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Screening of Antibacterial and Antifungal Activity of *Canthium coromandelicum* from various solvent extracts

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ABSTRACT

The present study was aimed to investigate the antibacterial and antifungal activity of aqueous, methanol, ethanol, petroleum ether and acetone extract of *canthium coromandelicum* using disc diffusion method. The antibacterial activity was detected against Gram-positive and Gram-negative bacteria, *Bacillus subtilis, Bacillus ceresu, Staphylococus aureus, Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa.* Different solvent extracts of *canthium coromandelicum* was taken in 100,150,200 and 250 µl/ml. The zones of inhibitions obtained were recorded and analyzed against standard controls, Streptomycin and Amphotericin. The methanolic extract of *canthium coromandelicum* was showed highest antibacterial activity than ethanolic and aqueous extracts. The higher concentration of methanolic leaves extract of *canthium coromandelicum* plant, maximum antibacterial activity was observed against to Gram-negative bacteria than Gram-positive bacteria and antifungal activity against to *Candida lbicans, Aspergillus niger.* The results indicate that *canthium coromandelicum* leaves also has potent antibacterial and antifungal activity.

Keywords: C*anthium coromandelicum;* Antibacterial; Antifungal activity.

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INTRODUCTION

The use of medicinal plants as a source for relief from illness can be traced back over five mellenia to written documents of the early civilization in China, India and the north east, but it is thoughtless as art as old as mankind [1]. The potential of higher plants as a source for new drugs is still largely unexplored. Among the estimated 250,000- 500,000 plant species, only a small percentage have been investigated phytochemically and the fraction submitted to biological or pharmacological screening. Compound of natural or synthetic origin has been the source of innumerable therapeutic agents[1,2,3] . Medicinal plants are rich sources of antimicrobial agents. Plants are used medicinally in different countries and are the source of potential and powerful drugs. A wide range of medicinal parts are used to get different rasayanas which possess different medicinal properties against different microbes.

Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens[4]. Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. In rural areas of the developing countries, they continue to be used as the primary source of medicine[5]. About 80% of the people in developing countries use traditional medicines for their health care[6].

In India, infectious diseases accounts for high proportion of health problems. Morbidity and mortality due to these infections continues to be a major problem, especially amongst children. Infections due to a variety of bacterial etiologic agents, such as pathogenic *Escherichia coli*, *Staphylococcus aureus*, *Shigella* sp., *Salmonella* sp., *Enterobacter* sp. are most common[7]. In the present time multiple drug resistance in microbial pathogens become a serious health problem to humankind worldwide [8]. It is aroused due to indiscriminate and repetitive use of antimicrobial drugs.

Canthium coromandelicum (Syn.*C. parviflorum*) of Family: Rubiaceae is a bushy thorny herb, native of India, found mainly in coast of the coramandel region of India. The plant is popularly recorded under the local name ie in Odisha state “Tutidi saga” (in Odia). All the genus of the family are economically important. Canthium is a genus of about 230 species of shrubs or small trees. An antioxidant, wound healing activity and antitumor acitivity were reported. D-mannitol, phenolic acid, phenolic compounds, carbohydrates, proteins were found from *Canthium coromandelicum* Pharmacological activities such as antimicrobial, antioxidant, antidiabetic, wound healing, diuretic, anti-

inflammatory, antinociceptive, antitumor and antipyretic from various species of Canthium has been reported [9]. Hence in this study, the antibacterial and antifungal activity of selected medicinal plant were tested against human pathogens

MATERIALS AND METHODS

Sample Collection

The plant samples of *Canthium coromandelicum* leaves were collected from Kolli Hills in Namakkal District, Tamil Nadu , India.

Preparation of Extract

The leaves were washed thoroughly with tap water and in distilled water and then dried the leaves at room temperature. The dried leaves were ground to a fine powder in a mechanic grinder. About 25gm of powdered plant material was uniformly packed into a thimble and extracted with 250ml of different solvents separately. Solvents used were Methanol, Ethanol, Petroleum ether, and Acetone. The process of extraction continues till the solvent in siphon tube of an extractor become colourless. Aqueous extract was prepared by the cold maceration methods .The extracts were filtered through Whatman No.1 filter paper and the solvent was removed by evaporating in a water bath, which gave rise to a solid mass of the extract.

Microorganisms Used

To assess the antibacterial properties of crude extracts of *Cephalendra indica*, Gram-positive bacteria *Bacillus subtilis, Bacillus cereus* and *Staphylococus aureus*, and Gram-negative trains of *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aureginosa* and antifungal *Candida lbicans , Mucor rouxii , Penicillium citrenigrum , Aspergillus niger , Fusarium oxysporum* were used. The organisms were maintained on nutrient agar slants at 4°C and sub-cultured into nutrient broth for 24 hrs before use.

Antibacterial Assay

Antibacterial activity of plant extracts was carried using cup-plate agar diffusion method with some minor modifications[10]

Antifungal Assay

The cup-plate agar diffusion method was adopted with some minor modifications to assess the antifungal activity of prepared extracts[10]

RESULTS AND DISCUSSION

Antibacterial activity

The presence of antifungal and antimicrobial substances in the higher plants is well established as they have provided a source of inspiration for novel drug compound as plant derived medicines have made significant contribution towards human the treatment of diseases as is done in cases of Unani and Ayurvedic system of medicines.

The antibacterial activity of leaf extracts of *Canthium coromandelicum* were determined against for bacterial strains. Antibacterial activity at different concentrations 100, 150 and 200 and 250 µl/ml of Aqueous, Methanol, Ethanol, Petroleum ether, and Acetone leaf extracts were presented in (Table 1).The antibacterial activity of leaf extracts were observed in dose dependent manner, 250 mg/ml showed more activity in tested microorganisms. The results were also compared with the standard antibiotic and all these six micro organisms are sensitivity to standard antibiotic (Table 1). Gram-positive bacteria *Staphylococus aureus* was most resistant in contrast to this Gram-negative bacteria *Escherichia coli* showed antibacterial activity at different concentrations of aqueous, Methanol, Ethanol, Petroleum ether, and Acetone leaf extracts. In the higher concentration of methanolic leaf extracts, maximum antibacterial activity was observed against to Gram-negative bacteria than Gram-positive bacteria. The minimum concentration (100mg/ml) of methanolic extract of *Canthium coromandelicum* also showed antibacterial activity against to Gram +ve and Gram –ve bacteria. But zone of inhibition was observed in 100mg/ml of leaf extract. Whereas high concentration of methanolic extract of *Canthium coromandelicum* showed minimum zone of inhibition was observed in less concentration. Higher concentrations of aqueous , Ethanolic,Petroleum ether, and Acetone extracts of *Canthium coromandelicum* were showed minimum antimicrobial activity against to microorganisms but zone of inhibition was observed in less concentration (Table1).

The present study shows that extracts of *Canthium coromandelicum* were effective inhibitors of bacteria growth. The methanolic extracts of *Canthium coromandelicum* were more effective against to Gram- negative bacteria than Gram-positive bacteria but not in the Aqueous ,Ethanol, Petroleum ether and Acetone extracts. This may be due to the ability of methanol to extract a wide range of chemical constituents of plant material[11]. The present result confirm the previous studies, the methanol is a better solvent for more consistent extraction of antimicrobial substances from medical plants as compared to other solvents[12,13,14,15]. In the present study leaf extract of *Canthium coromandelicum* showed a potent antibacterial activity. Similar type of results reported in different leaf extracts of plant, *Camellia sinensis*[16], *Tridax procumbens*[17], *Andrographis paniculata, Andrographis serpyllifolia*[18], *Sida spinosa* Linn[19], *Solanum nigrum* [15]

Table – 1 Antibacterial activity of selected solvent extracts of *Canthium coromandelicum*,

Mean Diameter Inhibition Zone(mm)																									
Standard Streptomycin (SM)	**Aqueous extract**					**Methanol extract**					**Ethanol extract**					**Petroleum ether**					**Acetone**				
Organisums	SM 20µl	100 µl	150 µl	200 µl	250 µl	SM 20µl	100 µl	150 µl	200 µl	250 µl	SM 20µl	100 µl	150 µl	200 µl	250 µl	SM 20µl	100 µl	150 µl	200 µl	250 µl	SM 20µl	100 µl	150 µl	200 µl	250 µl
Bacillus subtilis	7.5	5.5	6.5	7.0	9.0	14	9	13	15	17	13	7.5	9	13	14	6.5	4.0	5.0	5.5	6.0	9.0	6.5	7.0	7.5	8.5
Bacillus cereus	6.0	5.0	6.0	6.5	7.5	13.5	8.5	12	13	18	12	7.0	8.5	11	15	6.0	3.0	3.5	4.0	5.5	7.5	6.0	6.5	7.0	7.5
Staphylococus aureus	8.0	6.5	7.0	8.0	8.5	16	11	14	15	20	15	8.5	11	14	19	7.0	4.0	4.5	5.0	6.5	8.5	7.0	7.5	8.0	8.5
Escherichia coli	8.5	7.0	7.5	9.0	9.5	17	13	16	18	23	16	9.5	13	16	20	7.5	5.0	5.5	6.0	8.0	9.5	7.5	8.0	8.5	9.0
Klebsiella pneumonia	7.5	6.0	6.5	7.5	8.0	14.5	10	12	13	18	14	8.0	10	12	16	6.5	2.0	2.5	7.0	7.5	8.0	6.5	7.0	7.5	8.0
Pseudomonas aureginosa	6.5	6.0	6.0	6.5	7.0	14	10	11	13.5	18.5	13	7.0	10	11	14	6.0	3.0	3.5	4.0	5.5	7.0	6.0	6.5	7.0	7.5

Table 2: Antifungal activity of different solvent extracts of *Canthium coromandelicum*

Mean Diameter Inhibition Zone(mm)																									
standard Amphotericin (AT)	**Aqueous extract**					**Methanol extract**					**Ethanol extract**					**Petroleum ether**					**Acetone**				
Pathogenic fungi	AT20 µl	100 µl	150 µl	200 µl	250 µl	AT20µl	100 µl	150 µl	200 µl	250 µl	AT 20µl	100 µl	150 µl	200 µl	250 µl	AT20 µl	100 µl	150 µl	200 µl	250 µl	AT20 µl	100 µl	150 µl	200 µl	250 µ
Candida lbicans	6.5	3.5	4.5	5.0	6.5	12	5.5	6.5	8.5	13	11	4.5	6.0	8.5	12	5.5	2.0	2.5	6.5	6.0	8.0	5.5	6.0	7.5	8.5
Mucor rouxii	6.0	2.5	3.0	4.0	5.5	10	4.0	5.5	7.5	11	9.0	3.5	5.0	7.5	10	5.0	2.5	3.5	5.5	5.5	6.5	5.0	5.5	6.0	7.5
Penicillium citrenigrum	5.5	3.0	3.5	4.0	4.5	11	4.5	6.5	7.0	12	10	4.0	5.5	5.0	9.0	6.0	2.0	3.5	6.0	6.5	7.5	6.0	6.5	7.0	8.0
Aspergillus niger	7.5	5.5	6.5	7.0	8.5	15	6.0	7.5	9.5	18	13	5.5	7.0	10	14	6.5	3.5	4.5	7.0	8.0	8.5	6.5	7.0	7.5	9.0
Fusarium oxysporum	5.0	2.0	2.5	3.0	4.0	10.5	4.0	5.5	6.5	11	10	3.5	4.5	9.0	11	5.5	2.0	2.5	3.5	7.5	7.0	5.5	6.0	6.5	8.0

Antifungal activity

Antifungal activity of *Canthium coromandelicum* extract was assayed by agar well diffusion method. The result revealed that the extract of *Canthium coromandelicum* showed significant reduction in growth . Among the Methanol extract of *Canthium coromandelicum* exhibited maximum antifungal activity of 250mg/ml compared with other extract were presented in table 2. The results of antifungal effect of aqueous extract minimum concentrations of 100mg/ml showed activity against *Canthium coromandelicum*.Hydro-alcohol extract of *Valeriana jatamansi, Coleus barbatus, Berberis aristata, Asparagus racemosus, Andrographis paniculata, Achyranthes aspera, Tinospora cordifolia, Plantago depressa* showed maximum antifungal activity against *aspergillus niger* and *candida albicans*. Similarly [20] evaluated the antifungal activity of *Senna alata linn*. Crude leaf extract exhibited moderate activity against *Microsporum canis, Trichophyton jirrucosum, Trichophyton mentagrophytes* and *Epidermophyton jlorrcosum*[21] reported that the antifungal potential of Aqueous, Methanol, Ethanol, Petroleum ether, and Acetone extracts of *Canthium coromandelicum* *invitro* and *invivo* studies . To develop environment-friendly alternatives to synthetic fungicides for the control of fungal plant disease the interest on plant extracts has been increased. This study provides new scientific information on antifungal activity of *Aspergillus niger* against *Canthium coromandelicum* The extracts should be tested against the disease under the disease field conditions.

CONCLUSION

The present results showed that *Canthium coromandelicum* plant extracts possess certain constituents with antibacterial and antifungal properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. There is a further studies need to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

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