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Research Article

GC-MS analysis of methanol extract of *Acacia nilotica* (L.) Leaves

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Abstract

The aim of this study was to carry out for identification of various bioactive compounds from the methanol extract of *Acacia nilotica* L. leaves by Gas chromatography and Mass spectroscopy (GC-MS). A total of 77 compounds were observed that has been shown in the table below. The GC-MS analysis revealed the presence of various compounds like Calycanthidine, D Galactose, Linoleic acid, Catechine, Pepperdine carboxylic acid (Pipecolic acid), Erythritol, Malic acid and Octadeconic acid (Stearic acid) in the methanol extract of *A. nilotica* leaves. Further studies are needed to isolate active compounds of the extract as well as to explicate their exact mechanism of action in various disorders.

Keywords: *Acacia nilotica*, GC-MS analysis, methanol extract.

1. Introduction

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants [1].

Acacia nilotica (L.) commonly known as *Babool* belonging to family Fabaceae is very commonly growing medium sized tree. The leaves are bipinnate, with 3-6 pairs, of pinnulae and10-30 pairs of leaflets each, tomentose, rachis with a gland at the bottom of the last pair of Pinnulae [2].

*Acacia*is a pioneer species, relatively high in bioactive secondary compounds andis important for a variety of functions is economically used as a source of tannins, gums, timber, fuel and fodder. It is commonly known as '*Babul*' plant having various therapeutic uses such as: anti-cancer, anti tumour, antiscorbutic, astringent, anti-oxidant, antispasmodial, diuretic, intestinal pains and diarrhoea, nerve stimulant and is used for treatment of cold, congestion, coughs, dysentry, fever, hemorrhages, leucorrhea, ophthalmia and sclerosis [3].

Extraction is the main step for the recovery and isolation of bioactive phytochemicals from plant materials, before component analysis [4]. Hence, for the discovery of lead compounds for use as therapeutic drugs, the active principals in medicinal plants need to be identified [5]. GC-MS method can serve as an interesting tool for testing the amount of some active principles of herbs.It combines two analytical techniques to a single method of analyzing mixtures of chemical compounds. Gas chromatography separates the components of the mixture and mass spectroscopy analyzes each of the components separately.

Numerous studies on *A. nilotica* showed various interesting biological activities [6-8]. Hence, the present study was aimed to carry out the identification of bioactive compounds from the methanol extract of *Acacia nilotica* leaves by Gas chromatography and Mass spectroscopy (GC-MS).

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Figure 1: *Acacia nilotica* leaves and whole plant.

2. Materials and Method

2.1 Collection of plant material

Acacia nilotica leaves collected from the plant and washed with the distilled water then leaves of the plant materials were dried in an open air protected from direct exposure to sunlight. The dried plant materials were separately powdered to suitable size and made ready for extraction with the help of grander.

2.2 Preparation of plant extract

The powdered plant leaves (10 gm) were successively extracted with methanol. The extraction was done by hot continuous Soxhlet apparatus. The extracts were stored at -4 ℃ till further uses. Methanol extract was used for the present study.

2.3 GC-MS analysis

For the analysis of the non-aqueous soluble fractions of leaves by GC–MS, a volatile TMS derivative of the sample was prepared. About 5.0 mg of the sample was suspended in 50µl of methoxylamine hydrochloride solution in GC grade pyridine (20 mg ml^{-1}). The mixture was shaken for 2 hr. at 37^0C before adding 70 µl of MSTFA. Shaking was continued for another 30 min. Analysis of lipid content by GC–MS was performed using Thermo Trace GC Ultra coupled with Thermo fisher DSQ II mass spectrometers. Chromatographic separations of metabolites were carried out on 30 m x 0.25 mm Thermo TR50 column (polysiloxane column coated with 50% methyl and 50% phenyl groups). Xcalibur software was used to process the chromatographic and mass spectrometric data. The GC oven temperature was maintained at 70^0C for 5 min, then gradually raised at the rate of 5^0C min^{-1} to 290^0C and maintained for 5 min. The sample was injected in the split mode at a splitting ratio of 1:16. Helium was used as a carrier gas and set at a constant flow rate of 1 mL min^{-1}. The mass selective detector was run in the electron impact (EI) mode, with electron energy of 70 eV. The resulting GC–MS profile was analyzed using Replib, WILLY and NIST mass spectral library by matching the chromatogram with commercially available standards. A freely available mass spectral deconvolution algorithm (Automated Mass Spectral Deconvolution and Identification System, AMDIS32) was used for processing multiple datasets.

3. Results

3.1 GC-MS analysis

The analysis and extraction of plant material play an important role in the development, modernization and quality control of herbal formulations. Hence the present study was aimed to find out the bioactive compounds present in the methanol extract of *A.nilotica* by using Gas chromatography and Mass spectroscopy. The active compounds with their peak number, concentration (peak area %), and retention time (RT) are presented in Table 1 which shows the presence of 77 bioactive phytochemical Compounds in the methanol extract of *A. nilotica*. The percentage content of compounds Catechine (13.05), Calycanthidine (11.74), D Galactose (10.55), Malic acid (7.92), Linoleic acid (5.13), Erythritol (4.08), D-Glucitol (3.36), Pipecolic acid (3.17), 2,5 Dihydroxyacetophenone (2.84),Oxalic acid (1.44), Butanoic acid (1.38), Ribitol (1.32) and Palmitic acid (0.42). Due to the presence of above mentioned compounds in the chloroform extract of *A. nilotica* leaves, it can be used in various pharmaceutical and industrial applications.

Table: Showing GC–MS data about the different compounds present in *A. nilotica*.

Sr. No.	RT(min)	%Area	Metabolites	Mol. formula
1	10.46	1.44	Ethanediaicacid,Oxalic acid	$C_2H_2O_4$
2	10.65	0.89	Potassium chlorate	$KClO_3$
3	10.66	3.7	Glycerol	$C_{12}H_{32}O_3Si_3$
4	11.87	0.26	Malonic acid	$C_3H_4O_4$
5	11.88	1.84	Propanedioic acid	$C_3H_4O_4$
6	12.96	0.32	Serine	$C_3H_7NO_3$
7	12.97	0.31	Furazan	$C_2H_2N_2O$
8	13.22	0.9	Alanylalanine	$C_6H_{12}N_2O_3$
9	13.46	0.42	Propanoic acid	$C_3H_6O_2$
10	14.25	0.44	Urea	CH_4N_2O
11	14.89	3.17	Piperidine carboxylic acid (Pipecolic	$C_6H_{11}NO_2$
12	15.03	0.22	Butanedioic acid	$C_4H_6O_4$
13	15.57	0.12	Maleic acid	$C_4H_4O_4$
14	16.14	4.08	Erythritol	$C_4H_{10}O_4$
15	16.14	0.1	threital	$C_4H_{10}O_4$
16	16.57	0.06	2-Pentamethyldisilyoxypropane	$C_5H_{12}O_2$
17	18.39	7.92	Malic acid	$C_4H_6O_5$
18	18.41	1.38	Butanoic acid	$C_4H_8O_2$
19	18.87	0.38	Erythronic acid	$C_4H_8O_5$
20	18.9	0.39	L-Threnoic acid	$C_4H_8O_5$
21	19.43	0.27	2 Isopropylmalic acid	$C_7H_{12}O_5$
22	19.87	0.17	L-Theronic acid	$C_4H_8O_5$
23	20.45	2.53	D,L Alanine	$C_3H_7NO_2$
24	20.48	1.54	Arabinitol	$C_5H_{12}O_5$
25	20.48	11.74	Calycanthidine	$C_{23}H_{28}N_4$
26	20.5	1.32	Ribitol	$C_5H_{12}O_5$
27	20.85	3.36	D-Glucitol	$C_6H_{14}O_6$
28	21.46	0.42	Xylonic acid	$C_5H_{10}O_6$
29	21.67	1.88	Cyclohexane	C_6H_{12}
30	21.85	1.65	Proline	$C_5H_9NO_2$
31	22.16	0.65	Arabinose	$C_5H_{10}O_5$
32	22.29	0.4	Ribonic acid	$C_5H_{10}O_6$
33	22.34	0.89	Tartaric acid	$C_4H_6O_6$
34	22.38	1.03	Tris ethylene	$C_6H_{24}N_6Cl_3Co$
35	22.4	13.65	my inositol	$C_6H_{12}O_6$
36	22.91	60.09	inositol	$C_6H_{12}O_6$
37	23.21	0.81	L-Ascorbic acid	$C_6H_8O_6$
38	23.67	2.81	Asparginine	$C_4H_8N_2O_3$
39	23.9	0.97	D-Mannitol	$C_6H_{14}O_6$
40	24.29	8.59	D-fructose	$C_6H_{12}O_6$
41	24.88	10.55	D Galactose	$C_6H_{12}O_6$
42	25.17	1.55	Glucopyronose	$C_6H_{12}O_6$
43	25.94	0.47	Phytal acetate	$C_{22}H_{42}O_2$
44	26.2	0.02	β-D-Galactopyranoside	$C_7H_{14}O_6$
45	26.62	0.37	Acrylic acid	$C_3H_4O_2$
46	26.67	0.17	D-Glucose	$C_6H_{12}O_6$
47	28.55	2.84	2,5 Dihydroxyacetophenone	$(HO)_2C_6H_3C$
48	28.93	1.58	Benzoic acid	$C_7H_6O_2$
49	30.36	0.67	Hexadeconic acid	$C_{16}H_{32}O_2$
50	30.75	0.33	Mannose	$C_6H_{12}O_6$
51	31.48	0.06	Mangifenin	$C_{19}H_{18}O_{11}$
52	32.48	0.39	Glucoside	$C_{16}H_{32}O_6$
53	32.48	0.09	2-Pyrrolidinone	C_4H_7NO
54	33.88	0.57	Octadeconic acid (Stearic acid)	$C_{18}H_{36}O_2$
55	34.66	0.48	Linoleic acid	$C_{21}H_{40}O_2Si$

Table 1 Continue….				
56	34.68	5.13	Linoleic acid	$C_{21}H_{40}O_2Si$
57	34.76	0.42	D-Gluronic acid	$C_{21}H_{40}O_2Si$
58	34.76	0.3	β-D Lyxopyranose	$C_5H_{10}O_5$
59	36.22	0.45	melibiose	$C_{42}H_{70}O_{11}$
60	36.64	3.52	D Glucopyranoside	C7H14O6
61	37.02	0.66	d glucitol (Sorbitol)	$C_6H_{14}O_6$
62	37.23	0.06	undecanoic acid	C11H22O2
63	38.11	0.19	β-D Galactopyranoside	C7H14O6
64	38.12	0.19	Chloropropyl (Styrene)	$C_{11}H_{13}Cl$
65	38.13	1.18	N-Acetyl glucosamine	$C_8H_{15}NO_6$
66	38.57	0.05	Biphenyl	$C_{12}H_{10}$
67	43.33	0.16	Heptadecane	$C_{17}H_{36}$
68	43.45	0.42	Hexanedioic acid (Palmitic acid)	$C_6H_{10}O_4$
69	43.6	0.21	Melibiose,octakis	$C_{36}H_{86}O_{11}Si_8$
70	43.61	0.33	D-Glucopyranose	$C_6H_{12}O_6$
71	44.43	0.44	2H-1 Benzopyran	C_9H_8O
72	44.47	13.05	silane,Catechine	$C_{30}H_{54}O_6Si_5$
73	46.33	1.17	Trimethysilyl	C_3H_9Si
74	46.33	0.51	Methylaniline	C_6H_5NH
75	50.78	2.11	4H-1-Benzopyran-4 one,2-[3,4{(Trimethylsily)oxy}phenyl]-3,5,7-trisoxy	$C_{30}H_{50}O_7Si_5$
76	51.26	0.57	D Turanose	$C_{12}H_{22}O_{11}$
77	52.72	0.35	Androstan	$C_{19}H_{30}O_2$

4. Discussions and Conclusion

The demand in study of plants, which is one of the richest sources of promising versatile chemical compounds, is growing persistently throughout the world during the last few decades. Plant could play a great role in exploring new resources against the threats of new and recent diseases [9]. From this study it can be concluded that the *A.nilotica* may serve as a new potential source of medicines due to the presence of these phytochemicals and bioactive compounds.

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