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ORIGINAL ARTICLE **OPEN ACCESS**

Antibacterial Activity of *Herpestis monniera* Against Some selected Antibiotics

*** Chhaya Singh, Rishi Kumar Saxena, Pawan Tripathi**
Singhania University, Pacheribari, Jhunjhunu, Rajasthan, India
Department of Microbiology and Biochemistry, Bundelkhand University, Jhansi, Uttar Pradesh,
*E**mail id:** schhaya1801@gmail.com

ABSTRACT

Bacopa monniera is a small, creeping herb with light purple flowers. The plant is normally located in wet, damp, and marshy regions. Thyme-Leave Gratiola is delegated a "Medhya Rasayan" drug used to improve memory and the mind (Medhya). It has been utilized by Ayurvedic clinical experts in India for very nearly 3000 years.The present study was carried out to determine the antimicrobial activity of Bacopa monnieri (L)by using methanol extracts against three gram-positive and three gram-negative bacteria at 200µg/ml and 300µg/ml concentrations according to the pharmacopoeias method. An agar well diffusion assay was carried out to determine the antimicrobial effects of B. monnieri against Gram-positive (Staphylococcus aureus MTCC 916, Bacillus subtilis MTCC 1134, Streptococcus faecalis MTCC 439) and Gram-negative (Escherichia coli MTCC167, Salmonella typhimurium MTCC1264, Klebsiella pneumonia MTCC 530) bacterial strains. Only Staphylococcus aureus demonstrated significant sensitivity to both the 200 µg/ml and 300 µg/ml concentrations. Resistance was observed against all five bacterial strains when subjected to 200 µg/ml methanolic extract of Bacopa monnieri (L), including Bacillus subtilis, Streptococcus faecalis, Escherichia coli, Salmonella typhimurium, and Klebsiella pneumoniae. When the antibiotics ciprofloxacin and gentamicin are used in combination, the ZOI values for gram-positive bacteria ranged from 16-10 µg/ml and from 12-8 µg/ml for gram-negative bacteria. These findings show the plant extracts of Bacopa monnieri (L) are obviously strongly effective against all the tested bacteria. For all of the tested samples, the plant extracts indicated a definite inhibitory zone.

Keywords: *Bacopa monnieri (L), Medhya Rasayana, Antimicrobial activity, Pharmacopoeias method, Agar well diffusion assay*

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INTRODUCTION

InIndia, plants have been used since the Vedic period**.** According to WHO estimates, about 80% of the world's population living in rural areas relies on traditional herbal medicines as their primary healthcare. The increase in resistance to many commercially produced synthetic antimicrobial agents by microorganisms has increased with time, hence the need to search for new antimicrobial agents [1]. There are various methods of medicine, like Ayurveda, Homeopathy, and Unani, that utilize plant material for drug production. Etymologically, Ayurveda is born from the amalgam of the Hindi words "Ayar",meaning life, and "Veda", meaning science. Therefore, Ayurveda is regarded as the science of life. After many decades, recently medicinal plants have been given wide attention,with global recognition in biotechnology is an internationally [2-3].

Bacopa is a genus of 70–100 aquatic plants belonging to 146 species. The leaves of the Brahmi plant may be chewed (handiest 2-3 at a time), allowing you to relieve pressure and tension. The entire plant is being used as a nervous tonic[4]. The energetic components in this herb can affect hormonal balance inside the frame and undoubtedly affect the balance of stress hormones in our body, thereby inducing a relaxed, comfortable kingdom naturally, keeping off the side effects of conventional pharmaceutical options for stress and anxiety remedies.Also, it consists of betulinic acid, D- mannitol, stigmasterol, alanine, aspartic acid, glutamic acid, serine, and pseudo-jujubogen in glycoside [5]. Bacopa diet animated more is a charge of IgA and IgG in rodents' blood contrasted with Echinacea or Ashwagandha[6].

Bacopa effectively manages diseases involving chronic brain inflammation driven by the innate immune system [7]. Plant possess various biological activities such as neuroprotective activity [8], anti-depressant and anti-anxiety activity [9], anti- convulsant / anti-epileptic activity [10], antioxidant properties [11] and anti-cancer activity [12]. *Bacopa monnieri* was highly effective as an adaptogen; it

normalized acute and chronic stress-induced corticosterone changes in rats. It also normalized noradrenaline (NA), 5-HT and DA in cortex and hippocampus of rats in acute and chronic unpredictable stress [13].

The antibacterial properties of several parts of the plant were included in this activity. In any case, the combined effect of Ciprofloxacin (CPFX) and Gentamicin (GM) has not been accounted for so far against all these pathogens. Therefore, in this communication, we studied the action of CPFX in combination with GM to assess the GM-mediated suppression of CPFX-resistance. Diseases because microorganisms represent a health problem worldwide and their resistance mechanisms have added to the worsening ofclinical results. Consequently, the objective of the current review was to research, the antimicrobial action of *Bacopa monnieri*(L) extracts to utilize them as a potential source for new antimicrobial substances against human microbes.

MATERIAL AND METHODS

Plant Collection and Identification

Leaves, stems, and roots of *Bacopa monniera were* used in this study. For experimental purposes, all these plant materials were collected from the Ayurvedic Research Centre, Jhansi (U.P.). Collected parts of *Bacopa monniera* were identified by Mr. JagdeshArya, research officer, botany from the Central Council for Research in Ayurvedic Sciences, Jhansi, where an authenticated voucher number (28718) was deposited for the specimen.

Drying and Storage of Plant Samples

The samples were gathered and dried for test extraction. It was guaranteed that the plant was solid and uninfected. The leaves were washed under running tap water, followed by distilled water to take out residue and other unfamiliar particles, and shade dried for 15 days. Moisture was entirely removed by placing the leaves in an oven at a temperature below 300ºC to prevent the decomposition of thermolabile compounds. The dried leaves and roots of *Bacopa monniera* were powdered and put away in completely sealed holders until used at room temperature.

Dried Sample Grinding

The dried samples were grinded to a coarse powder with the help of a mortar and pestle and sieved with the help of a sieve (size0.36 mm) to obtain uniform particles. The powdered type of the sample facilitates and enhances the surface zone, thus improving the viability of the extraction process. Furthermore, the extraction interaction requires a lower measure of solvent.

Preparation and Extraction of Plant Extract

5 gm of the powder was filled into the thimble and extracted using 200ml of methanol using a Soxhlet extractor for 3 hours. The sample powder is kept in a porous material like muslin cloth or filter paper in a thimble. The extract was concentrated using a rotary shaker and evaporated or stored at 4 ºC in a water/airproof bottle until further use. The extract was used to determine its antibacterial potential.

Test Microorganisms

The test organisms were selected on the premise that they cause a lot of diseases in people. Six types of bacteria, especially three gram-positive microorganisms and three gram-negative microorganisms, were utilized in our laboratory. Restorative plant extricates demonstrate a broad assortment of antimicrobial activities in opposition to each bacterial pathogen. Other studies have proven a notable synergistic action of plant concentrates and spices used against pathogenic, probiotic, and meal spoilage pathogens such as *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli,* and other microorganisms, both Gram-positive and Gram-negative [14].

Gram-positive Bacteria

- *Staphylococcus aureus* MTCC 916
- *Bacillus subtilis* MTCC 1134
- *Streptococcus faecalis* MTCC 439

Gram-negative Bacteria

- *Escherichia coli* MTCC 167
- *Salmonella typhimurium* MTCC 1264
- *Klebsiella pneumoniae* MTCC 530

Preparation of Culture Media

The Muller Hinton Agar Media was prepared as per the standard composition given by HiMedia; that is, 38 gm of the media was suspended in 1ml of water, and the mixture was autoclaved at 121ºC and 15 psi for 15 minutes using an autoclave (Gentek India Pvt. Ltd.). The composition of premixed MHA was –

S/N	Ingredient	Amount (g/l)
1	Beef infusion	300 gm
2	Casein acid hydrolysate	17.50 gm
3	Starch	1.50 gm
4	Agar	17.00 gm

Maintenance of Bacterial Cultures

After sterilization, the media was allowed to cool at 45–50 ºC before being poured into sterile glass petri dishes under laminar airflow (Toshiba, India) using aseptic techniques.Each plate was poured with 20 ml of culture media. The plates were allowed to solidify properly, then the media was inoculated with the respective bacterial isolate, that is, *S. aureus, B. subtilis, S. faecalis, E. coli, S. typhimurium,*and *K. pneumoniae,* separately by the spread plate technique, for which 100 µl of the bacterial culture broth was added over the media and uniformly spread using a sterile glass rod.

Drugs used

For the antibacterial studies, Gentamicin and Ciprofloxacin were used as reference standards. Ciprofloxacin causes cardiovascular illness and neurotoxicity. Gentamicin may cause genuine kidney issues. Kidney issues may occur more frequently in older people or in people who have dried out. Yet, our Ayurveda is immense to the point that it furnishes treatment not exclusively to battle with the infection; additionally, it detoxifies the body from poisons delivered by present-day drugs. Herbs like Brahmi give security and detoxify the organs like the liver, kidney, and mind. The current paper deals with organ harmfulness brought about by present-day drugs and uses part of Ayurveda to check this issue.

Test for Antibacterial Activity

The methanol extract of leaves (BML01), stems (BMS01), and roots (BMR01) from *Bacopa monnieri* were prepared at two different concentrations, is 200 µg/ml and 300µg/ml. Ten minutes after spreading, wells were punched into the media plates using sterile micro tips, and then each well was loaded with 20 µl of the respective sample extract to be tested. The samples were allowed to diffuse through the wells into the media, and then the plates were sealed with paraffin and incubated at 37ºC for 24 hours. The plates had two wells of positive control with Gentamicin (C1) and Ciprofloxacin (C2) antibiotics at 500 µg/ml concentration each, and the negative control wells had methanol in them. On the next day after incubation, the plates were observed for the clear zone around the well, called the zone of inhibition, and the diameter of these zones was measured in mm.

RESULTS AND DISCUSSION

In general, stem crude extract indicated expressed control of the organism's development in the cultures compared with roots and leaves extract with various inhibition zones. The best activity against *S.aureus*, *B.subtilis, S.faecalis, S.typhimurium*, *E.coli,* and *K. pneumoniae* was shown by stem crude extract followed by leaves, roots crude extract of *Bacopa monnieri*. Details of the results are tabulated in Tables 1and 2.

For gram-positive bacteria, the stem crude extract showed the highest sensitivity against *Staphylococcus aureus,* which was 16 mm, followed by *B. subtilis* (12 mm). The lowest activity of 10 mm was found in *S. faecalis*. Regarding gram-negative bacteria, stem crude extract showed the highest sensitivity against *Salmonella typhimurium,* which was 12 mm, followed by *K. pneumoniae* (10mm) and on the other hand, it showed minimum activity against *Escherichia coli,* measured at 8 mm.

Using leaf crude extract showed the best MIC (zone of inhibition) of staphy*lococcus aureus* results with 15 mm, followed by *Salmonella typhimurium*(12mm), *B. subtilis* (11 mm), *S. faecalis*(10), *Escherichia coli* (10 mm) and *K. pneumoniae* (10 mm). In root extract, the resistance obtained for gram-positive bacteria ranged from 15 mm for *Staphylococcus aureus,* followed by *B. subtilis* (10 mm), *S. faecalis*(10 mm), and for gram- negative bacteria, the resistance ranged from 10 mm for *S. typhimurium,* followed by *Escherichia coli* (10 mm) and *K. pneumoniae* (10mm). Medicinally, this is also used for inflamed tissues[15].The consequences of the zone of hindrance study revealed the concentration-dependent nature of the extract with better viability against gram-positive microbes than gram-negative microbes. These observations are more likely to be due to an outer membrane in gram-negative bacteria, which acts as a barrier to many environmental substances, including antibiotics [16].

FIG. 1: ANTIBACTERIAL ACTIVITY TEST FOR THE LEAVES, STEMS, AND ROOTS METHANOLEXTRACTOF*B. MONNIERI* RESPECTIVELY AGAINST *S. AUREUS*

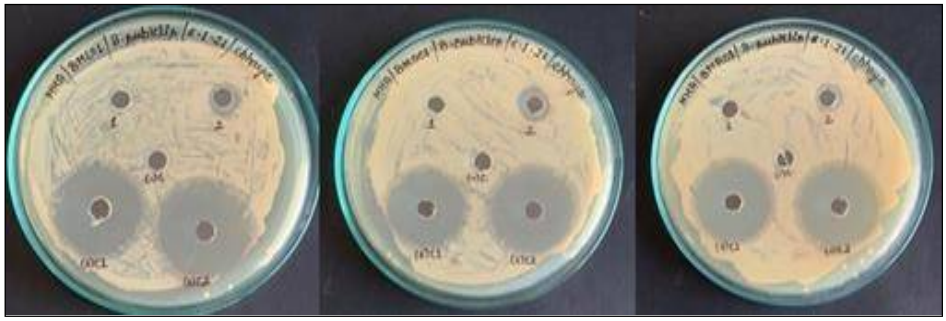

FIG. 2: ANTIBACTERIAL ACTIVITY TEST FOR THE LEAVES, STEMS, AND ROOTS METHANOL EXRACTOF *B. MONNIERI* RESPECTIVELY AGAINST *B. SUBTILIS*

FIG. 3: ANTIBACTERIAL ACTIVITY TEST FOR THE LEAVES, STEMS, AND ROOTS METHANOL EXTRACT OF *B. MONNIERI* RESPECTIVELY AGAINST *S. FAECALIS*

The development of resistance mechanisms is a never-ending process by microbes against known antibiotics [17]. Thus, new antimicrobial compounds are a huge concern in the medical field. The present study showed that the various extracts of *B. monnieri* possess antibacterial properties against human pathogenic organisms, which is in agreement with several studies reported [18-19].

TABLE 1: ZONE OF INHIBITION (MM) FOR GRAM-POSITIVE BACTERIA FOR THE DIFFERENT EXTRACTS OF *B. MONNIERI*

Leaf extract of *B.monnieri*				
S.No.	**Sample**	**Zone of Inhibition(mm)**		
		B.subtilis	***S.faecalis***	***S.aureus***
1	200µg/ml	10	Nil	Nil
2	300µg/ml	15	10	11
3	Positive control(C1)	19	27	13
4	Positive control(C2)	27	30	28
5	Negative control	-	-	-

Stem extract of *B.monnieri*				
S.No.	Sample	Zone of Inhibition(mm)		
		S.aureus	*B.subtilis*	*S.faecalis*
1	200µg/ml	11	Nil	Nil
2	300µg/ml	16	12	10
3	Positive control(C1)	19	27	13
4	Positive control(C2)	27	30	28
5	Negative control	-	-	-

Root extract of *B.monnieri*				
S.No.	Sample	Zone Of Inhibition(mm)		
		S.aureus	*B.subtilis*	*S.faecalis*
1	200µg/ml	Nil	Nil	Nil
2	300µg/ml	15	10	10
3	Positive control(C1)	19	27	13
4	Positive control(C2)	27	30	28
5	Negative control	-	-	-

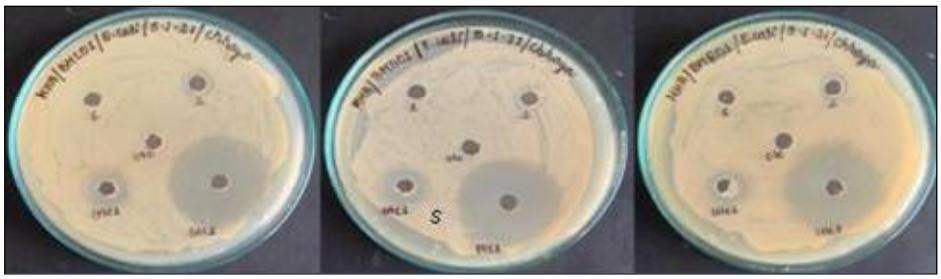

FIG. 4: ANTIBACTERIAL ACTIVITY TEST FOR THE LEAVES, STEMS, AND ROOTS METHANOL EXTRACT OF B. MONNIERI RESPECTIVELY AGAINST *S.TYPHIMURIUM*

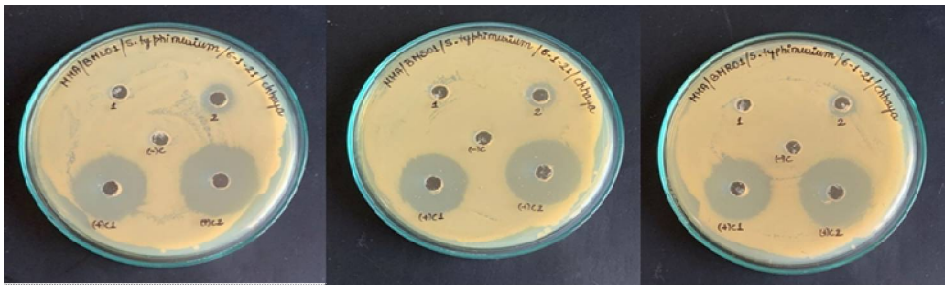

FIG. 5: ANTIBACTERIAL ACTIVITY TEST FOR THE LEAVES, STEMS, AND ROOTS METHANOL EXTRACTOF B. MONNIERI RESPECTIVELY AGAINST*K. PNEUMONIAE*

FIG. 6: ANTIBACTERIAL ACTIVITY TEST FOR THE LEAVES, STEMS, AND ROOTS METHANOL EXTRACTOF B. MONNIERI RESPECTIVELY AGAINST *E. COLI*

The wells labelled as 1, 2, (+) C1, (+) C2, and (-) C in the plates stand for the 200 µg/ml, 300 µg/ml concentration of extract, Gentamicin, Ciprofloxacin, and negative control (methanol), respectively.

TABLE 2: ZONE OF INHIBITION (MM) FOR GRAM-NEGATIVE BACTERIA FOR THE DIFFERENT EXTRACTS *OF B. MONNIERI*

Leaf extract of *B.monnieri*				
S.No.	**Sample**	**Zone of Inhibition(mm)**		
		S.typhimurium	***E.coli***	***K.pneumonia***
1	200µg/ml	Nil	Nil	Nil
2	300µg/ml	12	10	10
3	Positivecontrol(C1)	24	14	17
4	Positivecontrol(C2)	27	30	28
5	Negativecontrol	-	-	-

Stem extract of *B.monnieri*				
S.No.	**Sample**	**Zone of Inhibition(mm)**		
		S.typhimurium	***E.coli***	***K.pneumonia***
1	200µg/ml	Nil	Nil	Nil
2	300µg/ml	12	08	10
3	Positivecontrol(C1)	24	14	16
4	Positivecontrol(C2)	27	30	28
5	Negativecontrol	-	-	-

Root extract of *B.monnieri*				
S.No.	**Sample**	**Zone of Inhibition (mm)**		
		S.typhimurium	***E.coli***	***K.pneumonia***
1	200µg/ml	Nil	Nil	Nil
2	300µg/ml	10	10	10
3	Positivecontrol(C1)	24	14	16
4	Positivecontrol(C2)	27	30	28
5	Negativecontrol	-	-	-

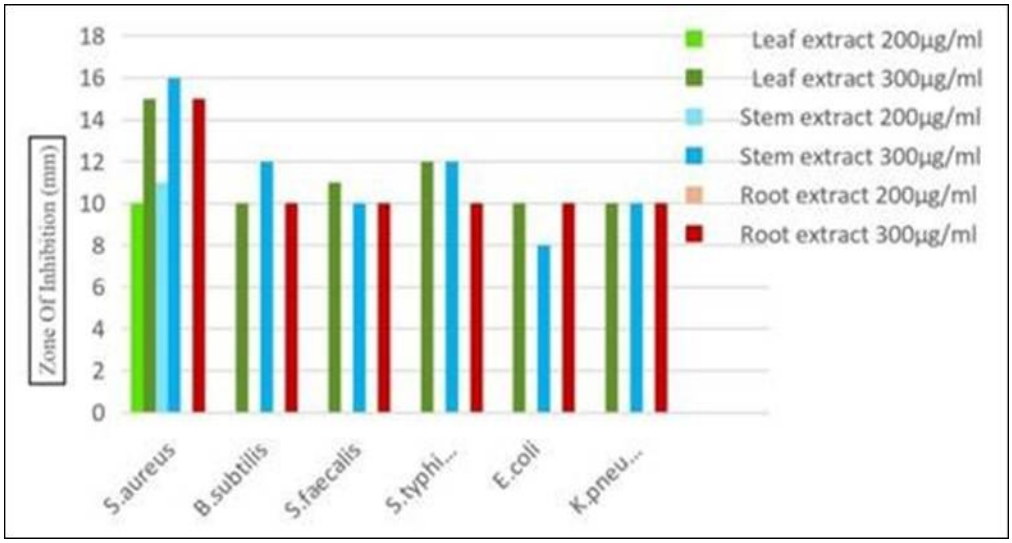

FIG. 7: AGAR WELL DIFFUSION ASSAY OF METHANOL EXTRACT OF *B. MONNIERI*

CONCLUSION

The therapeutic value of medicinal plants and the bioactivity of extracts lies in the various phytochemicals present in them. Plant-rich tannins have antimicrobial potential due to their basic characteristics that allow them to react with proteins to form stable water-soluble compounds, thereby

killing the bacteria by directly damaging its cell membrane [20]. *Staphylococcus aureus* with methanol stems crude extract of *B. monnieri*showedthebestzoneofinhibitionresultswith16mm as compared to leaves and roots. The results showed maximum activity against *S. aureus, followed by S. typhimurium, B. subtilis, and S. faecalis, as K. pneumoniae and E. coli* showed less activity.

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