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Determination of Antimicrobial Efficiency with Reference to *Bacopa monnieri* (L.) Phytochemical Evaluation

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Abstract

The *Bacopa monnieri* (L.) was one of the traditional medicinal plants. These are commonly called as "Brahmi" and the Tamil vernacular name is "Neerbrahmmi". The present study was focused on the analysis of phytoconstituents and antimicrobial activity of *B. monnieri* leaves. The phytochemical and antimicrobial activity was performed with different polar and non-polar solvents. Totally 13 phytochemical compounds were observed from *B. monnieri* extract. The following phytochemical compounds such as, alkaloids, amino acids, coumarins, flavonoids, glycosides, phenols, phlobatannins, quinones, reducing sugars, saponins, steroids, tannins and terpenoids were recorded respectively. Antibacterial activity was performed against clinical bacteria like *Clostridium butyricum, Escherichia coli, Staphylococcus aureus* and *Streptococcus pneumoniae* and similarly found the antifungal activity against clinical fungi such as, *Aspergillus flavus, A. niger, Aspergillus* sp. and *Penicillium* sp. The aqueous extract of *B. monnieri* was showed maximum zone of inhibition against *Clostridium butyricum* followed by *Escherichia coli, Staphylococcus aureus* and *Streptococcus pneumoniae.* The benzene extract of *B. monnieri* was expressed maximum zone of inhibition against *S. aureus* followed by *E. coli, S. pneumoniae* and *C. butyricum.* According to the antifungal activity of *B. monnieri* aqueous extract showed the maximum zone of inhibition against *Penicillium* sp. followed by *Aspergillus* sp., *A. flavus* and *A. niger.* In the benzene extract showed maximum zone of inhibition against *A. niger* followed by *Aspergillus* sp., *Penicillium* sp. and *A. flavus* at 100µl concentration.

***Key words*:** *Bacopa monnieri,* Aqueous and methanol, Phytocompounds, Antibacterial, Antifungal activity

The plants are considered as a primary source of pharmaceutical products [1]. The consumption of plant materials are showed significant health benefits for human and animals [2]. In the human history was had been better remedies for several disorders and diseases by plant [3]. Plants have long been regarded as a rich source of necessary and secure medicines. Around the world, herbal remedies have traditionally been regarded as the foundation of the basic healthcare system. Around 80% of people worldwide rely on conventional treatments [4-5]. An eighty percentage of contemporary medications, according to resources, are made either directly or indirectly from plant extract [6]. In past few centuries peoples were used the *B. monnieri* as a food and therapeutical agent against disorders and diseases [7-8].

Natural therapy have many benefits, such as less side effects, increased patient tolerance, lower costs, wide acceptance due to long-term usage histories, and sustainability. Studies on the phytochemistry and pharmacology of natural compounds have resulted in the discovery and development of numerous typical meditations [9-10]. In the past, medicinal plants have been known to have a wide range of elements with known therapeutic benefits [11]. In human society from time immemorial medicinal plants have played an important role in the prevention and control of diseases. Development of science and technology and the modern medicine has resulted in increased and effective usage of plant-based medicines [12]. The world's oldest system of medicine, ayurveda, has its roots in India and dates back more than 3,000 years. It is the most traditional and pure form of healthcare. Herbs and medications known as nootropics are used to improve memory, cognition, mood, and other aspects of mental functions [13].

Bacopa monnieri (L.)

The plant is classified as endangered because it is overused to make medicine [14]. *Bacopa monnieri* (L.) is an important medicinal plant of the family Plantaginaceae used in traditional medicine to treat various nervous disorders and for promoting memory and intellect. Some important medicinal uses of the plant *B. monnieri* for treatment of different diseases like memory enhancer [15], cardiac tonic, epilepsy, bronchial and diarrheal ailments, malaria, hair fall, headache and snakebite [16]. These properties are attributed to the active principles of the plant specially 'Bacosides' and many others. Bacoside- A, a saponin found in almost all the parts of the plant [17-20]. In last few decades have to caused several microbial infections and their advanced treatment also find. But the

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natural therapies were not done. So, the present investigation was aimed to natural treatment for microbial infections. The estimation of qualitative and quantitative phytochemicals of *B. monnieri* leaves extract with different solvents.

Bacopa monnieri (L.)

MATERIALS AND METHODS

Collection of plant material

Bacopa monnieri (L.) plant leaves were collected from the Campus of Periyar University (11.7188°N and 78.0779°E), Salem.

Preparation of plant extracts [21]

The fresh leaves of *B. monnieri* (L.) were washed immediately after collection and chopped into small pieces, air dried and ground into powder. The resulting powder was soaked in an Erlenmeyer flask with hexane, benzene, methanol and aqueous and left for seven days allowing occasional stirring of the flask. Filtrate obtained filtered through cheesecloth and Whatman filter paper No. 1 was concentrated under reduced pressure at the temperature below 50°C using a rotatory evaporator.

Qualitative phytochemcial analysis of B. monnieri (L.) [22]

The following phytochemicals such as alkaloids, amino acids, chalcones, coumarins, flavonoids, glycosides, phenols, phlobatannins, quinones, reducing sugars, saponins, steroids, tannins and terpenoids were determined in hexane, benzene, methanol and aqueous by the standard methodologies.

Quantitative phytochemcial analysis of Bacopa monnieri (L.)
Estimation of alkaloids [23]

A part of extract residue was dissolved in 2N HCL and then filtered. 1 ml of this solution was transferred to separatory funnel and washed with 10 ml chloroform. The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of Bromocresol green (BCG) and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and complex extracted with 4 ml chloroform by vigorous shaking, the extract was then collected in a 10 ml volumetric flask and diluted with chloroform.

Accurately measured aliquots of Atropine standard solution were transferred to different separatory funnels. Then 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution was taken and the mixture was shaken with extract with 4 ml of chloroform. The extracts were then collected in 10 ml volumetric flask and then diluted to adjust solution with chloroform. The absorbance of the complex in chloroform was measured at spectrum of 470 nm in UV-Spectrophotometer against the blank prepared as above but without Atropine.

Estimation of amino acids [24]

One ml of the *B.monnieri* extract was pipetted out into a test tube. A drop of methyl red indicator was added. The sample was neutralized with 1 ml of 0.1 N sodium hydroxide. To this, 1 ml of ninhydrin reagent was added and mixed thoroughly. The content of the test tube was heated for 20 minutes in a boiling water bath. Five ml of the diluents solution was added and heated in water bath for 10 minutes. The tubes were cooled under the running water and the contents were mixed thoroughly. Blank was prepared with 1 ml of distilled water or ethanol. The absorbance was read at 570 nm in a UV-spectrophotometer.

Estimation of coumarins [25]

Powdered material (2.5 g) was added to a beaker containing 25 ml of water, methanol, chloroform or n-hexane placed in a shaker water bath adjusted at 37°C for 24 hours. The extracts were filtered using Whatman No. 1 filter paper and the resulted solutions were concentrated under reduced pressure and weighed. Coumarins Stored in amber tightly closed containers apparently labelled and kept in the refrigerator until used for phytochemical screening tests.

Estimation of flavonoids [26]

The aluminum chloride colorimetric method was used for the determination of flavonoid content of the sample. The flavonoid determination, quercetin was used to make the standard calibration curve. Stock quercetin solution was prepared by dissolving 5.0 mg quercetin in 1.0 mL methanol, then the standard solutions of quercetin were prepared by serial dilutions using methanol (5–200μg/mL). An amount of 0.6 mL diluted standard quercetin solutions or extracts was separately mixed with 0.6mL of 2% aluminum chloride. After mixing, the solution was incubated for 60 min at room temperature. The absorbance of the reaction mixtures was measured against blank at 420nm wavelength with a Varian UV-Vis spectrophotometer. The concentration of total flavonoid content in the test samples was calculated from the calibration plot and expressed as mg quercetin equivalent (QE)/g of dried plant material. All the determinations were carried out in triplicate.

Estimation of glycosides [27]

One gram plant extract dissolved with 50mL of distilled water and filtered. Taken 1mL filtrate then added 4mL of alkaline pirate solution. The mixture was boiled for 5 min and allowed to cool. The absorbance was read at 490 nm.

Estimation of phenol compounds [28]

The determination of phenol content of the plant extract was performed using the Folin– Ciocalteu reagent with modifications. Two hundred and fifty µL of ethanolic extract were mixed with 2.5 mL of distilled water, followed by 125µL of 1N Folin–Ciocalteu reagent and stirred for 5 min. Finally, 375 µL of 20% Na_2CO_3 solution was added and kept up in dark

conditions for 2 hours at room temperature. Absorbance was read using spectrophotometer at 760 nm wavelength. Total phenolic contents were estimated using a gallic acid standard curve.

Estimation of quinones [29]

The extract (10.00g) was accurately weighed and added 30ml distilled water. The mixture was mixed and incubated at 15 minutes for water bath. The flask was allowed to cool, weighed, adjusted to the original weight with water and the mixture was centrifuged at 4000 rpm for 10 minutes. Twenty milliliters of the supernatant liquid was transferred to a separating funnel and acidified with 2MHCl. Fifteen milliliters of chloroform was added, the mixture was extracted and the chloroform layer was discarded. The extraction was done triplicate. The UV absorbance was measured at 515nm.

Estimation of reducing sugars [30]

100mg of sample was hydrolyzed in a boiling tube with 5ml of 2.5N HCl in a boiling water bath for a period of 3hrs. It was cooled at room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100ml by using distilled water. From this 0.2ml of sample was pipetted out and made up the volume to one ml with distilled water. Then one ml of phenol reagent was added and followed by 5.0ml of sulphuric acid. The tubes were kept at 25-30°C for 20min. The absorbance was read at 490nm.

Estimation of saponins [31]

One ml of test sample were mixed with 80% methanol in 2ml, then added 2ml of 72% sulphuric acid solution were added, then mixed well and heated on a water bath at 600˚C for 10 minutes, absorbance was measured at 544nm against reagent blank.

Estimation of steroids [32]

One ml of extract of different solvents like acetone, ethanol was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5ml). The mixture was heated in a water-bath maintained at 70±20°C for 30 minutes with occasional shaking and made up to the mark with distilled water. The absorbance was measured at 780nm against the reagent blank.

Estimation of tannins [33]

Five hundred mg of the sample was weighed into 100ml plastic bottle. 50ml of distilled water was shaken for one hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pipette out into a tube and mixed with 3ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120nm wavelengths, within 10 minutes. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannic acid.

Estimation of terpenoids [34]

Dried plant extract 100mg (wi) was taken and soaked in 9mL of ethanol for 24 hour. The extracts were filtered with 10mL of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and weighed for its complete drying (wf). The Ether was evaporated and the yield (%) of total terpenoids contents was measured by using the following formula.

$$(wi - wf / wi \times 100)$$

Antimicrobial activity [35]

Bacterial strains

The common clinical pathogens of bacteria like *Clostridium butyricum* (IBRI187), *Escherichia coli* (IBRI102), *Staphylococcus aureus* (IBRI112) and *Streptococcus pneumoniae* (IBRI195) were procured from Indian Biotrack Research Institute, Thanjavur – 613 005.

Fungal strains

The human clinical fungal pathogens of *Aspergillus flavus* (IBRI185), *A. niger* (IBRI144), *Aspergillus* sp. (IBRI131) and *Penicillium* sp. (IBRI199) were procured from Indian Biotrack Research Institute, Thanjavur – 613 005.

Preparation of B. monnieri leaves extract

The fresh leaves of *B. monnieri* were washed and chopped into small pieces, air dried and ground into powder. The resulting powder was soaked in an Erlenmeyer flask which is containing aqueous and benzene solvents for seven days allowing occasional stirring of the flask. Filtrate obtained through cheesecloth and Whatman filter paper No. 1 was concentrated under reduced pressure at the temperature below 50°C using a rotatory evaporator.

Media preparation for antimicrobial activity

Nutrient agar

In 1000ml distilled water containing peptone (5g), sodium chloride (5g), HM peptone B (1.5g), yeast extract (1.5g) and Agar (15g) dissolved medium was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 mins. The pH was adjusted to 7.4±0.2, cool to 45-50°C then mixed well and poured into sterile petri plates.

Potato dextrose agar

In 1000ml distilled water containing 200g potatoes infusion then added dextrose (20g) and agar (15g). The medium were maintained with pH 5.6±0.2 cool to 45-50°C then mixed well and poured into sterile petri plates.

Antimicrobial activity [36]

The antimicrobial activity was carried out with 24 hours bacterial cultures of *Clostridium butyricum*, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae* and 48 hours of fungal cultures *Aspergillus flavus*, *A. niger*, *Aspergillus* sp. and *Penicillium* sp., with different solvents of aqueous and benzene extracts of *Bacopa monnieri* was tested separately using Agar well diffusion method. The nutrient agar and Mueller Hinton agar plates were swabbed with bacterial and fungal strains were individually inoculated and maintained. A well 6mm diameter was made using a sterile cork borer. The different concentration (25, 50, 75 and 100µl) of plant extracts were introduced in the well. The plates were incubated at 37±2°C for 24hrs and antifungal assay plates were incubated at 28±2°C for 48hrs and every 24hrs the results were measured and tabulated.

Statistical analysis

Excel 2010 was used to initially analyze data and create a database for drawing related charts. Statistical analyses were performed using standard procedures for a randomized plot design.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The present investigation was aimed to determine the phytochemical compounds from the medicinal plant of *B. monnieri* (L.) with different solvents such as hexane, benzene, methanol and aqueous were estimated. Alkaloids, amino acids, coumarins, flavonoids, glycosides, phenols, quinones, reducing sugars, saponins, steroids, tannins and terpenoids were analyzed.

Hexane extract of *Bacopa monnieri* (L.) sample containing alkaloids, phenols, reducing sugars and saponins were observed. Benzene extract of *Bacopa monnieri* (L.) leaves has been alkaloids, coumarins, flavonoids, phenols and saponins were represented respectively. Methanol extract of *Bacopa monnieri* (L.) leaves containing alkaloids and flavonoids were recorded. An aqueous extract of *Bacopa monnieri* (L.) leaves contain alkaloids, amino acids, coumarins, flavonoids, glycosides, quinones, reducing sugars, saponins, steroids and terpenoids were founded (Table 1).

Table 1 Qualitative phytochemical analysis of *B. monnieri* with different solvents

Name of the phytoconstituents	Hexane	Benzene	Methanol	Aqueous
Alkaloids	+	+	+	+
Amino acids	-	-	-	+
Coumarins	-	+	-	+
Flavonoids	-	+	+	+
Glycosides	-	-	+	+
Phenols	+	+	-	-
Quinones	-	-	-	+
Reducing sugars	+	-	-	+
Saponins	+	+	-	+
Steroids	-	-	-	+
Tannins	-	-	-	-
Terpenoids	-	-	-	+

(+) Present; (-) Absent

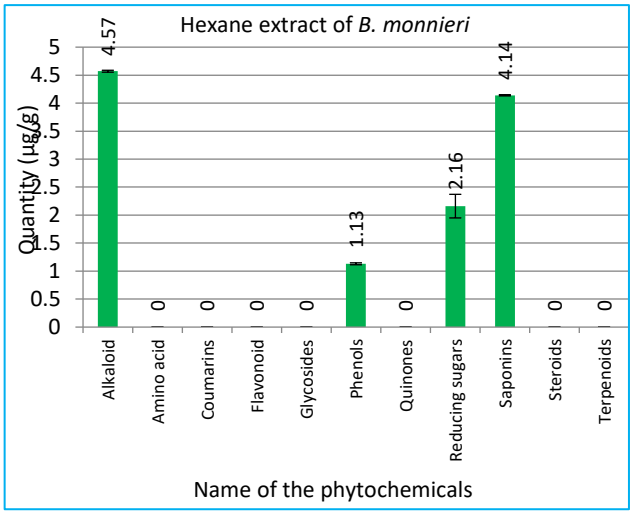

Fig 1 Quantitative phytochemical analysis of *B. monnieri* with hexane solvent

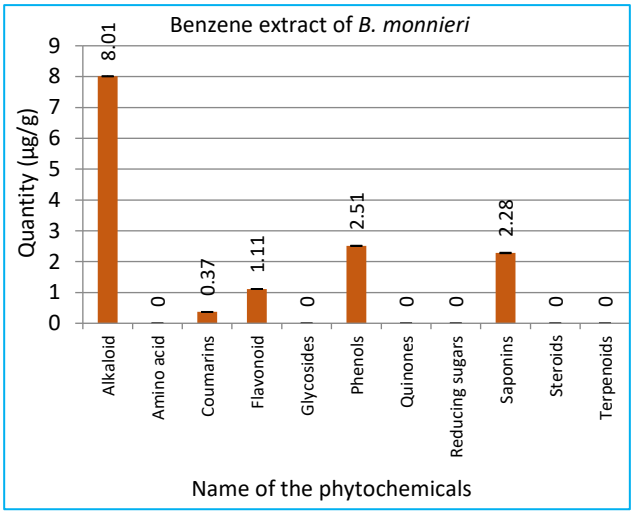

Fig 2 Quantitative phytochemical analysis of *B. monnieri* with benzene solvent

Quantitative phytochemical analysis

Alkaloids (4.57±0.017µg/g), phenols (1.13±0.017µg/g) and saponins (4.14±0.011µg/g) present in hexane extract of *Bacopa monnieri* (L.) (Fig 1). Alkaloids (8.01±0.012µg/g), coumarins (0.37±0.007µg/g), flavonoids (1.11±0.002µg/g), phenols (2.51±0.014µg/g) and saponins (2.28±0.017µg/g) present in benzene extract of *Bacopa monnieri* (L.) (Fig 2). Alkaloids (5.53±0.024µg/g) and flavonoids (0.51±0.001µg/g) were present in methanol extract (Fig 3). Alkaloids (4.29±0.005µg/g), amino acids (1.96±0.017µg/g), coumarins (1.21±0.021µg/g), flavonoids (1.21±0.021µg/g), glycosides (0.39±0.011µg/g), quinones (1.77±0.013µg/g), saponins (9.02±0.021µg/g), steroids (3.18±0.001µg/g) and terpenoids (2.06±0.003µg/g) present in aqueous extract of *Bacopa monnieri* (L.) were recorded respectively (Fig 4).

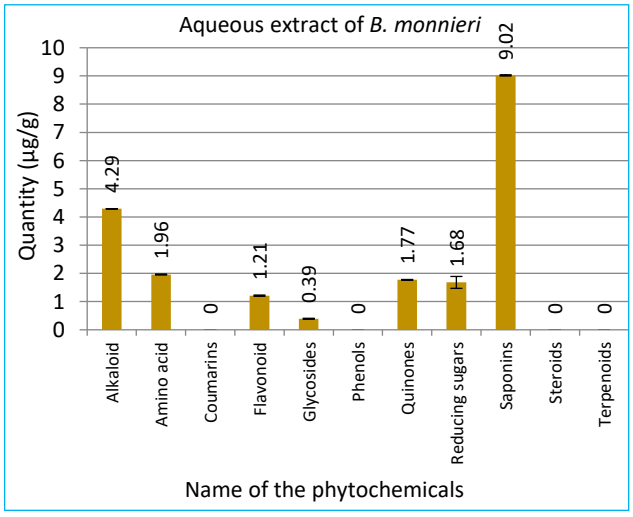

Fig 3 Quantitative phytochemical analysis of *B. monnieri* with methanol solvent

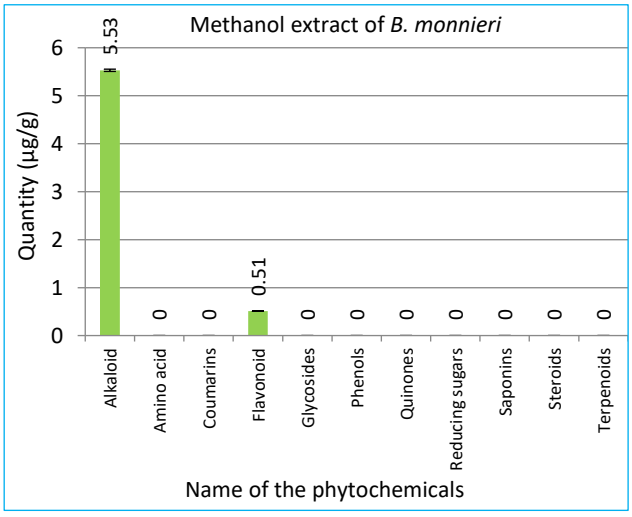

Fig 4 Quantitative phytochemical analysis of *B. monnieri* with aqueous solvent

Antimicrobial activity

Antibacterial activity

Antibacterial activity was done *B. monnieri* with different solvents like aqueous and benzene. In aqueous extracts of *B. monnieri* were performed against *Clostridium butyricum* (12.3±0.02mm), *Escherichia coli* (10.1±0.05mm), *Staphylococcus aureus* (9.33±0.16mm) and *Streptococcus pnemoniae* (8.33±0.16mm) with100µl, 200µl expressed following zone of inhibition *Clostridium butyricum* (10.2±0.33mm), *Escherichia coli* (12.3±0.16mm), *Staphylococcus aureus* (12.1±0.28mm) and *Streptococcus pneumoniae* (9.33±0.16mm), In 300µl performed *Clostridium butyricum* (19.5±0.02mm), *Escherichia coli* (14.5±0.00mm), *Staphylococcus aureus* (13.1±0.16mm) and *Streptococcus pneumoniae* (10.0±0.00mm) and 400µl *Clostridium butyricum* (20.2±0.03mm), *Escherichia coli* (17.5±0.28 mm), *Staphylococcus aureus* (14.8±0.16 mm) and *Streptococcus pnemoniae* (10.5±0.00mm) recorded respectively (Table 1).

Table 1 Effect of antibacterial activity using *Bacopa monnieri* (L.) with aqueous extract

Name of the bacteria	Zone of inhibition (mm)			
	100µl	200µl	300µl	400µl
Clostridium butyricum	12.3± 0.02	10.2±0.33	19.5±0.02	20.2±0.03
Escherichia coli	10.1±0.05	12.3±0.16	14.5±0.00	17.5±0.28
Staphylococcus aureus	9.33±0.16	12.1±0.28	13.1±0.16	14.8±0.16
Streptococcus pneumoniae	8.33±0.16	9.33±0.16	10.0±0.00	10.5±0.00

Values are expressed in standard and error mean

Table 2 Effect of antibacterial activity using *Bacopa monnieri* (L.) with benzene extract

Name of the bacteria	Zone of inhibition (mm)			
	100µl	200µl	300µl	400µl
Clostridium butyricum	10.0±0.02	12.0±0.03	13.8±0.02	20.0±0.00
Escherichia coli	12.3±0.16	13.3±0.16	14.1±0.16	17.5±0.28
Staphylococcus aureus	10.1±0.28	13.1±0.28	15.5±0.28	18.1±0.28
Streptococcus pneumoniae	8.16±0.16	10.1±0.16	13.3±0.16	14.1±0.16

Values are expressed in standard and error mean

In Benzene extracts *Bacopa monnieri* (L.)were performed against *Clostridium butyricum* (10.0±0.02mm), *Escherichia coli* (12.3±0.16mm), *Staphylococcus aureus* (10.1±0.28mm) and *Streptococcus pneumoniae* (8.16±0.16mm) with 100µl, 200µl expressed following zone of inhibition *Clostridium butyricum* (12.0± 0.03mm), *Escherichia coli* (13.3±0.16mm), *Staphylococcus aureus* (13.1±0.28mm) and *Streptococcus pneumoniae* (13.3±0.16mm), In 300µl performed *Clostridium butyricum* (13.8±0.02mm), *Escherichia coli* (14.1±0.16mm), *Staphylococcus aureus* (15.5±0.28mm) and *Streptococcus pneumoniae* (13.3±0.16mm) and 400µl *Clostridium butyricum* (20.0±0.00mm), *Escherichia coli* (17.5±0.28mm), *Staphylococcus aureus* (18.1±0.28mm) and *Streptococcus pneumoniae* (14.1±0.16mm) were inhibited found to be inhibited (Table 2).

Table 3 Effect of antifungal activity using *B. monnieri* with aqueous extract

Name of the fungi	Zone of inhibition (mm)			
	100µl	200µl	300µl	400µl
Aspergillus flavus	9.16±0.01	9.56±0.00	10.1±0.29	15.1±0.28
A. niger	8.62±0.03	9.91±0.21	10.4±0.03	10.6±0.03
Aspergillus sp.	12.3±0.59	15.6±0.24	16.7±0.37	17.3±0.52
Penicillium sp.	16.1±0.29	17.1±0.29	17.7±0.41	20.1±0.66

Values are expressed in standard and error mean

Table 4 Effect of antifungal activity using *B. monnieri* with benzene extract

Name of the fungi	Zone of inhibition (mm)			
	100µl	200µl	300µl	400µl
Aspergillus flavus	9.13±0.05	11.2±0.05	12.1±0.15	12.2±0.49
A. niger	9.33±0.01	15.1±0.29	17.1±0.29	19.1±0.03
Aspergillus sp.	13.1±1.07	13.9±0.61	14.1±0.28	16.8±0.31
Penicillium sp.	14.7±0.14	13.6±0.05	13.9±0.00	14.2±0.20

Values are expressed in standard and error mean

Antifungal activity

The antifungal properties of *Aspergillus flavus* (9.16±0.01mm), *A. niger* (8.62±0.03mm), *Aspergillus* sp., (12.3±0.59mm) and *Penicillium* sp. (16.1±0.29mm) by100µl concentration of plant extract respectively. The higher concentration 200µl expressed following zone of inhibition *Aspergillus flavus* (9.56±0.00mm), *A. niger* (9.91±0.21mm), *Aspergillus* sp., (15.6±0.24mm) and *Penicillium* sp (17.1±0.29mm), In 300µl performed which showed *Aspergillus flavus* (10.1±0.29mm), *A. niger* (10.4±0.03mm), *Aspergillus* sp., (16.7±0.37mm) and *Penicillium* sp. (17.7±0.41mm) and 400µl *Aspergillus flavus* (15.1±0.28mm), *A. niger* (10.6±0.03mm), *Aspergillus* sp., (17.3±0.52mm) and *Penicillium* sp. (20.1±0.66mm) zone of inhibition was observed in benzene extract of *Bacopa monnieri* (L.) were recorded respectively (Table 3).

The antifungal activities are exhibited the zone of inhibition against *Aspergillus flavus* (9.13±0.05mm),

Aspergillus niger (9.33±0.01mm), *Aspergillus* sp., (13.1±1.07mm) and *Penicillium* sp (14.7±0.14mm) with 100µl concentration were expressed. The 200µl concentration was performed in the zone of inhibition of *Aspergillus flavus* (11.2±0.05 mm), *Aspergillus niger* (15.1±0.29mm), *Aspergillus* sp., (13.9±0.61mm) and *Penicillium* sp (13.6±0.05mm), In 300µl performed *Aspergillus flavus* (12.1±0.15mm), *Aspergillus niger* (17.1±0.29mm), *Aspergillus* sp., (14.1±0.28mm) and *Penicillium* sp. (13.9±0.00mm) and 400µl *Aspergillus flavus* (12.2±0.49mm), *Aspergillus niger* (19.1±0.03mm), *Aspergillus* sp., (16.8±0.31mm) and *Penicillium* sp. (14.2±0.20mm) zone of inhibition was recorded in aqueous extract of *B. monnieri* were recorded respectively (Table 4).

Bacopa monnieri (L.) methanol and ethanol solvents showed the presence of carbohydrate, flavonoid, tannin, saponin, steroid, phyto-sterol and phenols. Similarly, the carbohydrate, flavonoid, saponin, steroid and Phyto steroid were observed from *B. monnieri* aqueous extract [37 & 38]. Alkaloids, amino acids, carbohydrates, flavonoids, phenolic compounds, protein, saponnin, steroids, tannins and terpenoids were determined from *B. monnier*. This study demonstrated the presence of high amount protein and tannin [39-41]. In methanol extract of *B. monnieri* have phenol, tannin, flavonoid, alkaloid, saponin, phlobatannin and cardiac glycoside [42]. *B. monnieri* aqueous extract had been following phytochemical compounds is carbohydrates, tannins, alkaloids, quinones, cardiac glycosides and phenols. These phytochemicals were considered as a potential phytochemical for drug related compounds. The minor phytochemical compounds of alkaloids, coumarins, flavonoids phenols and quinones were also founded [43].

Extraction of *Bacopa monnieri* in methanol, ethanol, aqueous, chloroform, acetone, dichloromethane, ethyl acetate and petroleum ether extract was determined. Phytochemical screening revealed that saponins, flavonoids, alkaloids, tannins, carbohydrates, proteins and steroids were presented in methanolic, aqueous and ethanolic extracts of *Bacopa monnieri*. The aqueous extract of *Bacopa monnieri* showed the presence of amino acids and methanolic, ethanolic extracts showed absence of amino acids. anthraquinone and glycosides were absent in methanolic, aqueous and ethanolic extracts of *Bacopa monnieri* and also reported the highest antifungal activity was observed in methanolic extract and maximum zone of inhibition was observed against *Aspergillus niger* and *Candida albicans* where as in aqueous extract no antifungal activity was observed. The zone of inhibition of methanolic extract was highest for *Candida albicans* at 1.25mg/ml concentrations, while for *Aspergillus niger* the highest zone of inhibition was observed at 2.5mg/ml and 1.25mg/ml concentrations. No antibacterial activity was observed against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* in aqueous and methanolic extracts of *Bacopa monnieri* L. in these concentrations [44].

The phytochemical analysis showed the presence of alkaloid and saponin in all three extracts. The acetone and ethanolic extract also showed the presence of glycosides and reducing sugars. Test for steroid and tannin was negative for some extract of *B. monnieri*. The test for resin showed negative result. Amino acid test was positive only in ethanolic extract of *B. monnieri* [45]. Phytochemical screening investigation indicated that the dry powder of whole plant contains tannin, phlobatannin, saponin, steroid, flavonoid, cardiac glycoside, phenol, carbohydrate and alkaloid [46]. The *B. monnieri* whole plant was subjected to preliminary phytochemical analyses, which revealed that it contains tannins, alkaloids, steroids, saponins, glycosides, flavonoids, resins, amino acids, carbohydrates, lipids, fixed oils, proteins and starch [47].

The aqueous and methanol extract of *Bacopa monnieri* leaves has been following phytochemicals such as alkaloids, flavonoids, saponins, phenols, resins, tannins, terpenoids, xanthoproteins, quinines and glycosides but steroids and carboxylic acid were not determined. Antibacterial activity also observed in aqueous and methanol extract were performed effectively against the *Bacillus subtilis* when compared *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* [48].

An *in-silico* approaches were used for anti-neurodegenerative property of *B. monnieri* phytoconstituents and the Vitamin E, Benzene propanoic acid, 3,5-bis (1,1-dimethylethyl)- 4hydroxy-, methyl ester (BPA), Stigmasterol and Nonacosane was acted as significant role in several neurodegenerative disorders [49]. Antibacterial activity of *Bacopa monnieri* plant extract was performed against *Staphylococcus aureus* and produced extraordinary zone of inhibition, but more zone of inhibition was produced by tetracycline and ampicillin antibiotics [50]. The infections were successfully combatted by all of the evaluated *Bacopa monnieri* extracts. This study shows that *Bacopa monnieri's* methanolic extract had a greater inhibitory impact on *Klebsiella pneumoniae* and *Staphylococcus aureus* [51].

Similarly, the *S. aureus* was showed minimum inhibitory concentration effect of ethanol and Diethyl ether extract of *B. monnieri* at a concentration of 300µg. Ethyl acetate and ethanolic extract has a moderate effect over *S. aureus*. But the aqueous extract does not show any inhibitory effect and also studied about the antifungal ability of various extracts of *B. monnieri* against *Candida albicans* and *A. niger*. Ethanolic extract was found to have maximum activity followed by diethyl ether extract. Aqueous extract of *B. monnieri* does not show any inhibitions [52]. Antibacterial activity was performed against UTI pathogens like *Klebsiella pneumoniae* and *Proteus mirabilis* had the highest zone of inhibition in methanolic extract followed by ethanol and aqueous extract. The antibacterial activity of methanolic solvents of *B. monnieri* leaves was showed maximum inhibition against *Klebsiella pneumoniae.* The *Proteus mirabilis* was also highly inhibited by methanolic extract than the ethanolic extract of *B. monnieri* [37-38].

Agar disc diffusion tests were carried out to determine the antimicrobial effects of ethanolic, diethyl ether, ethyl acetate and aqueous extracts of *B. monnieri* against Gram-positive (*Staphylococcus aureus*), Gram-negative (*Escherichia coli*) bacterial strains and antifungal strains (*Aspergillus flavus* and *Candida albicans*). Among the various extracts, diethyl ether extracts of *B. monnieri* has an antibacterial potency against *Staphylococcus aureus* (gram positive), and ethyl acetate extract showed effects on *E. coli* (gram negative) at higher concentrations of 300µg/mL^{-1}. The ethanolic extract has potent antifungal activity against the fungus (*Aspergillus flavus* and *Candida albicans*) compared to diethyl ether and ethyl acetate-ether. Both extracts (diethyl ether and ethyl acetate) have a minimum antifungal effect while these extracts showed more inhibitory effects aqueous the tested bacteria [53]. All the extracts (ethanol, methanol and acetone) of *B. Monnieri* were more or less effective against microbes. Overall, methanolic extract proved more effective than other two extracts zone of inhibition. In case of MDR-UTI strain of *E. coli* methanolic extract exhibited maximum inhibition [54]. The bactericidal and bacteriostatic activity was potent in the *B. monnieri* methanol solvent against UTI and RTI pathogens [55]. Similarly, most potent for MIC against the clinical pathogens

are bacteria as well as fungi with the MIC range of 500 – 7.81µg/ml. The MIC of *Bacopa monnieri* (L.) leaves extracts treated against bacterial strains were ranged 25 to 100µl/ml. Maximum the gram-positive bacteria was inhibited by *B. monnieri* extracts at lower concentration [49]. The present investigation revealed that not only gram-negative bacteria as well as gram positive bacteria also inhibited with minimum concentration of *B. monnieri* aqueous and methanol extracts. The propolis, rosemary, clove, capsaicin and cumin extracts showed the minimum inhibition concentration against the Staphylococcus aureus [56-57].

CONCLUSION

Maximum number of phytochemical compounds was observed from aqueous extract of *B. monnieri.* All the tested extracts showed the presence of high amount alkaloid and saponin. Both of the compounds are generally possessing the antimicrobial properties. These enormous amounts of phytochemical compounds were responsible for the antimicrobial properties of *B. monnieri.* In the experiment of antimicrobial activity was also demonstrated their antimicrobial efficiency of *B. monnieri*. The antimicrobial efficiency was gradually increased from lower concentration to higher concentration of plant extract. Hence, the present study was suggested the *B. monnieri* plant extract for the treatment of microbial infections on the human skin.

Authors contribution statement

Dr. R. Magalingam is designed and finalized the manuscript of study, Mrs. N. Amudha was collecting samples and analyzed the work and prepared the draft manuscript, Dr. K. Selvam helped for sample collection. All authors read and approve the final version of the manuscript.

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