/\text{mL}$). Butyrate hydroxytoluene (BHT) was utilized as a positive standard. Discoloration was calculated on 517 nm after hatching for 30 min. Estimations were taken at slightest in triplicate. The capacity to rummage the DPPH radical was calculated utilizing the taking after condition: DPPH rummaging impact (%) = $[A_{\text{DPPH}} - A_s / A_{\text{DPPH}}] \times 100$ where, A_{DPPH} is the UV absorbance of the DPPH arrangement and A_s is the UV absorbance of the arrangement when the test extricate is included. The extract concentration giving 50% restraint of radical-scavenging movement (IC_{50}) was calculated and communicated as mg/mL, d.w. (Blois, 1958).

2.6.2 Ferric diminishing control determination

2.5 ml phosphate buffer for pH maintaining (200 mM and pH 6.60) and 500 $\mu\text{g}/\text{mL}$ alcohol extracts were blended with and 2.5 ml 1% potassium ferricyanide. At that point the blend was brooded at 50 °C with twenty minutes. 2.5 ml 10% dilute trichloroacetic acid was included to over blend and centrifuged at 10000 revolutions per minute for ten minutes. 5 ml of the top surface was poured with distill water (5 ml) and 0.1% FeCl_3 (1 ml). The absorbance of the response blend was measured at 700 nm. The ultimate comes about were communicated as μg ascorbic acid counterparts / g based on dry weight of the extract (Zhao et al. 2008).

2.6.3 Hydrogen peroxide rummaging activity

Solution of H₂O₂ (43 mM) was made in buffer phosphate (0.10 M, pH 7.4). Extricates at concentration 50 µg/mL broken up in 3.4 mL phosphate buffer were included to a H₂O₂ arrangement (600µL). The absorbance of the response blend was recorded at 230 nm. The extricate concentration giving 50% of H₂O₂ rummaging movement (IC₅₀) was calculated and communicated as µg/mL based on test dry weight. (Shon et al. 2007).

2.6.4 Statistical analysis

The tests were carried out utilizing Completely Randomized Design (CRD). Triple readings were attempted in each test. Data are maintained as means ± standard deviation (SD). Analysis of variance and significant differences among means were tried by one-way ANOVA using the COSTAT computer package (Cohort Software, 1989). The least significant difference (LSD) at $P \leq 0.05$ level was included. Correlation coefficients (R^2) from regression investigation between phenolic, flavonoid substance and antioxidant activities were moreover premeditated.

2.7 Methods for antibacterial activity

2.7.1 Microbial strains

The microbial strains were taken from Division of Biotechnology, University of Mewar, Chittorgarh, India. The reference strains of microbes ought to be refined in supplement broth (Hi-media,) at 37°C, subcultured routinely (each 30 days) and put away in supplement agar inclines at 4°C as well as at -20°C by planning suspensions in 20% glycerol. Bacterial strains chosen for antimicrobial study are following

BS :*Bacillus subtilis*,

SA: *Staphylococcus aureus*,

EC: *Escherichia coli*,

PA: *Pseudomonas aeruginosa*

2.7.2 Formationand establishment of stock cultures

The microbial strains were inoculated on nutrient agar and maintainted during the night at 37°C. These cultures were set aside at 4°C. Fresh slope cultures were arranged every 3-4weeks.

2.7.3 Culture media:

2.7.3.1 Nutrient agar: Nutrient agar will be used for preparation of stock cultures on agar slopes. The formula was following

Peptone	5.00 gram
Sodium chloride quantity	5.00 gram
Beef extract quantity	1.50 gram
Yeast extract quantity	1.50 gram
Agar	1.50 gram
pH (at room temprature)	7.40 ± 0.2

2.7.3.2 Nutrient broth

Beef extract quantity	3.00 g
Peptone quantity	5.0 g
pH	6.80 ± 0.2

2.7.3.3 YEPD medium will be used as the basic liquid culture medium for growing overnight fungal cultures. The formula was following

Yeast extract quantity	3.0 g
Peptone quantity	10.0 g
Dextrose quantity	20.0 g
pH	7.0

2.7.3.4 Mueller Hinton broth (MHB) medium

It is utilized for determining the antibacterial susceptibility testing.

Beef extract powder quantity	2.00 gram
Casein quantity	17.50 gram
Soluble starch quantity	1.50 gram
pH	7.3± 0.1

2.8 Water Extraction

25 gm of discuss dried powder was set in hot refined water and bubbled for 30 min and kept undisturbed for 24hr. It was sifted off utilizing sterile channel paper (Whatman no. 1) into a clean cone shaped carafe. Watery dissolvable was expelled beneath weight employing a rotatory vaccum evaporator at 60°C. The

dried buildup of rough extricate was resuspended in 20% DMSO and after that put away in dim bottles at 4°C.

2.9 Solvent Extraction

Dried regenerated shoot powdered were extracted with solvent (Chloroform, acetone and 95% (v/v) ethanol) using soxhlet extractor. 25 gm powder was arranged in soxhlet thimble and 250 ml of solvent was poured to soxhlet flask then extracted at 40°C until the extract was apparent.

2.10 Preparation of bacterial inoculum:

Inoculums were prepared by of isolated bacterial colonies from 24 hr old culture and suspended in 5 ml of germ-free nutrient broth. The broth culture was maintained at 37°C for 4hr.

2.11 Agar well dissemination strategy [Murray et al. 1995]

Agar well-diffusion strategy was taken after to decide the antimicrobial action. Supplement agar (NA) plates were cultured with 8 hour ancient - broth culture of individual microbes. Wells (10mm breadth and approximately 2 cm a portion) were made in each of these plates utilizing sterile plug borer. Stock arrangement of each plant extricate was arranged at a concentration of 1 mg/ml in several plant extricates viz. Ethanol, chloroform, Water. Almost 100 µl of distinctive concentrations of plant dissolvable extricates were included sterile syringe into the wells and permitted to diffuse at room temperature for 2hrs. Control tests including inoculums without plant extricate were set up. The plates were hatched at incubation temperature 37°C for 18 to 24 h for bacteria and incubation temperature 28°C for 48 hours parasitic pathogens. The breadth of the hindrance zone (mm) was calculated and the action record was too calculated. Triplicates were kept up and the test was rehashed thrice.

2.12 Assurance of Minimum inhibitory concentration [Baron and Fingold ¹⁹⁹⁰].

Least amount inhibitory concentrations were decided by the tube weakening strategy. The MIC was decided utilizing the tube weakening strategy. A two- overlap serial weakening of the extract and divisions was carried out aseptically to grant changing concentrations extending from 30 mg/ml-0.23435 mg/ml. Each of the weakenings was immunized with 0.1 ml of the standardized inoculum and the tubes were brooded at 37 °C for 24 hours and microbial development watched as turbidity in tubes was looked out for. The most reduced concentration that appeared no development was considered the MIC. The complete strategy was replicated using Ampiclox and Zosyn at concentrations extending from 0.5 mg/ml-0.0004 mg/ml. A positive control tube containing broth with living beings without plant extricate and a negative control tube containing extricate without living beings were moreover hatched along. The MIC was taken as the slightest concentration that repressed the development of test organism.

3. Result

3.1 Phytochemical analysis

Table 2 indicate that methanol extracts of Leaves extracts of all samples showed presence of alkaloids, carbohydrates, phenolics and tannins, flavonoids, proteins and amino acids, saponins, steroids and terpenoids, whereas mucilage and anthraquinone glycosides were found absent in all the samples tested.

3.2 Total phenolics and flavanoid content in leaves samples of *Bacopa monnieri* (L.) Pennell from four different geographical regions

Table 3 indicates that maximum quantity of total phenolics was observed in leaves sample of Jammu whereas minimum quantity of phenolics was observed in leaves sample of Bangalore.

Table 4 indicate that maximum quantity of flavonoid content was observed in leaves sample of Jammu and minimum flavonoid content was observed in leaves sample of Delhi.

3.3 Relative in vitro antioxidant action of of leaves samples of *Bacopa monnieri* (L.) Pennell from different geographical regions.

3.3.1 DPPH free radical scavenging activity

The antioxidant activities of methanol extract of **leaves samples of *Bacopa monnieri* (L.) Pennell from different geographical regions** and standard antioxidant BHT were determined using the DPPH method. Table 5 shows leaf sample of Jammu has given relatively higher radical scavenging activity (40.63%) with IC₅₀ value (0.313 mg/mL) than other leaves samples. The rummaging activity of BHT as standard represented higher activity 45.63% with IC₅₀ value (0.613 mg/mL) than others leaves samples.

3.3.2 Ferric reducing power determination

Changing of the Fe³⁺ form in complex to the ferrous form due to the involvement of antioxidant phytochemical compounds in sample blend. The transformation of iron (III) to iron (II)-reducing activity in the leaves samples of methanol extracts was communicated as µg ascorbic acid equivalent/g sample on the basis of dry weight. Table 5 represents highest reducing power capacity (25.4 µg/g d.w.) was reported in leaves sample of Jammu as compared to the activity of control (30.4 µg/g d.w.).

3.3.3 Hydrogen peroxide scavenging activity

Hydrogen peroxide can enter to inside cell and may slowly oxidize a number of compounds. Thus, depleting of hydrogen peroxide and superoxide anion is very important for preservation of food systems. Table 5 shows leaves sample of Jammu gave the highest H₂O₂ scavenging activity (68.63%) with IC₅₀ value 81.65 µg/mL.

3.4 Evaluation of antibacterial activity of diverse solvent extracts of leaves samples of *Bacopa monnieri* (L.) Pennell from different geographical regions.

Experiment was performed on evaluation of antibacterial action of diverse solvent extracts of leaves samples against bacteria and all data are represented in **Table 6**.

The existence of zones of hindrance on the agar plates indicates that the plant extracts have antibacterial action on the tested organisms. Figure 1 shows highest zone of hindrance against bacteria *B. subtilis* was reported with ethanol extract of Jammu and minimum zone of hindrance against bacteria *B. subtilis* was observed with acetone extract of leaves sample from Bangalore.

Highest zone of hindrance was reported with ethanol extract of leaves samples of Jammu against bacteria *S. aureus* while minimum zone of hindrance was observed with acetone extract of leaves sample of Delhi against bacteria *S. aureus*.

Figure 2 shows aximum zone of hindrance was reported with ethanol extract of leaves sample of Jammu against bacteria *P. aeruginosa* and minimum zone of hindrance against was observed with chloroform extract of leaves sample of Delhi against bacteria *P. aeruginosa* whereas maximum zone of hindrance was reported with ethanol extract of leaves sample of Jammu against bacteria *E. coli* and minimum zone of hindrance was observed with chloroform extract of leaves sample of Delhi against bacteria *E. coli*.

4 Discussions

Reactive oxygen species (ROS) are formed as a natural by-product of the normal metabolism of oxygen and have important role in cell signaling. However, during stress ROS levels can increase dramatically, resulting in significant damage to cell structures. Hence antioxidants are employed to scavenge the excess ROS. DPPH acts as a steady free radical in methanol that easily accepts an electron or hydride radical and converts to a stable diamagnetic molecule by reacting with suitable reducing agents DPPH radicals formed into the corresponding hydrazine (Alam et al. 2012). The IC₅₀ of ascorbic acid and hydroethanolic extract of *Bacopa monnieri* (HEBM) were just about 28µg/ml and 270µg/ml with regression coefficients (r^2) of 0.93 and 0.89, respectively. The values of regression coefficients indicate a strong antioxidant potential of HEBM comparable to that of ascorbic acid. The antioxidant activity might be attributed to phenolic content, triterpenoids and flavonoid content of the tested plant. Somewhat similar finding were also reported by other workers in their *in vitro* studies (Ghosh et al. 2007; Volluri et al. 2011). In this present study, leaves sample of Jammu showed more prominent DPPH scavenging activity than other leaves samples.

The adequate amount of antioxidants in the extract of leaves samples from *Bacopa monnieri* yielded the reduction of Fe³⁺ and Fe²⁺ by utilizing an electron. The quantity of Fe³⁺ and Fe²⁺ ions can be identified by the Perl's blue colour observation and evaluated by the absorbance at 700nm [Akinpelu et al. 2010]. Phenolics compounds as antioxidant is related to reducing capacity [Thirugnanasampandan et al. 2008]. Reducing capacity is observed due to the presence of the high phenolic compounds of the plant [Yildirim et al. 2001]. Highest Ferric reducing power of was observed in leaves samples of Jammu due to the presence of highest phenolic content and flavanoids.

Involvement of low levels of H_2O_2 in biological systems may be important. Naturally found iron complexes inside the cell are generally interacted with H_2O_2 *in vivo* to produce highly reactive hydroxyl radicals which may be toxic for animal and human body [Miller et al. 2000]. Hydrogen peroxide is very responsive and may be toxic to cell because it may produce hydroxyl radical in the cells [Halliwell et al. 1987]. Thus, clearance of H_2O_2 is very important for safety of our food systems. Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2 , thus neutralizing it to water. Highest capacity for scavenging of hydrogen peroxide is given by the leaves sample of Jammu.

The present result showed that the maximum antibacterial activity is revealed by ethanol and chloroform extracts in leaves samples of Jammu from *B. monnieri* (Table 6). This suggests that this plant may be of clinical benefit for uses in different pathogenic diseases. Bioactive compounds in these extracts may be used in the development of novel antibacterial drugs. Pathogenic bacteria are known to develop resistance for antibiotics, thus search for new antibiotics is a never-ending process.

5. Conclusion

On the basis of our research, it was observed that higher quantity of total phenolics and flavanoids were reported in methanol extracts of leaves sample of Jammu from *Bacopa monnieri* (L.) Pennell. They give significant antioxidant activities to hydroxyl radical, superoxide radical, and DPPH radical. Content of flavonoids are directly related to antioxidant activity of sample molecules. Thus, the extract from leaves sample of Jammu from *Bacopa monnieri* (L.) Pennell could be used as an antioxidant herb for adjuvant therapy. As the synthetic antioxidant Butyl Hydroxy Toluene was forbidden being used in food due to its side effects on human, development of the natural antioxidants was meaningful and prospective.

Crude extracts of *B. monnieri*, especially ethanol and chloroform extracts may deserve further investigations to develop a new antibiotic that may help in combating several bacterial infirmities in tropical countries. In conclusion, *B. monnieri* may be effective for treatment in different pathogenic diseases. Our finding may be useful for development of antibacterial drugs from this plant *B. monnieri* (L.) Pennell.

Declarations

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Authors' contributions

AA was involved in the formation and design of the work, collection of sample, analysis and interpretation of data, and manuscript preparation. MS and RA contributed to the antimicrobial activity of

sample. SA and AS were concerned in the idea of work and review of manuscript. We ensure and hereby declared that all authors have read and approved the manuscript.

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Availability of data and materials

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Tables

Table 1: Places and addresses from where the drug has been collected

Scientific name	Family	Date of collection	Address (sources) for collection of sample	Geographic coordinates
<i>Bacopa monnieri</i> (L.) Pennell	Schropulariaceae	Jnuary 2019	Herbal garden of Jamia Hamdard, New Delhi, India	28.7041° N,77.1025°E
<i>Bacopa monnieri</i> (L.) Pennell	Schropulariaceae	Jnuary 2019	Kerala University,Kerala, Kerala	10.8505°N,76.2711E
<i>Bacopa monnieri</i> (L.) Pennell	Schropulariaceae	Jnuary 2019	University of Agriculture Science, Bangalore,India	12.9716°N, 77.5946°E
<i>Bacopa monnieri</i> (L.) Pennell	Schropulariaceae	Jnuary 2019	Regional Research Laboratory, Jammu,India	32.7329° N,74.4642°E

Table 2- Phytochemical analysis of four leaves samples of different geographical regions

S.No.	Constituents	Delhi	Banglore	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	+	+	+	+

Table3: Total phenolic contents in all leaf extract samples

Samples name	Absorbance (Mean $\pm$ SD)	Concentration (Mean $\pm$ SD) μ g/ml	% w/w
Banglore	0.249 $\pm$ 0.0015	76.00 $\pm$ 1.6520	0.09%
Kerala	0.623 $\pm$ 0.002	150.8 $\pm$ 1.5019	1.88%
Delhi	0.605 $\pm$ 0.0017	147.2 $\pm$ 0.8717	1.84%
Jammu	0.899 $\pm$ 0.0027	206.0 $\pm$ 1.0132	2.57%

Table 4: Total flavanoid content in all leaves sample

Samplesname	Absorbance (Mean $\pm$ SD)	Concentration (Mean $\pm$ SD) μ g/ml	% w/w
Kerala	0.876 $\pm$ 0.0016	107.40 $\pm$ 1.2063	2.67%
Banglore	0.456 $\pm$ 0.0018	23.40 $\pm$ 0.3055	0.59%
jammu	0.936 $\pm$ 0.0015	119.40 $\pm$ 0.8082	2.98%
Delhi	0.352 $\pm$ 0.0019	2.60 $\pm$ 0.1707	0.06%

Table 5: Relative *in vitro* antioxidant action of of leaves samples of *Bacopa monnieri* (L.) Pennell from different geographical regions.

Sample extract	*DPPH scavenging activity (%) (mean $\pm$ SEM)	*IC50 values (mg/mL, d.w.) of DPPH scavenging activity	*Ferric reducing power(μ g/g, dw)	*Hydrogen peroxide scavenging activity (%)	*IC50 values (μ g/mL, d.w.) of H ₂ O ₂ scavenging
Jammu	40.63 $\pm$ 0.3452	0.313 $\pm$ 0.5489	25.4 $\pm$ 0.9834	68.63 $\pm$ 0.3076	71.65 $\pm$ 0.0765
Delhi	35.31 $\pm$ 0.6732	0.291 $\pm$ 0.9834	18.8 $\pm$ 0.6875	61.34 $\pm$ 0.4590	63.43 $\pm$ 0.3054
Kerala	37.87 $\pm$ 0.2929	0.301 $\pm$ 0.8065	22.2 $\pm$ 0.3564	65.98 $\pm$ 0.1098	68.87 $\pm$ 0.2054
Banglore	34.31 $\pm$ 0.5698	0.293 $\pm$ 0.5042	20.5 $\pm$ 0.6754	60.54 $\pm$ 0.3076	62.43 $\pm$ 0.4021
Control(BHT)	45.63 $\pm$ 0.5052	0.613 $\pm$ 0.6489	30.4 $\pm$ 0.5434	78.63 $\pm$ 0.4066	81.65 $\pm$ 0.0665

***Mean $\pm$ standard error of three replicates**

Table 6: Evaluation of antibacterial activity of diverse solvent extracts of leaves samples of *Bacopa monnieri* (L.) Pennell from different geographical regions.

Leaves samples of different geographical regions	Solvent extracts	Name of gram positive bacteria		Name of gram negative bacteria	
		*Zone of Inhibition (mm)			
		BS	SA	EC	PA
Jammu	Water Extract	–	–	–	–
	Chloroform Extract	14 ± 0.6453	16 ± 0.2314	11 ± 0.6578	12± 0.4325
	Acetone Extract	13± 0.6754	14 ± 0.5429	–	–
	Ethanol Extract	15 ± 0.8765	18 ± 0.9812	13 ± 0.2367	14 ± 0.3478
Gentamycin (10µg/ml)		20 ± 0.8098	25± 0.5432	21 ± 0.9043	20 ± 0.3409
Kerala	Water Extract	–	–	–	–
	Chloroform Extract	13 ± 0.2309	15± 0.5612	10± 0.4310	11 ± 0.3208
	Acetone Extract	12 ± 0.7429	13 ± 0.4208	–	–
	Ethanol Extract	13 ± 0.9643	16 ± 0.6509	11 ± 0.5430	13 ± 0.4320
Delhi	Water Extract	–	–	–	–
	Chloroform Extract	12± 0.6320	14 ± 0.3108	09 ± 0.6501	10 ± 0.5430
	Acetone Extract	11 ± 0.8709	12± 0.6506	–	–
	Ethanol Extract	12 ± 0.4308	15± 0.4208	10 ± 0.4308	13 ± 0.3208
Banglore	Water Extract	–	–	–	–
	Chloroform Extract	11 ± 0.3098	13 ± 0.6403	10 ± 0.7605	11 ± 0.4508
	Acetone Extract	10± 0.4309	13 ± 0.5409	–	–
	Ethanol Extract	11 ± 0.8706	14 ± 0.3408	10 ± 0.2306	13 ± 0.5043

*Mean $\pm$ standard error of three replicates

Figures

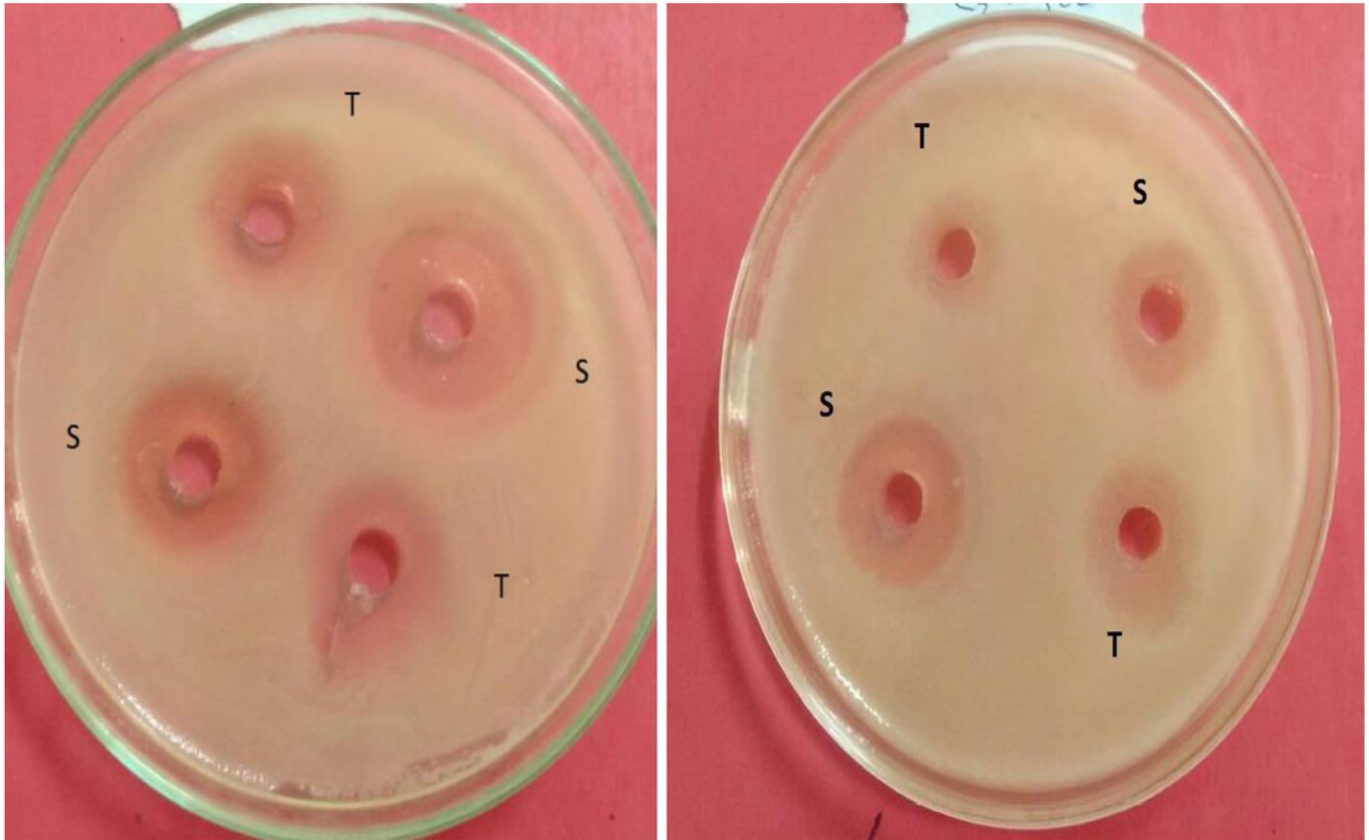


Figure 1

A) Ethanol extract (1000 μ g/ml) of leaves sample of Jammu against *B. subtilis*

B) Ethanol extract (1000 μ g/ml) of leaves sample of Jammu against *S. aureus*

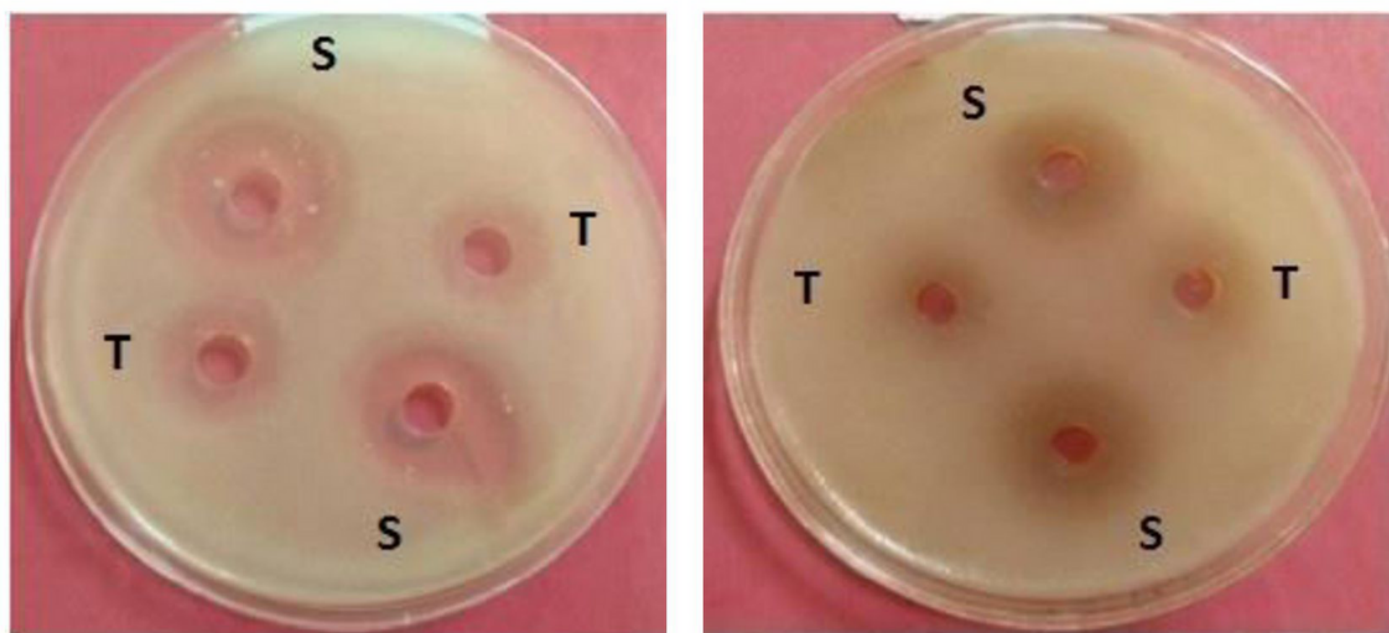


Figure 2

A) Ethanol extract (500µg/ml) of leaves sample of Jammu against *P. aeruginosa*

B) Ethanol extract (500µg/ml) of leaves sample of Jammu against *E. coli*