

“Phytochemical Screening and Antimicrobial Evaluation of *Bacopa Monneri* against Urinary Tract Infection – Causing Pathogen”

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Abstract- Urinary tract infections (UTIs) are common bacterial infections affecting the kidneys, bladder, ureters, and urethra, with higher prevalence among females. Common causative bacteria include *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., and *Pseudomonas* spp. Increasing interest in medicinal plants has encouraged research into alternative antimicrobial agents. *Bacopa monnieri* (L.), a medicinal herb widely used in traditional Ayurvedic medicine, contains bioactive phytochemicals, including saponins, flavonoids, and other secondary metabolites. These compounds have demonstrated various pharmacological properties, including antioxidant and anti-inflammatory activities. This study explores the potential antimicrobial activity of *Bacopa monnieri* against UTI-associated bacterial pathogens and its possible application in developing plant-based antimicrobial agents. This study investigated the antimicrobial potential of *Bacopa monnieri* against UTI-associated bacterial pathogens. Plant leaves were collected from the Forest Research Institute, Dehradun, and extracts were prepared using methanol, ethanol, and distilled water. Phytochemical screening revealed the presence of flavonoids, tannins, saponins, steroids, and phytosterols in the extracts. Bacterial isolates, including *Klebsiella pneumoniae* and *Proteus mirabilis*, were obtained from urine samples and evaluated for antimicrobial susceptibility. The results demonstrated zones of inhibition, particularly with ethanol and methanol extracts, indicating potential antibacterial activity. These findings suggest that *Bacopa monnieri* may be a promising source of plant-derived antimicrobial compounds. Further research is required to establish its efficacy, safety, and potential therapeutic applications against UTIs.

Keywords— Urinary tract infection (UTI), *Bacopa monnieri*, Antimicrobial activity, Phytochemical screening, *Klebsiella pneumoniae*, *Proteus mirabilis*, Medicinal plants, Plant extracts, Antibacterial activity.

I. INTRODUCTION

The normal daily production of urine is estimated to be about 1.4 liters and the urine is normally acidic with a pH of 5-8 and in color it may vary from pale yellow to amber. Normal urine should not contain bilirubin, RBCs, proteins and pus cells in it. But when there is an infection, changes are seen such as deep yellow color and high turbidity which can be associated with UTI. Urinary tract infections (UTIs) are among the most common bacterial infections acquired in the community and in hospitals. In individuals without anatomical or functional abnormalities, UTIs are generally self-limiting, but have a propensity to recur. [14] UTI is a non-contagious infection (i.e., it does not spread). In UTI, any part of the urinary system which includes-

kidneys, bladder, ureters, and urethra are infected. On the basis of infection area UTI is divided into two types: 1) Bladder infection (cystitis) - if the infection is in lower urinary tract. 2) Kidney infection (pyelonephritis) (pronounced: PIE-LOA-NEF-RIGHT-ISS)- if the infection affects the upper section of urinary tract. If we look at the ratio of infection then almost 10% of the total world population experiences UTI at least once in their lifetime. In females the ratio is 1:2 whereas in males the ratio which comes out to be at risk are 1:10. UTI is commonly observed in females as compared to the males in India and other developed and under developed countries like Uganda (East Africa), Kenya (USA), United Kingdom, etc

1. Reason for the cause of UTI

Low water intake/ day is one of the reasons for UTI, as low water intake leads to the less flushing out of toxic material from the urinary tract. Those who consume adequate amount of water during day time becomes less susceptible to the urinary tract infection as frequent urination cleans the urinary tract and reproductive organs making it less prone to the area for bacterial growth.

- Females are more susceptible to bacterial infection because of the anatomy of urinary tract reproductive organs. The location of urogenital tract opening is near to anus which is the most prone area of bacteria and helps the bacteria to transfer to urinary tract easily.
- In females living in villages, UTI is common because in some places still women use infected sanitary cloths during the menstrual cycle period.
- Another reason of UTI include - unhygienic conditions, lack of clinical diagnostic facilities and dearth of knowledge of self-sanitation.
- Pregnancy in women and prostate enlargement in men also becomes the factors for UTI.

2. Symptoms of UTI

Burning sensation while urination; increase in urgency of urination; blood in urine; pain in upper back and sides; fever; nausea; vomiting; foul smell in urine.

UTI causing bacteria include:- *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *staphylococcus* spp. etc.

Bacopa monnieri (L) belongs to the family Scrophulariaceae. It is a medicinal herb found throughout the Indian subcontinent in wet, damp and marshy area[1]. *Bacopa monnieri* (L) is used in the indigenous systems of medicine for the treatment of various nervous system ailments such as insomnia, anxiety, epilepsy, hysteria and in improving intellect, memory[2-4].

Medicinal plants are currently in considerable significance view due to their special attributes as a large source of therapeutic phytochemicals that may lead to the development of novel drugs. Most of the

phytochemicals from plant sources such as phenolics and flavonoids have been reported to have positive impact on health and cancer prevention [1].

Medicinal plants are the most important natural resource of life saving drugs. Secondary metabolites of plants possess biological properties such as antioxidant, antiapoptotic, antiaging, anticarcinogen, anti-inflammatory, antiatherosclerosis, cardiovascular protection, inhibition of angiogenesis and cell proliferation activity [1-7].

It has been reported that it is used in the treatment of epilepsy and asthma [10]. It has also been used as a cardio tonic, digestive aid and to improve respiratory function [11]. Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening is an important tool in bioactive compound analysis [13]. *Bacopa monnieri* (L.) Wettst. (Brahmi) is a medicinal plant used for enhancing memory and improving function of the brain in Ayurvedic medicines (Sivarajah & Balachandran, 1994). Recently, many studies have revealed its pharmacological roles as cognition-enhancer (Das et al., 2002; Singh & Dhawan, 1997; Sough et al., 2001; Sumathi et al., 2002), antidepressant (Sairam et al., 2002), antioxidant (Pawar et al., 2001; Russo et al., 2003), antiulcerogenic agent (Sairam et al., 2001), and calcium antagonist (Dar & Channa, 1999). Dammarane-type triterpenoid saponins, classified as pseudojubenogenin and jubenogenin glycosides were reported to be responsible for the cognition enhancing activity of this plant (Das et al., 2002; Singh & Dhawan, 1997; Stough et al., 2001; Sumathi et al., 2002). In recent studies, *B. monnieri* has been placed second in a priority list of the major Indian medicinal plants assessed on the basis of medicinal significance, potential candidate, and commercial value for further research and development [10,11]. Therefore, the current exploratory depression, diarrhoea, fever, skin disorders, hepatic diseases, and edema (Sonboli et al., 2006). The *Ziziphora* species are rich in essential oils, flavonoids and sterols (Zhaparkulova et al., 2015). The plant has an immense number of dynamic constituent

compounds including a few alkaloids and saponins (Fig.), with the fundamental dynamic mixtures being the steroidal saponins bacosides (a combination of bacoside A3, bacoside II, bacoside X, and bacosaponin C). Despite the fact that few other restorative properties of the plant including cardioprotective, hepatoprotective and anticancer exercises have been accounted for, *B. monnieri* has been broadly utilized in Ayurveda significantly for its memory boosting property (James et al., 2021).

II. MATERIALS AND METHODS

1. Collection of plants and extract preparation

To carry out the research the materials used were divided into sub categories, Plant material – Plant material used for the research purpose is *Bacopa Monnieri*

Solvent Used
Methanol
Ethanol
Mayer's reagent
Glacial Acetic Acid
Ferric Chloride
Concentrated sulphuric acid
Concentrated hydrochloric acid
Molish Reagent
Ammonia
Chloroform
Iodine
Distilled water

Bacteriological media used

Nutrient Agar Media

Table 1.-Composition of NAM

Agar	1.5%
Peptone	0.5%
Beef extract/yeast extract	0.3%
Salts	0.5% NaCl

Table -2 -Composition of MacConkey Agar

Agar	13.5%
Pancreatic digest of gelatin	17.0%
Peptones (meat and casein)	3.0%

Lactose monohydrate	10.0%
Bile salts	1.5%
Sodium chloride	5.0%
Crystal violet	0.001%
Neutral red	0.03

III. METHODOLOGY

Sample Collection

Bacopa monnieri plant was gathered from FRI (Forest examination of India), Dehradun, Uttarakhand, India under the direction of Dr. P.K Tamta.

Sterilization of Plant Material

The collected samples were first washed with tap water, followed by cutting the leaves from the stems. The leaves were rinsed with distilled water and left for 20 minutes to allow soil to settle. They were then treated with fungicides for 20 minutes to remove fungal growth and subsequently with bactericides to eliminate bacteria. After thorough rinsing with distilled water, the leaves were dried in a hot air oven at 50°C for 30 minutes and ground into a fine powder using a mortar and pestle.

Drying the sterilized plant for grinding

After the plant was properly sterilized, it was left inside the LAF for drying with the air flow kept on until it was completely dried. To aid the drying process was also kept inside the hot air oven with intermittent heating. After the plant was completely dried, it was powered in the mixer grinder keeping the particles as small as possible to ensure, maximum extraction.



Fig 1: *Bacopa Monnieri* left for drying

Grinding of Dried Plant material

After the plant was completely dried, it was powered in the mixer grinder keeping the particles as small as possible to ensure, maximum extraction.



Fig 2: Dry powder of Bacopa Monnieri

Table 3: Weight of leaves recorded for 5 continuous day

No. of days	Weight of the leaves (g)
0 Day	66.98
1 st day	45.28
2 nd day	38.65
3 rd day	24.25
4 th day	22.46
5 th day	22.25
After Hot Air Oven	22.23

Preparation of plant extract

The extract of Bacopa monnieri L. was prepared by traditional maceration method. Bacopa monnieri L. dried plant powder was dissolved in methanol, Ethanol and distilled water. The flask was covered with aluminium foil to avoid evaporation and was kept for 48 hours at 50 rpm shaker at room temperature. After 48 hours the solution was filtered by using Whatman filter paper and filtrate was collected in falcon tube. Then the prepared extract was stored at 40C for further use

Phytochemical study

Different test was done on extract dissolved with methanol, Ethanol and distilled water to check the presence of phytoconstituents such as Flavonoids, Tannins, Saponins, Steroids, Phytosterol and

phenolic were confirmed by the following procedure.

- Test for Flavonoids Dilute ammonia 5ml was added to the extract with concentrated sulphuric acid
- (H₂SO₄) of volume 1 ml was added A yellow coloration which fades on standby indicates the presence of Flavonoids in the extract.
- Test for Tannins 1ml of filtered extract was taken in test tube and 1ml of ferric chlorid was added in the extract. Formation of blue black precipitate indicates the presence of tannins in the extract.
- Test for Saponins 1ml of extract was added in 5ml of distilled water in a test tube. The solution was shaken vigorously and observed. The formation of stable persistence froth indicated the presence of saponins.
- Test for steroids To 0.5 ml of extract add 5ml of chloroform and 5ml of concentrated sulphuric acid. A coloured layer was formed with upper layer turns red, sulphuric acid layer forms yellow which indicates the presence of steroids
- Test for phytosterol 0.5 ml of extract was dissolved in 5 ml of chloroform and 5 ml of concentrated sulphuric acid along the side of the test tube. Presence of brown ring in the middle indicates the presence of phytosterol.
- Test for phenolic 1ml of extract was taken and 0.5 ml of ferric chloride was added on to all of the test tube, A bluegreen colour observed at the bottom of the test tube indicates the presence of phenol.

Table-4 - Phytochemical Test Analysis

1. Test for Flavonoids	Dilute ammonia 5ml was added to the extract with concentrated sulphuric acid (H ₂ SO ₄) of volume 1 ml was added	A yellow coloration which fades on standby indicates the presence of Flavonoids in the extract.
2. Test for Tannins	1ml of filtered extract was taken in test	Formation of blue black precipitate

	tube and 1ml of ferric chloridewas added in the extract.	indicates the presence of tannins in the extract.
3. Test for Saponins	1ml of extract was added in 5ml of distilled water in a test tube. The solution was shaken vigorously and observed.	The formation of stable persistence froth indicated the presence of saponins.
4. Test for steroids	To 0.5 ml of extract add 5ml of chloroform and 5ml of concentrated sulphuric acid.	A coloured layer was formed with upper layer turns red, sulphuric acid layer forms yellow which indicates the presence of steroids
5. Test for phytosterol	0.5 ml of extract was dissolved in 5 ml of chloroform and 5 ml of concentrated sulphuric acid along the side of the test tube.	Presence of brown ring in the middle indicates the presence of phytosterol.
6. Test for phenolic	1ml of extract was taken and 0.5 ml of ferric chloride was added on to all of the test tube,	A blue green colour observed at the bottom of the test tube indicates the presence of phenol.

Isolation of Bacteria from Urine Samples

Sample Collection

Urine sample was provided by DNA labs itself and sample were stored at 4 Degree Media Preparation

- MacConkey preparation: 49.53 in 1000 ml
- Blood Agar Preparation: 28.0 gm in 1000 ml
- Preparation of NAM: 28.0 in 1000 ml
- Mannitol salt Agar Preparation: 111gm in 1000 ml

Spreading of Urine Sample on different specified media to check the growth of suspected microbes

Method -1

- 100 ml of NAM were prepared using 2.80 gm of nutrient agar.
- 25 ml of media was poured in a petri dish and was left for solidification.
- 100 microlitre of urine sample was spread on NAM plate using L shaped spreader and placed in incubator for 48 hours at 37 C.

Method-2

- 100 ml of MacConkey agar media was prepared using 4.956 gm MacConkey agar.
- Approx. 25 ml was poured in petri dish and wait until it solidifies.
- 100 micro litre of urine sample was taken and spread on MacConkey plate using L shaped glass rod and the plate was placed in incubator for 48 hours at 37 C.

Method -3

- 100 ml of NAM was prepared by using 2.80 gm of nutrient agar
- 5 ml of blood was added to the NAM before it gets solidify
- Approx. 25 ml of media was poured in a petri dish and left until it solidifies.
- 100 micro litre of urine sample was spread on nutrient blood agar plate using L shaped spreader and placed in incubator for 48 hours at 37 C.

Microscopy

Gram staining is a technique which is used to determine the bacterial strain whether is gram positive bacteria or gram-negative bacteria.

For this technique 4 different stains are used:

- Crystal violet
- Gram iodine
- Decolourizer agent
- Safranin

Smear Preparation

- Before taking the sample, heat the wire loop and allow colling (to make sterile)
- Using the inoculum loop, take the sample and place the culture on to a clean, sterile slide.
- Gently spread the inoculum to about a cm in a diameter.

Fixing

- Air dry the slide for smear to dry.
- Pass the slide through the flame a few times smear slide up.

Gram staining

- Place the slide on a staining rack and flood the smear with crystal violet for 30 sec.
- Slightly tilt the slide and rinse with water gently.
- Flood the sample with iodine and allow the slide to rest for 30 seconds.
- Slightly tilt the slide again and rinse gently.
- Tilt the slide and decolorize.
- Flood the safranin for about 1 minutes.
- Wash with water and dry.

Then placed the slide under the microscope and observe it under 10 x, 45 x, 65 x, 100x. After the microscopy different biochemicals are performed for the conformation of suspected bacteria.

Simmons Citrate Agar

Methyl Red (MR) Voges Proskauer (VP) broth

Mannitol salt agar

Preparation of master plates of the Isolates

Method 1

- Step 1: 200 ml of NAM were prepared using 5.6 gm of nutrient agar media
- Step 2: Urine sample was streaked on a NAM plate using inoculation loop and was kept in a incubator for 48 hours at 370C
- Step 3: Mix bacterial colonies were obtained

Method 2

- Step1:200 ml of MacConkey agar was prepared using 11 gm of MacConkey agar media
- Step 2: MacConkey was poured in sterile 6 petri plates
- Step 3: After solidifying of MacConkey agar urine sample was spread using L Shaped spreader
- Step 4: After spreading plate was covered with parafilm Step 5: Plates was incubated for 48 hours at 370C

NOTE: Since, few of the bacteria does not grow on a selective media so again isolation process was repeated this time using method 3, i.e.; Blood agar method

Method 3

- Step 1: 200 ml of NAM were prepared using 5.6 gm of nutrient agar media
- Step 2: 10 ml of negative blood sample was added to the 200 ml of NAM solution
- Step 3: Urine sample was spread on a blood agar plate using L- shaped glass rod and was kept in a incubator for 48 hours at 370C
- Step 4: Bacterial colonies were obtained
- Step 5: Bacterial colonies were observed under the microscope
- Step 6: 200 ml of NAM and MacConkey media was made
- Step 7: After 5 minutes NAM and MacConkey media was poured in 8 plates
- Step 8: Different bacterial colonies were taken from inoculation loop and was streaked on the NAM, MacConkey media
- Step 9: Plates was kept for incubation for 48 hours at 370C.

IV. RESULT

Observation 1

Phytochemical test

Table-5 Phytochemical test results

Test	Solution	Result
1. Flavonoid test	Methanol	+ve
	Ethanol	+ve
	Distilled water	+ve
2. Tannin's test	Methanol	+ve

	Ethanol	+ve
	Distilled water	+ve
3. Saponins test	Methanol	+ve
	Ethanol	+ve
4. Steroid's test	Distilled water	+ve
	Methanol	+ve
	Ethanol	+ve
	Distilled water	+ve
5. Phytosterol test	Methanol	+ve
	Ethanol	+ve
6. Phenol test	Distilled water	+ve
	Methanol	+ve
	Ethanol	+ve
	Distilled water	-ve

+ ve – Presence of the phytochemical

-ve – Absence of the phytochemical

Antimicrobial Susceptibility Testing (AST)

ASP is a procedure or technique used to figure out which antibiotic a particular living being or microorganisms are vulnerable to. Antibiotic susceptibility is stated as the highest dilution or lowest concentration of antibiotic that suppresses the growth of organism.

Zone of inhibition (ZOI)

ZOI is a circular region form around the spot of antibiotic in which the microorganism does not show any growth. The zone can be used to measure the inability or defence lessness of the microorganisms towards the antibiotic.

Oservation 2

Antibiotics susceptibility test

Klebsiella pneumonia



Figure-ZOI in Klebsiella pneumonia

Table-6 Result of Klebsiella pneumonia in crude extract

S.no	Concentration (μl)	ZOI in Ethanol	ZOI in Methanol	ZOI in Distilled water
1	20	21mm	19mm	Negligible
2	40	24mm	16mm	Negligible

Table-7 Result of k. Pneumonia in DMSO

S.no	Concentration (μl)	ZOI in Ethanol	ZOI in Methanol	ZOI in Distilled water
1	20	16mm	24mm	Negligible
2	40	23mm	19mm	Negligible
3	60	19mm	21mm	Negligible

Table-8 Result of k. pneumonia in Master Solvent (MS)

S.no	Concentration (μl)	ZOI in Ethanol	ZOI in Methanol	ZOI in Distilled water
1	20	12mm	18mm	Negligible
2	40	14mm	20mm	Negligible
3	60	15mm	22mm	Negligible

Proteus mirabilis





Figure-ZOI in *Proteus mirabilis* (crude)
Figure- ZOI in *Proteus mirabilis* (DMSO, MS)

Table-9 Result of *Proteus mirabilis* in crude extract

S.no	Concentration (μl)	ZOI in Ethanol	ZOI in Methanol	ZOI in Distilled water
1	20	22	21	16
2	40	23	13	17
3	60	25	24	18

Table-10 Result of *Proteus mirabilis* in DMSO

S.no	Concentration (μl)	ZOI in Ethanol	ZOI in Methanol	ZOI in Distilled water
1	20	17	21	Negligible
2	40	18	23	20
3	60	20	25	15

Table-11 Result of *Proteus mirabilis* in MS

S.no	Concentration (μl)	ZOI in Ethanol	ZOI in Methanol	ZOI in Distilled water
1	20	16	17	Negligible
2	40	18	19	17
3	60	21	22	20

V. CONCLUSION

Many of the female in India as well as around the world are suffering from urinal tract infection and have become the victims of harmful microbes present like (*E. coli*, *Klebsiella pneumonia*, *Proteus*

mirabilis, *Staphylococcus aureus*, and *Enterococcus faecalis*). Although many different types of treatment are going on to cure this disease but are expensive. '*Bacopa Monnieri*' is a medicinal herb which also showed sensitivity against the bacteria. So, it is concluded that the bacteria resist while coming near the extract and formed zone.

It is evident from this study that '*Bacopa Monnieri*' or '*Brahmi*' could serve as a treatment of urinal tract infection but on a different concentrated solution like ethanol, methanol. So, on a conclusion from this study we can conclude that it can be well effective in the treatment of the better result much other experiment can be performed using different species of bacteria present in urinal tract infection.

REFERENCES

1. Al-Hadhrani, R., Al Muniri, R. and Hossain, M., 2016. Evaluation of antimicrobial and cytotoxic activities of polar solvent extracts from leaves of *Ammi majus* used by the Omanis. *Pacific Science Review A: Natural Science and Engineering*, 18(1), pp.62-65.
2. Egamberdieva, D., Wirth, S., Behrendt, U., Ahmad, P. and Berg, G., 2017. Antimicrobial Activity of Medicinal Plants Correlates with the Proportion of Antagonistic Endophytes.
3. Arunkumar, S., Muthuselvam (2009). Analysis of phytochemical constituents and antimicrobial activities of aloe vera L. against clinical pathogens. *World J. Agril. Sc.*, 5(5): 572-576
4. Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia, E.C. and Atangbayila, T.O. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South Western Nigeria. *Trop. J. Pharm. Res.*, 7: 1019-1024. *Frontiers in Microbiology*, 8. 3
5. Charoenphon, N., Anandsongvit, N., Kosai, P., Sirisidthi, K., Kangwanransan, N. and Jiraungkoorskul, W., 2016. *Brahmi* (*Bacopa monnieri*): Up-to-date of memory boosting medicinal plant: A review. *Indian Journal Of Agricultural Research*, 50(1).

6. Rai, K., Gupta, N., Dharamdasani, L., Nair, P. and Bodhankar, P., 2017. Bacopa Monnieri: A Wonder Drug Changing Fortune of People. International Journal of Applied Sciences and Biotechnology, 5(2), pp.127-132.
7. Singh HK, Dhawan BN. Neuropsychopharmacological effects of the Ayurvedic nootropic Bacopa monniera Linn. (Brahmi). Indian J Pharmacol 1997;29:S359-S365
8. Basak, A., Hossain, M. and Parvin, M., 2016. Evaluation of phytochemical and pharmacological activities of Bacopa monnieri (L.). International Journal of Scientific Reports, 2(10), p.242. 6
9. Phrompittayarat, W., Putalun, W., Tanaka, H., Jetiyanon, K., Wittaya-arekul, S. and Ingkaninan, K., 2013. Comparison of various extraction methods of Bacopa monnieri. Naresuan university journal: science and technology (NUJST), 15(1), pp.2934.
10. Sharma, A., Verma, R. and Ramteke, P., 2009. Antibacterial activity of some medicinal plants used by tribals against UTI causing pathogens. World Applied Sciences Journal, 7(3), pp.332-339
11. Aguiar, S. and Borowski, T., 2013. Neuropharmacological review of the nootropic herb Bacopa monnieri. Rejuvenation research, 16(4), pp.313-326.
12. Saha, P.S., Sarkar, S., Jeyasri, R., Muthuramalingam, P., Ramesh, M. and Jha, S., 2020. In vitro propagation, phytochemical and neuropharmacological profiles of bacopa monnieri (L.) wettst.: a review. Plants, 9(4), p.411.
13. Bharti, P., Bai, S., Seasotiya, L., Malik, A., and Dalal, S. (2012). Antibacterial activity and chemical composition of essential oils of ten aromatic plants against selected bacteria. Int J Drug Dev Res 4, 342–351.. Mkk, F., Sp, D. and Irfan, M., 2019. Antibacterial and antifungal activity of various extracts of Bacopa monnieri. arXiv preprint arXiv:1909.01856.
14. Foxman, B. The epidemiology of urinary tract infection. Nat Rev Urol 7, 653–660 (2010)