RESEARCH ARTICLE

Antibacterial and Antifungal Potential of Berberine Isolated from Stem of *Berberis aristata* DC

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ABSTRACT

Background: This research was conducted to evaluate berberine as an isolated bioactive compound from *Berberis aristata* DC for its activity against microbial infections. To examine the antibacterial activity using ethanol hot extraction procedure to verify the medicinal use of *B. aristata* DC in infection control and qualitatively estimate the phytochemical composition of the plant stem extract.

Objective: To isolate a bioactive phytoconstituent from *B. aristata* DC for its antimicrobial efficacy in managing human infections.

Methods: The antibacterial activity of berberine derived from the stem extract was examined against gram-ve and +ve multi-drug resistance examined by diffusion method using an agar-well isolated from the stem extract was examine by the agar-well using a 96-well microtitre plate. The minimum inhibitory concentration was calculated against each bacterium using both the microtitre plate technique and broth dilution of berberine

Results: The appearance of alkaloids in the methanol extract of the stems was demonstrated. The pathogenic bacteria used were *Shigella sp., Escherichia coli,* including some more standard bacterial strains. These multi-drug resistant (MDR) bacteria were reported with bacterial inhibition capability when methanol was used as a solvent. In addition, the effect of berberine against *Shigella sp.* was the maximum inhibition zone size (1.25 cm). Ciprofloxacin 30 µg/disc served as the reference/ +ve control, whereas 10% dimethyl sulfoxide (DMSO) was the -ve control. These chosen bacteria were reported minimum inhibitory concentration (MIC) of berberine values ranging from 200 to 1000 µg/mL.

Conclusions: *B. aristata* DC stem extract berberine showed antibacterial properties using hot methanol against all bacteria. In alternative and complementary medicine, berberine is used to treat diseases caused by many strain of bacteria

Keywords: *Berberis aristata* DC., Berberine, Gram-positive bacteria, MIC, Disk diffusion method, Gram-negative bacteria.

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INTRODUCTION

The plant world would be a wide source of organic components, some of which have been utilized as treatments for a variety of infectious and non-infectious disorders in the contemporary medical system. According to the WHO, almost 80% of the population in underdeveloped nations relies on traditional medicines, primarily herbal remedies, for basic health care.[1,2] Inrural India, raw herbal and herbal products in Ayurvedic medicine are largely sought after due to cheap availability and are also increasingly popular in urban areas for existing cultural nuances.[3] Furthermore, it is popular and there are many phyto drugs in preference to synthetic ones, for much safer and showing leas side effects.[4] As per the records, maximum viral infections are managed using bioactive phytoconstituents.

Several hundred plants are known in Indian ethnobotanical literature to have the potential to cure diseases and among one of popular them ones is from family Berberidaceae, *Berberis aristata* DC (syn. Daru haldi, Indian barberry; Sanskrit: "daruharidra").[5] This herb has traditionally been used to treat several forms of wound healing, inflammation, ENT infections, uterine, vaginal, indigestion and dysentery disorders and have immunopotentiation properties.[6] Berbamine, one of the active constituents, efficiently prevents chemically induced hepatocarcinogenesis.[7] According to preliminary findings, it possesses anticancer efficacy when tested against human hepatoma cells, colon cancer cells and murine leukemia L1210 cells. Its anticancer action is thought to be related to its COX-II inhibiting function.[8] Other uses of *B. aristata* DC are as a diaphoretic, purgative, cooling laxative, and useful

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in rheumatism. To cure ophthalmia and other eye problems dried root extract is administered topically to the eyelids. It is also used as a tonic, moderate laxative for treating fevers and ulcers.[9] *B. aristata* DC primary constituent is berberine, an astringent alkaloid.[10] Infections with both gram-ve (GN) and gram+ve (GP) bacteria are becoming clinically incurable as multidrug-resistant (MDR) strains arise.[11] GP pathogens include strains of methicillin-resistant *S. aureus* (MRSA), *S. aureus*, and strains of *Enterococcus* sp. are notable and vancomycin-resistant *S. aureus* (VRSA).[12] In addition, *E. coli*, *Klebsiella pneumoniae*, *Citrobacter freundi*, *Acinetobacter* sp., and *Pseudomonas aeruginosa* are commonly found in urinary tract pathogens, while *Shigella* sp, *K. pneumoniae, E. coli, Salmonella typhi* and *Chromobacterium violaceum* are of the gastrointestinal tract pathogens. According to numerous reports, these pathogens are currently too MDR.[13-16]

MATERIALS AND METHODS

B. aristata DC Stems was collected in month of February 2008 from the Mussoorie woods in Dehradun, India. The NBPGR received the herbarium of plant *B. aristata* DC for verification. The specimen from the National Bureau of Plant and Genomic Resources (NBPGR), Pusa Road, New Delhi, was verified by botanist Dr. K.C. Bhatt, Senior Scientist.

Extraction of Stem of *B. aristata* DC

The *B. aristata* DC stem was gently dead scraped, washed with water, and then cut into small pieces for air drying for a week at room temperature. A total of 200 g of finely ground plant material were extracted for 2 days using a soxhlet method with 500 mL of methanol. The extract was first filtered before being dried by rotary evaporation in a low-pressure, low-temperature environment using an R-114, Buchi, Switzerland, rotary evaporator. Solvents of the analytical grade were utilized Table 1.

Isolation of the Bioactive Compound from the BAME

Healthy stems of *in-vivo* plants material were collected, dried at shade and powdered mechanically. In a soxhlet system, 100 g of the powdered stem were extracted with methanol for 48 hours. The extract was concentrated under decreased pressure using a rotary flash evaporator (Büchi, Switzerland). Separately, 10 g of the extract were acidified with 2% HCl. The filtered acidic solution was extracted with dichloro methane (DCM) after being made basic (pH 8–9) using sodium bicarbonate (Na_2CO_3) and diethyl ether (Et_2O) to eliminate neutral components (CH_2Cl_2). In the desiccator, the CH_2Cl_2 solution was concentrated and dried. After being acquired, the isolated compound's crystals were pulverized and kept in an airtight amber container. By utilizing multiple solvents, including glacial acetic acid, n-butanol, and water in the proportions of 3: 12: 4, TLC chromatography was used to evaluate the crude extract and separated chemicals for the presence of alkaloids. Then Rf values for extract and separated chemicals were determined. Through the use of spectrum analyses, the isolated compound was characterized using IR, ^{1}H-NMR, ^{13}C-NMR and MASS spectroscopy study.

The FTIR spectrum was captured using KBr pellets and a Perkin-Elmer 1710 FTIR spectrophotometer. With the use of a Bruker AMX (400 MHZ) spectrophotometer, the ^{1}H-NMR spectra were collected. For the ^{13}C-NMR and ^{1}HNMR, the solvents utilized were CDC_{l3} and DMSO and, respectively. At the Indian Institute of Technology in Delhi, India, a Bruker micrOTOF-Q II 10262 ESI spectrophotometer was used to record the mass spectrum.

In-vitro Antibacterial Activity

The isolated *B. aristata* DC methanol extract (BAME) compound's MIC and zone of inhibition were evaluated in relation to several gram+ve bacteria categories of *Staphylococcus, Bacillus,* gram -ve bacteria, *Pseudomonas, Escherichia*, and *Salmonella* by the double serial dilution technique in triplicates. The dose of the isolated compound was chosen as 10, 25, 50, 100 µg/mL for MIC, whereas 50 and 100 µg/mL were chosen to estimate the zone of inhibition against the selected bacterial strains. Bacterial strains that showed a positive response to BAME were selected for the antibacterial study of the isolated compound from the *B. aristata* DC strain. The present investigation was carried out to test whether there is any antimicrobial activity and to find out the MIC and zone of inhibition of the isolated compound against some gram-ve, +ve bacteria. Finally, the isolated compound's antimicrobial activity was correlated with standard antibiotic ciprofloxacin at a dose of 100 µg/mL Table 2 and 3.

Antifungal Activity

The isolated compound was tested for their MIC against standard fungal microorganisms namely *Phaenorochaete chrysporium* MTCC 787, *Candida albicans* 5, *Penicillium chrysogenum* MTCC 2725, ATCC 10231, *Aspergillus niger* MTCC 281, using entropha231 technique and RMT5thon twice entropha15. Comparison was done with standard drug Nystatin and isolated compound from plant at 100 µg/mL dose. This research was carried out to test whether there is any antifungal activity and to find out the MIC of the isolated compound against some fungal strains. The dose of the isolated compound was chosen as 100, 200, 400, 800 and 1000 µg/mL for MIC, whereas 100 µg/mL were chosen to estimate the zone of inhibition against selected fungal strains.

Procurement of Test Microorganisms

The clinical isolates of the microorganisms were procured from Jadavpur University, Kolkata and Woodlands Nursing Home, Kolkata. The test bacteria used include *Shigella flexneri type* 36 NK 381, *S. flexneri* type BCH 995, *S. flexneri* type 6B 999, *S. boydii* 22461, *S. sonnei* F11001, *S. sonnei* 1, *S. sonnei* NK 840, *S. boydii* 8, *S. sonnei* BCH 397, *S. sonnei* E08869, *S. dysenteriae* 9, *S. sonnei* BCH 937, *S. boydii* 16552, *S. sonnei* DN_3, *S. sonnei* NK 29, *S. dysenteriae* 1, *Vibrio cholerae* 452, *V. cholerae* BD 1/81, *V. cholerae* DN6, *V. cholerae* 1023, *V. cholerae* 756, *V. cholerae* 1341, *V. cholerae* A 26, *V. cholerae* 1033, *V. cholerae* 575, *V. cholerae* 675, *V. cholerae* 1311, *Escherichia coli* 306, *E. coli* K88, , *E. coli* 383, *E. coli* 872,

E. coli 18/9, *E. coli* RH 07/12*E. coli* Ap600, *E. coli* 597, *E. coli* 798, *S. aureus* ML 267, *S. aureus* ATCC 6538, *Bacillus subtilis* MTCC 441, *B. cereus* MTCC 1305, *B. pumilus* 8241, *P. putida* MTCC 2252, *E. spp* AP596 , *P. aeruginosa* AP585 NLF, *K. pneumoniae*, *S. aureus* 381, *S. aureus* MTCC 96, *S. typhi* Type (2), *P. vulgaris* AP679 NLF, *E. coli* 35B.

The Institute of Microbial Technology in Chandigarh and Jadavpur University in Kolkata provided the fungal strains. The test fungi employed were *Ralstonia eutropha* MTCC 285, *Aspergillus niger* MTCC 281, *Candida albicans* ATCC 10231, *C. albicans* 5, *Penicillium chrysogenum* MTCC 2725, *Phanerochaete chrysosporium* MTCC 787, and *P. chrysogenum* ATCC 10231.

Two fold serial dilution technique

In-vitro antimicrobial activity was performed against 50 bacterial strains, which were 24-hour-old cultures, and the medium used was double-strength nutrient broth (Hi-media). The isolated compound from the extract was tested in the 50 to 400 µg/mL concentration range to determine their MIC and solutions were developed by liquefying in DMSO. Tubes used for the antibacterial test were incubated at 37 ± 1°C for 1-day under aseptic conditions. The MIC of the isolated compound from the extract was set on. The MIC is the minimum concentration of the extract that totally inhibited the extension of the test organism after 24 hours of incubation at 37°C.

Preparation of Test and Standard stock solution

Standard drug ciprofloxacin and berberine were dissolved in DMSO to obtain concentrations from 50 to 400 and 100 µg/mL, respectively.

In-vitro Antifungal Activity

An isolated compound from the BAME was tested for its MIC against standard organisms. *A. niger, C. albicans* 5, *P. chrysporium* MTCC 787, MTCC 281, *R. entropha* MTCC1255, *C. albicans* ATCC 10231, *P. chrysporium* MTCC 787, by two-fold serial broth dilution technique in triplicates, Nystatin was used as standard. Additionally, the zone of inhibition that berberine produced against the fungus strains was measured and contrasted with that of the industry-standard medication Nystatin.

The present investigation was done to test the antifungal and MIC of berberine against different fungal strains. Finally the antifungal efficacy of the berberine was assured by the standard method (agar dilution of disc diffusion technique) against different fungal strains and so with the standard drug Nystatin.

Two-fold serial dilution technique

The 1 day old cultures of several fungi were tested for the *in-vitro* antifungal property, and the medium used was double strength malt yeast extract broth. Berberine was tested at the concentration range of 800 to 1000 µg/mL to determine their MIC and solutions were prepared by diluting in solvent DMSO. The test tubes used for antifungal activity were incubated at 37 ± 1°C for 1 day and used aseptic condition. MIC of the berberine was acquired.[17]

Preparation of test stock solution and Standard stock solution

Nystatin and berberine wrere diluted in solvent DMSO to give a concentration of 100 µg/mL.

MIC Determination Using the Serial Dilution Method

Antibacterial activity

A stock solution of berberine isolated from the stem extract was renovated with a small quantity of solvent (DMSO does not show any antibacterial and antifungal activity. Volume of stock solution was calculated and mixed in nutrient agar medium and with each dilution of different conceration of 50, 100, 200, 400, 800, 1000 µg/mL berberine to prepare a final volume of 30 µg/mL to form a different dilution of berberine. These media then mixed into pre-sterilized petri dish (70 mm) to form sterile nutrient agar plates. These plates were refrigerated at a temperature 4°C for 1-day and ensured even diffusion dried the plate before inoculation at 25°C for 2 hours by checkboard technique. The cultured bacterial strain was added to suspension in each quadrant MIC value were acquired and spotted plates were inoculated Table 4 adn 5.

Antifungal activity

In this method, the calculated volume of the stock solution of the isolated bioactive group from the BAME (reconstituted with minimal DMSO) were dispersed in a series of McCartney bottles previously containing a calculated volume of sterile chilled molten SDA medium (40–45°C) to prepare a final volume of 5 mL each at dilutions of 400, 800, 1000, 1500 and 2000 µg/mL molten media containing different concentrations of berberine were aseptically poured into pre-sterilized tubes to form sterile SDA slopes. These slopes were then stored in a refrigerator at 4°C for 24 hours to ensure uniform diffusion of berberine. Then these plates were dried at 25°C for 2 hours before inoculation. One loop of overnight suspensions of fungal strains (10 5 CFU/mL) was inoculated into the respective slope. These slants were incubated at 25°C for 3 to 7 days and the MIC value was obtained.

Zone of inhibition by disc diffusion method

Prepration of stock solution (each of 1-mg/ mL) of ciprofloxacin and bereberin was prepared. Different dilutions of berberine using solvent DMSO and ciprofloxacin (solvent: sterile distilled water) were prepared from these stock solutions. Sterile nutrient agar plates were prepared and incubated at 37ºC for 24 hours to check for the presence of any sort of contamination. These were then flooded with 5 mL of bacterial suspension (Overnight 18 hours grown culture) of a test organism, the excess of which was removed by a sterile Pasteur pipette and plates were again dried at 37ºC for 30 to 45 minutes. These were then ready to apply the disc impregnated with the test drug berberine and the standard ciprofloxacin. The sterile Whatman filter paper disc (6 mm), soaked in different dilutions of the berberine and the drug, were placed in the appropriate position

Table 1: Percentage yield, slvent system and Rf value of molecule derived from BAME

Name of extract	*Yield in %*	*Solvent system*	*R_f Value*	*Inference*
Isolated compound	2.8% w/w from stem of *B. aristata* on dry wt. basis	n-Butanol: Glacial acetic acid: Water (12:3:4)	0.55	A single R_f value indicates a single compound may be isolated

of the plates marked as quadrant at the back of petri dishes with forceps. The disc was laid on the plates[18] apart from each other so around them clear zone of inhibition was produced in solution of berberine all disc with plate were soaked. All the flooded plates with corresponding paper discs was soaked and incubated for 1-day at 37°C. A similar course of action was with drug ciprofloxacin comparison was done after mesauring zone diameter in mm and compared. Sterilized paper discs without berberine or antibiotic were used as control.[19]

Isolation of the bioactive compound from the BAME

Healthy stems were constructed from the *in-vivo* plant, dried in the shade, and mechanically ground into powder. In a soxhlet device, 100 g of powdered stem was extracted with methanol for two days. A rotary flash evaporator (Buchi, Switzerland) was used to concentrate the extract under low pressure, and 10 g of the extract underwent individual 2% HCl acidification. To eliminate neutral components from the filtered acid solution it was extracted with diethyl ether (Et_2O), basified (pH 8–9) with sodium bicarbonate (Na_2CO_3), and extracted with dichloromethane (CH_2Cl_2). A desiccator was used to concentrate and dry the (CH_2Cl_2) solution. Crushed crystal was obtained and kept in an amber-colored container.

To test the presence of alkaloid extract and isolated components by TLC method using three different solvents n-butanol:water: glacial acetic acid: in the ratio 12:4 :3v/v. The Rf values and characterization was done using different spectral studies FTIR, MASS HPTLC, ^{1}H-NMR, HPLC and ^{13}C-NMR Figure 1, 2 and 3.

Isolation of compound was done by HPLC at Jubilant Chemsys Ltd., Noida. HPLC Water with 3 µL injection used for 10 minutes run time. By using Perkin-Elmer 1710 FTIR spectrophotometer. FTIR spectrum was recorded for KBr pelletes and obtained with the help of AMX spectrophotometer frequency (400 MHz) 'Bruker' H-NMR spectra. using solvent DMSO and $CDCl_3$ in ^{13}C-NMR and ^{1}H-NMR. At the Indian Institute of Technology, Delhi, India.using spectrophotometer Bruker micro OTOF-Q II 10262 ESI the mass spectrum was recorded

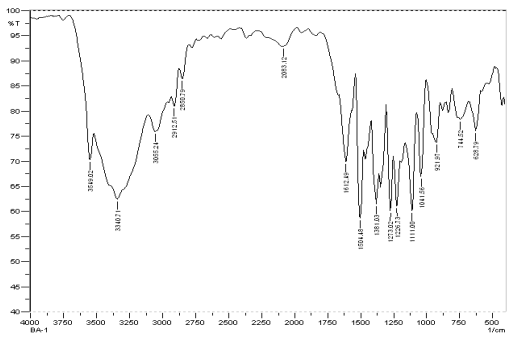

Figure 1: Graph of FTIR

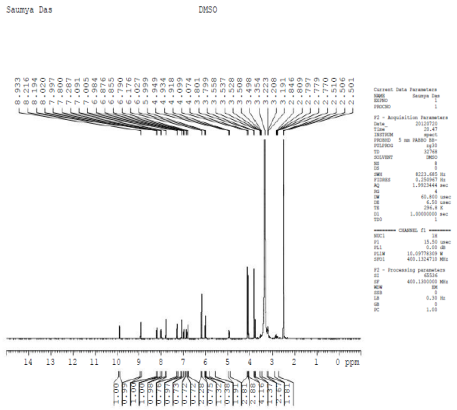

Figure 2: ^{1}H-NMR peak

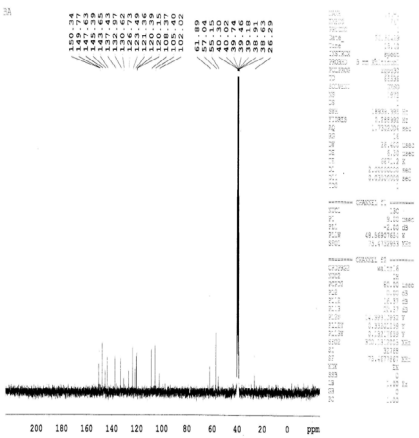

Figure 3: ^{13}C-NMR peak

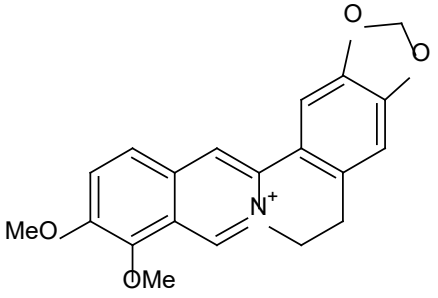

Figure 4: Chemical structure of berberine

RESULTS AND DISCUSSION

The %therapeutic yield of BAME was obtained to be 12.8%w/w on dry weight basis. The appearence of BAME was brownish yellow colored dried powder.

Isolation of Bioactive Molecule from the BAME

The derivative compound was procured as yellowish needle shaped crystals.

Table 2: *In-vitro* antibacterial activity of berberine obtained from BAME

S. No.	*Bacteria*	*Growth in nutrient agar containing different concentrations of berberine*				
		0 µg/mL*	*10 µg/mL*	*25 µg/mL*	*50 µg/mL*	*100 µg/mL*
1	*S. aureus* ML267	+	±	±	±	-
2	*S. sonnei* NK 840	+	±	±	-	-
3	*K. pnemoniea*	+	+	+	+	+
4	*B. subtilis* MTCC 441	+	±	±	±	-
5	*S. dysenteriae* 9	+	+	+	±	-
6	*V. cholerae* 1023	+	±	±	-	-
7	*Proteus vulgaris* AP 679NLF	+	+	±	±	-
8	*V. cholerae* 1033	+	+	+	±	-
9	*E. coli* 383	+	+	+	-	-
10	*S. sonnei* F11001	+	+	±	±	-
11	*E. coli* 798	+	±	±	-	-
12	*Enterobacter spp* AP596	+	+	+	±	-
13	*S. typhii* Ty (2)	+	+	±	-	-
14	*V. cholerae* 1341	+	+	±	-	-
15	*S. boydii* 22461	+	±	±	±	-
16	*S. aureus* 381	+	+	+	-	-
17	*P. auriginosa* AP 585 NLF	+	+	+	±	-
18	*B. pumilus* 8241	+	+	±	-	-
19	*S. sonnei* BCH 937	+	±	±	-	-
20	*E. coli* Ap600	+	±	±	±	-

* Control (plate without drug),
± Reduced Growth,
± IC Isolated Colony,
+ Growth,
– No Growth.
All determinations were done in triplicates.
MIC values of berberine as opposed to different bacterial strains were 50 to 100 **µg**/mL.

TLC Analysis of a Bioactive Molecule Derived from BAME

Spectral Studies

- *MASS spectra*

In ESI mass spectrum the molecular peak of the isolated compound was [M+1] 336 suggesting the molecular formula $C_{20}H_{18}NO_4$. For conforming theat berberine alkaloid present in BAME IR values and MASS values to identify other parameters like functional group, molecular structure, atomic stretching,etc. After analyzing and correlating them it was concluded that the compound that was derived is berberine with a condensed formula $C_{20}H_{18}NO_4$.

The antibacterial study of berberine from the stem extraction using méthanol *B. aristata* DC against E. *coli*, *S. boydii, Sh. soneii, Asiatic cholera* and *Sh. soneii* appeared to have greatest activity. All the MDR-selected micorbial species showed prominant antimicrobial activity. The effect of berberine against *Shigella sp.* own the benefit of having biggest hindrance zone-size (1.25 cm). MIC values of berberine ranged from 200 to 1000 µg/mL for this selected bacterial sp. The outcome of preliminary *in-vitro* antimicrobial studies of

Table 3: *In-vitro* antifungal activity of derivative berberine

S. No.	*Fungi*	*Growth in various concentrations (µg/mL) of Berberine*					
		*0**	*100*	*200*	*400*	*800*	*1000*
1	*R. entropha* MTCC1255	+	±	±	±	±	±
2	*C. albicans* ATCC 10231	+	±	±	IC	IC	-
3	*P. chrysporium* MTCC 787	+	+	±	±	IC	IC
4	*C. albicans* 5	+	-	-	-	-	-
5	*P. chrysogenum* MTCC 2725	+	+	+	±	±	±
6	*A. niger* MTCC 281	+	-	-	-	-	-

* Control (plate without drug),
± Reduced Growth,
±IC Isolated Colony,
+ Growth,
– No Growth.
All determinations were done in triplicates.
The isolated *B. aristata* DC stem compound had a MIC of 100 g/mL against various fungal strains.

Table 4: *In-vitro* antibacterial activity by disc diffusion method of derived compound Berberine

S. No.	Microorganisms	Zone if inhibition, (mm) of the isolated component (µg/mL)		Ciprofloxacin Std.Zone of inhibition, (mm)
	Bacteria	50 µg/mL	100 µg/mL	100 µg/mL
1	*S. boydii* 22461	7.0	14.5	25.5
2	*S. sonnei* NK 840	14.5	19.5	25.0
3	*S. sonnei* F11001	6.5	14.5	18.5
4	*S. sonnei* BCH 937	7.0	9.0	12.5
5	*S. dysenteriae* 9	10.5	12.5	19.0
6	*V. cholerae* 1023	16.5	18.0	22.5
7	*V. cholerae* 1341	12.0	14.6	18.0
8	*V. cholerae* 1033	14.5	16.9	21.0
9	*E. coli* Ap600	8.5	11.5	22.0
10	*E. coli* 383	13.8	15.5	20.5
11	*E. coli* 798	11.0	14.5	22.0
12	*Enterobacter spp* AP596	8.6	11.5	15.0
13	*S. typhii* Ty (2)	8.9	15.5	22.5
14	*S. aureus* ML267	10.5	13.9	21.5
15	*S. aureus* 381	13.0	14.5	20.0
16	*B. subtilis* MTCC 441	10.4	11.5	19.5
17	*P. auriginosa* AP 585 NLF	9.5	13.5	21.5
18	*B. pumilus* 8241	14.0	17.5	22.5
19	*K. pnemoniea*	10.0	18.9	25.0
20	*P. vulgaris* AP 679NLF	8.0	11.5	21.5

All determinations were measured three time for each sample (triplicate) and the outcome were demonstrated as Mean.

Table 5: *In-vitro* antifungal activity by disc diffusion method of isolated compound berberine

S.No.	Fungi	Isolated compound (zone of inhibition, mm)	Nystatin Std. (zone of inhibition, mm)
		(100 µg/mL)	(100 µg/mL)
1	*A. niger* MTCC 281	12.5	18.5
2	C. albicans5	11.0	21.8
3	*P. chrysogenum* MTCC 2725	11.0	16.0
4	*P. chrysporium* MTCC 787	6.0	22.5
5	*C. albicans* ATCC 10231	7.5	18.5
6	*R. entropha* MTCC1255	6.0	16.5

All determinations were measured three time for each sample (triplicate) and the outcome were demonstrated as Mean.

the isolated bioactive phytoconstituent affirm its restorative capability usage, as illustrated in the literature. Further studies are required for active constituent berberine to investigate its molecular and cellular mechanism in inhibiting microbial sp.

CONCLUSION

This research paper contains data on isolated bacteria like gram -ve and +ve. *B. aristata* DC has for the first time, scientifically confirmed as antimicrobial. *B. aristata* DC also has been reported traditionally for controlling various infectious disorders. This is also reported in the present study that antimicrobial efficacy gives multi-drug resistance in various bacterial strains. Efficacy has been approved of isolated compounds by MIC value. *B. aristata* DC has many phytoconstituents responsible for various activities people in India use these to treat diarrhea and infectious disease. Scientific data by phytochemical analysis prove that the have potent antibacterial activities. The gamètes were isolated from the hospital clinical sample. In today's time search of MDR pathogens of non-microbial antimicrobials is important. India, being diverse with forest and herbal, has unique sources and herbal treatments suited for controlling MDR microbial sp.

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