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Research Article

ANTIBACTERIAL EVALUATION OF METHANOLIC RHIZOME EXTRACT FROM AN *IN VIVO* AND *IN VITRO* GROWN PTERIDOPHYTE, *DRYNARIA QUERCIFOLIA* (LINN.) J SMITH

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ABSTRACT

Objective: *Drynaria quercifolia* (Linn.) J. Smith has been used in many herbal folklore medicines in Ganjam - Gajapati districts of Odisha state. Study pertains to its *in vivo* and *in vitro* antibacterial activities were undertaken to scientifically validate one of such therapeutic claim raised in the literatures, foreseeing the vulnerable state of the species.

Methods: Methanolic extract from both the *in vivo* grown plant and *in vitro* grown callus were prepared and tested for their antibacterial efficacy against a wide range of bacterial pathogens concerned. Both agar well diffusion and disk diffusion methods were followed for evaluation of antibacterial activities. Development of callus was done using modified MS media supplemented with 20/gl sucrose and varied concentrations of auxins and cytokinins and the extract was prepared thereof. Antibacterial potency of both the extracts was measured in terms of zone of inhibition and statistical analysis was performed using Graph pad prism 6 software.

Results: Methanolic extracts from both *in vivo* and *in vitro* samples had broad spectrum antibacterial activity on series of bacteria; however, bacterial species like *Salmonella typhi, Shigella flexinneri, Streptococcus pneumoniae* were resistant to both the samples. Further, the *in vitro* sample showed a higher degree of inhibition compared to the *in vivo* sample, was revealed.

Conclusion: Study explored that the plant species is a potential source of antibacterial activity suggesting its *in vitro* culture for conservation and to obtain higher degree of antibacterial efficacy as well.

Keywords: *In vitro* culture, Therapeutic claim, Folklore medicine, *In situ*/*ex-situ* conservation.

INTRODUCTION

Plants bear numerous active compounds, each identified with specific biological activities among which antimicrobial activity is the most important one, which protects the plants from vulnerable state of microbial infections. Many such active compounds like secondary metabolites such as flavonoids [1], phenolics and polyphenols [2], tannins [3], terpenoids [4], and sesquiterpenes [5], etc., are found to act as effective antimicrobial compounds against a wide range of microorganisms. The lower phyto group especially the pteridophytes too possess similar such active principles for which they are not infected by microbial pathogens and hence survived for more than 350 million years. [6]. Screening for antibiotic activity in the extracts of 114 species of pteridophytes belonging to 61 genera from 27 families was surveyed. 67 ferns and 6 fern allies, representing 64% of the samples examined, were actively antibiotic. The active amenable substances in most cases were antibacterial and only 3 possessed antifungal activities [7]. Based on these reports it is said that pteridophytes constitute, a good source of antimicrobial compounds among which the species *Drynaria quercifolia* Linn. was screened for evaluation of the antibacterial activity. As these plants are in a threatened and vulnerable state due to habitat destruction including cutting down of forest species in which these grow as epiphytes, it urged its comparative bioactivity study on *in vivo* and *in vitro* grown plant parts locally used as medicinal. The other important reason for screening of its antibacterial activity is to put restriction in increasing failure of chemotherapeutics and antibiotic resistance exhibited by microbial pathogens; thus, establishing its antimicrobial potency [8-10] indirectly providing herbal source with less cost effective with no side effects.

The main objective of the present study envisaged to investigate the effects of methanolic rhizome extracts of a pteridophytic species *D. quercifolia* (Linn.) J. Smith against a wide range of bacteria and to evaluate the effective concentration of the crude extracts in inhibiting the bacterial pathogens concerned.

METHODS

Bacterial strains

Standard strains of Gram-positive and Gram-negative bacteria were used for the study. While selecting microbes, their role for causing infection both in animals and plants were given due importance. Gram-positive bacteria include *Staphylococcoccus aureus* (MTCC 96), *Staphylococcus epidermidis* (CI), *Streptococcus pneumoniae* (MTCC 2672), *Streptococcus pyogenes* (CI), *Bacillus subtilis* (MTCC 121). The Gram-negative strains used were *Escherichia coli* (MTCC 390), *Salmonella typhimurium* (MTCC 98), *Shigella flexineri* (MTCC 1457), *Shigella sonnei* (MTCC 2957), *Pseudomonas aerugenosa* (MTCC 1688), and *Proteus vulgaris* (MTCC 1771) and *Proteus mirabilis* (CI). All the standard strains were obtained from Institute of Microbial Technology, Chandigarh - 160 036, India vide customer No. 2075 dated 20/4/2010 and 19/01/2011 and clinical isolates were obtained from MKCG Medical College, Berhampur vide letter No. CPS/MSc.-Bs/10-11/109, respectively.

Growth medium

For routine use, the cultures were maintained on nutrient Agar (Hi media) plates. For long term storage, glycerol stocks were prepared and stored at −20°C. Glycerol stocks were prepared by inoculating a single colony into Luria Bertani media/Nutrient Agar media (as the case may be) and incubated at 37°C for 16 hrs. From this prepared culture, 0.85 ml was taken and 0.15 ml of 50% sterile glycerol was added. After thorough mixing, the above mixture was stored at −20°C in a deep freezer.

Standard drugs used as reference for activity studies

Chloramphenicol procured from Himedia, Mumbai, India was used as standard drugs in the present work for antibacterial activity study.

Processing of the *in vivo* plant sample and preparation of the extract

The herbal, available as an epiphyte inhabited on arboreal phorophytes or growing on rocks, was collected from the hill tops of Mahendragiri hills spread through Kerandimals of undivided Ganjam district at Taptapani - Chandragiri areas. Based on the folklore use, the rhizomes of the herbal only were collected. Because secondary metabolites normally are not distributed uniformly throughout the plant and may be accumulated/synthesized in specific or some part of the plant which varies depending on developmental stage of the plant or seasonal state pertains to surrounding biotic and abiotic factors, etc. The collected rhizomes were washed thoroughly removing the humus adhered and shade dried for 3 weeks. These were powdered mechanically for size reduction and then was subjected to successive extraction with solvents like n-Hexane, petroleum ether, chloroform and methanol and finally with distilled water in the increasing order of polarity using soxhlet extractor. The yields of the concentrated crude extracts were estimated and were subjected to preliminary phytochemical screening [11] and the chemical tests concerned thereof were carried out. The results so obtained, were tabulated. The herbal after collection from its venue was identified and authenticated by the taxonomist Dr S. K. Dash, Professor and Head, PG Department of Biosciences, CPS and BSI, Howrah, Kolkata vide letter no. CNH/TECH/2014/187. Both voucher herbarium specimens (vide no. Ranjan/08/2008) and live specimens were deposited in the Museum of College of Pharmaceutical Sciences, Berhampur of Ganjam district, Odisha, for future reference and for undertaking *ex situ* conservation and *in vitro* studies of the specimen.

***In vitro* callus development, processing and preparation of the extract**

The collected rhizomes were cleaned by removing the rhizoidal mats with humus adhering to its surface. These were washed thoroughly with tap water and then surface sterilized with fungicide miconazole (20/mgl) followed by mercuric chloride and sodium dodecyl sulfate solution (0.1%) for 10 minutes [12]. These surface sterilized rhizomes were then taken to the laminar air hood and rinsed thoroughly in sterile distilled water. After thoroughly rinsing the explants (rhizome tips) were cut into small sized explants pieces of 1-2 cm in length, which were inoculated in the test tubes containing culture media for initiation of callus.

The prepared explants were placed in simpler modified MS semi-solid media [13] supplemented with 20/gl sucrose and varied concentration of auxin: 2, 4- dichlorophenoxyacetic acid (2, 4D), Indole 3-butyric acid and cytokinins i.e. 6-benzyl amino purine (BAP), and 2-isopentyl adenine (2 iPA). Standardization of the media for better induction of callus culture was screened using different combinations. Before inoculation of the explants, the media was sterilized by autoclaving at 15 lb/inch^2 pressure (=121°C) for 20 minutes. The pH of the basal medium was also adjusted to 5.6 and cultures were brought to culture room maintained at temperature 25±2°C and humidity 70±5% under 16 hrs photoperiod provided by cool white fluorescent lamps (Philips, India). Initially, the media was poured to culture tubes (25 mm × 15 mm) plugged with non-absorbent cotton and autoclaved for sterilization at 121°C for 20 minutes. The explants were inoculated in the laminar air hood, which was maintained in standard conditions at least for 6-8 weeks until further subculturing. After a period of 8 weeks 250 ml basal medium supplemented with 3 mg/lit of BAP and 1 mg/lit 2 iPA was dispensed in a flask and approximately 30 mg of the morphogenetic calluses from the test tubes were withdrawn and inoculated in the flask containing the above media devoid of agar. These flasks were also maintained in standard conditions as above. The flasks containing media were incubated in a shaker incubator at 100 rpm for 8-10 hrs per day for 3 weeks and observed for development of callus cells. Time to time (i.e., in every 6-7 days intervals) callus cells were taken to study for morphogenesis. Upon subculturing after 6-7 weeks, the cells were put on a filter paper and its fresh weight and dry weight was estimated. Dry weight was estimated by drying the filtered cells in the hot air oven at 60°C for 1 hr. In the 2nd phase of subculturing, minimum basal medium supplemented with auxin: 2, 4-D (3 mg/lit), 2-iPA 3 mg/lit and sucrose 15 g/lit were taken.

To determine the antimicrobial susceptibility of microbes, the most reliable method of diffusion tests (disk and well diffusion methods) were followed. For disk diffusion test Kirby–Bauer method and for well diffusion test agar well diffusion method were followed. Initially, antimicrobial susceptibility was determined by disk and well diffusion methods and later on confirmed with the determination of minimum inhibitory concentration (MIC).

Agar well diffusion method

A single colony was suspended in 1 ml of sterile saline, added into 20 ml of media at 45°C, mixed thoroughly and poured into plates. After solidification, wells of 6 mm diameter were cut in it and different doses of the drug were loaded into the wells. The plates were left at room temperature for 1 hr for drug diffusion into the media and then incubated overnight at 37°C.

Disk diffusion method

Standardized filter paper disk agar diffusion procedure was followed which was standardized by WHO in 1961. In this method, presence or absence of an inhibitory area or zone around the disk identifies the bacterial sensitivity to the drug [14].

Determination of MIC

The agar well diffusion and disk diffusion methods were used for the antibacterial activity of the crude methanolic drug. However, for further confirmation, the MIC of the test drug and standard drug were also determined. This was carried out taking a stock solution of the test drug; activated standard test bacterial cultures and sterile broth. The stock solution was prepared by dissolving 100 mg of the methanolic extract in 2 ml of dimethyl sulfoxide (DMSO) and volume was adjusted to 20 ml with sterile distilled water that brought the sample concentration to 5 mg/ml. Sets of test tubes (each single set for one type of bacterium) were made ready, poured with 1ml of sterilized broth into which 1 ml of stock solution was added and mixed thoroughly. After mixing, 1 ml of the mixed stock solution from the first tube was transferred to the next tube containing fresh sterile broth. In this way, the dilution procedure was continued for the subsequent tubes to attain a series of dilutions of 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, and 1/1024, respectively. To each test tube of every set, 50 µl of the respective bacterial broth culture inoculums was added. These test tubes were then incubated at 37°C for 24 hrs and monitored for turbidity as growth and non-turbidity for no growth. Tubes as negative control containing 10% DMSO with culture medium and positive control tube of chloramphenicol dissolved in 10% DMSO with bacterial culture were maintained separately.

RESULT

The preliminary phytochemical investigation of the methanolic extracts revealed the presence of flavonoids, saponins, and tannins like important

Table 1: Preliminary phytochemical screening of methanolic extract obtained from rhizome of *Drynaria quercifolia* (Linn.) J. Smith

Test for the phytochemicals	Methanolic extract of the rhizome
Alkaloids	-ve
Flavonoids	+ve
Phenols	+ve
Proteins and amino acids	-ve
Saponins	+ve
Tannins	+ve
Steroids	+ve
Carbohydrates	-ve

+ve sign indicates presence and –ve sign indicates absence of the phytochemicals in the extract

phytochemicals (Table 1). Both the disk and well diffusion methods were performed along with determination of MIC for the assessment of antibacterial activity of the methanolic extract and the results were presented (Tables 2-4). The results showed that the methanolic extract had antibacterial activity on the series of selected strains of Gram-positive and Gram-negative bacteria. Gram-positive bacteria, sensitive to the extract were *Staphylloccus aureus, S. epidermidis, B. subtilis, and S. pyogenes*; while *S. pneumoniae* was resistant as observed in both disk and well diffusion methods. The susceptibility of the selected strains was precisely marked comparing to results of disk and well diffusion methods. The susceptibility of all the selected strains were precisely marked comparing to results of disc and well diffusion methods with respect to the control (Figs. 1-12). Due to the application of both the methods, it could be possible to test the effectiveness of the crude drug ranging from lower to maximum concentration. It also helped in encountering diffusion problem and working on the micro flora that prefers anaerobic deep-seated environment. Similarly, diffusion tests pertains to activities of Gram-negative were evaluated which also showed significant activities at par. Here *E. coli* was found to be the most sensitive strain followed by *Pseudomonas aeruginosa* and *S. typhimurium* while *S. flexineri* seemed to be a drug-resistant strain evident from its zone of inhibition compared to other strains. For further

Table 2: Antibacterial activity of the methanolic rhizome extract on tested bacteria (CI and MTCC strains) by agar well diffusion method

Test organism	Positive control chloramphenicol (100 µg/ml)	Concentration of methanolic extract/well		
		10 mg/ml	20 mg/ml	40 mg/ml
Gram-negative bacteria				
E coli MTCC 390	35.5±1.342	10.33±0.441**	16.17±1.167*	29.33±1.364
P. aeruginosa MTCC 1688	28.0±1.155	7.164±0.441**	12.33±0.441**	26.67±0.88
P. aeruginosa MTCC 741	28.83±0.726	7.33±0.6**	12.67±0.441**	22.83±0.6
P. vulgaris MTCC 1771	9.66±0.881	4.66±0.441*	7.83±0.726	14.33±0.441
P. mirabilis (CI)	8.66±0.441	5.33±0.6	7.33±0.6*	15.83±1.66**
S. typhimurium MTCC 98	34.83±1.202	8.83±0.6**	17.83±0.441*	28.83±0.441
S. flexineri MTCC 1457	6.167±0.441	5.167±0.166	10.50±0.288*	14.33±0.166**
Gram-positive bacteria				
S. aureus MTCC 96	29.67±0.881	9.83±0.441**	20.83±0.441*	28.33±0.333
S. epidermidis (CI)	25.33±2.205	7.5±0.288*	15.83±0.441	25.83±0.166
S. pneumonia MTCC 2672	25.33±1.878	5.167±0.441**	6.33±0.333*	12.50±0.288*
S. pyogenes (CI)	28±1.443	10.33±0.333*	15.5±0.5*	23.5±0.288
B. subtilis MTCC 121	29.83±0.726	9.833±0.166**	17.67±0.333*	22±0.577*

The results are expressed as mean±SEM values, n=3, the values represent the diameter of growth inhibitory zones in mm and probability values of ****p<0.0001, ***p<0.001, **p<0.01 and *p<0.05 were compared with control, SEM: Standard error of the mean, *B. subtilis: Bacillus subtilis, S. pyogenes: Streptococcus pyogenes, S. pneumonia: Streptococcus pneumonia, S. epidermidis: Staphylococcus epidermidis, S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, S. flexineri: Shigella flexineri, S. typhimurium: Salmonella typhimurium, P. mirabilis: Proteus mirabilis, P. vulgaris: Proteus vulgaris, E. coli: Escherichia coli*

Table 3a: Antibacterial activity of the methanolic rhizome extract on tested Gram-positive bacteria (CI and MTCC strains) by disk diffusion method

Gram-positive bacteria					
	***S. aureus* MTCC 96**	***S. epidermidis* Clinical Isolate (CI)**	***S. pneumonia* MTCC 2672**	***S. pyogenes* clinical isolate (CI)**	***B. subtilis* MTCC 121**
Concentration	Methanolic extract				
5 mg/ml	4.66±0.33**	3.16±0.16***	0.83±0.16***	4.83±0.33**	5.16±0.16**
10 mg/ml	7.83±0.16**	7.16±0.44***	2.66±0.16***	8.667±0.16**	9.66±0.16**
20 mg/ml	18.17±0.6*	12.17±0.33**	8.833±0.16***	14.17±0.44**	15.5±0.28**
Concentration	Standard drug - chloramphenicol				
100 µg/ml	27.83±1.302	21.67±0.166	28.33±0.33	26.83±0.92	31.1±0.60

The results are expressed as mean±SEM values, n=3, the values represent the diameter of growth inhibitory zones in mm and probability values of ****p<0.0001, ***p<0.001, **p<0.01 and *p<0.05 were compared with control. -: No inhibition, *B. subtilis: Bacillus subtilis, S. pyogenes: Streptococcus pyogenes, S. pneumonia: Streptococcus pneumonia, S. epidermidis: Staphylococcus epidermidis, S. aureus: Staphylococcus aureus*

Table 3b: Antibacterial activity of the methanolic rhizome extract on tested Gram-negative bacteria (CI and MTCC strains) by disk diffusion method

Gram-negative bacteria								
	***E coli* MTCC 390**	***P. aeruginosa* MTCC 1688**	***P. aeruginosa* MTCC 741**	***P. vulgaris* MTCC 1771**	***P. mirabilis* (CI)**	***S. typhimurium* MTCC 98**	***S. sonnei* MTCC 2957**	**S. flexineri MTCC 1457**
Concentration	Methanolic extract							
5 mg/ml	8.5±0.33	5.4±0.06	5.1±0.08	1.8±1.06	-	4.4±0.08	3.4±0.10	-
10 mg/ml	14.6±0.53	9.8±0.02	8.3±0.52	4.5±0.02	-	10.2±0.33	7.1±0.31	2.2±0.01
20 mg/ml	24.3±0.12	16.5±0.04	15.2±0.47	7.3±0.14	3.5±0.16	16±0.07	10.2±0.34	3.5±0.22
Concentration	Standard drug - chloramphenicol							
100 µg/ml	34.3±2.04	29.3±2.17	27.6±2.08	8.3±0.75	14.1±0.	35.3±0.43	7.2±0.64	6.2±0.11

The results are expressed as mean±SEM values, n=3, the values represent the diameter of growth inhibitory zones in mm and probability values of ****p<0.0001, ***p<0.001, **p<0.01 and *p<0.05 were compared with control, -: No inhibition. *S. sonnei: Shigella sonnei, P. aeruginosa: Pseudomonas aeruginosa, S. flexineri: Shigella flexineri, S. typhimurium: Salmonella typhimurium, P. mirabilis: Proteus mirabilis, P. vulgaris: Proteus vulgaris*

confirmation of the activities, the MIC value was also determined. This value of the test drug ranged from 0.31 mg/ml to 1.25 mg/ml against Gram-positive bacteria and 0.62 mg/ml to 1.25 mg/ml against Gram-negative bacteria respectively. This indicated that the methanolic extract worked effectively on both Gram-positive and Gram-negative category; thus, have a broad spectrum of activity on a wide range of bacteria. Further, it was also observed that Gram-negative bacteria were more sensitive to the methanolic extract compared to Gram-positive though there were certain bacteria which were of intermediate or moderately sensitive type. Among Gram-negative category, *P. vulgaris* and *S. flexineri* were moderately sensitive while *Streptococcus pneumoniae* was least sensitive or resistant to the extract.

Antibacterial activity of the *in vitro* grown plant callus was also evaluated by disk diffusion method (Table 5). The methanolic extract prepared from the callus showed a higher degree of inhibition compared to *in vivo* methanolic rhizome extract, evident from the results so obtained (Figs. 12-24).

Table 4: Minimum inhibitory concentration (mg/ml) of the standard and methanolic rhizome extract of *Drynaria quercifolia* Linn on tested bacteria (CI and MTCC strains)

Test organism	MIC of methanolic extract (mg/ml)	MIC of standard drugs (µg/ml)	
		Chloramphenicol (100µg/ml)	Amoxylin
E. coli MTCC 390	0.625	?500	15.63
P. aeruginosa MTCC 1688	1.25	?500	?500
P. aeruginosa MTCC 741	1.25	?500	?500
P. vulgaris MTCC 1771	1.25	?500	125
P. mirabilis (CI)	1.25	?500	125
S. typhimurium MTCC 98	0.625	?500	15.63
S. sonnei MTCC 2957	1.25	1.25	?500
S. flexineri MTCC 1457	2.5	?500	?500
Gram-positive bacteria			
S. aureus MTCC 96	0.312	125	3.12
S. epidermidis (CI)	0.625	125	125
S. pneumonia MTCC 2672	1.25	125	?500
S. piogenes (CI)	0.625	?500	?500
B. subtilis MTCC 121	1.25	?500	?500

Results are mean±SEM values, n=3, the values represent the diameter of growth inhibitory zones in mm. CI: Clinical isolate, SEM: Standard error mean, MIC: Minimum inhibitory concentration, *D. quercifolia: Drynaria quercifolia, S. sonnei: Shigella sonnei, P. aeruginosa: Pseudomonas aeruginosa, S. flexineri: Shigella flexineri, S. typhimurium: Salmonella typhimurium, P. mirabilis: Proteus mirabilis, P. vulgaris: Proteus vulgaris, B. subtilis: Bacillus subtilis, S. aureus: Staphylococcus aureus, E. coli: Escherichia coli*

Table 5a: Antibacterial activity of the methanolic rhizome extract on tested Gram-positive bacteria (CI and MTCC strains) by disk diffusion method

Gram-positive bacteria					
	***S. aureus* MTCC 96**	***S. epidermidis* (CI)**	***S. pneumoniae* MTCC 2672**	***S. piogenes* (CI)**	***B. subtilis* MTCC 121**
Concentration	Methanolic extract				
In vivo sample (20 mg/ml)	18.17±0.6*	12.17±0.33**	8.833±0.16***	14.17±0.44**	15.5±0.28**
In vitro callus sample (20 mg/ml)	28.33±0.333	20.83±0.441*	12.67±0.441**	26.67±0.88	23.5±0.288
Concentration	Standard drug - chloramphenicol				
100 µg/ml	27.83±1.302	21.67±0.166	28.33±0.33	26.83±0.92	31.1±0.60

The results are expressed as mean±SEM values, n=3, the values represent the diameter of growth inhibitory zones in mm and probability values of ****p<0.0001, ***p<0.001, **p<0.01 and *p<0.05 were compared with control. -: No inhibition. *B. subtilis: Bacillus subtilis, S. pyogenes: Streptococcus pyogenes, S. pneumonia: Streptococcus pneumonia, S. epidermidis: Staphylococcus epidermidis, S. aureus: Staphylococcus aureus*, SEM: Standard error of mean, *E. coli: Escherichia coli*

Table 5b: Antibacterial activity of the methanolic rhizome extract on tested Gram-negative bacteria (CI and MTCC strains) by disk diffusion method

Gram-negative bacteria								
	***E. coli* MTCC 390**	***P. aeruginosa* MTCC 1688**	***P. Aeruginosa* MTCC 741**	***P. vulgaris* MTCC 1771**	***P. mirabilis* (CI)**	***S. typhimurium* MTCC 98**	***S. sonnei* MTCC 2957**	***S. flexineri* MTCC 1457**
Concentration	Methanolic extract							
In vivo sample (20 mg/ml)	14.6±0.53	9.8±0.02	8.3±0.52	4.5±0.02	-	10.2±0.33	7.1±0.31	2.2±0.01
In vitro callus sample (20 mg/ml)	24.3±0.12	16.5±0.04	15.2±0.47	7.3±0.14	3.5±0.16	16±0.07	10.2±0.3	3.5±0.22
Concentration	Standard drug – chloramphenicol							
100 µg/ml	34.3±2.04	29.3±2.17	27.6±2.08	8.3±0.75	14.1±0.33	35.3±0.43	7.2±0.64	6.2±0.11

The results are expressed as mean±SEM values, n=3, the values represent the diameter of growth inhibitory zones in mm and probability values of ****p<0.0001, ***p<0.001, **p<0.01 and *p<0.05 were compared with control. -: No inhibition, *S. sonnei: Shigella sonnei, P. aeruginosa: Pseudomonas aeruginosa, S. flexineri: Shigella flexineri, S. typhimurium: Salmonella typhimurium, P. mirabilis: Proteus mirabilis, P. vulgaris: Proteus vulgaris, B. subtilis: Bacillus subtilis*, SEM: Standard error of mean, *E. coli: Escherichia coli*

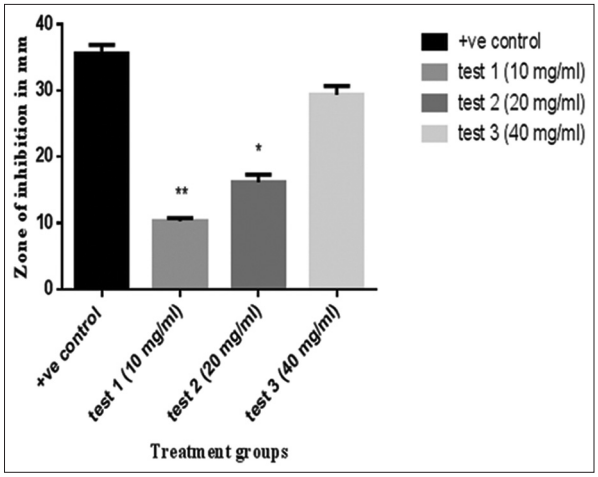

Fig. 1: Antibacterial activity of MEDQ on *Escherichia coli* (MTCC 390) by agar well diffusion method

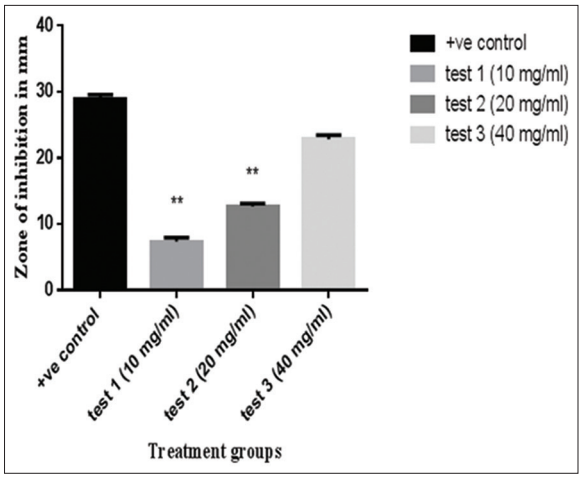

Fig. 2: Antibacterial activity of MEDQ on *Pseudomonas aeruginosa* (MTCC 741) by agar well diffusion method

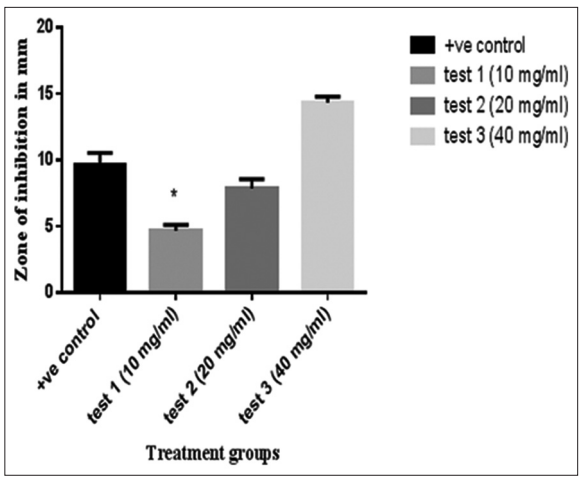

Fig. 3: Antibacterial activity of MEDQ on *Proteus vulgaris* (MTCC 1771) by agar well diffusion method

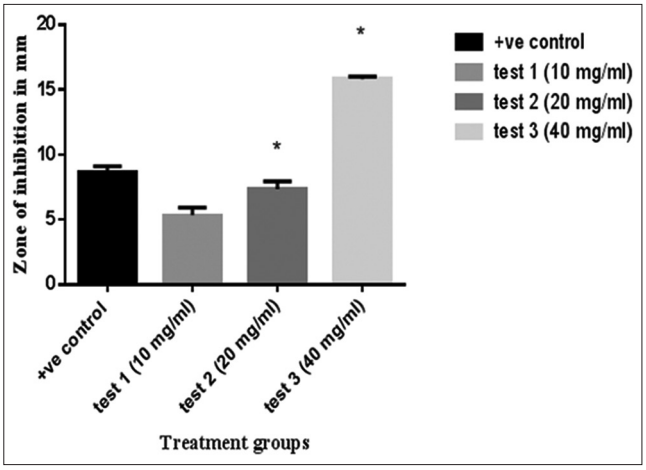

Fig. 4: Antibacterial activity of MEDQ on *Proteus mirabilis* (CI) by agar well diffusion method

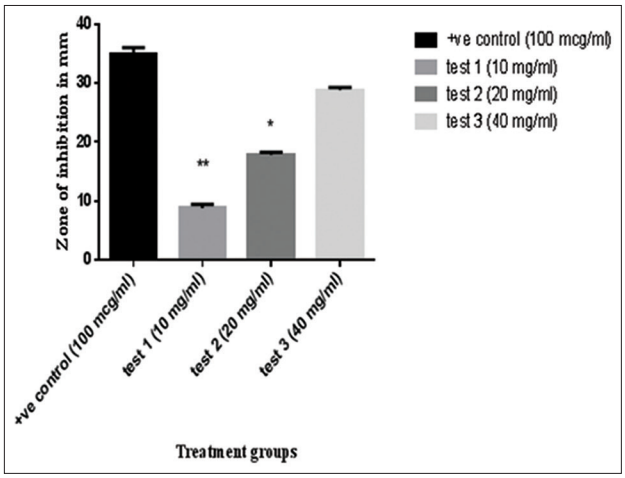

Fig. 5: Antibacterial activity of MEDQ on *Salmonella typhimurium* (MTCC 98) by agar well diffusion method

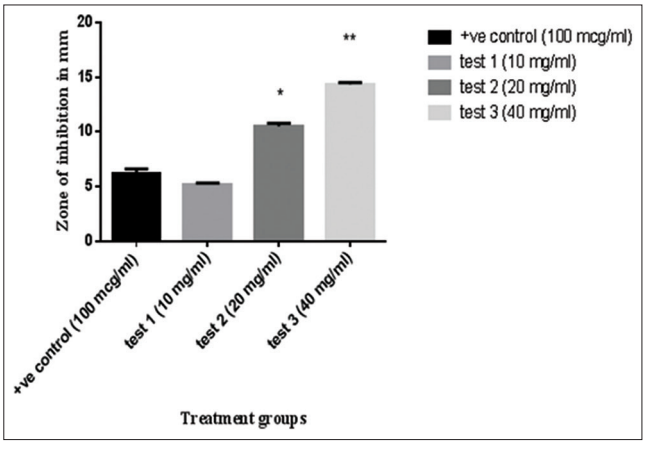

Fig. 6: Antibacterial activity of MEDQ on *Shigella flexineri* (MTCC 1457) by agar well diffusion method

DISCUSSION

The results explored that the extracts could inhibit the growth of the pathogenic microbes efficiently and this might be due to many active principle deposited in the form of phytochemicals. The various such phytochemicals present in the extract were saponins, tannins, steroids, flavonoids, and phenols (Table 1) known to have antimicrobial property [15] against many resistant strains of bacterial flora. It is also

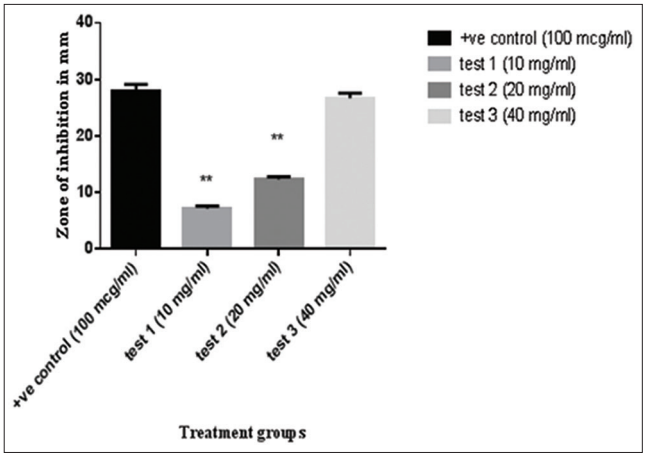

Fig. 7: Antibacterial activity of MEDQ on *Pseudomonas aeruginosa* (MTCC 1688) by agar well diffusion method

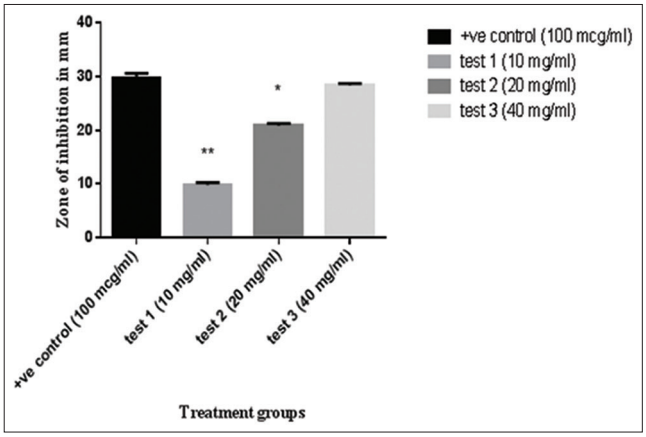

Fig. 8: Antibacterial activity of MEDQ on *Staphylococcus aureus* (MTCC 96) by agar well diffusion method

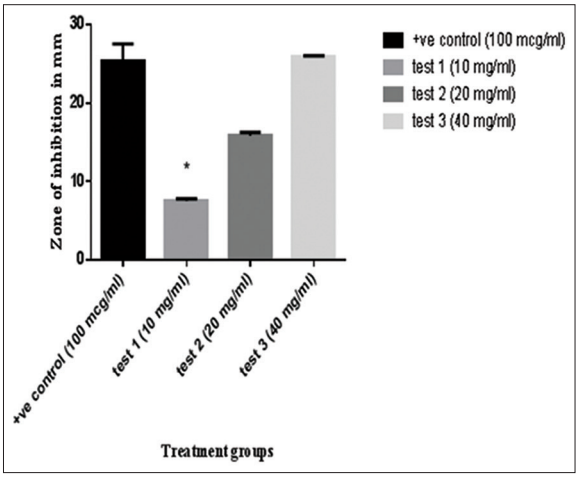

Fig. 9: Antibacterial activity of MEDQ on *Staphylococcus epidermidis* (CI) by agar well diffusion method

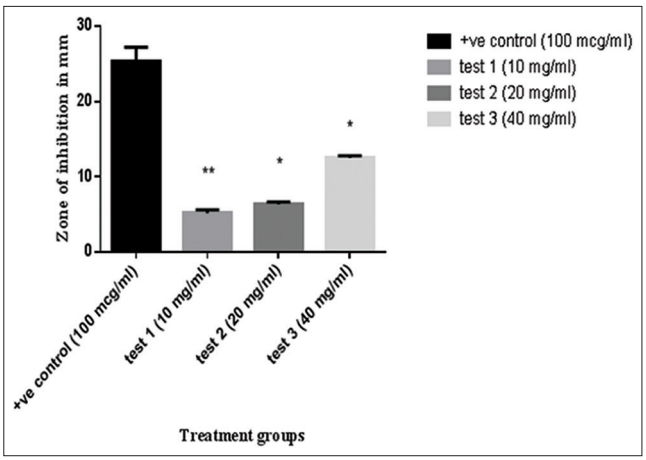

Fig. 10: Antibacterial activity of MEDQ on *Streptococcus pneumonia* (MTCC 2672) by agar well diffusion method

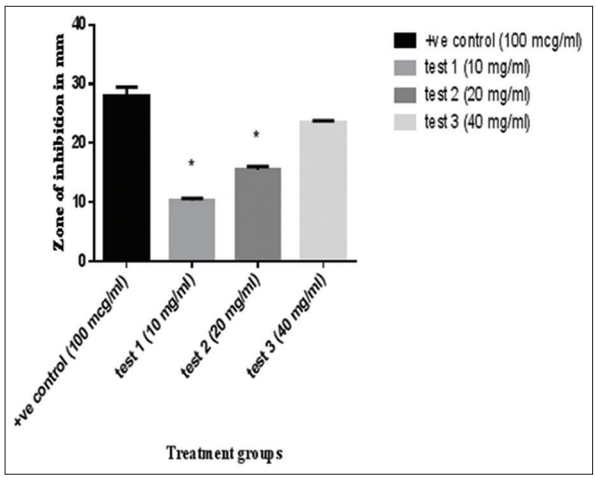

Fig. 11: Antibacterial activity of MEDQ on *Streptococcus pyogenes* (CI) by agar well diffusion method

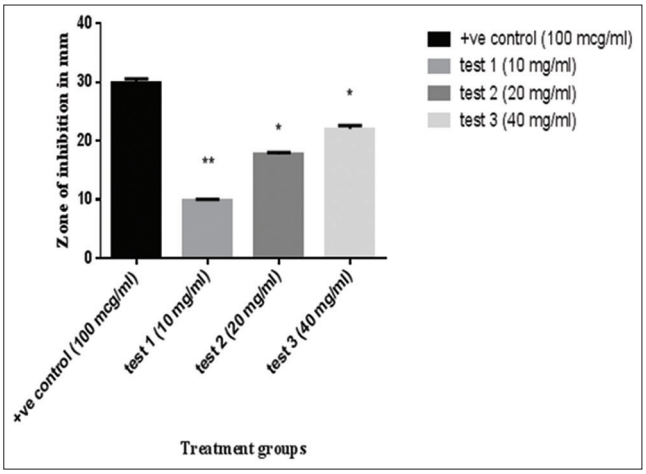

Fig. 12: Antibacterial activity of MEDQ on *Bacillus subtilis* (MTCC 121) by agar well diffusion method

reported that a broad spectrum of antibacterial activity is also due to saponin like phytochemicals. The antibacterial activity of the methanolic extract of *D. quercifolia* is significant from the point that it was sensitive against *P. aeruginosa;* but resistant to most of the antibiotics used in the clinical practice [16]. Investigation on the antibacterial activity of *in vivo* plant of *D. quercifolia* is at par with earlier findings [17-19]. In the antibiotic sensitivity test, it was found that the test organisms were multidrug resistant; but, they were sensitive to methanolic extract and chloramphenicol. Out of the 12 different bacterial strains selected for the study, 2 were in the Gram-negative category such as

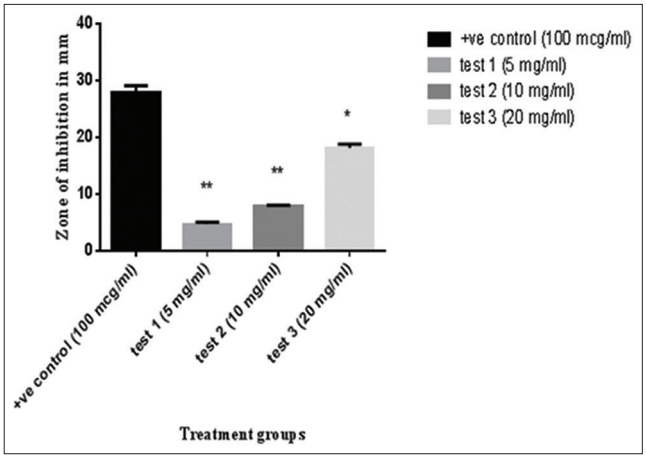

Fig. 13: Antibacterial activity of MEDQ on *Staphylococcus aureus* (MTCC 96) by disk diffusion method

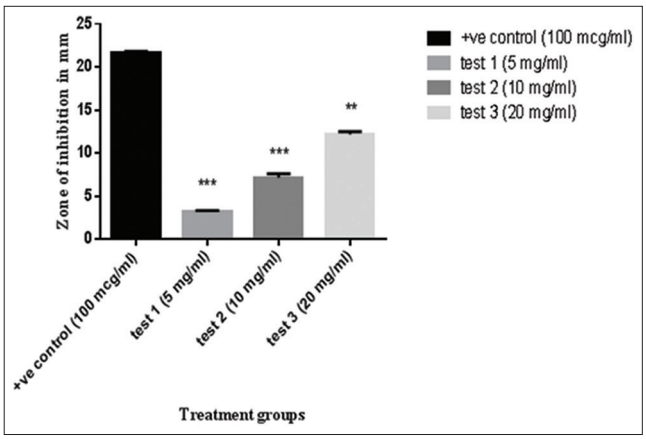

Fig. 14: Antibacterial activity of MEDQ on *Staphylococcus epidermidis* (CI) by disk diffusion method

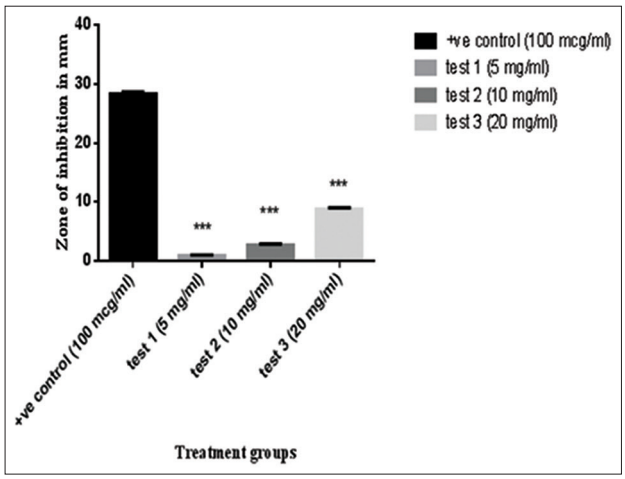

Fig. 15: Antibacterial activity of MEDQ on *Streptococcus pneumonia* (MTCC 2672) by disk diffusion method

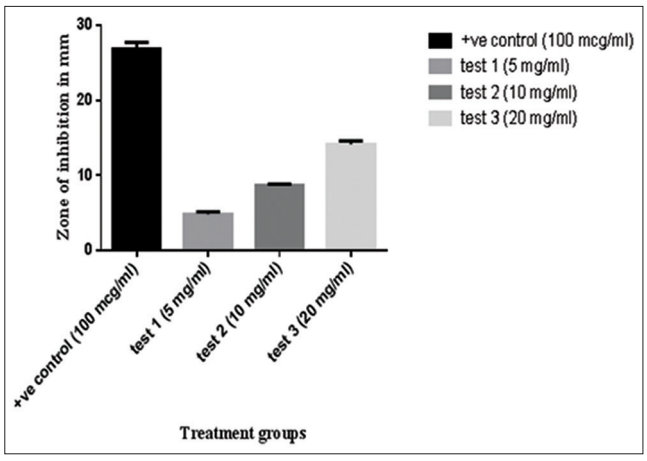

Fig. 16: Antibacterial activity of MEDQ on *Staphylococcus epidermidis* (CI) by disk diffusion method

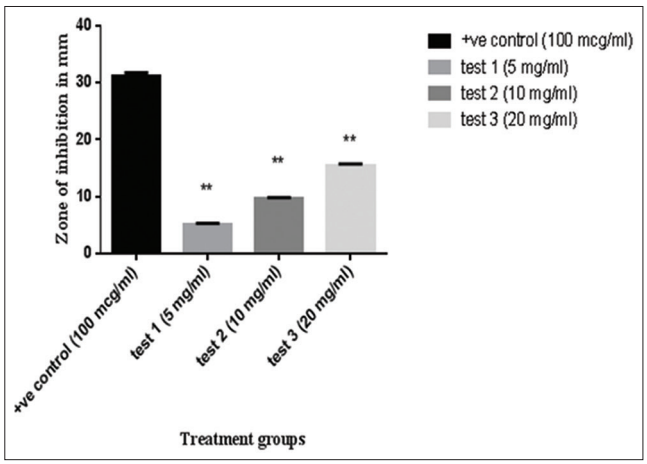

Fig. 17: Antibacterial activity of MEDQ on *Bacillus subtilis* (MTCC 121) by disk diffusion method

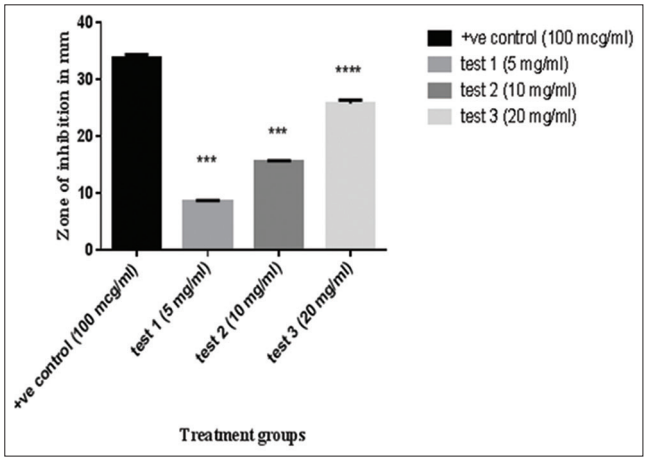

Fig. 18: Antibacterial activity of MEDQ on *Escherichia coli* (MTCC 390) by disk diffusion method

S. flexineri and *P. vulgaris* and one in the Gram-positive group such as *S. pneumoniae* were found to be resistant although were sensitive to the antibiotic chloramphenicol.

The stronger antibacterial activity of the *in vitro* callus extract was observed which might be due to factors like supply of the plant growth regulators in the culture medium, production, and accumulation of compounds more in undifferentiated callus tissue than in the normal differentiated plant cells etc. [20,21].

The results of the present investigation enriched the medico folklore database pertains to antibacterial potential of the plant species.

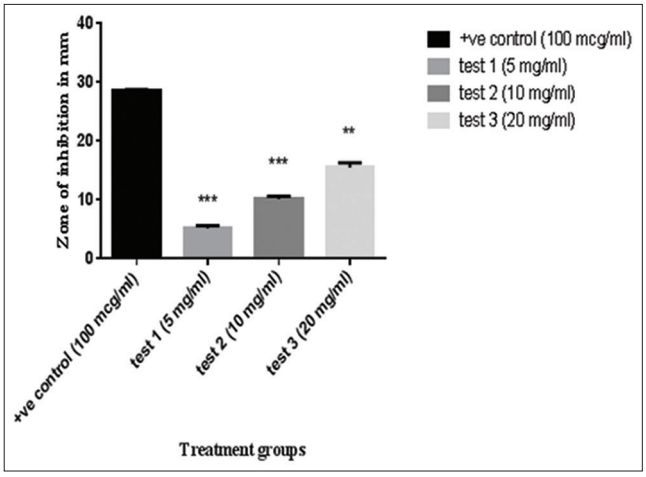

Fig. 19: Antibacterial activity of MEDQ on *Pseudomonas aeruginosa* (MTCC 1688) by disk diffusion method

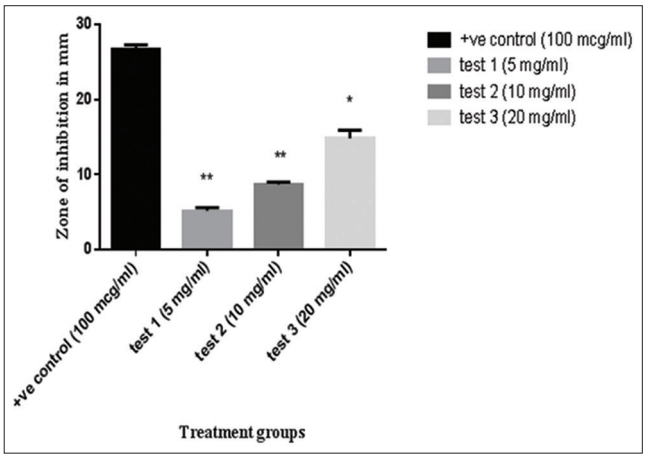

Fig. 20: Antibacterial activity of MEDQ on *Pseudomonas aeruginosa* (MTCC 741) by disk diffusion method

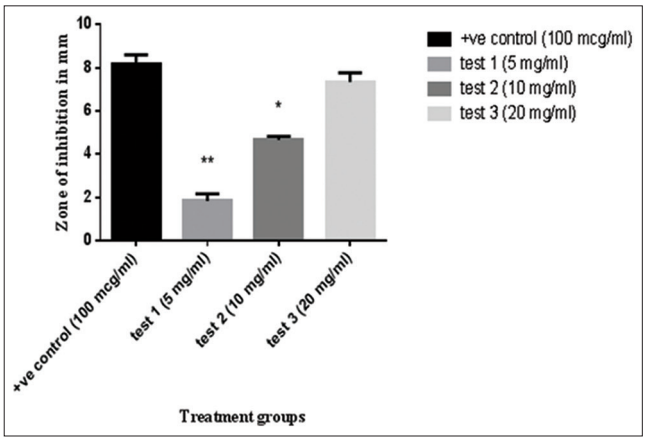

Fig. 21: Antibacterial activity of MEDQ on *Proteus vulgaris* (MTCC 1771) by disk diffusion method

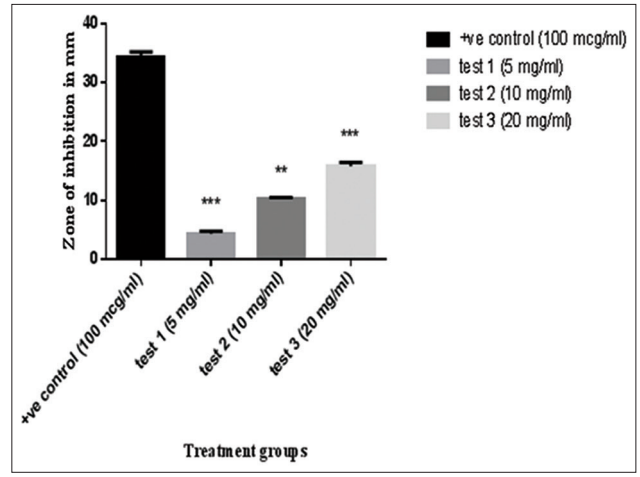

Fig. 22: Antibacterial activity of MEDQ on *Salmonella typhimurium* (MTCC 98) by disk diffusion method

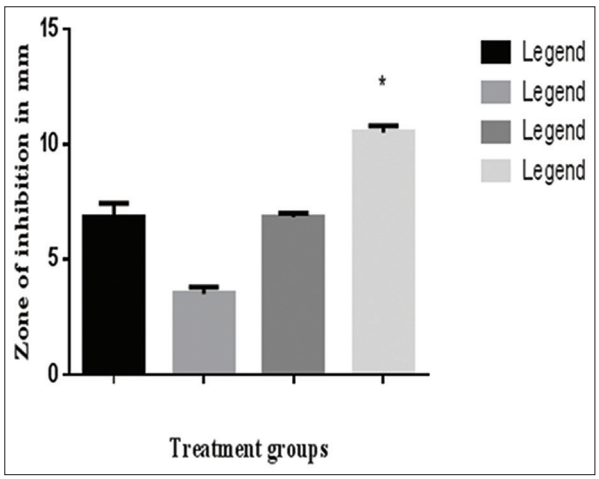

Fig. 23: Antibacterial activity of MEDQ on *Shigella sonnei* (MTCC 2957) by disk diffusion method

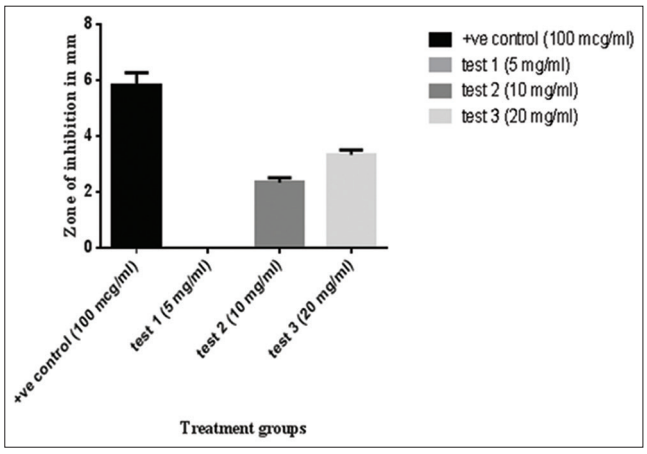

Fig. 24: Antibacterial activity of MEDQ on *Shigella flexineri* (MTCC 1457) by disk diffusion method

Further, the extracts of the *in vitro* grown callus offered better and higher degree of antibacterial activity compared to *in vivo* grown plants. This becomes encouraging in the development of novel antibacterial therapy against varied bacterial diseases and ex-situ conservation of the species as well.

ACKNOWLEDGMENTS

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REFERENCES

1. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, *et al.* Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant Staphylococcus aureus. J Ethnopharmacol 1996;50(1):27-34.
2. Mason TL, Wasserman BP. Inactivation of red beet beta glucan synthase by native and oxidized phenolic compounds. Phytochemistry 1987;26:2197-202.
3. Ya C, Gaffney SH, Lilley TH, Haslam E. Carbohydrate – polyphenol complexation. In: Hemingway RW, Karchesy JJ, editors. Chemistry and Significance of Condensed Tannins. New York: Plenum Press; 1988. p. 553.
4. Scortichini M, Pia RM. Preliminary *in vitro* evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora* (Burrill). J Appl Bacteriol 1991;71:109-12.
5. Gören N, Woerdenbag HJ, Bozok-Johansson C. Cytotoxic and antibacterial activities of sesquiterpene lactones isolated from Tanacetum praeteritum subsp. praeteritum. Planta Med 1996;62(5):419-22.
6. Sharma BD, Vyas MS. Ethnobotanical studies on the ferns and fern-allies of Rajasthan. Bull Bot Surv India 1985;27:90-1.
7. Banerjee RD, Sen SP. Antibiotic activity of pteridophytes. Econ Bot 1980;34(3):284-98.
8. Samy RP, Ignacimuthu S. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. J Ethnopharmacol 2000;69(1):63-71.
9. Gutmann L, Billot-Klein D, Williamson R, Goldstein FW, Mounier J, Acar JF, *et al.* Mutation of Salmonella paratyphi A conferring cross-resistance to several groups of antibiotics by decreased permeability and loss of invasiveness. Antimicrob Agents Chemother 1988;32(2):195-201.
10. Mohanasundari C, Natarajan D, Srinivasan K, Umamaheswari SA, Ramachandran A. Antibacterial properties of *Passiflora foetida* (L) - A common exotic medicinal plant. Afr J Biotechnol 2007;6(23):2650-3.
11. Kokate CK. Practical Pharmacognosy. 4th reprinted ed. New Delhi: Vallabh Prakasan 1999. p. 107, 124.
12. Tynan JL, Conner AJ, Macknight RC, Poulter RT. Miconazole: An effective antifungal agent for plant tissue culture. Plant Cell Tissue Organ Cult 1993;32(3):293-301.
13. Murashige T, Skoog TA. Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 1962;15:473-97.
14. Bauer AW, Kirby MD, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disc diffusion method. Am J Clin Pathol 1966;45:493-6.
15. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12(4):564-82.
16. Tanira MO, Bashir AK, Dib R, Goodwin CS, Wasfi IA, Banna NR. Antimicrobial and phytochemical screening of medicinal plants of the United Arab Emirates. J Ethnopharmacol 1994;41(3):201-5.
17. Ashour SM and Kheralla ZH. Study of antimicrobial activity by some medicinal plants. Egypt J Microbiol 1995;30:445-69.
18. Azu NC, Onyeagba RA, Nworie O, Kalu J. Antibacterial activity of *Allium cepa* Onion and *Zingiber officinale* (ginger*) on Staphylococcus aureus and Pseudomonas aeruginosa* isolated from high vaginal swap. Internet J Trop Med 2007;3(2):110-5.
19. Kandhasamy M, Arunachalam KD, Thatheyus AJ. *Drynaria quercifolia* (L.) J. Sm: A potential resource for antibacterial activity. Afr J Microbiol Res 2008;2:202-5.
20. Choi KT, Ahn IO, Park JC. Production of ginseng saponin in tissue culture of ginseng (*Panax ginseng* C. A. Mayer). Russ J Plant Physiol 1994;41:784-8.
21. Takahashi D, Fjita Y. Cosmetic materials. In: Komamine A, Misawa M, Dicosmo F, editors. Plant Cell Culture in Japan. Tokyo: CMC Co Ltd.; 1991. p. 72-8.