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Antimicrobial activity of various extracts from various parts of *Calophyllum inophyllum* L.

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ABSTRACT

The Indian flora is extensively utilized as source of any drugs mentioned in the traditional systems of medicine. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Therefore, researchers are increasingly turning their attention to folk medicine; looking for new leads to develop better drugs against microbial infections. The aim of the current study was to investigate antimicrobial activity of the various extracts of *Calophyllum inophyllum L.* An ethanol and ethyl acetate extracts of *Calophyllum inophyllum L.* (Family: *Clusiaceae*) were evaluated for antimicrobial activity against clinically important bacterial and fungal sp. The results obtained in the present study suggest that the ethanol and ethyl acetate extracts of *Calophyllum inophyllum* revealed a significant scope to develop a novel broad spectrum of antibacterial and antifungal herbal formulation.

Key words: Antimicrobial activity, *Calophyllum inophyllum*, *Clusiaceae*, ethanol extract, ethyl acetate extract.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Cragg and Newmann, 2001). Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Their role is twofold in the development of new drugs: (1) they may become the base for the development of a medicine, a natural blue print for the development of new drugs or; (2) a phytomedicine to be used for the treatment of diseases (Iwu, 1993 and Irobi et al., 1994) The World Health Organisation (WHO) estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for their primary health care needs. Also, modern pharmacopoeia contains at least 25% drugs derived from plants. *Calophyllum inophyllum* belongs to the family *Clusiaceae* and is a tree that can grow 8 to 20 meter tall with a broad spreading crown of irregular branches which exudes white latex when bruised (Da silva, 2001). The leaves have opposite arrangements, and are petiolate, thick and shiny with numerous parallel secondary veins (Ito, 2003 and Ito, 2002). The flower is 25 millimetres (0.98 in) wide and occurs in racemose or paniculate inflorescences consisting of 4 to 15 flowers. Flowering can occur year-round, but usually two distinct flowering periods are observed, in late spring and in late autumn. The fruit is a purplish black globoid-to-ovoid drupe when mature with a single seed. When ripe, the fruit is wrinkled and its color varies from yellow to brownish-red. This species is globally distributed in the Paleotropics (Ito, 2002). Within India, it is distributed in the coastal regions of Orissa, Andhra Pradesh, Maharashtra, Karnataka, Kerala, Tamil Nadu and the Andamans.

MATERIALS AND METHODS

Plant material

Calophyllum inophyllum L was collected from Kollimalai hills at the west of pachaimalais in Namakkal district of Tamilnadu, India. The fresh leaves, fruit, stem, flower and root were collected separately, washed with tap water, chopped into smaller pieces with a knife and then kept in the shade for 30 days to dry and then crushed using mortar and pestle, then further reduced to powder using electric blender and then stored in airtight closed bottles until required. The powder materials were passed through sieve number 40 and used for further studies

Figure 1. Various parts of *Calophyllum inophyllum L.* plant.

Preparation of extract

The shade dried (100 g) powder of plant materials (leaves, fruit, stem, flower and root) were filled separately in the thimble and extracted successively with 500 ml each of ethanol and ethyl acetate using a Soxhlet extractor for 48 hours. Each extracts were concentrated at reduced pressure using rotary evaporator. The extracts were stored in airtight vials and kept refrigerated (2-8 °C) until further analysis for antimicrobial activity studies.

Collection of strains

The lyophilized form of different strains of microorganism like *Salmonella typhi* (MTCC 733), *Pseudomonas aeruginosa* (MTCC 1934), *Staphylococcus aureus* (MTCC 3160), *Escherichia coli* (MTCC 1652), *Vibrio cholera* (MTCC 3904), *Candida albicans* (MTCC 183), *Aspergillus niger* (MTCC 281), *Candida tropicalis* (MTCC 184), *Aspergillus fumigates* (MTCC 2550) and *Alternaria tenuissima* (MTCC 2802) were used for the anti microbial study. The above strains were obtained from Microbial Type of Culture Collection (MTCC), Institute of Microbial technology (IMTECH), Chandigarh.

Growth and maintenance of the culture

Primary bacterial cultures were prepared by inoculating the lyophilized cultures of bacteria in Nutrient Broth (Peptic digest of animal tissue -5.00gm/lit, Sodium chloride-1.50gm/lit, Beef extract -1.50gm/lit, Yeast extract-1.50gm/lit) and fungal culture in Potato Dextrose medium (potato infusion 200 g/lit, dextrose 20 g/lit) was incubated according to their instructions. The cultures were maintained by subculture process done once in 30 days.

Screening for Antibacterial Properties

Antibacterial activities of various plant extracts were tested by cup-plate method with some modifications (James Cappuccino and Natalie Sherman, 2009). The culture plates were prepared by pouring 30 ml of Mueller Hinton Agar medium (Hi-media, Mumbai) into sterile petri plates. The inoculums suspension was spread uniformly over the agar medium using L-spreader to get uniform distribution of microorganisms. Using a flamed cork borer, well of 5 mm diameter was made in the media at a distance of 1-2 cm from the periphery of the plates. These plates were labelled and 100 µl of each plant extract (at different concentration of extracts i.e. 100, 200, 300 and 400 µg/100 µl) was added aseptically into the well. Then the plates were incubated for 24 to 48 h at 37 ˚C. The affectivity of these extracts was recorded by measuring the diameter of inhibition zone by Antibiotic zone reader. Triplicate was performed and the experiment was repeated thrice and the average values were recorded. The above result was compared with the zone of inhibition produced by standard antibiotic disc like Tetracycline (30µg/disc- Hi media, Mumbai).

Screening for Antifungal Properties

Antifungal activities of various plant extracts were tested by cup-plate method (James Cappuccino and Natalie Sherman, 2009). The culture plates were prepared by pouring 30 ml of Potato Dextrose Agar medium (Hi-media, Mumbai) into sterile petri plates. The inoculums suspension was spread uniformly over the agar medium using L-spreader to get uniform distribution of microorganism [15]. Using a flamed cork borer, well of 5 mm diameter was made in the media at a distance of 1-2 cm from the periphery of the plates. These plates were labelled and 100 µl of each plant extract (at different concentration of extracts i.e. 100, 200, 300 and 400 µg/100 µl) was added aseptically into the well. Then the plates were incubated for 24 to 48 h at 25˚C. The affectivity of these extracts was recorded by measuring the diameter of inhibition zone by Antibiotic zone reader. Triplicate was performed and the experiment was repeated thrice and the average values were recorded. The above result was compared with the zone of inhibition produced by standard antifungal disc like Fluconazole (10µg/disc- Hi media, Mumbai).

RESULT & DISCUSSION

The ethno botanical efficacy of various parts like leaf, fruit, stem, flower and root of ethanol and ethyl acetate extracts against various clinically important pathogenic bacteria and fungal species by cup –plate method. Results of the antimicrobial screening of the different concentrations of the extract on the bacterial and fungal sp. were observed. Among treatments, maximum in vitro inhibitions of tested bacteria's were shown in Table 1and fungal species in Table 2. Here ethanol extracts were

Table 1. Antibacterial activity of Ethanol and Ethyl acetate extracts of leaf, fruit, stem, flower and root of Calophyllum inophyllum by cup-plate method.

Plant parts	solvent used	concentration of Extract µg/100 µl	Zone of inhibition in diameter (mm)				
			Salmonella typhi MTCC 733	*Pseudomonas aeruginosa* MTCC 1934	*Staphylococcus aureus* MTCC 3160	*Escherichia coli* MTCC 1652	*Vibrio colerae* MTCC 3904
Leaf	Ethanol	100	6.2	7.4	6.3	7.4	7.4
		200	9.8	10.2	10.4	11	9.7
		300	14.2	14.4	15.6	16.2	14.2
		400	18.6	19.6	18.8	19.8	18.7
	Ethyl acetate	100	7.1	7	6.4	6	6.4
		200	12.3	9.8	8.2	9.2	10.2
		300	15.8	13.5.	14.6	13.8	16.3
		400	18.6	18.2	17.2	18.2	19.2
Fruit	Ethanol	100	8.2	6.3	7	6.4	7.4
		200	10.8	11.7	11.2	10.4	11.2
		300	14.8	13.8	15.3	16.4	16.3
		400	20.4	19.8	21.2	18.3	19.5
	Ethyl acetate	100	10.2	7.6	6.1	6.6	8
		200	12.8	11.2	9.9	10.4	9.2
		300	16.2	15.4	13.8	15.3	12.2
		400	19	19.4	17.4	18.2	16.4
Stem	Ethanol	100	6.1	6.2	6.3	6.4	6.4
		200	7.4	8.6	11.2	8.3	10.3
		300	12.9	13.4	16.8	14.5	15.2
		400	18.4	18.6	19.2	17.2	16.4
	Ethyl acetate	100	6.8	7.6	6.3	8	8.8
		200	8.7	12.3	10.2	11.2	10.2
		300	12.2	14.7	15	13.7	15.2
		400	13.6	18.2	17.6	15.3	17.2
Flower	Ethanol	100	7.8	6.5	6.8	7.2	8.2
		200	12.1	11.7	10.4	9.5	12.2
		300	14.3	16.5	15.3	16.2	15.4
		400	18.6	20.2	18.2	18	17.2
	Ethyl acetate	100	7.6	7.7	6.4	6.2	6.6
		200	10.7	11.2	11.5	10.3	9.4
		300	13.8	14.5	15.2	14.8	14.2
		400	16.8	18.4	18.3	17.2	16
Root	Ethanol	100	9.4	7.8	6.2	7.8	7.4
		200	12.9	11.3	12.2	10.3	10.7
		300	16.3	14.7	15.9	16.9	16.1
		400	19.8	17.4	21.2	20.8	19.7
	Ethyl acetate	100	8.9	10.6	9.6	9.8	10.2
		200	13.9	14.8	13.3	13.8	15.7
		300	16.6	17.1	19.6	17.2	19.2
		400	20.2	18.2	18.6	20.2	20.8
Standard disc	Tetracycline	30µg/disc	22.1	18.8	23.6	20.2	22.3
Control	……	……	……	……	……	……	……

more effective and produced more zone of inhibition when compared to ethyl acetate extracts. Although the various parts of the plant differ significantly in their activities against the micro-organisms tested. The results were shown that while increases in concentration of extract increased the zone of inhibition of the microorganisms. From the above data is evident that ethanolic and ethyl acetate extracts were more active against both gram positive, gram negative bacterial and fungal organisms. The results of present research highlights, the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium (Benkeblia, 2004). This tends to show that the active ingredients of the plant parts are better extracted with alcohol than other solvents (Bustamante and Bauer, 2003). The alcohol extracts contain alkaloids, coumarins and tannins (Okemo, 1996). Coumarins and tannins have antibacterial and antihelminthic properties (Hedberg et al., 1983) found that alcohol was more efficient than ethyl acetate, acetone in extracting phytochemicals from plant materials (Eloff, 1998 and Cowan, 1999).

CONCLUSION

Plant based antimicrobial compounds have enormous therapeutically potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials (Priyanka Vijay and Rekha Vijayvergia, 2010). The genus *Calophyllum (Clusiaceae)* is composed of about 180 - 200 different species confined to the warm humid tropics of the world (Stevens, 1980). Some of these species are frequently employed in folk medicine to treat several injuries (Sartori et al., 1999). Extensive chemical investigation of this genus has resulted in the isolation of a wide variety of natural products, including xanthones, coumarins, biflavonoids, chalcones, benzofurans and triterpenes (Da Silva et al., 2001; Ito et al., 2002, 2003; Oger et al., 2003; Isaias et al., 2004). The results of present study supports the traditional usage of the studied *Calophyllum inophyllum* plants and suggests that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in

Table 2. Antifungal activity of Ethanol and Ethyl acetate extracts of leaf, fruit, stem, flower and root of *Calophyllum inophyllum* by cup-plate method.

Plant parts	solvent used	concentration of Extract µg/100 µl	Zone of inhibition in diameter (mm) *Candida albicans* MTCC 183	*Aspergillus niger* MTCC 281	*Candida tropicalis* MTCC 184	*Aspergillus fumigates* MTCC 2550	*Alternaria tenuissima* MTCC 2802
Leaf	Ethanol	100	6.2	7.2	8.1	7.3	9.2
		200	11.4	13.8	11.5	10.6	15
		300	15	15.3	14.2	16.7	17.4
		400	16.2	17.8	17.7	18.3	18.5
	Ethyl acetate	100	6.6	6.2	7.7	7	7.3
		200	8	8.8	10.1	14.8	13.5
		300	14.2	13.5	15.8	16.3	15.3
		400	15.4	17.1	16.3	18.3	17.4
Fruit	Ethanol	100	8.2	7.4	6.2	8.1	7.5
		200	11.3	14.1	14.7	12.7	11.9
		300	15.7	17.8	16.6	16.2	17.3
		400	19.6	20.2	19.2	19.5	21.2
	Ethyl acetate	100	7.2	6.8	8.2	7.7	8.8
		200	10.1	11.5	10.1	12.6	11.2
		300	16.4	15.3	14.7	15.2	14.3
		400	19.8	18.6	18.9	18.4	19.5
Stem	Ethanol	100	6.3	7.5	7.9	8.2	7.8
		200	10.8	13	11.3	12.6	11.4
		300	15.2	15.7	13.4	14.7	15.8
		400	18.8	17.2	16.8	18.5	18.6
	Ethyl acetate	100	7.2	8.3	6.2	9	6.8
		200	8.3	9.4	7.8	14.2	7.8
		300	14.6	13.7	13.5	15.6	14.9
		400	16.9	16.5	17.5	17.7	18.4
Flower	Ethanol	100	7	6.4	6.2	7.2	6.8
		200	11.3	9.2	9.4	13.8	8.2
		300	14.7	13.8	13.8	14.5	15.6
		400	15.2	16.2	15.8	17.2	18.5
	Ethyl acetate	100	6.7	6.2	6.1	5.9	7.5
		200	11.7	9.4	12.8	9.4	8.3
		300	14.7	14.7	15.2	14.8	11.7
		400	16.5	16.9	17.8	17.1	16.7
Root	Ethanol	100	8.2	9	8.4	7.4	6.5
		200	11.6	13.5	13.2	11.5	11.3
		300	17.2	16.2	17.8	15.3	17.2
		400	19.8	18.6	18.2	21.2	20.8
	Ethyl acetate	100	7	7.5	8.4	6.8	7.1
		200	11.8	11.4	13.2	14.3	11.3
		300	15.3	15.9	16.5	16.6	15.2
		400	16.9	17.8	18.2	19.8	20.4
Standard disc	Fluconazole	10µg/disc	23.2	22.8	20.9	21.2	22.1
Control	……	……	……	……	……	……	……

new drugs for the therapy of infectious diseases caused by pathogens.

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