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Antimicrobial activity and GC-MS analysis of Chloroform extract of Acacia concinna (Willd). DC.

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Abstract:
The ethanol extract of fruits of Acacia concinna (Willd). DC. was analyzed for antimicrobial activity against fungal isolates like Candida albicans, Microsporum audouinii, Trichophyton rubrum, Trichophyton mentagrophytes and the bacterial isolates of Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa and Bacillus pumilus by disc diffusion method (Zone of Inhibition in mm at 100 µg / disc). It was observed that ethanol extract was positive against Trichophyton rubrum (zone of inhibition 9 mm). GC-MS analysis study of ethanol extract of fruit shows the presence of twelve phytochemical compounds.

Introduction:

Fabaceae, also called **Leguminosae,** is the third largest family among the angiosperms consists of more than 700 genera and about 20,000 species of trees, shrubs, vines, and herbs and is worldwide in distribution. *Acacia concinna* (Willd). DC. belongs to Fabaceae is a large climbing shrub, armed with numerous small hooked prickles, Pods and Leaves of *Acacia concinna* (Willd). DC. were used as anti-dandruff. Pods were used for some skin diseases. It acts as anti-dermatophytic (Natarajan and Natarajan 2009). The Pods contains secondary metabolites such as alkaloids, flavonoids, phytosterols, saponin, tannins, phenolic compounds, gums. In present investigation antimicrobial activity of chloroform extract of *Acacia concinna* (Willd) DC used in the treatment of Skin Disease was analyzed against eight clinically significant organisms and GC- MS analysis of *Acacia concinna* (Willd) DC. was studied to detect chemical constituents.

Material and Methods:

1. Successive solvent extraction of plant material:

The pod of *Acacia concinna* (Willd). DC**.** was collected and washed thoroughly and air dried under shade. After complete shade drying the fruit was grinded. The extraction was done by using Soxhlet's extraction method with analytical grade refluxing solvents like ethanol

2. Antimicrobial Activity:

The extract was used for antimicrobial activity against pathogens e.g. Fungal isolates *Candida albicans, Microsporum audouinii, Trichophyton rubrum, Trichophyton mentagrophytes* and the bacterial isolates of *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Bacillus pumilus* by disc diffusion method (Zone of Inhibition in mm at 100 µg / disc).

3. GC-MS (Gas Chromatography and Mass Spectroscopy):

The samples were subjected to GC-MS analysis from Central Instrumentation

Laboratory (CIL), Punjab University Chandigarh. GC-MS analysis of the samples were carried out using Perkin Elmerclarus 680 with mass spectrometer clarus 600 (EI) using TurboMass ver 5.4.2 Software with NIST – 2008 Library ver. Mass spectra were recorded over 35-650 amu range with electron impact ionization energy 70 eV; a scan interval of 2 min and fragments from 50 to 600 Da. The chemical components form the different extract of plant were identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

Result and Discussion:

Table 1.1: Antimicrobial activity of fruit extracts of *Acacia concinna* (Willd). DC**.** by disc diffusion method (Zone of Inhibition in mm at 100 µg / disc)

Sr. No.	Micro-organism	Ethanol
1	*Staphylococcus aureus*	00
2	*Streptococcus pyogenes*	00
3	*Pseudomonas aeruginosa*	00
4	*Bacillus pumilus*	00
5	*Trichophyton rubrum*	**9 mm**
6	*Trichophyton mentagrophytes*	00
7	*Microsporangium audouinii*	00
8	*Candida albicans*	00

*Data represented in mean of three replicates.

The zone of inhibition of 9 mm against pathogen *Trichophyton rubrum* was observed in ethanol extract. Ethanol extracts showed positive microbial zone of inhibition against *Trichophyton rubrum*. Ethanol extracts was found non-reactive to other test organisms.

2.1. H1: GC-MS analysis of *Acacia concinna* (Willd). DC.

GC-MS was carried out to study and to determine the possible chemical components from pods of *Acacia concinna* (Willd). *DC.* The ethanol of ethanol extract clearly shows the presence of twelve peaks indicating presence of twelve phytochemical compounds detected was shown in Fig. 2.1 H1. The twelve phytoconstituents were characterized and identified on comparison of the mass spectra of the constituents provided by NIST library. The ethanol extract of *Acacia concinna* (Willd). DC. pods analyzed by GC-MS shows the presence of compounds like Cyclohexasiloxane, dodecamethyl-, Phenol,2,4-bis(1,1-dimethylethyl)-, Dodecanoic acid, Tetradecanoic acid, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, n- Hexadecanoic acid, Palmetic anhydrade, (E)-9-Octadecanoic acid ethyl ester, n-Tetracosanol, Oleic acid, eicosyl ester, 17-Pentatriacontene, Milbemycin,b 13-Chloro-5-demethoxy-6,28-epoxy-5-(hydroxylmino)-25-(1-methylethyl)-(6R,13R,25R). The active compound with their retention time (RT), % peak area, Compound analyzed, molecular formula, probable structural formula and activity reported are presented in Table- . 2.2 H1.

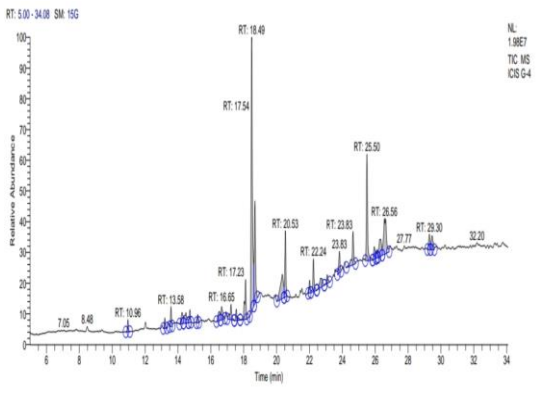
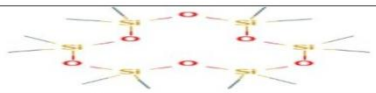
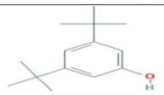
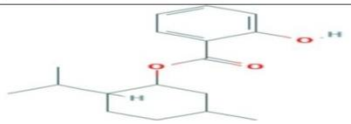
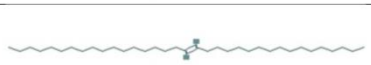
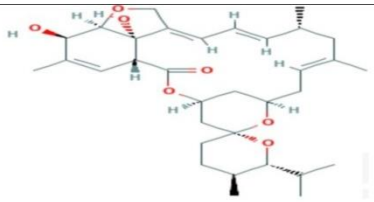


Fig. 2.1 H1 : GC-MS chromatogram of *Acacia concinna* (Willd). DC.

Table 2.2 H1 : GC-MS Analysis of *Acacia concinna* (Willd). DC.

Sr. No.	Retention Time	Peak area %	Compound Analyzed	Molecular formula	Probable Structural Formula	Activity reported
1	10.96	0.81	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$		Antiperspirant, Glow skin and hair
2	13.58	1.36	Phenol,2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$		Antibacterial, Antifungal
3	14.25	1.56	Dodecanoic acid	$C_{12}H_{24}O_2$		Antifungal
4	16.52	1.45	Tetradecanoic acid	$C_{14}H_{28}O_2$		Skin cleanzer
5	18.12	3.87	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$		Antiallergic, Antibacterial, Anti-inflammatory, Demulcent
6	18.49	25.38	n- Hexadecanoic acid	$C_{16}H_{32}O_2$		Anti-itching, Antiirritant
7	18.69	8.94	Palmetic anhydrade	$C_{32}H_{62}O_3$		Antiirritant, Demulcent
8	20.34	5.25	(E)-9-Octadecanoic acid ethyl ester	$C_{20}H_{38}O_2$		Skin hydrator
9	20.53	5.00	n-Tetracosanol	$C_{24}H_{5_0}O$		Antiallergic, Antidermatitic, Antiedemic, Anti-inflammatory
10	22.01	0.93	Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$		Skin hydrator
11	23.83	2.14	17-Pentatriacontene	$C_{35}H_{70}$		Antiseptic
12	26.11	0.85	Milbemycin,b 13-Chloro-5-demethoxy-6,28-epoxy-5-(hydroxylmino)-25-(1-methylethyl)-(6R,13R,25R)	$C_{33}H_{46}ClNO_7$		Antiitching, Antiallergic, Antidermatitic, inflammatory

Conclusion:

The plant species *Acacia concinna* (Willd). DC. (Fruits), extract was positive against *Trichophyton rubrum.* The data obtained after GC-MS analysis of plant used in skin diseases revealed the presence of chemical constituents like Cyclohexasiloxane, dodecamethyl- which is used as antiaging skin and hair conditioner, lubricant, antiperspirant scalp treatment, deodorant and skin lightener agents. N-Hexadecanoic and Palmetic anhydrade, obtained from GC-MS analysis of plants used as anti-inflammatory, antiallergic, absorbent, demulcent, antiitch and antidermatic agents. The study would be helpful for treating skin diseases.

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