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Research Article

ANTIMICROBIAL ACTIVITY OF GARDENIA LATIFOLIA AIT

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A B S T R A C T

The crude extracts (aqueous and ethanol) of the leaves of *Gardenia latifolia* were studied for their antibacterial activity by Disc diffusion method against bacterial strains *Staphylococcus aureus, Bacillus subtilis, Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae, Shigella flexneri, Pseudomonas fluroscence* and fungal strains *Candia albicans* and *Aspergillus niger*. It was observed that, ethanol extract showed the significant antibacterial activity against *Staphylococcus aureus* followed by other microorganisms. The MIC values were obtained by serial dilution techniques.

INTRODUCTION

Infectious diseases are the number one among all causes of death, accounting approximately one-half all deaths throughout the world. About 50-75% of hospital deaths are reported due to infectious diseases (Gnanamani *et al*., 2003). These numbers are still increasing due to development of resistance in microorganisms to the existing first line drugs. Scientists from divergent fields are investigating plants with a new eye for their antimicrobial usefulness and as an alternative source to existing drugs. Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agents with general as well as specific activity (Evans and Trease, 1996). In recent years focus on use of non-traditional approaches to treat diseases has been revived worldwide. The evidence collected till now shows immense potential of medicinal plants used in traditional systems. The world health organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. Hence the present study was taken up with an objective to evaluate the antimicrobial potential against the microorganisms.

Gardenia latifolia (Rubiaceae) is commonly known as Indian boxwood or Ceylon boxwood, is a densely foliaceous small tree that occurs throughout the greater parts of Indian common in deciduous forests along the streams. The stem bark and fruits are reported to be used in the treatment of various ailments such as snake bite, skin diseases, stomach pais, caries in humans and ephemeral fever in live stocks (Reddy *et al*., 2006; Madava Chetty *et al*, 2008; Dr.Duke's) Fruits are used for making perfumes (Chandra Prakash, K.2009).

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MATERIALS AND METHODS

Plant collection

The fresh aerial plant parts were collected from Kolli hills, Namakkal District, Tamil Nadu, India. The collected plant is identified by Botanical Survey of India (BSI/SRC/5/23/2013/Tech-795 & Serial No. 1), Coimbatore and the voucher specimens were deposited at the herbarium of Department of Botany, National College (Autonomous), Tiruchirappalli-1.

Preparation of extracts

Plant material

Fresh and health leaves were collected from Kolli hills, Tamilnadu, India. The leaves were washed thoroughly in distilled water and the surface water was removed by air drying under shade. The leaves were powdered with the help of mechanical blender and used for extraction.

Aqueous extract

Ten grams of powdered leaves were macerated with 100 ml of sterile distilled water in a blender for 24 hrs. The macerate was filtered through Whatmann no.1 filter paper to get pure extract. The extract was preserved aseptically in brown bottles at 4^{o}C until further use.

Ethanol extract

Air dried powder of 10 g was placed in a conical flask containing 100 ml of ethanol plugged with cotton and then kept on a rotary shaker at 200 rpm for 24 hrs. Later, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated to make volume one fourth of its original volume.

Antibacterial Activity

Medium Preparation

The ingredients (Muller Hinton Agar - Hi-media) were dissolved in distilled water with the aid of heat and pH was adjusted to 7.0 using dilute alkali or dilutes acid. Mueller-Hinton agar was prepared and autoclaved at a pressure of 15 psi (121ºC) for not less than 15 minutes. The sterilized medium was transferred into sterile petridish.

Microorganisms

Microorganisms were obtained from the Microbial Type Culture Collection centre (MTCC), Chandigarh, India. Amongst nine microorganisms investigated, seven were bacterial strains viz., *Staphylococcus aureus* MTCC 3160, *Escherichia coli* MTCC 598, *Salmonella typhi* MTCC 3917 and *Shigella flexneri* MTCC 1457, while the other two were fungal strains viz. *Aspergillus nige*r and *Candida albicans*. The remaining microbial strains (*Bacillus subtilis*, *Stpahylococcus aureus* and *Pseudomonas fluorscence*) were obtained from Department of Microbiology, Kamaraj College, Tuticorin. All the microorganisms were maintained at 4°C on nutrient and potato dextrose agar slants.

Disc diffusion method

Antimicrobial activity was carried out by the disc diffusion method. The antimicrobial assays of aqueous and ethanolic extracts were performed by Bauer *et al*. (1966). Each plant extracts were tested at two different concentrations (100 & 200 mg/ml) to see their inhibitory effects against microbial pathogens. Sterile paper discs (6 mm in diameter) prepared from Whatman No. 1 filter paper was impregnated with drug, containing solution placed on the inoculated agar. Negative and Positive controls used DMSO and Chloramphenical. The inoculated plates were incubated at 37^0C for 24 h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone for the test microorganisms.

Minimum inhibitory concentration (MIC)

For determination of MIC, 1 ml of broth medium was taken into 10 test tubes for each bacterium. Different concentrations of plant extracts ranging from 0.125 to 8 µg/ml-1 concentration were incorporated into the broth and the tubes were then inoculated with 0.1 ml of inoculums of respective bacteria (10^5 CFU ml^{-1}) and kept at 37°C for 24 h. The test tube containing the lowest concentration of extract which showed reduction in turbidity when compared with control was regarded as MIC of that extract (Muhamed *et al*., 2011).

Minimum Bactericidal Concentration (MBC)

The MBC is defined as the lowest concentration where no bacterial growth is observed (bacteriocidal concentration). In this technique, the contents of the test tubes resulting from MIC was streaked using a sterile wire loop on agar plate free of bacteria and incubated at 37ºC for 18 hours. The lowest concentration of the extract which showed no bacterial growth was noted and recorded as the MBC.

Total activity (TA) determination

Total activity is the volume at which test extract can be diluted with the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract or compound isolated and is expressed in ml/g (Sharma and Kumar, 2009).

AI = Activity Index (IZ developed by extract/IZ developed by standard).

Antifungal activity

The potato dextrose agar plates were inoculated with each fungal culture by point (10 days old cultures) inoculation. The filter paper discs impregnated with 100 and 200 mg/ml concentrations of the extracts were placed on test organism seeded plates. The activity was determined after 72 h of incubation at 28ºC. The diameters of the inhibition zones were measured in mm (Taylor *et al*., 1995). The antifungal activity was evaluated by measuring the diameter of the inhibition zone for the test microorganisms.

RESULTS AND DISCUSSION

Medicinal plants are an important source for the therapeutic remedies of various ailments. Although there are numerous classes of drugs that are routinely used to treat various diseases in humans, pathogenic microorganisms are constantly developing resistance to these drugs because of indiscriminate use of antibiotics. The use of higher plants and preparations made from them to treat infections is a longstanding practice in a large part of the population, especially in the developing countries, where there is dependence on traditional medicine for a variety of diseases. Interest in plants with antimicrobial properties increased because of current problems associated with the commercial antibiotics. Recently, the antimicrobial effects of various plant extracts against certain microbial pathogens have been reported by a number of researchers.

Both ethanol and aqueous extracts of Gardenia latifolia exhibited varying degrees of antimicrobial activities against the test organisms. The ethanol extract showed significant antibacterial activity at 200 mg/ml against *Staphylococcus aureus, Bacillus subtilis* and *Salmonella typhi*. Standard drug (Chloramphenicol) exhibited maximum zone of inhibition 22 mm at 10mcg concentration against *Staphylococcus aureus*. In the case of *Staphylococcus aureus* the zone of inhibition values were from 10mm in the aqueous extract at 100 mg/ml concentration to 13 mm recorded in the ethanol extract at 200 mg/ml concentration as well in the ethanol extract exhibited significant activity against clinically isolate *Staphylococcus aureus* (12 mm) at 200 mg/ml concentration (Table 1). The significant activity was shown in ethanol extract against (8mm) *Shigella flexneri* at 200 mg/ml concentration. Standard Chloramphenicol exhibited maximum zone of inhibition (20 mm) at 10 mcg concentration. Moderate activity was seen in ethanol extract against (7mm) *Pseudomonas fluroscence*. Against *Salmonella typhi* minimum zone of inhibition 7 mm was recorded in the methanol and aqueous extracts at 100 mg/ml concentration (Table 1). The zone of inhibition of leaf extract was found to be 8 mm for *Candida albicans*. The widest zone of inhibition was produced by ethanol extract of

G.latifolia while and aqueous extract had the no zone of growth inhibition (Table 1).

Table 1 Effect of ethanol and aqueous extracts of *G.latifolia* on microbes

S.No.	Name of the Strains	Zone of Inhibition (mm)				Chloramphenicol
		Ethanol (µg/ml)		Aqueous (µg/ml)		
		100	200	100	200	
1	*Staphylococcus aureus*	12	13	10	11	22
2	*Staphylococcus aureus*	10	11	8	10	20
3	*Bacillus subtilis*	10	8	-	8	17
4	*Escherichia coli*	-	8	-	7	21
5	*Pseudomonas fluroscence*	-	7	-	-	18
6	*Salmonella typhi*	7	10	7	8	16
7	*Shigella flexneri*	-	8	-	-	20
	Antifungal activity					**Fluconazole**
8	*Candida albicans*	-	8	-	-	15
9	*Aspergillus niger*	-	-	-	-	17

Ethanol extract showed least MIC value i.e. 0.125 µg/ml (MBC = 0.250 mg/ml) against *S.aureus* while aqueous extract has shown moderate activity against *S.aureus* at 0.250 µg/ml (MBC = 0.500 µg/ml) concentration (Table 2).

Table 2 The MIC_{Index} of ethanol and aqueous extract of *G.latifolia*

S.no	Name of the Strains	Ethanol			Aqueous		
		MIC (µg/ml)	MBC (µg/ml)	MIC_{Index}	MIC (µg/ml)	MBC (µg/ml)	MIC_{Index}
1	*Staphylococcus aureus*	0.125	0.250	2	0.250	0.500	2
2	*Staphylococcus aureus*	0.125	0.250	2	0.250	0.500	2
3	*Bacillus subtilis*	0.500	1.0	2	0.500	1.0	2
4	*Escherichia coli*	0.500	1.0	2	1.0	2.0	2
5	*Pseudomonas fluroscence*	1.0	2.0	2	1.0	2.0	2
6	*Salmonella typhi*	2.0	4.0	2	1.0	2.0	2
7	*Shigella flexneri*	2.0	4.0	2	2.0	4.0	2
8	*Candida albicans*	4.0	-	-	-	-	-
9	*Aspergillus niger*	4.0	-	-	-	-	-

Aqueous extract has shown highest activities against *S.aureus*, *B.subtilis* and *E.coli* at 0.500, 1.0 & 2.0 µg/ml concentration respectively (Table 2). TA was also calculated and recorded (Table 3). TA was highest for both the extracts (ethanol and aqueous) (1.4 & 0.72 ml/g) against *S. aureus*. On a general note, ethanolic extract exhibited higher degree of antibacterial activities than the aqueous extract. Amongst the gram-positive and gram-negative bacteria, gram-positive bacterial strains were more susceptible to the extracts when compared to gram negative bacteria. This may be attributed to the fact that these two groups differ by its cell wall component and its thickness. For ethanol extract of the tested plants MIC values recorded were very low, indicating strong bioefficacy of the plant.

Table 3 Antimicrobial activity index and Total activity of extracts of *G.latifolia*

S.No.	Name of the Strains	Ethanol			Aqueous		
		Activity index		Total activity (ml/g)	Activity index		Total activity (ml/g)
		100	200		100	200	
1	*Staphylococcus aureus*	0.545	0.590	1.44	0.454	0.500	0.72
2	*Staphylococcus aureus*	0.500	0.550	1.44	0.400	0.500	0.72
3	*Bacillus subtilis*	0.588	0.470	0.36	-	0.470	0.36
4	*Escherichia coli*	-	0.380	0.36	-	0.333	0.18
5	*Pseudomonas fluroscence*	-	0.388	0.18	-	-	0.18
6	*Salmonella typhi*	0.437	0.625	0.09	-	0.500	0.18
7	*Shigella flexneri*	-	0.400	0.09	-	-	0.09
8	*Candida albicans*	-	0.533	0.045	-	-	-
9	*Aspergillus niger*	-	-	0.045	-	-	-

Both the extracts of *G.latifolia* exhibited varying degrees of antimicrobial activities against the test organisms. At 200 mg/ml, ethanolic extract had higher antibacterial activity with zone of inhibition 13 mm (AI = 0.590), 12 mm (AI = 0.545), 11 mm (AI = 0.550) and 10 mm (AI = 0.588) than aqueous extract with zone of inhibition 8 mm (AI = 0.470) against *S.aureus*, *S.aureus* (clinical isolates), *B.subtilis* and *E.coli*, respectively (Table 3).

The presence of antibacterial activity in leaf extracts implies that are would be possibilities of substituting leaves for other plant parts which utilizing the plants for microbial related infections. However, of leaves for medicinal purposes is more sustainable compared to harvesting of plant parts such as stem, flower, bark and roots. Similarly, the ethanolic extract showed considerably more bacterial activity than the water extract. This is interesting in that the traditional method of treating a bacterial infection was by administrating a decoction of the plant or a part there of by boiling it in water, whereas according to our results an organic solvent is better, hence this may be more beneficial. Several reports have shown the antimicrobial activity of plant extracts under laboratory conditions (Sasikumar *et al*., 2006; Doss *et al*., 2012; Doss and Anand, 2013). Normally gram positive bacterial strains are found to be more susceptible to the extracts than gram negative strains. This is attributed to the fact that these two groups differ by their cell wall components and their thickness (Doss *et al*., 2009). From the above results it can be concluded that plant extracts have great potential as antibacterial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant microorganisms.

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