SCREENING OF ANTIMICROBIAL ACTIVITY OF LEAVES EXTRACT AND ISOLATED COMPOUNDS OF ACACIA CATECHU (L.) WILLD AND ACACIA AURICULIFORMIS A.CUNN

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Abstract
The leaves extract and isolated compounds of *Acacia catechu* (L.) Willd and *Acacia auriculiformis* A.Cunn were screened for antimicrobial activities against some pathogens Extracts and isolated compounds were found to produce significant inhibition against all the pathogens. Results were compared with the standard drug and it was revealed that isolated compounds were more potent than extract in both the plants extract.

Key-words: *Acacia species,* Leaves, Anti-microbial activity

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Introduction

Microbial infections are a leading cause of morbidity and health care expenditures in persons of all ages. Several other populations, including elderly persons and those who are living in unhygienic conditions, are also at major risk. Keeping all these aspects in mind the search of natural anti-microbial agents is essential. [1-2] The plant *Acacia catechu* (L.) Willd and *Acacia auriculiformis* A.Cunn belongs to family Fabaceae is an indigenous plant grown under wild condition in some parts of our country and was chosen for the present investigation. These plants were used in traditional system of medicine for the treatment of bacterial infection, fungal infection, diabetes, liver disorders etc. [3-4] The scanty availability of information on this plant facilitates the study on it. The attempt was made to study anti-microbial activity of extract and isolated compounds.

Material and Methods

Collection of herbs and their authentication

The leaves of *Acacia catechu* (L.) Willd and *Acacia auriculiformis* A.Cunn were collected in the months of July-December 2019 from the Southern region of India and identified & authenticated by Dr. Smruti Sohani, Professor, Faculty of Life Sciences, SAGE University, Indore (M.P.) and was deposited in our Laboratory. Voucher specimen No. SU/LS-ACL36 & SU/LS-AAL37 was allotted.

Successive Extraction of selected herbs

Sample were shattered and screened with 40 mesh. The shade dried coarsely powdered plant material (250gms) were loaded in Soxhlet apparatus and was extracted with n-hexane, pet. ether, benzene, chloroform, ethyl acetate, ethanol and water until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. [5]

Isolation of Compounds

The various extract obtained after extraction were subjected for CC for isolation of compounds and isolated compounds were tested for anti-microbial activity. [6]

Screening of Anti-Microbial Activity [7-8]

Preparation of microorganisms for experiment

The microorganism strains employed in anti-microbial investigations came from pathological lab, Indore. The organisms were sub-cultured in nutrient broth, nutrient agar, Macconky agar, and Blood agar medium for use in research. In the testing of antibiotic sensitivity, Muller Hinton agar was employed.

Preparation and application of disks for experiment

By reconstituting the extracts/isolated compounds with DMSO, various concentrations (10–60 g/ml) were created. By using the streaking plate method, the test microorganisms were transferred to Muller Hinton agar medium. After streaking, flame-sterilized forceps were used to transfer the autoclaved filter paper discs (5 mm in diameter) soaked with extracts onto plates. The antibacterial assay plates underwent a 24-hour incubation period at 37°C. Amoxycillin/Cefitaxime (60 g/ml) was used as a positive control, and DMSO was utilized as a negative control.

Observation of results

Results were noted as either a zone of inhibition was present or not. It was determined that the inhibitory zone surrounding the test paper discs was positive (growth inhibition was seen), and that the absence of the zone was negative. To ensure that the results were reliable, the test was conducted three times over a 24-hour period. The inhibitory zones' sizes were measured in millimeters (after subtraction the diameter of disc i.e.5mm).

Statistical analysis

One-way analysis of variance (ANOVA) and Dunnett's test were used to statistically examine all the values. Significant differences (*P 0.01) were determined between the control and drug-treated groups. Every value is expressed as the mean SEM.

Results and Discussion

In this study the results of the investigations show that extract and isolated compounds i.e., Lupeol and Lupenone from two *Acacia* species viz., *Acacia catechu* (L.) Willd and *Acacia auriculiformis* A.Cunn possess antimicrobial activities against selected micro-organism organisms (Table 1 and 2). Isolated compounds (Table 3) showed more potent anti-microbial activity than leaves extract of the selected plant as compare to the standard.

Table 1: Antimicrobial activity of various extracts of *Acacia catechu* (L.) Willd

Bacterial Strain	Treatments								
	C	SD (µg/ml)	ACL						
			HEACL	PEEACL	BEACL	CEACL	AcEAACL	EEACL	AEACL
Bacillus	-	20.12±0.16	5.01± 0.1	9.19± 0.1	5.41± 0.3	7.60± 0.5	8.32± 0.4	18.69± 0.4	17.18± 0.1
Proteus	-	18.78±0.49	3.11± 0.6	11.21±0.9	11.04±0.6	12.03± 0.4	13.11± 0.5	14.84± 0.5	13.39± 0.6
Pseudomonas	-	24.16±0.72	11.10±0.6	15.12±1.0	13.22±0.7	18.29± 0.4	19.10± 0.4	22.34± 0.4	21.22± 0.8
E. coli	-	22.50±0.76	10.22±1.2	16.04±0.8	18.46±0.2	20.42± 0.2	18.20± 0.3	20.28± 0.3	19.98± 0.3
S. aureus	-	25.16±0.72	16.43±0.8	19.49±0.6	19.23±0.4	21.42± 0.7	20.18± 0.2	22.27± 0.2	21.45± 0.5
Enterobacter	-	19.50±0.28	7.11± 0.2	10.12±0.1	17.33±0.03	10.08± 0.2	11.28± 0.6	17.06± 0.6	16.16± 0.7
Enterococci	-	23.90±0.21	11.10±0.8	17.22±0.4	11.02±0.7	18.42± 0.3	19.32± 0.9	20.19± 0.9	19.28± 0.7
Klebsiella	-	20.41±0.23	5.21 ± 0.2	12.12±0.2	13.29±0.6	19.12± 0.8	18.11± 0.2	18.34± 0.2	17.22± 0.2\2

Values are expressed as Mean (X) ±SEM, n=3 ; **Abbr**.: C: Control (DMSO), SD= Standard (Amoxycillin)

Table 2: Antimicrobial activity of various extracts of *Acacia auriculiformis* A.Cunn

Bacterial Strain	Treatments								
	C	SD (µg/ml)	AAL						
			HEAAL	PEEAAL	BEAAL	CEAAL	AcEAAL	EEAAL	AEAAL
Bacillus	-	20.12±0.16	4.11± 0.3	8.11± 0.6	6.31± 0.5	8.04± 0.2	9.25± 0.3	18.24± 0.5	17.04± 0.4
Proteus	-	18.78±0.49	3.18± 0.5	10.01±0.4	10.18±0.7	11.36± 0.2	11.10± 0.4	14.10± 0.7	13.02± 0.3
Pseudomonas	-	24.16±0.72	9.11±0.7	12.22±0.5	11.39±0.9	17.10± 0.6	15.19± 0.4	22.20± 0.7	21.10± 0.3
E. coli	-	22.50±0.76	11.02±1.8	15.14±0.3	17.23±0.8	21.32± 0.8	17.29± 0.9	20.04± 0.9	19.11± 0.6
S. aureus	-	25.16±0.72	12.13±0.6	18.19±0.7	18.20±0.6	20.37± 0.4	19.11± 0.6	21.21± 0.4	21.03± 0.8
Enterobacter	-	19.50±0.28	8.10± 0.6	9.10±0.6	16.30±0.04	11.11± 0.5	12.27± 0.3	16.09± 0.6	15.11± 0.0
Enterococci	-	23.90±0.21	10.10±0.4	16.02±0.3	10.11±0.4	17.29± 0.9	18.28± 0.4	20.11± 0.8	19.38± 0.1
Klebsiella	-	20.41±0.23	6.22 ± 0.5	11.32±0.3	12.20±0.3	17.39± 0.6	17.39± 0.3	18.20± 0.3	17.26± 0.1

Values are expressed as Mean (X) ±SEM, n=3; **Abbr**.: C: Control (DMSO), SD= Standard (Amoxycillin)

Table 3: Antimicrobial activity of Lupeol and Lupenone

Bacterial Strain	Treatments			
	C	SD (µg/ml)	Lupeol	Lupenone
Bacillus	-	20.12±0.16	19.28± 0.6	18.89± 0.3
Proteus	-	18.78±0.49	18.21± 0.4	17.39± 0.3
Pseudomonas	-	24.16±0.72	22.38± 0.2	21.98± 0.6
E. coli	-	22.50±0.76	21.30± 0.2	21.14± 0.7
S. aureus	-	25.16±0.72	22.38± 0.3	21.09± 0.9
Enterobacter	-	19.50±0.28	18.29± 0.5	17.83± 0.3
Enterococci	-	23.90±0.21	21.11± 0.7	21.12± 0.2
Klebsiella	-	20.41±0.23	19.27± 0.2	19.22± 0.1

Values are expressed as Mean (X) ±SEM, n=3; **Abbr**.: C: Control (DMSO), SD= Standard (Amoxycillin)

Conclusion

In this investigation anti-microbial activity of leaves extract from two *Acacia* species viz., *Acacia catechu* (L.) Willd and *Acacia auriculiformis* A.Cunn and isolated compounds i.e., Lupeol and Lupenone were screened and was found that isolated compound possess more potent activity than extract.

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