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Antimicrobial Studies of Phyllanthus maderaspatensis And Celosia argentea

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----------ABSTRACT----------
*Phyllanthus maderaspatensis***,** a dominant weed in Jowar fields and *Celosia argentea***,** a dominant weed in Redgram fields were identified by the IVI studies conducted in the test fields of Kurnool district, Andhra Pradesh. Though they are weed plants they have medicinal value**.** Their Allelopathic influence was noted down by petriplate and pot studies on crops like Jowar and Redgram. Their growth and biochemical parameters were tested. *Phyllanthus sps* are used traditionally for the treatment of viral, bacterial and parasitic infections. *Celosia argentea* seeds are used medicinally as an ophthalmic, antiphlogistic and astringent in conjunctivitis. Being the test weeds are known ethanobotanicals in the area, their solvent extracts were subjected to antimicrobial screening which resulted that Alcohol and Ethyl acetate extracts have more significant inhibitory fraction.

Keywords : *Phyllanthus maderaspantensis, Celosia argentea,* MIC, MBC.

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I. INTRODUCTION

Phyllanthus maderaspantensis and *Celosia argentea* both are dominant weed plants in crops of Jowar and Redgram respectively.

Interestingly both are ethanomedicinals in the test area and their antimicrobial studies were carried out.

P. maderaspatensis herb is bitter in taste, possess several medicinal properties like astringent, debostruent, stomachic diuretic, febrifugeal and antiseptic properties.The leaves are expectorant, diaphoretic useful in strangury and sweats. The seeds have a bad taste used as carminative laxative, astringent to the bowels**,** tonic to the liver**,** diuretic useful in bronchitis, earache, griping**,** ophthalmia and ascites.

The leaves and shoots of *Celosia argentea* are consumed as vegetable. The seeds are used medicinally as an ophthalmic, antiphologistic and astringent in conjunctivitis or retinal haemorrhage. The leaves are antipyretic, aphrodisiac, reduce inflammations, and strengthen the liver, seeds**,** leaves and roots are used for curing urinary diseases and stomach disorders.

Phyllanthus maderaspatensis

Celosia argentea

In the present study antibacterial activity of different parts of plant, extracted with different solvents is compared. The purpose of this work was to perform the chemical prospection of the different extracts and to evaluate the antibacterial activity of *Phyllanthus maderaspatensis and Celosia argentea extracts.*

Materials and Methods
Antimicrobial Studies

Plant material

Samples of (root and shoot in *Phyllanthus***;** root, stem, leaf and infloresence in *Celosia*) were collected in study area, washed with clean water, chopped into small pieces and shade dried. The dried samples were ground into coarse powder.

Preparation of Crude extracts :

Known quantities of plant material (each 100 g) were extracted with different polar solvents with increasing polarity with the help of soxhlet apparatus. The extracts were filtered and concentrated under reduced pressure at 40^0 C using rotoflash evaporator. The extraction is carried with different polar solvents viz., ethyl acetate, ethyl alcohol and distilled water and the extracts were filtered and concentrated under vaccum (Waltor, 1971). The residues were collected and percentage of the yield was quantified for each extract.

Preparation of Discs for Antimicrobial Assay

Different concentrations of crude extracts were dissolved in dimethyl sulfoxide (DMSO) and used for biological assay, 20-25 µl of the stock solutions were applied to each sterilized filter paper disc of 6 mm. Later, the filter paper discs were carefully taken out and dried in the laminar air flow. Thus completely dried discs were used for antimicrobial studies. A control disc was maintained always in the solvent used for assay.

Microorganisms used

The following human pathogenic microorganisms were obtained from the microbial type culture collection centre, Institute of Microbial Technology (IMTECH) Chandighar, India and maintained on their respective media in slants at 4^0 C and used as test organisms for antimicrobial activity of the crude extracts.

Gram $^+$ - *Staphylococcus aureus*
Gram $^-$ - *Klebsiella pneumoniae*
Pseudomonas aeruginosa
Salmonella type- murium
Fungal - *Candida albicans*

Antimicrobial assay

The antimicrobial activity was performed by employing the pour plate disc diffusion methods adopted by Bauer et al (1966), was measured and expressed in millimeters. Each test was performed in three replicates and repeated twice to get the average values. Model values were selected.Discs containing antibiotics (30 mg/disc) like (vancomycin) served as standard obtained from Hi-media Bombay.

Minimum inhibitory concentration (MIC):

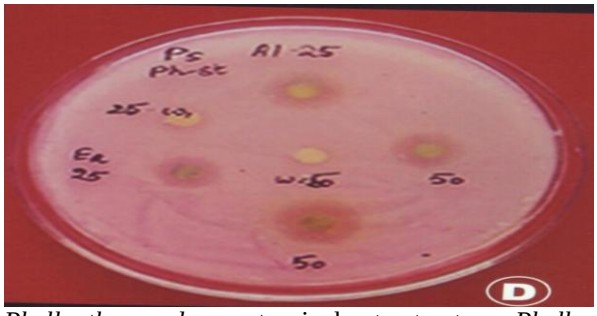
The extracts showing significant zones of inhibition were used for determining minimum inhibitory concentration (MIC). The MIC of crude extracts was determined by the broth micro-dilution methods (Koneman, 1995, Camporese, 1997 NCCLS, 2001) using 96 – well micro titer plates. Initially the extracts were dissolved in dimethylsulfoxide medium to create a concentration of 10 mg/ml of stock solution. About 100 ml of the solution was serially diluted (two fold dilution) to obtain different concentrations to ml of the previously prepared different microbial suspensions (10^5 CFU/ml) were added to each well plates were incubated for 18 hours at 37^0 C and then were examined with Cruickshank (1968). The crude extracts of each sample dissolved in (DMSO) and the concentrations of 25 to 50/ml were prepared 20-25 µl of each sample was applied to sterile whatmann filter paper discs.

The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. The diameter of the zones of inhibition around each disc measured and recorded at the end of incubation period.

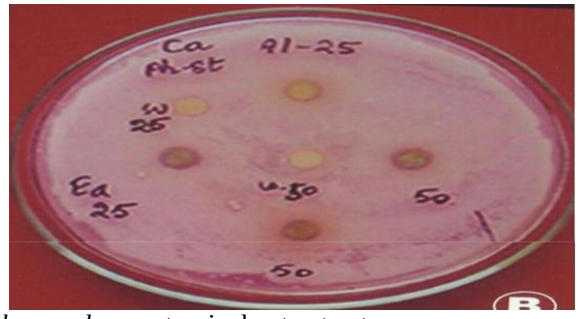
The diameters of inhibition zones Elisa reader (TEALAN, Sunrise, China) at 620 mm and the lowest concentration of each extract showing growth was taken as its minimum inhibitory concentrations (MIC).

Minimum Bacterial Concentration (MBC):

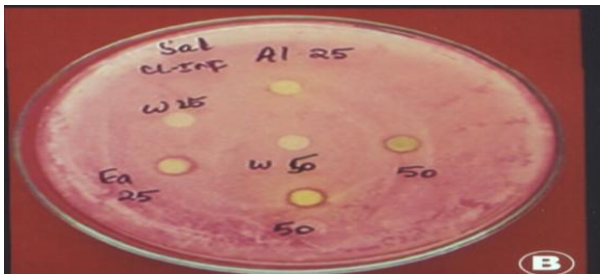
Minimum bacterial concentration (MBC) is defined as the concentrations at which killing of 99.9% of starting inoculum occur**s**. The minimum bacterial concentration was determined by adopting standard methods (NCCLS, 1999, Y u et al 2004). To determine MBC, broth was taken from each well and incubated in Nutrient agar at 37^0C, for 24 hours for bacterial or in Sabourand dextrose agar at 30^0C for 48 hours for the yeasts. The least concentration showing no visible growth on agar subculture was taken as MBC value. This is the lowest concentration, expressed in mg/ml. Each test was performed in three replicates and repeated twice to get the average value.



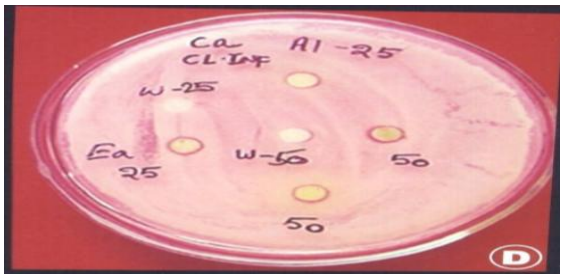
Phyllanthus maderaspatensis shoot extract against *Pseudomonas*



Phyllanthus maderaspatensis shoot extract against *Candida*



Celosia orgentia Inflorescence extract against Salmonella



Celosia orgentia Inflorescence extract against Candida

Results

Table:1 - Antimicrobial activity of *phyllanthus maderaspatensis* shoot

Organisms	Inhibition Zone												
	EtoAc				EtOH				Water				Standards
	mg/ml		MIC	MBC	mg/ml		MIC	MBC	mg/ml		MIC	MBC	µg/disc
	25	50	µg/ml	mg/ml	25	50	µg/ml	mg/ml	25	50	µg/ml	mg/ml	
Salmonella typhimurium MTCC98	8	8	312	0.625	8	8	625	1.25	-	-	-	2.5	25^V
Staphylococcus aureus MTCC 737	8	10	625	1.25	8	12	625	1.25	-	8	625	1.25	25^V
Pseudomonas aeruginosa MTTC 1688	11	13	312	1.25	14	16	312	1.25	7	9	312	2.5	25^V
Klbsiella pneumoniae MTCC 109	9	11	625	2.5	11	12	312	2.5	-	9	625	1.25	25^V
Candida albicans MTTC 183	8	8	625	2.5	8	8	625	1.25	8	8	625	1.25	25^V

Table: 2 - Antimicrobial activity of *Celosia argentea* Inflorescence

Organisms	Inhibition Zone												
	EtoAc				EtOH				Water				Standards
	mg/ml		MIC	MBC	mg/ml		MIC	MBC	mg/ml		MIC	MBC	µg/disc
	25	50	µg/ml	mg/ml	25	50	µg/ml	mg/ml	25	50	µg/ml	mg/ml	
Salmonella typhimurium MTCC98	8	7	312	1.25	7	9	1250	1.25	-	-	625	1.25	25^V
Staphylococcus aureus MTCC 737	-	8	625	2.5	7	9	1250	1.25	-	-	-	-	25^V
Pseudomonas aeruginosa MTTC 1688	8	8	625	2.5	8	9	625	0.625	7	7	625	2.5	25^V
Klebsiella pneumoniae MTCC 109	-	-	625	1.25	-	-	625	0.625	-	-	625	1.25	25^V
Candida albicans MTTC 183	8	8	625	1.25	8	8	1250	1.25	-	7	625	1.25	25^V

EtoAC : Ethyl acetate; EtOH: Ethanol; MIC: Minimum Inhibitory Concentration**;** MBC: Minimum Bacterial Concentration

Discussion

Out of all the extracts *Phyllanthus* shoot (Methanol) and *Celosia* inflorescence (Ethanol) exhibited maximum antimicrobial activity with maximum inhibition zones of 16mm and 7-9mm respectively. The *in vitro* antimicrobial screening results revealed that among the test extracts the alcohol and ethyl acetate have exhibited significant inhibition on pathogens than water extracts. *Pseudomonas* sp was found to be more sensitive. The inhibition zones were increased with increase in drug concentration revealed that the inhibitory property is concentration dependent.

Conclusion

Being the test weeds are known ethnobotanicals in the area, the solvent extracts were subjected to antimicrobial screening which resulted alcohol and ethylacetate extract as more significant inhibitory fraction.

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