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ANTIMICROBIAL ACTIVITY OF LEAF, CAPSULE AND ROOTS OF *CORCHORUS AESTUANS*

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ABSTRACT

The leaf, capsule and root extracts of *Corchorus aestuans* were tested for antibacterial and antifungal activity, six *Gram* (+Ve) and six *Gram* (-Ve) bacteria showed potent activity. The leaf and root extracts of *corchorus aestuans* showed positive activity than compared to *C.aestuans* capsule extract and for fungi, the methanolic extracts showed moderate activity.The petroleum ether, chloroform and methanolic extracts of *C.aestuans* showed potent antimicrobial activity.

Keywords: Antimicrobial, *Corchorus aestuans*

INTRODUCTION

The genus *Corchorus* (*Tiliaceae*), comprises certain herbs and under shrubs. It contains more than 100 species which are distributed in the tropics and subtropics, chiefly in South-East Asia and South America [1-3]. Biologically *Corchorus* species are used as diuretic, chronic cystitis, gonorrhoea and dysuria antihistaminic, anti-inflammatory, antimicrobial, cardiotonic, and also to increase the viscosity of the seminal fluid [4-5]. Several important bioactive molecules were reported which includes cardiac glycosides, their aglycones and polysaccharides, triterpenoids, phenolics, sterols and fatty acids.

Collection and Preparationof Plants

The leaf, capsule and root extracts of *Corchorus aestuans* were collected from Warangal, in September 2007 (each one 2kg) and was authenticated by Prof. V.S. Raju, Department of Botany, Kakatiya University, Warangal. A specimen was deposited in the Herbarium (Voucher specimen number (CA-7) leaf, capsule and roots were collected from the plant and dried under shade.

Antimicrobial activity of leaf, capsule and root extract of *C.aestuans*

An extensive review of literature has revealed that some species of the genus *Corchorus* and their exhibited antimicrobial activity against a wide range of micro-organisms. Based on these reports the plant extracts of the leaf, capsule and roots of *Corchorus aestuans* were tested for antimicrobial activity against bacteria and fungi strains.

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MATERIALS AND METHODS

1.1 Tested Materials

1. Chloroform extract of *C.aestuans* leaf (100mg/ml) and (200mg/ml)
2. Methanolic extract of *C.aestuans* leaf (100mg/ml) and (200mg/ml)
3. Chloroform extract of *C.aestuans* capsule (100mg/ml) and (200mg/ml)
4. Methanolic extract of *C.aestuans* capsule (100mg/ml) and (200mg/ml)
5. 5.Chloroform extract of *C.aestuans* root (100mg/ml) and (200mg/ml)
6. Methanolic extract of *C.aestuans* root (100mg/ml) and (200mg/ml)

1.1.1 Standards

Chloramphenicol (10µg/ml) and Nystatin (10µg/ml)

1.1.2 Vehicle

Dimethyl sulphoxide (DMSO)

1.2 Method:

The antimicrobial activity was determined by Agar cup-plate Method [6-11].

1.2.1 Cup-Plate Method / Well-Plate Method:

In this method 4-5mm diameter wells were made with metal borer on the agar plate In this well the chemotherapeutic agent and the plant extracts were added. The antimicrobial agent diffuses from the well through the agar medium to an extent so that the growth of the added microorganism is inhibited entirely around the well producing a clear zone. The antimicrobial activity is expressed as the diameter of zone of inhibition in millimeters, which is measured with a zone reader

In the cup plate method, the antimicrobial substance diffuses from the cup through a solidified agar layer in a petridish to an extent so that the growth of added micro-

organism is inhibited entirely in a circular area or zone around the cavity containing the solution of a known quantity of antimicrobial substance. The antimicrobial activity is expressed as the zone of inhibition in millimeters, which is measured with a zone reader.

Test Organisms:

The microorganisms used for the experimental work were procured from MTCC (Microbial Type Culture Collection, IMTECH (Institute of Microbial Technology), Chandigarh,Punjab, India).

***Gram* (+) ve organisms:**

Bacillus subtilis,
Bacillus pumilis,
Bacillus cereus,
Staphylococcus aureus

***Gram* (-) ve organisms:**

Escherichia coli,
Psuedomonasaeuriginosa,
Psuedomonas vulgaris,
Serratia marceseans

Fungal strains:

Aspergillusniger,
Rhizopusstolonifer,
Saccharomyces cervisiae.

Standardization of micro-organisms:

- One loop-full of micro-organisms were inoculated into 100 ml of sterile medium and incubated for 24 hrs at 37°C for bacterial culture and for 48 hrs at 27°C for fungal culture.
- After 24 h/48 h of incubation, 1 ml of broth containing the micro-organisms was added to 9 ml of peptone water.
- 10 fold serial dilutions were made in the range of 10^{-1} to 10^{-10}.
- 100 µl of the dilutions ranging from 10^{-5} to 10^{-8} were spread over the sterile nutrient agar (SDA) plates and kept at 37 and 27°C for 24 / 48 hours respectively.
- The number of colony forming units (CFU) was counted and number of micro-organisms per 1 ml of stock culture was calculated.

Preparation of test and standard solutions:

The stock solution of test compounds was prepared by dissolving the dried extracts at a concentration of 5 and 10mg/ml in dimethyl sulphoxide (DMSO) respectively. The stock solution of reference standards (chloramphenicol and nystatin) was prepared at a concentration of 0.6 mg/ml in sterile water. Antimicrobial activity was screened by adding 0.05 ml stock solution to each cup by micropipette.

Culture medium:

The following media were used for our antimicrobial studies.

Nutrient broth for bacteria:

Beef extract	-	0.35%
Sodium chloride	-	0.5%
Peptone	-	0.5%

The above ingredients weighing 37 g were dissolved in distilled water (1000 ml). pH was adjusted to 7.2-7.4 and sterilized by autoclaving at 15 lbssq.inch for 20 min.

Sabouraud's dextrose agar medium for fungi (SDA):

Dextrose	-	4.0%
Peptone	-	1.0%
Agar	-	2.5%

The above constituents were dissolved in distilled water and pH was adjusted to 5.6 ± 0.2 and then sterilized by autoclaving at 15 lbs sq. inch for 20 min.

Sterilization:

Sterilization of the media, water, etc., was carried out by autoclaving at 15 lbs/inch^2 for 20 minutes. The glassware like syringes, petridishes, pipettes, empty test-tubes were sterilized by dry heat in an oven at a temperature of 160°C for one hour.

Nutrient agar for bacteria:

Beef extract	-	0.3%
Sodium chloride	-	0.5%
Peptone	-	0.5%
Agar	-	2.0%
pH	-	7.2-7.4

The sterilized medium was cooled to 40°C and poured into the petridishes to contain 6 mm thickness. The media was allowed to solidify at room temperature.

Evaluation of antibacterial and antifungal activity:

Determination of zone of inhibition by cup plate method [7]

The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test compounds.

A sterile borer was used to prepare four cups of 6 mm diameter in the agar medium spread with the micro-organisms and 0.1 ml of inoculum was spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each concentration and reference standards were added to the cups with a micropipette.

All the plates were kept in a refrigerator at 2 to 8°C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of dimethyl sulphoxide and water which were used as a vehicle. The experiments were performed three times. The diameter of the zone of inhibition was measured and recorded.

Antifungal activity

The extracts tested for antibacterial activity were also evaluated for antifungal activity.

Procedure

The nutrient PDA medium (Hi-media) was prepared and inoculated with 0.5 ml of aqueous suspension of the above mentioned test organisms, which were prepared from 48 hour cultures, are thus transferred into sterile petri dishes. The medium in the plates were allowed to set at room temperature for about 10 minutes. 5 cups of 5mm diameter were made in each plate at equal distances. Stock solutions of the test residual extract were prepared in concentrations of 100mg/ml and 200mg/ml. 100µg/ml of each of the above stock concentrations were placed in the cups by means of sterile pipettes. In each plate one cup was used for each control and standard. Nystatin (10µg/ml) is used as reference standard. The petridishes thus prepared were incubated for 48 hours at 37° C and were later examined by measuring the zones of inhibition and the results were tabulated below.

Table 1: Antibacterial activity of *C.aestuans* leaf, capsule and root extract

Plant material	Concentration (mg/ml)	zone of inhibition* (diameter in mm)							
		Gram (+)ve				Gram (–)ve			
		B.s.	***B.c***	***B.p***	***S.a***	***E.c***	***P.a***	***P.v***	***S.m***
***C.aestuans* leaf extract:**									
chloroform extract	100	19	18	22	15	13	18	17	16
	200	22	24	22	16	12	18	14	15
methanolic extract	100	23	27	27	19	17	24	17	20
	200	26	30	25	19	16	26	15	18
***C.aestuans* capsule extract:**									
chloroform extract	100	12	20	18	13	13	12	14	12
	200	17	15	18	14	15	16	14	18
Methanolic extract	100	24	20	22	18	19	16	14	22
	200	24	25	21	17	14	18	16	16
***C.aestuans* root extract:**									
chloroform extract	100	19	20	18	13	21	15	12	14
	200	20	20	20	14	22	18	14	16
methanolic extract	100	21	20	22	15	23	17	18	18
	200	22	21	22	16	22	16	19	14
Standard:									
Chloramphenicol (µg/ml)	10	25	32	22	18	26	19	18	18
Vehicle:									
DMSO		-	-	-	-	-	-	-	-

*Zone of inhibition in millimeters, cup diameter: 6mm

B.s (*Bacillus subtilis*), *B.c*(*Bacillus cereus*), *B.p*(*Bacillus pumilis*), *S.a*(*Staphylococcus aureus*),*E.c*(*Escherichia coli*), *P.a*(*Psuedomonasaeuriginosa*), *P.v*(*Psuedomonas vulgaris*), *S.m*(*Serratiamarceseans*)

#Values are the average of triplicate

Table 2: Antifungal activity of *C.auestans* leaf, capsule and root extract

Plant Material	Concentration (mg/ml)	Zone of inhibition* (diameter in mm)			
		A.n	***R.s***	***S.c***	***P.c***
***C.aestuans leaf* extract:**					
chloroform extract	100	23	22	19	20
	200	22	20	20	21
methanolic extract	100	22	23	20	21
	200	23	22	22	20
***C.aestuans capsule extract*:**					
chloroform extract	100	20	17	18	18
	200	21	18	19	19
methanolic extract	100	20	18	21	20
	200	21	19	22	20
***C.aeustuans* root extract:**					
chloroform extract	100	18	16	13	10
	200	20	20	18	14
methanolic extract	100	20	19	21	18
	200	22	20	19	20
Standard:					
Nystatin	10	25	21	20	22
Vehicle:					
DMSO		-	-	-	-

*Zone of inhibition in millimeters, cup diameter: 6mm

A.n(*Aspergillusniger*), R.s (*Rhizopusstolonifer*),*S.c*(*Sacharomycescerevisiae*)*P.c*(*Pencilliumchrysogenum*)

#Values are the average of triplicate.

RESULTS AND DISCUSSION:

The chloroform and methanolic extracts of the leaf, capsule and roots of *Corchorus aestuans* were screened for antimicrobial activity against a wide spectrum of micro-organisms and the activity was compared with reference standards (Chloramphenicol for both Gram (+Ve) positive and *Gram* (-Ve) organisms and Nystatin for fungal strains). Micro-organisms were grown in nutrient agar medium. Dimethyl sulphoxide (DMSO) was used as control. The chloroform and methanolic extracts of different parts of leaf, capsule and root extracts of *C.aestuans* were used in two dose levels of 100mg/ml and 200mg/ml. Chloramphenicol (10µg/ml) is standard drug for both *Gram* (+Ve) and *Gram* (-Ve) organisms, Nystatin (10µg/ml) for fungal strains.

From the above observations, both the chloroform and methanolic extract showed moderate activity against *Gram* (+Ve) and *Gram* (-Ve) organisms are *B.subtilis, Bacillus cereus,B.pumilis, B.cerus, S.aureus, E.coli, P.aeruginosa, P.vulgaris, S.marcens*

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