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Original Research Article

Antimicrobial efficacy of Tejpata

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ABSTRACT

Treatment with antibiotics became so common that people start using these drugs even for the little of ailment resulting in misuse of antibiotics. This leads to decrease in susceptibility of microorganisms to the present antibiotics or antimicrobial agents. Currently, in addition to antibiotics and chemically synthesized drugs, the trend to look out for alternative medicines such as natural or herbal medicines is increasing because of fewer side effects or toxicity owing to their natural sources. Therefore, the need for novel / more effective antimicrobial drugs, is an absolute necessity and remains a very important area of research against pathogens. In the present study, antifungal and antibacterial effect of *Cinnamomum tamala* leaves has been evaluated against three fungal and five bacterial strains via methanolic extract. *C. tamala* showed moderate growth inhibition against the fungal strain *Bipolarisspecifera.* The methanolic extract of the leaves of the plantalsoshowed average activity against the three bacterial strains viz., *Bacillus subtilis, Escherichia coli* and *Klebsiella pneumoniae*.

1. Introduction

C. tamala, a moderate sized evergreen tree that attains a height of 8 m., a girth of 150 cm., is found in tropical / sub-tropical Himalayas, Khasi / Jaintia hills, Eastern Bengal and in Northern India. Leaves of the plant are commonly used for flavouring food and widely used in pharmaceutical preparations because of hypoglycemic, carminative and stimulant properties (Amma *et al.*, 2013).[1] Traditionally, leaf extracts are used in colic and diarrhoeal preparations (Warrieret al., 1994).[2] The leaves have been reported to possess antidiabetic, antioxidant (Kar et al., 2003; Chakraborty and Das, 2010),[3,4] antidiarrhoeal (Rao etal., 2008),[5] antihyperlipidemic (Dhulasavant *et al*., 2011),[6] antioxigenic (Semwalet al., 1999),[7] anti-inflammatory (Gambhireet al., 2009),[8] acaricidal (Reddy, 2009), hepatoprotective / gastroprotective (Selvam, 2010; Eswaran et al., 2010)[9,10] properties. Antimicrobial activity of bark extracts (aqueous, methanol and chloroform) against bacterial and fungal clinical isolates like *B. subtilis, S. aureus, E. coli, A. niger and C. albicans* was determined. The result showed that methanolic extract had better antibacterial and antifungal activity. The most susceptible bacterial and fungal strains were *B. subtilis and A. nigerrespectively.* The methanolic extract showed MIC value of 2.5 mg/ml for B. subtilis and 5 mg/ml for A. niger (Varalakshmi et al., 2014).[11] In view of the above, in vitro antifungal and antibacterial potential of plant leaves has been investigated in the present research work.

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2. Materials and Methods

2.1. Plant material and preparation of extract

C. tamala leaves were authenticated at site and enough quantity of fresh leaves were collected. The freshly collected leaveswere chopped, shade-dried and ground into powdered form. The methanolic extractwas prepared by percolating the dried ground plant material (100 g) with 99% methanol and then concentrating it to dryness under reduced pressure (Kandil et al., 1994)[12]

2.2. Determination of antibacterial activity

Qualitative analysis for evaluating antimicrobial activity of test material was carried out by agar–well diffusion method with modification. Two gram positive *(Bacillus subtilis*, MTCCC 2389and *Staphylococcus aureus* MTCC7443) and threegram negative (*Micrococcus luteus,* MTCC4821., *Escherichia coli*, MTCC2127., *Klebsiella pneumoniae*, MTCC7162) bacterial strains were used in the present study.20 mL of sterilized nutrient agar was inoculated with 100 μL of bacterial suspension (10^8CFU/mL) and then poured on to sterilized petri plates. The agar plates were left to solidify at room temperature. A well of 6 mm diameter was aseptically bored into the agar plates and 20 μL of the essential oil (diluted with DMSO, 1:1) was added in each well. Chloramphenicol (10 μg) was used as a positive reference to determine the sensitivity of bacteria and DMSO as negative reference. The plates were kept at 4 ^{0}C for 2 h to allow the dispersion and then incubated at 37 ^{0}C for 24 h.

2.3. Determination of MIC by broth dilution method

Broth dilution technique was used to determine the minimum inhibitory concentration of the test material against bacterial strains. One millilitre of nutrient broth was kept in each tube and autoclaved. The essential oil diluted with DMSO (1:1) was filtered with 0.22 μm filter disk before use and then added to each tube to keep the final concentration ranging from 62.5 μg/mL–2000 μg/mL. The test bacterial suspension was added into each tube to yield bacterial density of 10^6 CFU/mL and the inoculated tubes were incubated at 37 ^{0}C for 24 h. Tubes containing nutrient broth without essential oil served as positive control, whereas those without bacteria as negative control. After incubation, 50μL of 0.2 mg/mL p-iodonitrotetrazolium violet (INT) was added in each tube to indicate the bacterial growth. The tubes were again incubated for 30 min at 37 ^{0}C. Development of pink colour in the tube (due to reduction of dye) indicated the bacterial growth whereas tubes without colour indicated no active bacterial growth. The lowest concentration at which no bacterial growth was observed corresponds to the minimum inhibitory concentration (MIC). All the assays were performed in triplicate.

2.4. Determination of antifungal activity

The antifungal activity of the test samples was determined by Poisoned Food Technique (a type of agar dilution method) against three pathogenic fungal strains viz., *Alternaria alternata, Curvularia lunata and Bipolaris specifera* (procured from Division of Plant Pathology, SKUAST-Jammu). Different concentrations of test component were prepared in sterilized potato dextrose agar and poured in 9 cm petri plates. After this, 5 mm bit of test fungus was inoculated in the centre of the agar plate (mycelia surface of the bit was placed upside down) followed by incubation of petri plates at 26 ^{0}C.[13] The extension diameter (mm) of hyphae from the center to the dish was measured at 24 h interval, till the growth of fungus in the plate without test component (control) reached the edge of the plates. The experiment was repeated thrice and results were expressed as average of three replicates. Fungal growth diameter in each plate containing concentrations of test component was determined to calculate per cent growth inhibition.

The antifungal indices were calculated as:

Antifungal index (%) = $(1\text{-}D_a/D_b) \times 100$

D_a = Diameter of growth zone in the experiment dish (mm)

D_b = Diameter of growth zone in the control (mm)

Maximum growth inhibition by test material as indicated by IC_{50} value is given in bold numbers

Values indicating maximum zone of inhibition of test component are given in bold numbers

CMP^+: Chloramphenicol; DMSO: Dimethylsulfoxide

Mark (-) indicates no activity

3. Results and Discussion

The extract of *C. tamala* showed moderate inhibition effect on fungal strains as the IC_{50} values were observed as 1.34 ± 0.012, 1.35 ± 0.013 and 1.2 ± 0.018 mg/mL for *A. alternata, C. lunataand B. specifera* respectively (Table 1). The antibacterial analysis of *C. tamala* leaves showed average activity against *B. subtilis* (13±0.51 mm), *E.coli* (13±0.5 mm), *K. pneumoniae* (13±0.16 mm). *M. luteus* (12±0.2mm) and *S. aureus* (12±0.3mm) were also inhibited (Table 2). The report "*Antimicrobial Resistance: Global Report on Surveillance"* notes that the resistance occurring across many different infectious agents and has special focus on antibiotic resistance in seven different bacteria responsible for common serious diseases such as sepsis, diarrhoea, pneumonia, urinary tract infections and some sexually transmitted diseases like gonorrhea. Pneumonia is responsible for 2^{nd} highest number of deaths among children under five year age. So, screening of new complex antimicrobial agents poses a huge challenge and is required especially in the era of drug resistant microbial strains. Like animals, plants are also serious victims of pathogenic

Table 1: Growth inhibitory effect of *Cinnamomum tamala* leaves on fungal strains

Extract	Conc.(mg/mL)	Phytopathogenic fungi		
		Alternaria alternata	*Curvuleria lunata*	*Bipolarisspecifera*
		Growth Inhibition (%)		
Methanolic	0.5	28.75	26.25	25.8
	1	43.75	37.5	48.75
	2	65	56.25	69.5
	IC_{50}	1.34±0.012	1.35 ±0.013	1.2±0.018
	Conc. (μg/mL)		Growth Inhibition (%)	
Amphotericin B (positive control)	10	48.5	46.20	50.75
	20	65.00	61.00	71.50
	40	83.60	81.60	85.69
	IC_{50}	9.5±0.1	12.1±0.4	5.7±0.2

Table 2: Antibacterial analysis of *Cinnamomum tamala* leaves

Extract	Conc. (mg/mL)	Bacterial strains				
Methanolic	100 mg/mL (10μl)	*B. subtilis* MTCC 2389	M. luteus MTCC 4821	*S. aureus* MTCC 7443	*E. coli* MTCC 2127	*K. pneumoniae* MTCC 7162
		Zone of inhibition (mm)				
		13±0.51	12±0.2	12±0.3	13±0.5	13±0.16
MIC	62.5 μg/ml-2000 μg/ml	250	500	500	500	250
Positive control CMP^+	1mg/mL (10 μl)	36±1.3	35.6±1.2	26.2± 0.9	35±1.1	35±1.3
Negative control DMSO	10 μl	-	-	-	-	-

microorganism causing diseases. Fungal diseases presently destroy at least 125 million tonnes of the top five food crops - rice, wheat, maize, potato, soybean, each year, which could otherwise be used to feed those who do not get enough to eat. These crops are the major source of calories consumed by people. Rice blast, corn smut in maize, stem rust in wheat, soybean rust and late blight in potatoes are some diseases affecting productivity of the crops plants. Most frequently, chemicals / fungicides are used to control the diseases caused by plant pathogens. However, there is a serious problem in the effective use of these chemicals due to the development of resistance by the fungi (Zhang et al., 2009).[14] To overcome this problem, higher concentrations of these chemicals are used, but this increases the risk of high level of toxic residues in the products. This problem is a natural consequence of the adaption of infectious pathogens to antimicrobials used in several areas including medicine, food, animals, crop production and disinfectants in farms, hospital / households. This has forced the researchers to search new antimicrobial compounds from natural origin like medicinal plants which are more effective and less toxic to human health and environment.

To conclude, leaves of *C. tamala* were collected from Darhal, Rajouri, Jammuand tested against different fungal and bacterial strains. The methanolic extract from the leaf part of plantpossesses antimicrobial efficiency and forms a good basis for selection of this plant part for phytochemical and pharmacological analysis.

4. Source of Funding

None.

5. Conflict of Interest

None.

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