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Research Article

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Analysis of Phytoconstituents and Antimicrobial Properties of *Bacopa monnieri* (L.) Against Clinical Pathogen

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Abstract: There are numerous therapeutic uses for medicinal plants in treating and managing numerous diseases. Traditional medicinal plants were mostly needed in the current scenario. The world's warmer and wetlands regions are home to the medicinal herb *Bacopa monnieri* (L.). It has been utilized for ages in Ayurvedic systems. The *Bacopa monnieri* was one of the traditional medicinal plants. These are commonly called "Brahmi," and the Tamil vernacular name is "Neerbrahmmi." It is easy to cultivate and grow in all seasons. These peculiar active ingredients, also known as memory enhancement and phytoconstituents were enriched in this medicinal plant. The present study focused on analyzing phytoconstituents and the antimicrobial activity of *Bacopa monnieri* leaves. The efficacy of phytoconstituents on the antimicrobial activities concerning minimum inhibitory concentrations was performed with different polar and non-polar solvents of the respective plant. The phytoconstituents such as alkaloids, amino acids, coumarins, flavonoids, glycosides, phenols, phlobatannins, quinones, reducing sugars, saponins, steroids, tannins, and terpenoids were recorded, respectively. Antibacterial activity performed with following human clinical bacteria like *Clostridium butyricum, Escherichia coli, Staphylococcus aureus,* and *Streptococcus pneumoniae,* and the antifungal activity against human clinical such as *Aspergillus flavus, A.niger, Aspergillus* sp., and *Penicillium* sp. were determined. Among the two solvent extractions, the aqueous extract showed a more significant result than the benzene extract. According to the MIC, the antimicrobial properties of *B.monnieri*with different extracts were analyzed. The very minimum concentration has shown better inhibitions against microbes. The present study explored the antimicrobial properties of human clinical pathogens. So, the *B.monnieri*aqueous extract of leaves was suggested for the treatment of microbial infections.

Keywords: *Bacopa monnieri,* Different Solvents, Phytochemicals, Antimicrobial activity, MIC.

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1. INTRODUCTION

The primary source of pharmaceuticals, plants are essential to the global healthcare system[1]. The plant extracts acted as potential roles in the pharmacology field. The continued use of herbal plants demonstrates that these plants have important therapeutic characteristics that are beneficial for treating people and animals[2]. The Bible, the Rig Veda (4500-1600 BC), the Elliad, the Odyssey, and Herodotus' history all mention the use of plants to treat various human maladies[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].Compared to the root portion is used in various ways for about 30% of the plant[5]. According to resources, eighty percent of contemporary medications are made directly or indirectly from plant extract[6]. Sushruta Samhita, Charak Samhita, and Atharva Veda, which make up the Indian System of Medicine, refer to herbs that have a Prabhava (particular action) on the mind and memory as Medhya Rasayana*Bacopa monnieri* (L.) Penn. and *Herpestis monnieri*, sometimes known as water hyssop, are regarded as two of the most significant medicinal plants in India[7].Drug utilization is the marketing, distribution, prescribing, and use of pharmaceuticals in society with a focus on the subsequent medical. Identifying ineffective drug therapy and identifying remedies to therapy-related problems were essential[8].Natural therapy has many benefits, including fewer 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].Medicinal plants have been known to have a wide range of elements with known therapeutic benefits. The widely held belief that "green medicine is safe" appears to be the primary cause of the increasing interest in treatments derived from plants[11].In human society, from time immemorial, medicinal plants have played an important role in preventing and controlling diseases. WHO has confirmed that herbal medicines serve the health needs of about 80 percent of the world's population, especially for millions of people in the vast rural areas of developing countries. Developing science, technology, and 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 mental functioning[13].

1.1. 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 to promote memory and intellect. Some important medicinal uses of the plant *B. monnieri* are treating diseases like memory enhancer[15], cardiac tonic, epilepsy, bronchial and diarrheal ailments, malaria, hair fall, headache, and snakebite[16].It holds a very prominent place in the indigenous system of medicine due to its remarkable medicinal properties. These properties are attributed to the active principles of the plant, especially 'Bacosides' and many others. Bacoside- A, a saponin found in almost all the parts of the plant[17,18].*Bacopa monnieri* has anti-inflammatory, analgesic, antipyretic, sedative, antiepileptic, antioxidant, immuno-modulatory, memory-improving, anti-stress, anti-anxiety, and anticancer, among other therapeutic characteristics[19-20].In the last few decades, they have caused several microbial infections, and their advanced treatment has also been found. But the natural therapies were not done. So, the present investigation was aimed at natural treatment for microbial infections. Estimating qualitative and quantitative phytochemicals of *B.monnieri*leaf extract with different solvents. And its efficacy of antimicrobial properties against some clinical pathogens and a determination of MIC for the test plant leaves.

2. MATERIALS AND METHODS

2.1. 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, in June 2021 (Plate – 1).

plate 1: Collection of *Bacopa monnieri* (L.)

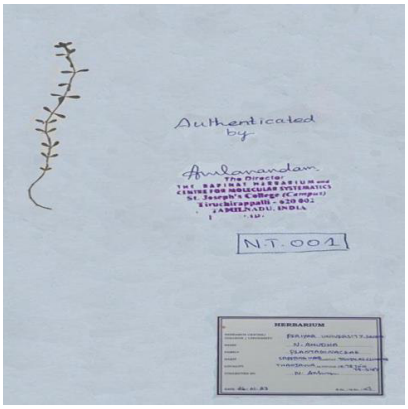

plate2: Taxonomy identification of *Bacopa monnieri* (L.)

Bacopa monnieri (L.) is the most valuable source of medical benefit in many respects. Plant collection and identification: The plant has been authenticated by Rapinat herbarium, St. Joseph's College (Autonomous), Trichy (Herbarium No. NT001) (Plate - 2).

2.2. *Preparation of Plant extract*[21]

The fresh leaves of *B. monnieri* (L.) were washed immediately after collection, 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. The filtrate obtained was filtered through cheesecloth, and Whatman filter paper No. 1 was concentrated under reduced pressure below 50°C using a rotatory evaporator (Plate – 3).

2.3. *Qualitative phytochemical 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 following standard methodology (Table – 1).

Table - 1: Methodology for phytochemical determination

Tests	Description	Inference
Alkaloids	Five ml of the *B.monnieri* (L.) extracts were taken into a flask and stirred with 5 ml of 1% aqueous hydrochloric acid in a steam bath. Then, 1 ml of that filtrate was treated with a few drops of Dragendorff's reagent.	Appear blue or black color.
Aminoacid	The *B.monnieri*extracts and added 1%ninhydrin solution. The reduction product obtained from ninhydrin then reacts with NH_3.	Appear blue color
Coumarins	To 1ml of extract, 1ml of 10% NaOH was added.	The formation of a yellow color indicated the presence of coumarins
Flavonoids	When 5 ml of diluted ammonia solution was added to the aqueous filtrate of the test sample, followed by the addition of concentrated H_2SO_4.	Appeared yellow colour
Glycosides	2ml of *B.monnieri*extract with dilute HCl and 2ml sodium nitrate in pyridine and sodium hydroxide solution were added.	Formation of pink to blood red color
Phenols	To 1ml of the extract, 2ml of distilled water and 10% ferric chloride was added.	The formation of blue or green color indicated the presence of phenols
Quinones	To 1ml of extract, 1ml of concentrated Sulphuric acid was added.	The formation of red color indicated the presence of quinines
Reducing sugars	About 3ml of the plant extract was weighed into a 150ml beaker, and a small quantity of anthrone and a few drops of concentrated sulphuric acid were added to the mixture.	The formation of green coloration indicated the presence of sugar.
Saponins	Five ml of the *B.monnieri*extracts and 5 ml distilled water in a test tube.	Appeared of Frothing on the tube
Steroids	Two ml of acetic anhydride was added to 0.5 ml of *B.monnieri* extract, and on the sides of the test tube, 2 ml of sulphuric acid was added.	Appeared violet or blue-green
Tannins	Five ml of the *B.monnieri* extracts and 100 ml of distilled water were filtered, and ferric chloride reagents were added.	Appeared blue-black or blue-green precipitated
Terpenoids	Two ml of chloroform was added to 1 ml of the extract, and Conc. H_2SO_4 (3ml) was added to form a layer.	Appeared reddish brown

2.4. Quantitative phytochemical analysis of Bacopa monnieri(L.)

2.4.1. Estimation of Alkaloids[23]

Some of the extract residue was dissolved in 2N HCL and then filtered. 1 ml of this solution was transferred to a 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 separating 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 a 10 ml volumetric flask and diluted to adjust the solution with chloroform. The absorbance of the complex in chloroform Powdered material (2.5 g) was added to a beaker containing 25 ml of water, methanol, chloroform, or n-hexane and 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 resulting solutions were concentrated under reduced pressure and weighed. Coumarins are Stored in amber, tightly closed containers labeled and kept in the refrigerator until used for phytochemical screening tests.

2.4.4. Estimation of Flavonoids[26]

The aluminum chloride colorimetric method was used for the determination of the 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 were separately mixed with 0.6 mL 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 a 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.

2.4.5. 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.

2.4.6. Estimation of Phenol compounds[28]

Determining the 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 in dark conditions for 2 hours at room temperature. Absorbance was was measured at a spectrum of 470 nm in a UV-Spectrophotometer against the blank prepared as above but without Atropine.

2.4.2. 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 diluent solution was added and heated in a 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.

2.4.3. Estimation of Coumarins[25]

read using a spectrophotometer at 760 nm wavelength. Total phenolic contents were estimated using a gallic acid standard curve.

2.4.7. Estimation of Quinones[29]

The extract (10.00g) was accurately weighed and added 30ml distilled water. The mixture was mixed and incubated for 15 minutes in a water bath. The flask was allowed to cool, weighed, and 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 in triplicate. The UV absorbance was measured at 515nm.

2.4.8. Estimation of Reducing sugars[30]

100mg of the sample was hydrolyzed in a boiling tube with 5 ml of 2.5N HCl in a water bath for 3 hours. It was cooled at room temperature, and added solid sodium carbonate until effervescence ceases. The contents were centrifuged, and the supernatant was made to 100ml 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, followed by 5.0 ml of sulphuric acid. The tubes were kept at 25-30°C for 20min. The absorbance was read at 490nm.

2.4.9. Estimation of Saponins[31]

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

2.4.10. 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.

2.4.11. Estimation of Tannins[33]

Five hundred mg of the sample was weighed into a 100ml plastic bottle. 50ml of distilled water was shaken for one hour in a mechanical shaker. It was filtered into a 50ml volumetric flask and made up to the mark. Then, 5 ml of the filtrate was pipette out into a tube and mixed with 3 ml 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 color developed and read at the same wavelength. A standard was prepared using tannic acid.

2.4.12. Estimation of Terpenoids[34]

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

(wi-wf/wi×100).

2.5. Antimicrobial activity[35]

2.5.1. Bacterial strains

The human clinical pathogens of bacteria like *Clostridium butyricum* (MTCC 11078), *Escherichia coli* (MTCC 10312), *Staphylococcus aureus* (MTCC 10787), and *Streptococcus pneumoniae* (MTCC 2672) were procured from Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh.

2.5.2. Fungal strains

The human clinical fungal pathogens of *Aspergillus flavus* (MTCC 10281), *A.niger*(MTCC 12988), *Aspergillus* sp. (MTCC 6974), and *Penicillium* sp. (MTCC 12996) were procured from Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh.

2.5.3. 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 containing aqueous and benzene solvents for seven days, allowing occasional stirring of the flask. Using a rotatory evaporator, the Filtrate obtained through cheesecloth and Whatman filter paper No. 1 was concentrated under reduced pressure at a temperature below 50°C.

2.5.4. Media Preparation for Antimicrobial Activity

2.5.5. 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, cooled to 45-50°C, then mixed well and poured into sterile petri plates.

2.5.6. Potato Dextrose Agar

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

2.6. Antimicrobial activity[36]

The antimicrobial activity was carried out with 24 hrs bacterial cultures of *Clostridium butyricum, Escherichia coli, Staphylococcus aureus* and *Streptococcus pneumoniae* and 48 hrs 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 and were individually inoculated and maintained. A well of 6mm diameter was made using a sterile cork borer. The different concentrations (25, 50, 75, and 100µl) of plant extracts were introduced into 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.

2.7. Determination of Minimum Inhibitory Concentration (MIC)[37]

The microdilution method determined Minimum inhibitory concentration using serially diluted (2 folds) plant extract according to the National Committee for Clinical Laboratory Standards. The dilution of B. monnieri determined the MIC of the extract with concentrations of 500, 250, 125, 62.5,31.25, 15.62, and 7.81µg/ml. An equal volume of each extract and nutrient broth was mixed in a test tube. Specifically, 0.1 ml of standardized inoculums (1-2 × 107 cfu/ml) was added to each tube. The tubes were incubated aerobically at 37°C for 18-24 h. Two control tubes were maintained for each test batch. These included antibiotic control (a tube containing extract and growth media without inoculums) and organism control (a tube containing the growth medium, saline, and inoculums). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) was considered as MIC.

2.8. 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.

3. RESULTS

3.1. Qualitative phytochemical analysis

The present investigation 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.

Table 2: Qualitative phytochemical analysis of *Bacopa 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

An aqueous extract of *B. monnieri* leaves containing alkaloids, amino acids, coumarins, flavonoids, glycosides, quinones, reducing sugars, saponins, steroids, and terpenoids was found. Benzene extract of *B.monneiri*leaves has been Alkaloids, coumarins, flavonoids, phenols, and saponins were represented respectively. The hexane extract of respective samples containing Alkaloids, phenols, reducing sugars, and saponins were observed, and the methanol extract of *B.monnieri*leaves containing Alkaloids and Flavonoids were recorded respectively (Table 2).

3.1.1. Quantitative phytochemical analysis

Table 3: Quantitative phytochemical analysis of *Bacopa monnieri* with different solvents

Name of the Phytoconstituents	Quantity (µg/g)			
	Hexane	Benzene	Methanol	Aqueous
Alkaloid	4.57±0.017	8.01±0.012	5.53±0.024	4.29±0.005
Amino acid	-	-	-	1.96±0.017
Coumarins	-	0.37±0.007	-	-
Flavonoid	-	1.11±0.002	0.51±0.001	1.21±0.021
Glycosides	-	-	-	0.39±0.017
Phenols	1.13±0.017	2.51±0.014	-	-
Quinones	-	-	-	1.77±0.013
Reducing sugars	2.16±0.211	-	-	1.68±0.211
Saponins	4.14±0.011	2.28±0.017	-	9.02±0.021
Steroids	-	-	-	-
Terpenoids	-	-	-	-

Values are expressed in Standard and Error Mean.

The quantitative phytochemicals of *B.monnieri*with hexane extract found moderate alkaloids, phenols, reducing sugars, and saponins. The benzene extract of *B.monnieri*leaves has maximum alkaloid content followed by saponins, phenols, flavonoids, and coumarins. As per the methanol extract, quantitative phytochemicals of very mixed amounts of coumarins and alkaloids were observed. The organic solvent of aqueous was recorded as maximum phytochemicals such as alkaloids, amino acids, flavonoids, glycosides, quinines, reducing sugars, and saponins (Table 3).

3.2. Antimicrobial activity

3.2.1. Antibacterial activity

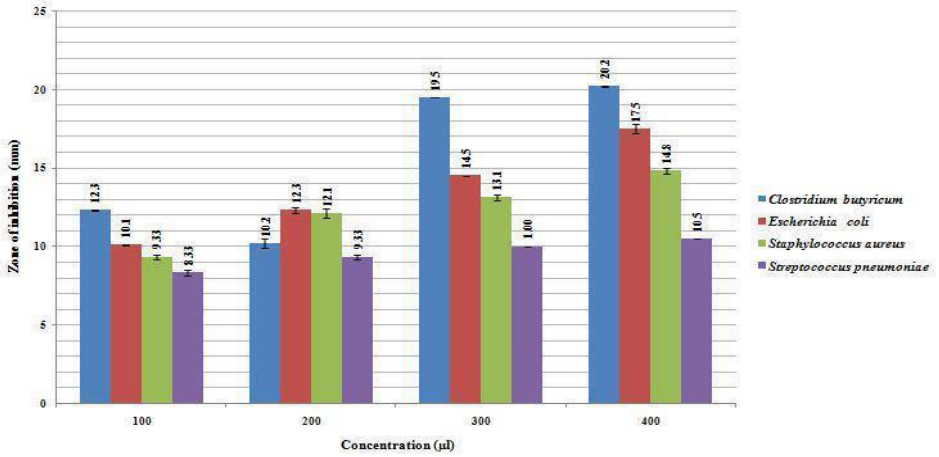

Fig 1: Effect of antibacterial activity using *Bacopamonnieri*with Aqueous extract

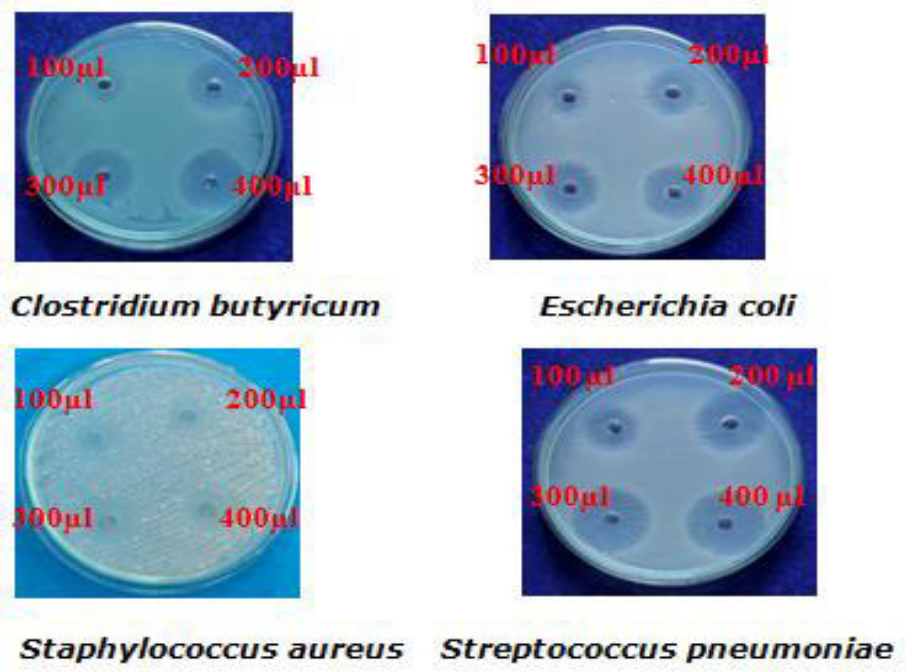

Plate 4: Effect on antibacterial activity of *Bacopa monnieri* Aqueous leaf extract

Antibacterial activity of *B.monnieri*was performed against *Clostridium butyricum, Escherichia coli, Staphylococcus aureus,* and *Streptococcus pneumoniae*. The maximum zone of inhibition was observed at 100µl of *B.monnieri*aqueous extract. Each concentration of benzene extracts showed gradually increased antibacterial activity. The maximum antibacterial activity was shown against *Clostridium butyricum,* followed by *Escherichia coli, Staphylococcus aureus,* and *Streptococcus pneumoniae* (Figure 1; Plate 4).

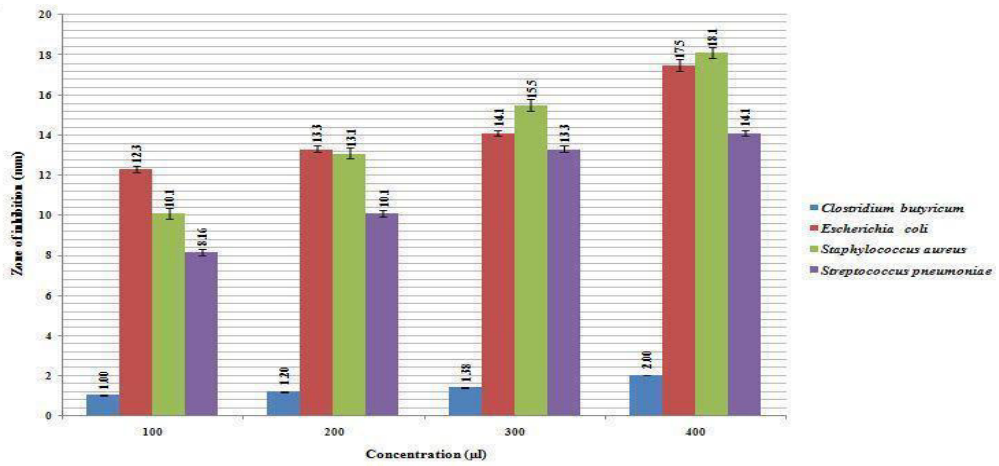

Fig 2: Effect of antibacterial activity using *Bacopa monnieri* with Benzene extract

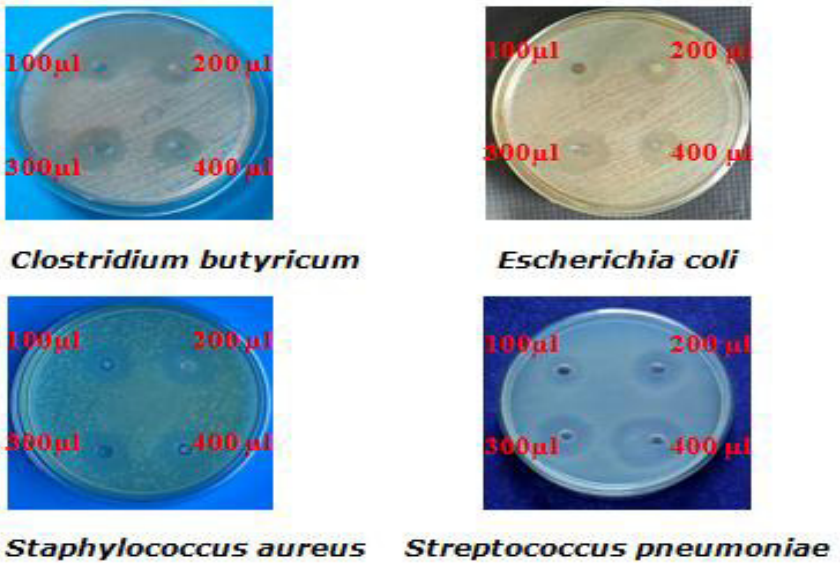

Plate 5: Effect of antibacterial activity of *Bacopamonnieri* Benzene leaf extract

The aqueous extracts showed almost similar range of resistance against the pathogenic bacteria at 100µl of benzene extract. The maximum zone of inhibition observed from *Clostridium butyricum* was followed by *Staphylococcus aureus, Escherichia coli,* and *Streptococcus pneumoniae,* respectively (Figure 2; Plate 5).

3.3. Antifungal activity

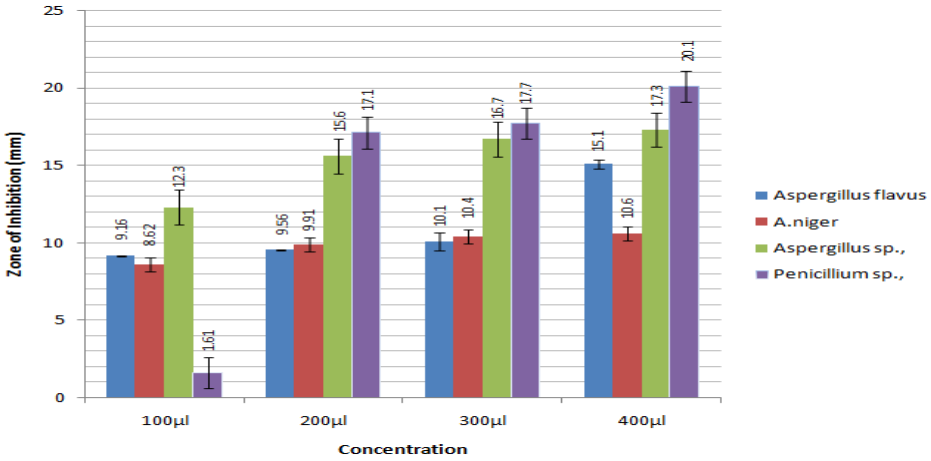

Fig 3: Effect of antifungal activity using *Bacopa monnieri* with Aqueous extract

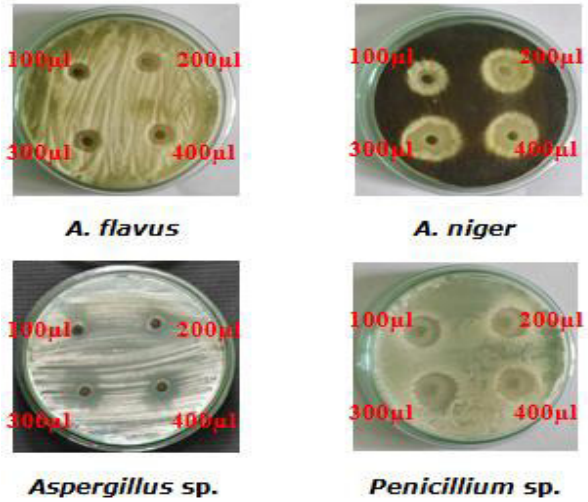

Plate 6: Effect of antifungal activity of *Bacopamonnieri* Aqueous leaf extract

Antifungal activity was performed with benzene and aqueous extract of *B.monnieri*against the *Aspergillus flavus, A.niger, Aspergillus* sp., and *Penicillium* sp. According to the antifungal activity of *B.monnieri*benzene extract, the maximum antifungal activity was observed from *A.niger*, followed by *Aspergillus* sp., *Penicillium* sp., and *Aspergillus flavus,* respectively (Figure 3; Plate 6).

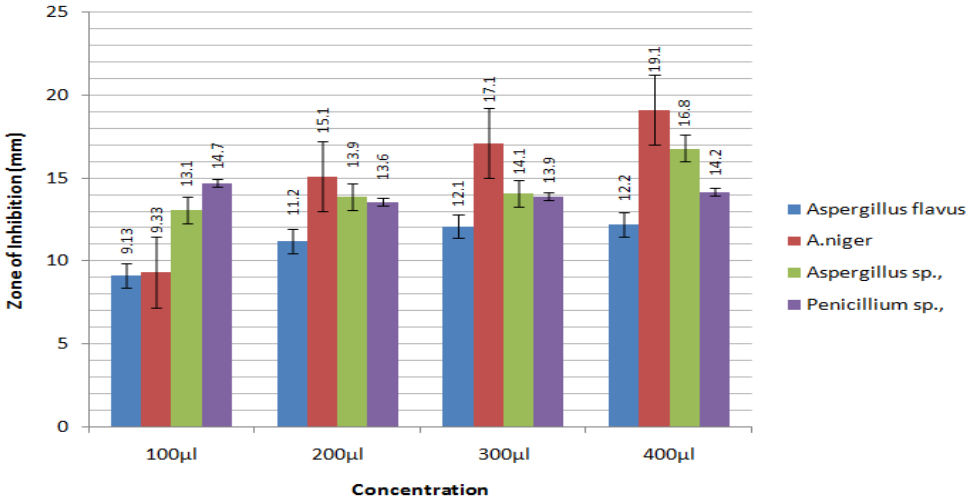

Fig 4: Effect of antifungal activity using *Bacopa monnieri* with Benzene extract

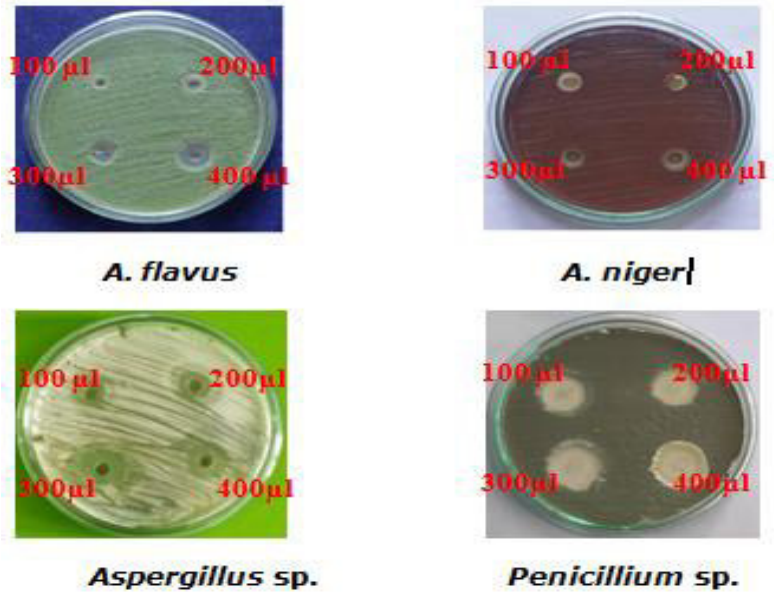

Plate 7: Effect of antifungal activity of *Bacopamonnieri* Benzene leaf extract

Maximum antifungal activity was observed from *Penicillium* sp. followed by *Aspergillus* sp., *Aspergillus flavus,* and *A.niger* at 100µl of *B.monnieri* aqueous extract. Aqueous extract was more effective than the benzene extract (Figure 4; Plate 7).

3.4. MIC

Table 2: Determination of bacterial resistance with different concentrations of *B. monnieri* against bacteria

Test bacteria	Solvents	Positive control (Ampicillin)	Negative control	500µg/ml	250µg/ml	125µg/ml	62.5µg/ml	31.25µg/ml	15.62µg/ml	7.81µg/ml
Clostridium butyricum	Aqueous	18.91± 0.00	-	1.612±0.04	0.312±0.05	0.276±0.02	0.510±0.09	0.593±0.03	0.432±0.11	0.316±0.08
	Benzene	21.3± 0.00	_	1.810±0.12	1.551±0.17	1.676±0.13	0.923±0.05	0.856±0.07	0.661±0.09	0.721±0.01
Escherichia coli	Aqueous	15.72±0.00	_	1.780±0.02	0.765±0.07	0.702±0.01	1.326±0.00	1.609±0.15	0.924±0.02	0.864±0.03
	Benzene	16.35±0.00	_	2.109±0.08	1.241±0.03	1.204±0.01	0.523±0.06	2.130±0.05	0.546±0.00	0.621±0.15
Staphylococcus aureus	Aqueous	17.82±0.00	_	0.722±0.21	0.755±0.05	1.081±0.11	0.571±0.06	0.316±0.02	1.157±0.09	1.076±0.10
	Benzene	19.32±0.00	_	1.732±0.14	1.146±0.02	2.130±0.21	1.921±0.03	0.891±0.07	1.576±0.05	1.390±0.12
Streptococcus pneumoniae	Aqueous	23.55±0.00	_	1.798±0.21	1.671±0.30	1.045±0.17	1.394±0.03	1.654±0.07	_	_
	Benzene	17.24±0.00	_	0.561±0.00	0.434±0.17	0.423±0.32	0.267±0.03	0.510±0.23	0.710±0.01	0.351±0.15

The values are expressed with Standard and Error Mean.

The minimum inhibitory concentration of *B. monnieri* aqueous and benzene were determined against *Clostridium butyricum, Escherichia coli, Staphylococcus aureus,* and *Streptococcus pneumoniae* bacterial pathogens. The aqueous and methanol extract of *B.*

monnieri was treated against the *C. butyricum* and found the minimum inhibition at 7.81µg/ml had been produced 0.316±0.08 and 0.721±0.01% of a zone of inhibition. The 7.81µg/ml aqueous and benzene extracts performed minimum inhibitory against the *E. coli* 0.864±0.03 and 0.621±0.15% zone of inhibition observed. The *S. aureus* also showed minimum inhibitory at 7.81µg/ml of aqueous and methanol extract, producing 1.076±0.10 and 1.390±0.12% zone of inhibition. The aqueous extract of *B. monnieri* showed minimum inhibitory at 31.25µg/ml against *Streptococcus pneumoniae,* showing a 1.654±0.07% zone of inhibition. Still, benzene extract showed 0.351±0.15% minimum inhibition at 7.81µg/ml (Table 2).

Table 3: Determination of fungal resistance with different concentrations of *B. monnieri* against fungi

Test bacteria	Solvents	Positive control (Nystatin)	Negative control	500 µg/ml	250 µg/ml	125 µg/ml	62.5µg/ml	31.25µg/ml	15.62 µg/ml	7.81µg/ml
Aspergillus flavus	Aqueous	10.53±0.00	-	0.798±0.02	0.890±0.04	0.723±0.03	0.912±0.01	0.658±0.08	0.547±0.01	0.413±0.08
	Benzene	11.15±0.00	-	1.127±0.16	1.013±0.19	0.982±0.04	1.149±0.01	0.891±0.02	0.689±0.09	0.598±0.03
A.niger	Aqueous	13.64±0.00	-	0.851±0.03	0.794±0.02	0.671±0.03	0.621±0.10	0.431±0.01	-	-
	Benzene	17.23±0.00	-	0.876±0.07	0.802±0.06	0.685±0.05	0.832±0.11	0.542±0.03	0.658±0.04	0.587±0.03
***Aspergillus* sp.**	Aqueous	12.34±0.00	-	0.732±0.32	0.634±0.37	0.519±0.08	0.415±0.04	0.303±0.00	-	-
	Benzene	15.42±0.00	-	0.756±0.04	0.676±0.01	0.529±0.07	0.457±0.08	0.379±0.05	0.245±0.00	-
***Penicillium* sp.**	Aqueous	19.89±0.00	-	1.032±0.08	0.721±0.04	0.691±0.00	0.571±0.14	0.711±0.05	0.921±0.06	0.543±0.09
	Benzene	21.43±0.00	-	1.419±0.23	0.998±0.07	0.832±0.16	0.721±0.04	0.612±0.13	1.142±0.16	0.671±0.18

The values are expressed with Standard and Error Mean.

Similarly, the determination of minimum inhibitory concentration was performed against clinical fungus. The *B. monnieri,* aqueous, and benzene extracts showed minimum inhibitory concentration at 7.81µg/ml, producing 0.413±0.08 and 0.598±0.03% zone of inhibition against *A. flavus.* The aqueous extract of *B. monnieri* was not resistant at 15.62, and 7.81µg/ml concentration against the *A. niger*, but 0.431±0.01% inhibition was observed at 31.25µg/ml. The benzene showed minimum inhibition concentration against the *A. niger* at 7.81µg/ml of concentration produced 0.587±0.03%. The *Aspergillus* species also showed minimum inhibition at 31.25µg/ml of aqueous extract of *B. monnieri*with 0.303±0.00% inhibition. Still, the benzene extract showed minimum inhibitory concentration at 15.62µg/ml against *Aspergillus* sp. with 0.245±0.00% inhibition. The 7.81µg/ml of aqueous and benzene extracts showed minimum inhibition like 0.543±0.09 and 0.671±0.18% (Table 3).

4. DISCUSSION

A methanolic and ethanolic extract of *Bacopa monnieri* (L.) contains carbohydrates, flavonoids, tannins, saponins, steroids, phytosterols, phenolic compounds, and aqueous extract of *B.monnieri*have carbohydrates, flavonoids, saponins, steroids, and phytosterols reported. Also, antibacterial activity 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 showed maximum inhibition against *Klebsiella pneumoniae.* The *Proteus mirabilis* was also highly inhibited by methanolic extract than the ethanolic extract of *B. monnieri*[38].Alkaloids, amino acids, carbohydrates, flavonoids, phenolic compounds, protein, saponins, steroids, tannins, and terpenoids were qualitatively determined from *B.monnieri.* Among the phytochemicals, the protein and tannins are high[39,] and the Mexican *B. monnieri* had been lipid, and chlorophyll and carotenoids were reported[40].Ethyl acetate and methanol extracts showed the most activity, followed by aqueous, benzene, and petrol extracts. The phytochemical examination revealed the presence of alkaloids, flavonoids, and saponins[41]. The methanol extract of *B.monnieri*has phenol, tannin, flavonoid, alkaloid, saponin, phlobatannin, and cardiac glycoside[42]. The main phytoconstituents found in the extract were carbohydrates, tannins, alkaloids, quinones, cardiac glycosides, and phenols. The minor constituents include flavonoids and coumarins. Secondary metabolites, such as alkaloids, quinones, and phenols, in *Bacopa monnieri*were reported[43].The extractive value 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 and ethanolic extracts showed the 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*. In contrast, 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. In contrast, *Aspergillus niger*'s highest inhibition zone 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 a negative result. The amino acid test was positive only in the 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* compared *to Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa*[48].*In-silico* approaches were used for anti-neurodegenerative properties 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 an extraordinary zone of inhibition, but more 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, *S. aureus* showed a minimum inhibitory concentration effect of ethanol and Diethyl ether extract of *B.monnieri* at a concentration of 300µg. Ethyl acetate and ethanolic extract have a moderate effect on *S. aureus*. However, the aqueous extract does not show any inhibitory effect and also studied 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 inhibitory[52].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* have 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 than the tested bacteria[53].All the extracts (ethanol, methanol, and acetone) of *B. Monnieri* were more or less effective against microbes. Overall, the methanolic extract proved more effective than the other two extract zones of inhibition. In the case of the 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, the most potent MIC against the clinical pathogens are bacteria and fungi, with a MIC range of 500 – 7.81µg/ml.The MIC of *B. monnieri* leaf extracts treated against bacterial strains ranged from 25 to 100µl/ml. Maximum the gram-positive bacteria was inhibited by *B.monnieri* extracts at lower concentration[49]. The present investigation revealed that gram-negative and gram-positive bacteria were inhibited with a minimum concentration of *B. monnieri* aqueous and methanol extracts.The propolis, rosemary, clove, capsaicin, and cumin extracts showed the minimum inhibition concentration against the *S. aureus*[56]. However, the present investigation revealed that the *B. monnieri* 7.81µg/ml concentration showed extraordinary inhibition against *S. aureus*.The microtiter plate technique was used to assess biofilm formation. The antifungal activity was assessed using the broth microdilution method. The safranin staining technique was used to evaluate the anti-biofilm activity. The optical densities of the biofilms formed by all C. neoformans isolates ranged from 0.16 to 0.89. Most of the tested C. neoformans isolates produced significant amounts of proteinase (5/8) and phospholipase (7/8). The strongest antifungal activity was demonstrated by plumbagin, with minimum inhibitory concentration values ranging from 4 to 16 g/mL, followed by thymol, with minimum biofilm inhibitory concentration values ranging from 8 to 64 g/mL. With minimal biofilm inhibitory concentration and minimum biofilm eradication concentration values ranging from 4 to 16 g/mL and 32 to 256 g/mL, respectively, plumbagin also demonstrated the best antibiofilm action[57].

5. CONCLUSION

The phytochemical compounds of *B.monnieri*were maximum in aqueous extract and their significant role in antimicrobial activities against various clinical pathogens. In contrast, the minimum inhibitory concentrations were also determined at lower concentrations of *B.monnieri*extracts in benzene and aqueous solvents. Therefore, phytochemicals play a significant role in biological activity. Medicinal plants are considered a rich resource of ingredients that can be used in drug development in the pharmacopoeial. Traditional Indian medicine is becoming increasingly popular, with many conditions responding well. A detailed knowledge of the action of food and medicinal plants is needed in order to understand this potential influence (or) bioactive compounds play a major role in the human ailments therapeutical process. The various parts of plants have also proven effective therapy against human ailments. The *Bacopa monnieri* (L.) medicinal plant is an excellent source for treating human disease .

6. AUTHORS CONTRIBUTION STATEMENT

Dr. R. Magalingam designed and finalized the manuscript of the study, Dr. V. Ambikapathy provided valuable suggestions for this work, Mrs. N. Amudha collected samples and analyzed the work and prepared the draft manuscript, Dr. K. Selvam helped with sample collection, Mr. P. Prakash was helped analysis and alignment of the manuscript. All authors read and approve the final version of the manuscript.

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8. CONFLICT OF INTEREST

Conflict of interest declared none.

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