WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 2, 1253-1258. Research Article ISSN 2277– 7105

EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF BARLERIA CRISTATA- AN INVITRO STUDY

Darling Chellathai[1], Punnagai Gunasekaran[2], Aparna Mani[3]

[1] Professor, Dept. of Pharmacology, SRMC & RI, Porur, Chennai.

[2] Associate Professor, Dept. of Pharmacology, SRMC & RI, Porur, Chennai.

[3] Senior Resident, Dept. of Pharmacology, SRMC & RI, Porur, Chennai.

Article Received on
08 Dec 2014,

Revised on 29 Dec 2014,
Accepted on 21 Jan 2015

***Correspondence for Author**
Dr. Darling Chellathai
Dept. of Pharmacology,
Sri Ramachandra
Medical College and RI,
Porur, Chennai.

INTRODUCTION

Infectious Diseases constitute a major proportion of the Global Disease burden and continues to be the foremost leading cause of mortality in the developing countries.[1] Over the past few decades tremendous advancements in the scientific field have led to the development of many antibiotics. However most of the available drugs have their own drawbacks in terms of adverse effects and high cost. The emergence of antibiotic resistance also poses a major threat to the patients and the treating physicians.[2] Thus, strategies to develop better and newer antibiotics is the need of the hour. This has forced the attention of researchers towards plant products. Plants have long since been used as potential sources of many drugs including antibiotics. Several studies have indicated that plants have active metabolites such as alkaloids, flavanoids, tannins and phenolic compounds which show good antimicrobial activity.[3,4,5] Plant derived drugs are generally well tolerated, with minimal side effects and can be sourced more easily.[6] Barleria cristata is an ornamental shrub belonging to Acanthaceae family widely found in Central and South India. It has been used as a traditional herbal medicine for treating various disorders including anaemia, toothache, cough, fever, asthma, bronchitis and diabetes.[7] The roots and leaves were found to have anti inflammatory properties and used for swelling and local inflammation.[8] Earlier studies have reported antibacterial activity of leaf extract of B.cristata mainly against gram positive organisms.[9,10] The current research focuses on the antibacterial activity against commonly occurring gram negative organisms and also to evaluate the anti fungal activity of the ethanolic leaf extracts of B. cristata.

MATERIALS AND METHODS

Collection of Plant Material: Barleria cristata plant was collected from Maduranthagam, Kancheepuram district. The plant was identified and certified by Research Officer Pharmacognosy, Siddha Central Research Institute, Chennai. The fresh plant leaves were washed free of sand, dried at room temperature and coarsely powered .

Preparation of Extract: The powdered leaf material (50 g) was subjected to successive solvent extraction (250ml) with ethanol and water using soxhlet apparatus and the extract was filtered using what man No.1 filter paper. The crude extract was further concentrated and used for further studies.

Test Organisms: Ten strains of bacteria (eight gram negative and two gram positive) viz. E. coli, Vibrio cholera, Pseudomonas aeruginosa, Vibrio parahaemolyticus, Salmonella, Aeromonas, Klebsiella , Proteus Staphylococcus aureus, Bacillus subtilis and five strains of fungi such as *Candida albicans, Aspergillus flavus , Penicillium spp, Aspergillus niger and Trichophyton spp.* were used for testing antimicrobial activity. The bacterial and fungal strains were obtained from MTCC, Chandigarh.

Antibacterial and Antifungal Activity: Antimicrobial activity was determined by disc diffusion method.[11] Bacteria were cultured using Muller Hinton Agar medium. Different concentration of the extract 1000µg/ml, 750µg/ml, 500µg/ml was prepared and MHA medium was poured into the petridish. After the medium solidified the inoculums of bacteria was spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc was placed on the MHA plates and 20µl of the extract sample was added to the disc. Ampicillin 1mg/ml was used as a positive control and DMSO was used as a negative control. Each bacteria was tested for the effect of different concentration of the plant extract, ampicillin and DMSO. The plates were incubated at 37˚C for 24 hours.

Antifungal activity was evaluated by preparing stock cultures which were maintained at 4°C on Sabouraud Dextrose agar slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing Sabouraud Dextrose broth , that were incubated at 48hrs at 37ºC. Each fungal innocula was spread evenly on Sabouraud Dextrose Agar (SDA) using a sterile swab. The Amphotericin B (20µl/ disc) standard and 20 µl of sample (Concentration: 1000µg, 750µg and 500 µg) were placed in the disc and incubated at 37ºC for 24 hrs. Finally, the antibacterial and antifungal

activities was determined by measuring the diameters of zone of inhibition in the MHA and SDA plates.

RESULTS

The antibacterial and antifungal activities of the ethanolic extract of Barleria cristata in terms of diameter of zone of inhibition is depicted in Table 1.

Against the ten bacteria evaluated, the plant extract showed potent antibacterial activity against Vibrio spp with inhibition zone diameter of 15mm followed by Staphylococcus aureus with 14 mm diameter at 1000 µg/ml concentration. At the same concentration, moderate antibacterial activity was seen against E-coli, Pseudomonas, Vibrio parahaemolyticus, Salmonella and Aeromonas spp, ranging between 6 to 8 mm inhibition zone diameters. The plant extract had least antibacterial property aginst Klebsiella and Proteus species.

Fungal spp were found to be comparatively more resistant than the bacteria to the ethanolic extract of B.cristata. Among the five fungal spp. studied, the maximum zone of inhibition was shown by Aspergillus niger species which was only around 6mm diameter at 1000µg/ml. All the other fungal species showed moderate susceptibility with inhibition zones ranging between 3 to 5 mm in diameter.

Table 1: Results of antimicrobial screening of ethanolic extract of B.cristata determined by the agar diffusion method (inhibition zone)

Organisms	Zone of Inhibition (mm)			Antibiotic (1mg/ml)	DMSO (20µl)
	Concentration(µg/ml)				
	1000	750	500		
Bacteria					
E.coli	7 mm	6 mm	5 mm	15 mm	-
Vibrio spp.	15 mm	10 mm	6 mm	20 mm	-
Staphylococcus aureus	14 mm	13 mm	8 mm	17 mm	-
Pseudomonas aeroginosa	8 mm	-	-	21 mm	-
Bacillus spp.	7 mm	6 mm	-	15 mm	-
Vibrio parahaemolytics	6 mm	7 mm	5 mm	17 mm	-
Salmonella spp.	6 mm	5 mm	7 mm	16 mm	-
Aeromonas spp.	7 mm	-	-	14 mm	-
Klebsiella spp.	5mm	3mm	1mm	19mm	-
Proteus spp.	4mm	2mm	-	17mm	-
Fungi					
Candida albicans	4mm	3mm	-	7mm	-

Aspergillus flavus	5mm	2mm	1mm	8mm	-
Penicillium spp	3mm	2mm	1mm	7mm	-
Aspergillus niger	6mm	5mm	2mm	13mm	-
Trichophyton	4mm	2mm	1mm	8mm	-

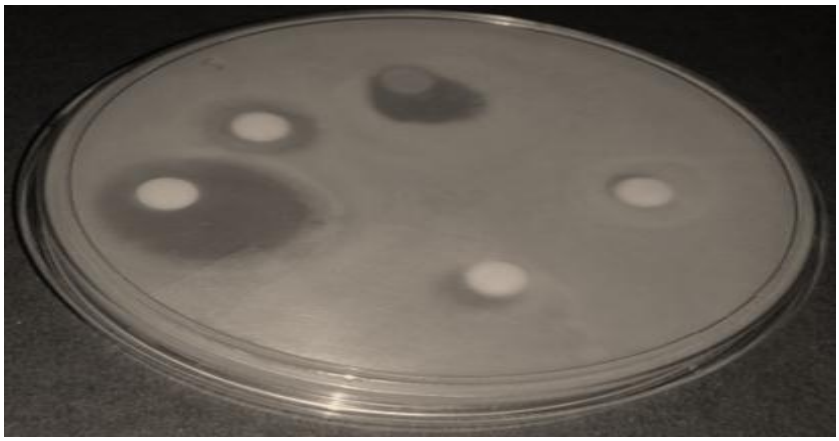

Fig 1: Ethanol extract of Barleria cristata produced highest zone of inhibition (15mm) against Vibrio spp.

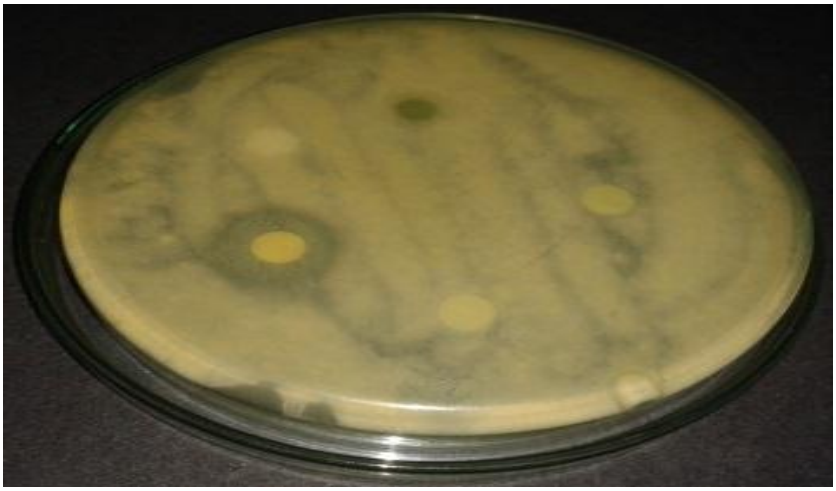

Fig 2: Ethanol extract of Barleria cristata produced highest zone of inhibition (6mm) against Aspergillus niger

DISCUSSION

Antibiotic resistance is an emerging global threat to mankind with numerous microorganisms now developing resistance to majority of the antibiotics available in the market[12]. Researchers have started focusing on alternate resources for the development of safer and more effective antimicrobials. The rich ecosystem of our planet still has more than 250,000 undiscovered plants with untapped medicinal properties.[13] Several studies are being conducted to study the antibacterial and antifungal properties of plant sources. This study was undertaken to assess the antimicrobial property of Barleria cristata against common

human pathogenic microorganisms. In the present study, ethanolic leaf extract of B.cristata showed significant antibacterial activity against mainly Vibrio spp. and Staphylococcus aureus. The zone of inhibition for Vibrio spp. is indicated in Fig1. Similar activity against Staphylococcus aureus was obtained in the study conducted by Joseline et al, 2013 where the ethanolic extract of B.cristat bark was studied.[9] In yet another study by Amudha and Doss, the saponin fraction from the leaves of B.cristata showed significant antibacterial activity against Klebsiella and Staphylococcus spp.[14] This is in contrast to our study, where there was negligible activity against Klebsiella spp. This could be attributed to enhanced antibacterial activity of the purified saponin fraction of B.cristata leaves. The overall antifungal activity of the crude ethanolic extract was found to be comparatively lesser when compared to the antibacterial effect. As depicted in Fig 2, Aspergillus niger showed maximum sensitivity, followed by Aspergillu flavus which correlates with the antifungal activity studied by Amudha and Doss. The antimicrobial activity of B.cristata is mainly due to the bioactive phytochemicals which include alkaloids, tannins, flavanoid, glycosides and phenols.[15]

CONCLUSION

The crude ethanloic extract of Barleria cristata was found to posses significant antimicrobial activity against human pathogens**.** Further studies are required to identify the active principles responsible for the antimicrobial activity thus paving way for more research to elucidate the exact mechanism of action by which it exerts its antimicrobial property. This study also suggests that the active compounds of B.cristata can be used as therapeutic agents in the development of novel drugs to combat infectious diseases.

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