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GC-MS Analysis of Chromatographic Fractions of *Acacia Nilotica* Leaves

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***Abstract*: *Acacia nilotica* is the member of the family *Mimosaceae*; *Acacia nilotica* is multipurpose nitrogen fixing tree legume. It is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India. The aim of this research work was to carry out for identification of various bioactive compounds from chromatographic fractions of *Acacia nilotica* leaves by Gas chromatography and Mass spectroscopy (GC-MS). A total of 40 compounds were observed that has been shown in the table 3.1. The GC-MS analysis revealed the presence of various compounds like 2,6-Lutidine-3,5-dichloro-4-dodecylthio, 10-Methyldodecan-4-olide, Octanoic acid, Urs-12-ene, Bis-2-ethylhexyl phthalate and Phenol-2,4-bis(1,1-dimethylethyl) in the chromatographic fractions. Further studies are needed to isolate active compounds of the fractions as well as to explicate their exact mechanism of action in various disorders.**

***Keywords*: *Acacia nilotica*, Chromatographic fractions, GC-MS analysis.**

1. Introduction

Plants were the main source of therapeutics for human since ancient times and until the current era. Although, pharmaceuticals (mostly synthetic drugs) are the dominant drugs in modern medicine, but phyto medicinal drugs (mostly plant derivatives) are more popular. The WHO reported that, up to 80% of the world population is depending on drugs derived from plants particularly in the developing countries [1]. However, the approach for drug development from plants is very complicated and expensive, each new drug costs about 100-360 million US dollars and at least 10 years of intensive work in a form of multi-disciplinary and integrated activities including many fields like botany, chemistry, pharmacology, biomedical sciences, biotechnology and even anthropology [2]. If we put in consideration that, there are about 250,000 to 500,000 species of plants on earth, human and animals are consuming not more than 10% of these plant species [3], that means screening for bioactivity of medicinal plants is important and worthwhile in order provide the basic knowledge for discovery of new drugs. Two main constituents are produced from plants; primary compounds such as sugars, proteins and chlorophyll; secondary compounds (phytochemicals) such as flavonoids, terpenoids and phenolic compounds; the bioactive properties of some plants are attributed to the secondary or phytochemical compounds [4], [5].

Acacia nilotica (*Mimosaceae*) is an imperative multipurpose plant [6]. *A. nilotica* occurs naturally and is imperative in traditional rural and agro-pastoral systems [7]. It is a tree 5–20 m high with a dense spheric crown, stems and branches usually dark to black colored, fissured bark, grey pinkish slash, exuding a reddish low quality gum. The tree has thin, straight, light, grey spines in axillary pairs, usually in 3 to 12 pairs, 5 to 7.5 cm (3 in) long in young trees, mature trees commonly without thorns. Flowers in globules heads 1.2–1.5 cm in diameter of a bright golden-yellow color set up either axillary or whorly on peduncles 2–3 cm long located at the end of the branches. Pods are strongly constricted, white-grey, hairy and thick [8]. The species can withstand extremely hot temperature (>50°C) and can also endure floods. The species is sensitive to frost when it is young [9]. Trees can flower and fruit two to three years after germination, but after high rainfall it is more quickly, usually between March and June [10]. The bark has a tinge of orange and/or green (young tree), but older trees have dark, rough bark and tend to lose their thorns [11].

Extraction is the main step for the recovery and isolation of bioactive phytochemicals from plant materials, before component analysis [12]. Hence, for the discovery of lead compounds for use as therapeutic drugs, the active principals in medicinal plants need to be identified [13]. 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 [14,15,16]. Hence, the present study was aimed to carry out the identification of bioactive come pounds from the chromatographic fractions of *Acacia nilotica* leaves by Gas chromatography and Mass spectroscopy (GC-MS).

2. Materials and Methods

1) Sample Collection and Identification

Mature leaves of *Acacia nilotica* were collected from Nasarawa Area Aliero Local Government, Kebbi State,

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Nigeria. The sample was later identified and authenticated by Professor Dhramandrah Singh a plant taxonomist at the Department of Plant Science and Biotechnology Kebbi State University of Science and Technology, Aliero a Sample was deposited in the laboratory and was given (Voucher number of 284).

2) Preparation of the Leaves Methanol Extract

The plant leaves were pulverized to powdered using pestle and mortar. 100g pulverized plant materials were mixed with 1 liter of 95% methanol. The mixture was kept at room temperature for 72 hours and then filtered with a Whatman filter paper No. 1 of pore size 11µm. The filtrates were evaporated at 45ºC using water bath. The extracts were stored at -4ºC till further uses.

3) Column Chromatography

Silica gel was suspended in n-hexane and stirred gently until the formation of slurry. This slurry was poured carefully into a cleaned, uprightly fitted; glass-wool sealed open glass column (2.5cm inner diameter and 75cm length) till it was about three-fourths filled, with tapping the walls with a cushioned rod in order to avoid air-bubbles. n-Hexane was poured continuously into the column and allowed to drain until the silica particles packed. The quantity collected was poured back into the column to ensure proper packing of the column. The extract was slowly added into the packed column.

The methanol fraction (3g) was then loaded into the packed column. The column was eluted with hexane: chloroform (100:0, 90:10, 80:20, 70:30, 50:50, 30:70 and 0:100 %v/v, 100ml each), followed by chloroform: methanol (98:02, 95:05, 90:10, 80:20 and 70:30 % v/v, 100ml each). The eluted fractions were collected into small bottles (20ml each). The fractions obtained were pooled based on their TLC profile.

4) Thin Layer Chromatography (TLC)

Precorted TLC plates were used. One (1) cm from the base of the TLC plate, marked with a pencil and labeled. Capillary tube was used to spot the plates with the column eluents by putting in a lidded tank containing the solvent system, Chloroform: Methanol (9:1). The level of solvent system in the tank was about 1cm beneath the origin. The solvent was allowed to move up the plate by capillary action till it reached the solvent front, marked by a straight line across. The developed plates were allowed to dry and detection of compounds was aided by spraying the plates with 10% H2SO4 [17]. The plates were heated at 100˚C for 5-7minutes for optimal colour development was calculated by making use of the distance moved by the solvent and the distance moved by the component as follows:

$$RF = \frac{Distance\ travelled\ by\ the\ Component}{Distance\ travelled\ by\ the\ Solvent}$$

5) GC-MS

GC-MS was performed using the GC clarus 500 Perkin Elmer equipment compound was separated on Elite 5MS (5%) Diphenyl / 95% Dimethyl polysiloxane, 30X0.25mmX0, 25umdf capillary column. Sample were injected with a split ratio of 10:1 with a flow rate of helium 1m /min (carrier gas). Mass detector used was Turbo mass gold Perkin Elmer. Software used was Turbo mass 5.2 2ul of sample was injected. Other condition monitored was as oven temperature up to 110^0C – 2min hold, up to 200^0c at the rate of 100C/min on hold. Injector temperature was maintained at 250^0C. The constituents were identified after comparison those available in the computer library (NIST version 2005), attached to the GC-MS instrument and documented.

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns, the spectrum of the unknown component was compared with the spectrum of the known component stored in the NIST library. The names, molecular formula, molecular weight, and structures of the component of the tested materials were ascertained.

The chemical compounds in the fraction from the leaves of *A. Nilotica* were identified based on GC retention time on VF-5 capillary column. The chromatogram was interpreted using NIST library consisting two hundred thousand compounds. The name, molecular weight and percentage of unknown compounds were evaluated by software.

3. Results and Discussion

1) Column Chromatography

The pools were collected from the column based on the color separation using some dry clean containers with plastic covers labeled serially as 1,2,3,4,5,6,7,8,9,10….120, the containers were ¾ filled up with the eluate having different colors by the solubility of the solvent in the extract at mobile phase and affinity to stationary phase. The eluate was concentrated by opening the bottle and allowed to dry at room temperature to ¼ of the total volume and were coded respectively then stored in refrigerator pending the needs. The fraction obtained were arrange and coded as A1 contains (1-15 eluate), A2 contains (16-27 eluate), A3 contains (28-41 eluate), A4 contains (42-52 eluate), A5 contains (53-71 eluate), A6 contains (72-91 eluate), A7 contains (92-105 eluate) and A8 contains (106-120 eluate).

2) Thin Layer Chromatography

The fractions obtained from Column were subjected to Thin Layer Chromatography to obtain the pure compounds. The fractions which show a similar RF value were pooled together and labeled as isolates A1, A2, A3……A8. The fractions were used for the determination of bioactive compounds using GC-MS.

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 chromatographic fractions of *A. nilotica* leaves by using Gas chromatographic and Mass Spectroscopy. The active compounds with their peak number, concentration (peak area %), retention time (RT) and molecular weight are presented in table 3.1which shows the presence of 40 bioactive phytochemical Compounds in the chromatographic fractions of

Table 1
Summary of the GC-MS Spectrum of A. nilotica Column Isolate

S. No.	rt (min)	%Peak area	Metabolites	MW
1	7.059	0.10	Octanoic acid, methyl ester	158
2	8.264	0.09	Nonanoic acid	158
3	9.888	0.35	Decanoic acid, methyl ester	186
4	10.592	0.47	Dodecanoic acid	200
5	10.864	0.13	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	212
6	11.347	0.07	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	212
7	11.502	0.05	Tridecanoic acid	214
8	11.645	0.19	1-Dodecanol	186
9	11.963	0.80	Phenol, 2,4-bis(1,1-dimethylethyl)-	206
10	12.117	10.79	Dodecanoic acid, methyl ester	214
11	12.573	8.72	Dodecanoic acid	200
12	12.876	0.55	Eicosanoic acid	312
13	13.877	7.28	Methyl tetradecanoate	242
14	14.212	2.33	Tetradecanoic acid	228
15	14.504	0.71	n-Heptadecanol-1	256
16	14.641	0.32	Methyl 9-methyltetradecanoate	256
17	15.017	0.32	Oxirane, hexadecyl-	268
18	15.196	0.50	7-Hexadecenoic acid, methyl ester, (Z)-	268
19	15.292	0.33	7-Hexadecenal, (Z)-	238
20	15.364	15.07	Hexadecanoic acid, methyl ester	270
21	15.903	0.68	1-Nonadecene	266
22	16.028	0.33	Methyl 9-methyltetradecanoate	256
23	16.351	0.15	10-Methyldodecan-4-olide	212
24	16.452	2.88	9,12-Octadecadienoic acid, methyl ester	294
25	16.484	9.55	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	292
26	16.511	15.38	9-Octadecenoic acid, methyl ester, (E)-	296
27	16.625	0.63	Phytol	296
28	16.667	4.72	Methyl stearate	298
29	16.984	1.03	7,10-Octadecadienoic acid, methyl ester	294
30	17.153	0.75	n-Tetracosanol-1	354
31	17.848	1.65	Eicosanoic acid, methyl ester	326
32	18.804	2.70	Octadecane, 1-chloro-	288
33	18.944	2.62	Bis(2-ethylhexyl) phthalate	390
34	19.339	1.44	i-Propyl 9-tetradecenoate	268
35	19.939	2.03	V Urs-12-ene	410
36	20.017	1.17	Tetracosanoic acid, methyl ester	382
37	20.109	0.40	2,6-Lutidine 3,5-dichloro-4-dodecylthio-	375
38	20.647	1.95	Squalene	410
39	21.286	0.44	Hexacosanoic acid, methyl ester	410
40	22.044	0.33	2,6-Lutidine 3,5-dichloro-4-dodecylthio-	375

RT = Retention time (min), %Peak Area = Percentage peak area, MW = Molecular weight.

A. nilotica. The identified bioactive compounds are Octanoic acid, methyl ester with percentage peak area of 0.10 and molecular weight of 158, Nonanoic acid with percentage peak area of 0.09 and molecular weight of 158, Decanoic acid with percentage peak area of 0.35 and molecular weight of 186, Dodecanoic acid with percentage peak area of 0.47 and molecular weight of 200, 9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione with percentage peak area of 0.13 and molecular weight of 212, 9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione with percentage peak area of 0.07 and molecular weight of 212, Tridecanoic acid with percentage peak area of 0.05 and molecular weight of 214, 1-Dodecanol with percentage peak area of 0.19 and molecular weight of 186, Phenol, 2,4-bis(1,1-dimethylethyl)- with percentage peak area of 0.80 and molecular weight of 206, Dodecanoic acid with percentage peak area of 8.72 and molecular weight of 200, Eicosanoic acid with percentage peak area of 0.55 and molecular weight of 312, Methyl tetradecanoate with percentage peak area of 7.28 and molecular weight of 242, Teteradecanoic acid with percentage peak area of 2.33 and molecular weight of 228, n-Heptadecanol-1 with percentage peak area of 0.71 and molecular weight of 256, Methyl 9-methyltetradecanoate with percentage peak area of 0.32 and molecular weight of 256, Oxirane, hexadecyl- with percentage peak area of 0.32 and molecular weight of 268, 7-Hexadecanoic acid, methyl ester, (Z)- with percentage peak area of 0.50 and molecular weight of 268, 7-Hexadecenal, (Z)- with percentage peak area of 0.33 and molecular weight of 238, Hexadecanoic acid, methyl ester with percentage peak area of 15.07 and molecular weight of 270, 1-Nonadecene with percentage peak area of 0.68 and molecular weight of 266, Methyl 9-methyldidenoate with percentage peak area of 0.33 and molecular weight of 256, 10-Methyltetradecanoate with percentage peak area of 0.15 and molecular weight of 212, 9,12-Octadecadienoic acid, methyl ester with percentage peak area of 2.88 and molecular weight of 294, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- with percentage peak area of 9.55 and molecular weight of 292, 9-Octadecanoic acid, methyl ester, (E)- with percentage peak area of 15.38 and molecular weight of 296, Phytol with percentage peak area of 0.63 and molecular weight of 296, Methyl stearate with percentage peak area of 4.72 and molecular weight of 298, 7,10-Octadecadienoic acid, methyl

ester with percentage peak area of 1.03 and molecular weight of 294, n-Tetracosanol-1 with percentage peak area of 0.75 and molecular weight of 354, Eicosanoic acid, methyl ester with percentage peak area of 1.65 and molecular weight of 326, Octadecane, 1-chloro- with percentage peak area of 2.70 and molecular weight of 288, Bis (2-ethylhexyl) phthalate with percentage peak area of 2.62 and molecular weight of 390, i-Propyl 9-tetradecenoate with percentage peak area of 1.44 and molecular weight of 268, Urs-12-ene with percentage peak area of 2.03 and molecular weight of 410, Tetrecosanoic acid, methyl ester with percentage peak area of 1.17 and molecular weight of 382, 2,6-Lutidine 3,5-dichloro-4-dodecylthio- with percentage peak area of 0.40 and molecular weight of 375, Squalene with percentage peak area of 1.95 and molecular weight of 410, Hexacosanoic acid, methyl ester with percentage peak area of 0.44 and molecular weight of 410, and 2,6-Lutidine 3,5-dichloro-4-dodecylthio- with percentage peak area of 0.33 and molecular weight of 375. Due to the presence of above mentioned compounds in the chromatographic fractions of *A. nilotica* leaves, it can be used in various pharmaceutical and industrial applications.

4. Conclusion

Plant could play a great role in exploring new resources against the threats of new and recent diseases [18]. From this study it can be concluded that the *A. nilotica* may serve as anew potential source of medicine due to the presence of these bioactive compounds. This study also encourages cultivation of the highly valuable plants in large scale to increase the economic status of cultivators in the country. The obtained results may provide a support to use of the plant in traditional medicine.

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