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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *ACACIA ALBIDA* (DEL.) A. CHEV. (MIMOSOIDEAE) FIXED OIL

Prof. Abdel Karim. M.*[1], Hashim H.[2] and Khalid M. S.[3]

[1]Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.
[2]Omdurman Islamic University,Faculty of Pharmacy.
[3]International University of Africa, Faculty of Pharmacy.

***Corresponding Author: Prof. Abdel Karim. M.**
Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.

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ABSTRACT

This study was designed to identify the constituents of *Acacia albida* fixed oil, which find many applications in herbal medicine, and to evaluate its antimicrobial activity. The GC-MS analysis showed the presence of 22 components**.** Major constituents are: Z, Z-9, 12-Octadecadienoic acid methyl ester (38.29%), Z-9-Octadecenoic acid methyl ester(22.31%) , Hexadecanoic acid methyl ester(19.55%) and methyl stearate(5.59%). In the diffusion bioassay, the oil showed excellent activity against the bacterial strain *Bacillus subtilis* in the concentration range: 100-50mg/ml.It also exhibited activity against all test organism at 100mg/ml except for *Aspergillus niger* which gave a partial activity.

KEYWORDS: *Acacia albida* oil, GC-MS analysis, Antimicrobial activity.

INTRODUCTION

Over centuries, medicinal plants, which contain bioactive constituents, have been used by humans in primary health care. Now there is a renewed interest in the constituents of medicinal plants which find diverse application in herbal medicine. The multi-drug resistance which became lately a matter of concern, triggered extensive studies in phytochemistry and pharmacology in an attempt to discover new molecules for drug discovery and drug design. Evidently medicinal plants are the best candidates for such leads.

Acacia albida (also known as *Faidherbia albida*) is a thorny tree with deep penetrating roots and dull grey bark[1] This species which belongs to the Luguminaceae family may reach 20m in height[1] Within the fruits are pod-bearing seeds. Seeds are brown with a tough seed coat often eaten by animals[2-4] Phytochemical screening revealed the presence of many secondary metabolites including among others: flavonoids, alkaloids, tannins and saponins[5-10] *Acacia albida* is rich in saturated and unsaturated fatty acids including stearic, oleic ,linoleic and palmitic acids[11]

The plant is used traditionally against a wide spectrum of diseases including : diarrhea[12,13] ,asthma, leprosy and skin diseases[14] The plant has also anti-inflammatory[5] and antihaemorrhagic[12] properties. Furthemore, the antipyretic[5] antimicrobial[15] antimalarial[16] and antidiabetic properties have also been reported.

Seeds are eaten by humans in time of famine [12,17] and the plant is considered as a nitrogen fixer increasing soil fertility.[18,19]

MATERIALS AND METHODS

Plant material

The seeds of *Acacia albida* were collected from Khartoum state, Sudan. The plant was authenticated by direct comparison with a herbarium sample.

Test organisms

The following standard bacterial pathogens were used to assess the antimicrobial potency of *Acacia albida* oil: *Bacillus subtilis* (Gram +ve), *Staphylococcus aureus* (Gram +ve), *Pseudomonas aeroginosa* (Gram –ve), *Escherichia coli* (Gram –ve) and the fungi *Candida albicans* and *Aspergillus niger.*

Methods

Extraction of fixed oil from *Acacia albida* seeds

Dry-powdered seeds of *Acacia albida* (400g) were exhaustively macerated with n-hexane at room temperature for 48h..The solvent was removed under reduced pressure leaving the oil. For GC-MS analysis, a methanolic solution of sodium hydroxide and a methanolic sulphuric acid were used to esterify the oil .

GC-MS analysis

Acacia albida fixed oil was analyzed by gas chromatography – mass spectrometry. A Shimadzo Ultra instrument was used with RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness). Analytical grade helium (purity; 99.99 %) was a carrier gas. Oven temperature program and other chromatographic conditions are displayed below:

Table 1: Oven temperature program.

Rate	Temperature(C)	Hold time ($mim.^{-1}$)
-	60.0	0.00
10.00	300.0	0.00

Table 2: Chromatographic conditions.

Column oven temperature	60.0 °C
Injection temperature	280.0 °C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	93.1KPa
Total flow	50.0ml/ min
Column flow	1.50ml/sec
Linear velocity.	44.7cm/sec
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial assay

Diffusion method was used for screening the oil. Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungi respectively. The media were prepared according to the manufacturer's instructions

One ml aliquots of 24 hours broth culture of the test microorganisms were aseptically distributed onto nutrient agar slopes and then incubated at 37°C for 24 hours. The harvested bacterial growth was washed off using sterile normal saline , then it was suspended in (100 ml) of normal saline to give about 108-109 colony forming units per ml. Using the surface viable counting technique , the average number of viable organism per ml of the stock suspension was determined . Serial dilutions of the stock suspension were made in sterile normal saline. (0.02 ml) of the appropriate dilutions were transferred onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature and then incubated at 37°C for 24 hours. Fungal cultures were accomplished on Sabouraud dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed off with sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing for antibacterial activity

(2ml) of the standardized bacterial stock suspension were mixed with (200 ml) of sterile molten nutrient agar which was maintained at 45°C. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle. Each plate was divided into two halves. In each half two cups (10mm in diameter) were cut using sterile cork borer (No 4). Each half was designed for a test solution.

Agar discs were removed, alternate cups were filled with(0.1 ml) samples of each test solution and allowed to diffuse at room temperature for two hours. The plates were then incubated at 37°C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured.

RESULTS AND DISCUSSION

GC-MS analysis

GC-MS analysis of *Acacia albida* oil was conducted and the identification of the constituents was accomplished by comparison with the MS library (NIST). The observed fragmentation pattern was also interpreted. The analysis revealed the presence of 22 components (Table 3). The typical total ion chromatogram (TIC) of hexane extract is shown in Fig.1.

Table 3: Constituents of *Acacia albida* oil.

eak#	R.Time	Area	Area%	Name
1	4.746	246320	0.29	Butylated Hydroxytoluene
2	7.928	71604	0.09	Methyl tetradecanoate
3	9.357	47106	0.06	6-Octadecenoic acid, methyl ester
4	9.878	50496	0.06	Tridecanoic acid, methyl ester
5	11.526	851549	1.02	9-Hexadecenoic acid, methyl ester, (Z)-
6	11.958	16339955	19.55	Hexadecanoic acid, methyl ester
7	13.578	73755	0.09	7-Hexadecenoic acid, methyl ester, (Z)-
8	14.071	73538	0.09	Hexadecanoic acid, 15-methyl-, methyl est
9	15.566	32010548	38.29	9,12-Octadecadienoic acid (Z,Z)-, methyl e
10	15.678	18649465	22.31	9-Octadecenoic acid (Z)-, methyl ester
11	15.782	3125696	3.74	11-Octadecenoic acid, methyl ester
12	16.198	4673018	5.59	Methyl stearate
13	19.356	1576868	1.89	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethyl
14	19.677	479614	0.57	Oxiraneoctanoic acid, 3-octyl-, methyl este
15	19.815	212262	0.25	11-Eicosenoic acid, methyl ester
16	20.341	1447742	1.73	Methyl 18-methylnonadecanoate
17	20.647	134714	0.16	9,12,15-Octadecatrienoic acid, methyl este
18	22.238	84701	0.10	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
19	22.328	168977	0.20	Heneicosanoic acid, methyl ester
20	24.257	2186882	2.62	Methyl 20-methyl-heneicosanoate
21	26.128	205630	0.25	Tricosanoic acid, methyl ester
22	27.930	890606	1.07	Tetracosanoic acid, methyl ester
		83601046	100.00	

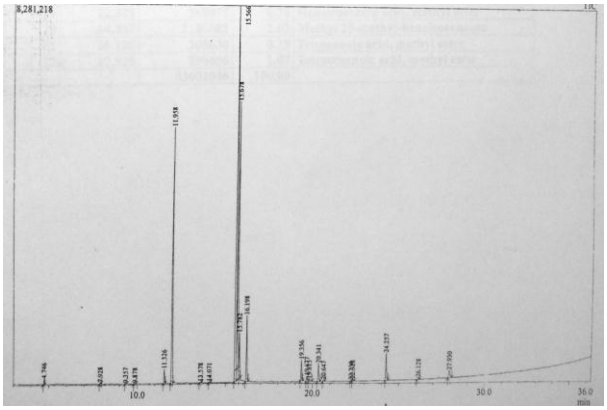

Fig. 1: Cromatograms of *Acacia albida* seed oil.

The following were detected in the chromatograms as major components:

Z, Z-9, 12-Octadecadienoic acid methyl ester (38.29%)

Fig. 2 shows the EI mass spectrum of 9, 12-octadecadienoic acid methyl ester.The peak at m/z294 (R.T. 15. 566), corresponds $M^{+}[C_{19}H_{34}O_2]^{+}$.The signal at m/z263 corresponds to loss of a methoxyl function.

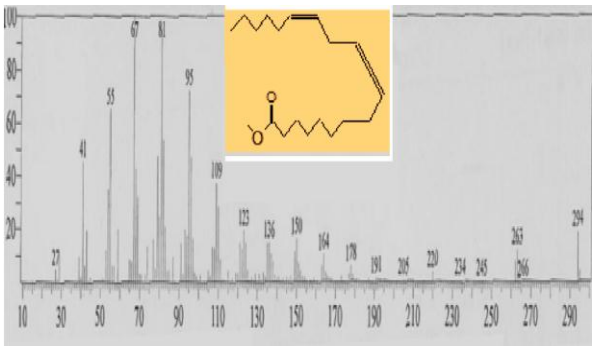

Fig. 2: Mass spectrum of 9, 12-octadecadienoic acid methyl ester.

Z-9-Octadecenoic acid methyl ester (22.31%)

Fig.3 displays the mass spectrum of 9-octadecenoic acid methyl ester.The peak at m/z 296, which appeared at R.T. 15.678, corresponds $M^{+}[C_{19}H_{36}O_2]^{+}$, while the peak at m/z266 accounts for loss of a methoxyl.

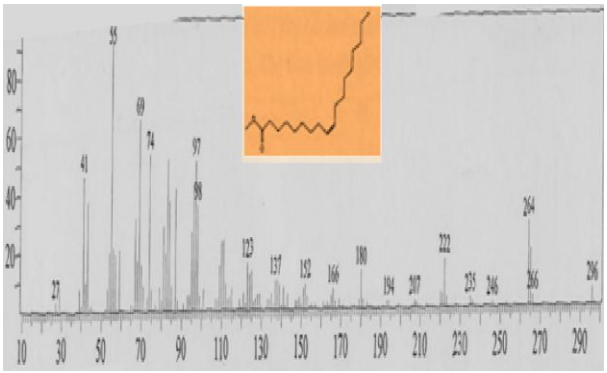

Fig. 3: Mass spectrum of 9-octadecenoic acid methyl ester.

Hexadecanoic acid methyl ester (19.55%)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig. 4.The peak at m/z 270(R.T. 11.958) corresponds $M^{+}[C_{17}H_{34}O_2]^{+}$, while the signal at m/z239 is attributed to loss of a methoxyl group.

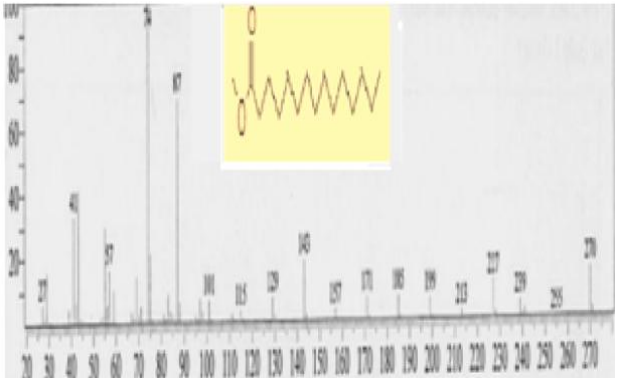

Fig. 4: Mass spectrum of hexadecanoic acid methyl ester.

Methyl stearate (5.59%)

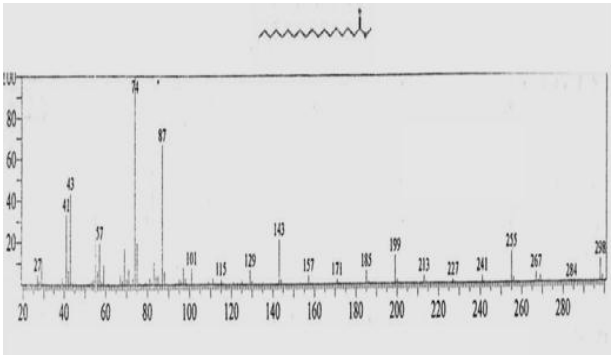

Fig. 5: Mass spectrum of methyl stearate.

The EI mass spectrum of methyl stearate is shown in Fig. 5.The signal which appeared at m/z 298 (R.T. 16.198) corresponds $M^{+}[C_{19}H_{38}O_2]^{+}$, while the peak at m/z267 corresponds to loss of a methoxyl.

Antimicrobial activity

Acacia albida fixed oil was screened for antimicrobial activity against six standard human pathogenic bacteria. The results are depicted in Table (4) .The results were interpreted in the following conventional terms : (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .Tables (5) and (6) represent the antimicrobial activity of standard antibacterial and anifungal chemotherapeutic agents against standard bacteria and fungi respectivel.

Table 4: Antimicrobial activity of *Acacia albida* oil.

Type		Sa	Bs	Ec	Ps	Ca	An
Oil	100	13	20	15	15	13	9
	50	10	17	12	12	--	--
	25	--	14	13	12	--	--
	12.5	--	12	10	10	--	--
	6.25	--	10	7	--	-	--

Table 5: Antibacterial ctivity of standard drugs.

Drug	Conc.(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 6: Antifungal activity of standard drug.

Drug	Conc.(mg/ml)	An	Ca
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

The oil showed excellent activity against the bacterial strain *Bacillus subtilis* in the concentration range: 100-50mg/ml. It also exhibited activity against all test organism at 100mg/ml except for *Aspergillus niger* which gave a partial activity.

REFERENCES

1. Keay RJ, Onochie CA, Stanfield DP. Nigerian Trees, Nigerian National Press Ltd., Apapa, Lagos, 1999; 495.
2. Wood PJ. The Botany and Distribution of Faidherbia albida. In: Vandenbeldt, R. J.(ed.), Faidherbia albida in the West African Semi-arid Tropics, Proceedings of a Workshop, 22-26 April, 1991, Niamey, Niger; ICRISAT and ICRAF, Patancheru, A. P. 502324, India, 1992; 206.
3. Coates PM. Trees of Southern Africa, 3rd ed., Stuix, Cape Town, South Africa, 2002; 23-24.
4. Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. Agroforestry Database: a Tree Reference and Selection Guide, Version 4. 0, World Agro Forestry Centre, Kenya, 2009.
5. Tijani AY, Uguru MO and Salawu OA. Anti-pyretic, Anti-inflammatory and Anti-diarrhoeal Properties of Faidherbia albida in Rats. *Afr. J.Biotech*, 2008; 7(6): 696 –700.
6. Tijani AY, Uguru MO, Salawu OA, Abubakar A, Onyekwelu NO and Akingbasote JA. Effect of Faidherbia albida on Biochemical Parameters of Rats Infected with Trypanosoma brucei, *Afr. J. Pharm Pharmacol*, 2009; 3(1): 26 –30.
7. Salisu Y, Agunu A, Abubakar MS and Ibrahim G. Hypoglycemic effects of Acacia albida del. (Mimosaceae) Methanolic Root Bark Extract. *Nig. J. Pharm. Sci*, 2009; 8(1): 66–67.
8. Calvalho LH, Brandao MGL, Santos-Filho D, Lopes JLC and Krettli AU. Antimalarial Activity of Crude Extracts from Brazilian Plants Studied in-vivo in Plasmodium berghei- infected Mice and in-vitro Against Plasmodium falciparum in Culture. *Braz. J. Med. and Biol. Res*, 1991; 24(11): 1113 –1123.
9. Almahy HA, Nasir OD. Phytochemical and Mineral Content of the Leaves of Four Sudanese Acacia species. *J. Stored Products and Post- harvest Res*, 2011; 2(11): 221 –226.
10. Osuntokun OT, Olajubu FA. Comparative Study of Phytochemical and Proximate Analysis of Seven Nigerian Medicinal Plants. *Appl. Sci. Res. J*, 2014; 2(1): 10 –26.
11. Gunstone FD, Taylor GM, Cornelius JA and Hammonds TW. New Tropical Seed Oils II. Component Acids of Leguminous and Other Seed Oils, *J Sci. Food and Agric*, 1968; 19(12): 706 –709.
12. Wickens GE, Seif El Din AG, Guinko S, Ibrahim N. Role of Acacia Species in the Rural Economy of Dry Africa and the Near East, FAO-Conservation Guide, 1995; 27.
13. Muhammad S, Mushtaq A and Ashfaq A. Chemistry of the Medicinal Plants of the Genus Acacia. *Hamdard-medicus*, 1998; 4(1): 63 – 67.
14. Gill LS. Medicinal Uses of Trees and Plants in Africa, University of Benin Press, Benin, Nigeria, 1999; 27.
15. Kubmarawa D, Ajoku GA, Enwerem NM and Okorie DA. Preliminary Phytochemical and Antimicrobial Screening of 50 Medicinal Plants from Nigeria, *Afr. J. Biotech*, 2007; 6(14): 1690 –1696.
16. Salawu O, Tijani, AB, Nwaeze AA and Agbakwuru A. Antimalarial Activity of Ethanolic Stem Bark of Faidherbia albida (Del.) a. Chev (Mimosidae) in Mice. *Scholars Research Library Archives of Applied Sci. Res*, 2010; 2(5): 261-268.
17. Murunda CT. Use of Seed of Faidherbia albida (syn. Acacia albida) for Human Consumption During Famine Periods in the Gokwe Communal Lands of Zimbabwe, Australian Dry Zone Acacias for Human Food, CSIRO, Canberra, Australia, 1992; 93-98.
18. Payne WA, Williams JH, MaiMoussa KA and Stern RD. Crop Diversification in the Sahel Through Use of Environmental Changes Near Faidherbia albida (Del) A. chev., *Crop Sci*, 1998; 38(6): 1585 –1591.
19. Vandenbeldt RJ. Faidherbia albida in the West African Semi-arid Tropics (Vandenbeldt, R. J. Ed.). Proceedings of a Workshop, 22-26 April, 1991, Niamey, Niger, ICRISAT and ICRAF, Patancheru, A. P. 502324, India, 1992; 9 –17.