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Research Article

Screening and antimicrobial activity of *Canna Indica* against Clinical pathogens bioactive

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Abstract

To study the antimicrobial activity and phytochemical characterization of essential oil isolated from the rhizome of *Canna indica* against pathogenic bacteria and fungi. Fresh rhizomes of *C. indica* were subjected to hydro distillation process to obtain essential oil. The essential oil was evaluated for antibacterial and antifungal activity against ten pathogenic bacteria and seven fungi by the disc diffusion method. The antimicrobial activity of the oil showed significant inhibitory activity against the human pathogenic bacteria, no activity was observed against the fungi *Aspergillus aculeatus* and *Fusarium oxysporum*. The findings of the present study indicate that the rhizome extract of *C. indica* possesses secondary metabolites and potential to develop antimicrobial drugs.

Keywords : Antimicrobial activity, *Canna indica*, Phytochemistry, *Aspergillus aculeatus, Fusarium oxysporum,* rhizomes, pathogenic bacteria and fungi

Introduction

Nature has been a source of medicinal agents for thousands of years. Various medicinal plants have been used for years in daily life to treat disease all over the world (Nair *et al.,* 2005). Antibacterial constituents of medicinal plants and their use for the treatment of microbial infections as possible alternatives to synthetic drugs to which many infectious microorganisms have become resistant seem to be very much promising (Bari *et al.,* 2010). Different extracts from medicinal plants were tested and some natural products were approved as new anti- bacterial drugs (Chehregani *et al.,* 2007). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body (Anpin Raja *et al.,* 2011). The most important of these biologically active constituents of plants are alkaloids, flavonoids, tannins and phenolic compounds (Kiruba *et al.,* 2011).

Canna indica belongs to family cannaceae. *Canna indica* is a native of tropical America and is a very popular ornamental and medicinal plant throughout the tropical world. *C. indica* is an upright perennial rhizomatous herb**.** The plant is used in the treatment of women's complaints. A decoction of the root with fermented rice is used in the treatment of gonorrhea and amenorrhea. The plant is also considered to be demulcent, diaphoretic and diuretic (Joshi and Pant, 2010). The main objective of the investigation was to determine the phytochemical constituents and antibacterial activity of the rhizome extracts of *C. indica*.

Materials and Methods

Plant collection and Oil extraction

Rhizome of *C. indica* was collected from the tropical forest of Bonocaud in the Agasthya-malai Hills of Kerala, India. The fresh rhizomes were shade dried and powdered in a mechanical

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blender. The powdered rhizome was subjected to hydro-distillation using a modified Clevenger-type glass apparatus for 6 hours for isolation of oils separately.

Bacterial isolates

Isolates of bacteria (*Bacillus subtilis, Lactobacillus lactis, Lactobacillus acidophilus, Staphylococcus aureus, Klebsiella pneumoniae, Acetobacter pasteurianus, Agrobacterium rhizogenes, Bradyrhizobium species, Escherichia coli, Flavo-bacterium species*) and fungi (*Aspergillus aculeatus*, *Aspergillus awomori, Aspergillus niger*, *Candida albicans, Fusarium oxysporum, Rhodotorula species, Trichoderma virideae*) were obtained from American Type Culture Collection (ATCC) ATCC. Fresh plates of the test bacteria were made from the isolate cultures obtained on agar slants. Discrete colonies of fresh cultures of the different bacterial isolates were then picked and suspended in 5 ml nutrient broth (NB, Oxoid), and incubated for 24 h at 37°C prior to antimicrobial susceptibility testing.

Determination of antimicrobial activity

The antimicrobial activity of the oil extracts of the plant sample was evaluated by the cup plate agar diffusion method (Murray *et al.,* 2009). Bacterial cultures were adjusted to 0.5 McFarland turbidity standards and inoculated onto Mueller Hinton agar (MHA, Oxoid) plates. A sterile cork borer was used to make a well (6 mm in diameter) on the MHA plates. Aliquots of 100 µl of extract were applied in each of the wells in the culture plates previously seeded with the test organisms. The cultures were incubated at 37°C for 24 h. A well was made in each of the culture plates and filled with 20 µl of 10 mg/ml of ciprofloxacin and streptomycin as positive controls, and sterile filter paper soaked in sterile glycerol served as a negative control. Antimicrobial activity was determined by measuring the zone of inhibition around each well. For each extract, three replicate trials were conducted against each organism.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC was determined by broth dilution methods. The multidrug resistant *B. subtilis, S. aureus* and *E. coli* were used for the determination of MIC and MBC. The 96 well microtitre plates were filled with 0.1 mL of varying concentration of active fractions prepared in Muller Hinton Broth. The microtitre plates were incubated at 37°C for 18 h. One row served as positive control (antibiotics) and another as negative control (methanol). After incubation, the OD was read at 610 nm in an ELISA reader. For measuring MBC, all the MIC cultures were plated on Muller Hinton Agar and incubated at 37°C for 24 h. The reduction in the number of viable colonies compared with the culture of the initial inoculum was noted. The ratio of MBC/MIC was calculated as an index of bacetriostatic and bactericidal activity.

Results

Antimicrobial activity of oil extract

The antimicrobial activity of essential oil extracts from *C. indica* rhizome was tested against ten pathogenic bacteria and seven fungi. In terms of antibacterial activity, the essential oil showed remarkable antibacterial activity with zone of inhibition of 15mm each against *E. coli* and *B. subtilis*, followed by *K. pneumoniae* (12mm), *A. pasteurianus* (10mm) and *A. rhizogenes* (11mm). Three bacteria *L. lactis*, *L. acidophilus* and *S. aureus* displayed the inhibition zone of 8mm each and *Bradyrhizobium species Flavobacterium species* showed each 7mm of inhibitory activity against the essential oil isolated from the rhizome of *C. indica*.

In order to find out the antifungal activity of chemicals present in the rhizome of seven

species of fungus were tested. Of these, *A. aculeatus* and *A. awomori* exposed the maximum and minimum inhibitory zones of 10mm and 7mm respectively. *A. niger, C. albican, F. oxysporum, Rhodotorula species* and *T. virideae* showed the inhibitory zone of 12mm each (Table - 1). The overall inhibitory effect of *C. indica* extract revealed a better activity against the pathogenic bacteria than fungus.

MIC and MBC

A 7µl dilution of crude extract of *C. indica* showed minimum inhibitory concentration (in OD) against *B. subtilis* (0.006nm), *S. aereus* (0.004 nm) and *E. coli* (0.002 nm). The optical densities of all the tubes were detected at 520nm by using nutrient broth as suitable blank. Minimum bactericidal concentrations of oil extract against *B. subtilis, S. aureus* and *E.coli* were determined (Table - 2).

Discussion

The antimicrobial activity of oil extracted from *C. indica* could be attributed to the broad spectrum of bioactive chemical compounds. On hydro distillation of fresh rhizomes, about 0.66% of white coloured, pleasant smelling oil was obtained from *C. indica*. In the present study the essential oil showed remarkable antibacterial activity with zone of inhibition of 15mm each against *E. coli* and *B. subtilis*, followed by *K. pneumoniae* (12mm), *A. pasteurianus* (10mm) and *A. rhizogenes* (11mm). Three bacteria *L. lactis*, *L. acidophilus* and *S. aureus* displayed the inhibition zone of 8 mm each and *Bradyrhizobium species Flavobacterium species* showed each 7mm of inhibitory activity against the essential oil isolated from the rhizome of *C. indica.* In order to find out the antifungal activity of chemicals present in the rhizome of *C. indica* seven species of fungus were tested. Of these, *A. aculeatus* and *A. awomori* exposed the maximum and minimum inhibitory zones of 10mm and 7mm respectively. *A. niger, C. albican, F. oxysporum, Rhodotorula species* and *T. virideae* showed the inhibitory zone of 12mm each. The overall inhibitory effect of *C. indica* extract revealed the better activity against the pathogenic bacteria than fungus. Similar findings are also reported elsewhere (Indrayan *et al.,* 2011).

Table -1. Antimicrobial activity of *Canna indica* oil against pathogenic bacteria and fungi

Sl. no	Microorganisms	Zone of Inhibition (mm)
Bacteria		
1	*Bacillus subtilis*	15
2	*Lactobacillus lactis*	8
3	*Lactobacillus acidophilus*	8
4	*Staphylococcus aureus*	8
5	*Klebsiella pneumoniae*	12
6	*Acetobacter pasteurianus*	10
7	*Agrobacterium rhizogenes*	11
8	*Bradyrhizobium species*	7
9	*Escherichia coli*	15
10	*Flavobacterium*	7
Fungi		
1	*Aspergillus aculeatus*	10
2	*Aspergillus awomori*	7
3	*Aspergillus niger*	12
4	*Candida albicans*	12
5	*Fusarium oxysporum*	12
6	*Rhodotorula species*	12
7	*Trichoderme virideae*	12

Table - 2: Minimum bactericidal concentration (MIC) of oil extracts of *Canna indica*

Zone of inhibition in nm			
	5	**6**	**7**
Bacillus subtilis	0.1	0.01	0.006
Staphylococcus aureus	0.2	0.03	0.004
Escherichia coli	0.2	0.04	0.002

The 7µl dilution of crude extract of *C. indica* showed minimum inhibitory concentration against *E. coli* (0.07nm) and *C. albicans* (0.08nm). In this study it was observed that the MIC of the active oil extract are lower than the MBC and MFC,

suggesting that the oil extract were bacteriostatic at lower concentrations and bactericidal at higher concentration (Aliyu *et al.,* 2008).

Plants have rich sources of biologically active metabolites with novel chemical structures, including cytotoxic and anticancer compounds. This investigation reveals a detailed schematic isolation and identification of bioactive compounds from selected plants. This information may help to develop potential purified bioactive compounds in the pharmaceutical industry for the development of drugs. With the advanced molecular biological tools, target-oriented screens have become available that will accelerate the quest for new sponge-derived drugs.

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