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Research Article

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GC-MS Analysis and Antimicrobial Activity of Sudanese *Acacia nubica* Benth. (Fabaceae) Seed Oil

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Abstract The present study was carried out to characterize the constituents of *Acacia nubica* seed oil and to assess the antimicrobial activity.

Twenty five components were detected by GC-MS analysis being dominated by: 9,12-octadecanoic acid methyl ester (31.54%); 9-octadecanoic acid methyl ester(15.86%); hexadecanoic acid methyl ester (15.50%); methyl stearate (13.92%) and 9-octadecanoic acid methyl ester (12.11%).

The antimicrobial activity of the oil was evaluated using the diffusion assay against: Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseudomonasa aeruginos*a and the yeast *Candida albicans*. The oil showed significant activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and moderate inhibitory effect against *Pseudomonas aeruginosa*. However, it failed to exhibit anticandidal properties.

Keywords *Acacia nubica*, Oil, GC-MS, Antimicrobial activity

Introduction

Acacia genus is a large genus- about 1350 species- in the family Fabaceae [1]. These species are considered as substantial source of gallic and ellagic acids [2]. Most of these species contain flavonoids beside other phenolics [3]. In ethnomedicine some Acacia species are used as antidiabetic, hypotensive, antiamoebic, antidiarrhoeic, anti-inflammatory [4].

The antimicrobial potential of many *Acacia* species has been documented [5]. In Sudanese system of medicine *Acacia nilotica* is used against cough, sore throat, malaria, intestinal worms and wounds [6-9]. Pods of *Acacia nilotica* are used commercially in Sudan for leather tanning [10]. The effect of the aqueous extract of *Acacia nilotica* pods on diabetic models has been studied. Diabetic rats exhibited hypoglycemia,significant increase in lipid peroxidation and elevated serum urea and creatinine [11]. The gum from *Acacia seyal*- Gum Arabic- finds many traditional uses including kidney disorders. Though the pharmhacological effects of Gum Arabic were extensively investigated in animal models, there is paucity of data regarding quantified use in humans [12]. Acacia gum has been used as demulcent in pharmaceutical preparations. The gum has been used traditionally for healing wounds and has been shown to inhibit early deposition of plaque [13]. The antioxidant capacity of the medicinally important species *Acacia auricoliformis* has been evaluated [14]. The ethanol extract of *Acacia aroma* showed significant activity against Gram positive bacteria [15].

Acacia nubica **Benth**. is a herb reaching a height of 1-5m. It is distributed in Egypt, Sudan, Saudi Arabia and Iran. However , Little information is available about *Acacia nubica*. The present study deals with the characterization of seed oil constituents and the antimicrobial potency of *Acacia nubica*.

Materials and Methods

Materials

Plant material

Seeds of *Acacia nubica* were collected from a forest reserve around Damazin (Sudan) and authenticated by direct comparison with a herbarium sample.

Instruments

GC-MS analysis was conducted on a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness)**.**

Test organisms

Acacia nubica oil was screened for antibacterial and antifungal activities using the standard microorganisms: *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Escherichia coli* and the fungal species *Candida albicans*.

Methods

Extraction of oil

Powdered shade-dried seeds of *Acacia nubica* (500 g) were exhaustively extracted with n-hexane at room temperature. The solvent was removed *in vauo* to give the oil.

GC-MS analysis

The constituents of *Acacia nubica* seed oil were investigated by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness). Chromatographic conditions are as follows: column oven temperature: 150.0 ^{o}C; injection temperature: 300.0 ^{o}C; injection mode: split; flow mode: linear velocity; pressure: 139KPa; total flow: 50.0 ml/min; column flow: 1.54 ml/sec.; linear velocity: 47.2cm/sec.; purge flow: 3.0 ml/min.; split ratio:-1.0. Oven temperature program is presented below:

Rate	Temperature (^{o}C)	Hold Time (min.$^{-1}$)
-	150.0	1.00
4.00	300.0	0.00

In vitro antimicrobial assay

Muller Hinton agar and Sabouraud dextrose agars were used for bacterial and fungal cultures respectively. The disc diffusion method was used to determine the antimicrobial activity of the oil. Fresh cultures of microorganisms grown for 24 h were used and diluted to 10^{-1} with sterile physiological saline solution (0.85% NaCl). 100 µl of test microorganisms containing 2.0×10^{6} colony forming units (CFU/ml) for bacteria were inoculated on the surface of agar plates. Sterile discs with a diameter of 6 mm were placed onto each agar plate containing microorganisms. Then the test solution was dropped onto discs under sterile conditions and incubated at 37 °C for 24 h. (for bacteria), for fungi the incubation continued for 3 days at 25^{o}C. After incubation, the diameters of inhibition zones were measured in millimetres. All experiments were repeated two times. Ampicillin, gentamycin and clotrimazole were used as positive controls, while DMSO was used as negative control. Control discs were tested on the same microorganisms under the same conditions.

Results and Discussion

The GC-MS analysis

GC-MS analysis of *Acacia nubica* fixed oil was conducted and the identification of the constituents was accomplished by comparison of retention times and through the MS library (NIST). The GC-MS analysis of the studied oil revealed the presence of 25 constituents (Table 1).

The following constituents were detected in the chromatogram as major constituents:

9,12-Octadecanoic acid methyl ester (31.54%)

The EI mass spectrum of 9,12-octadecanoic acid methyl ester is shown in Fig. 1. The peak at m/z 294, which appeared at R.T. 17.528 in total ion chromatogram, corresponds $M^+[C_{19}H_{34}O_2]^+$. The signal at m/z 263 corresponds to loss of a methoxyl function.

9-Octadecanoic acid methyl ester (15.86%)

The mass spectrum of 9-octadecanoic acid methyl ester is displayed in Fig. 2.The peak at m/z 296, which appeared at R.T. 17.587 corresponds $M^+[C_{19}H_{36}O_2]^+$ .The signal at m/z 265 accounts for loss of a methoxyl function.

Hexadecanoic acid methyl ester (15.50%)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig. 3. The signal at m/z 270 (R.T. 15.865) corresponds $M^+[C_{17}H_{34}O_2]^+$. The signal at m/z 239 is due to loss of a methoxyl.

Methyl stearate (12.11%)

Fig. 4 shows the mass spectrum of methyl stearate. The signal at m/z 298 (R.T. 17.784) corresponds $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z 267 corresponds to loss of a methoxyl .

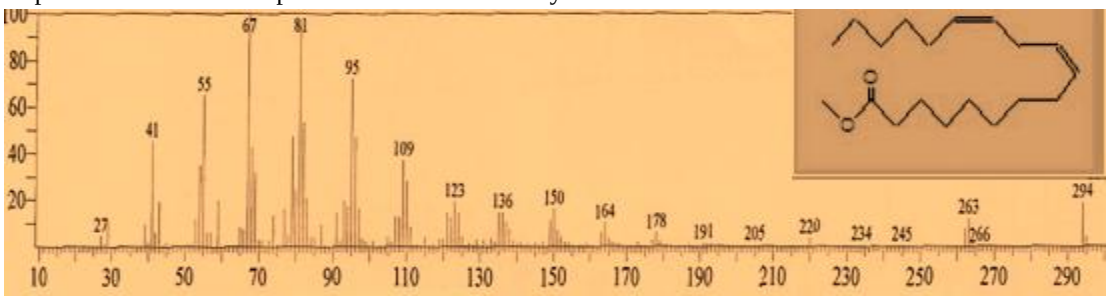

Figure 1: Mass spectrum of 9,12-octadecanoic acid methyl ester

Table 1: Constituents of *Acacia nubica* oil

No.	R.T.	Area %	Name
1	11.160	0.10	Beta-curcumene
2	11.261	0.04	Alpha-Farnesene
3	11.326	0.03	Beta-Bisabolene
4	11.419	0.02	Dodecanoic acid, methyl ester
5	11.532	0.06	Cyclhexene,3-(1,5-dimethyl-4-hexenyl)-6-
6	13.742	0.19	Methyl tetradecanoate
7	14.819	0.06	Pentadecanoic acid, methyl ester
8	15.656	0.55	9-Hexadecenoic acid, methyl ester
9	15.865	15.50	Hexadecanoic acid, methyl ester
10	16.242	2.01	Pentadecanoic acid
11	16.620	0.23	Cis-10--Heptadecenoic acid, methyl ester
12	16.829	0.32	Heptadecanoic acid, methyl ester
13	17.528	31.54	9,12-Octadecadienoic acid(Z,Z)- , methyl ester
14	17.587	15.86	9-Octadecenoic acid(Z)-, methyl ester
15	17.784	12.11	Methyl stearate
16	19.171	3.63	Tridecanedial
17	19.295	1.54	Oxiraneoctanoic acid ,3-octyl-, methyl ester
18	19.329	0.71	11-Eicosenoic acid, methyl ester
19	19.531	5.12	Eicosenoic acid, methyl ester
20	19.5889	1.18	BGHI , methyl ester
21	19.698	1.33	9,12,15-Octadecatrienoic acid ,-2,3-dihydro-, methyl ester
22	20.975	0.35	13-Docosenoic acid ,methyl ester
23	21.154	4.59	Docosenoic acid ,methyl ester
24	21.916	0.43	Tricosanoic acid , methyl ester
25	22.656	2.5	Tetracosanoic acid , methyl ester

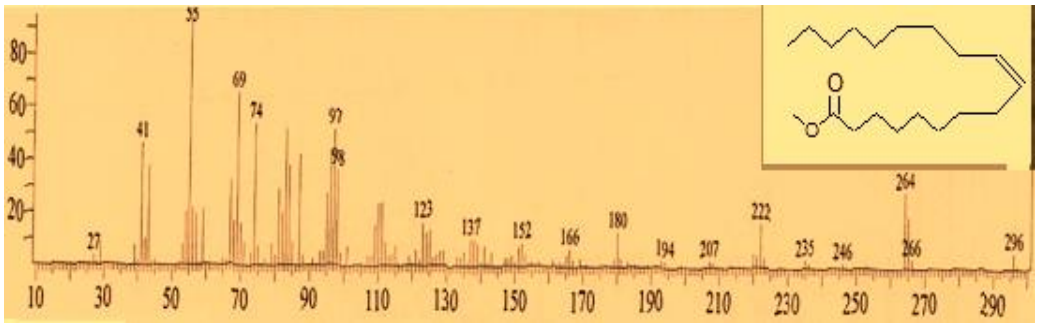

Figure 2: Mass spectrum of 9-octadecanoic acid methyl ester

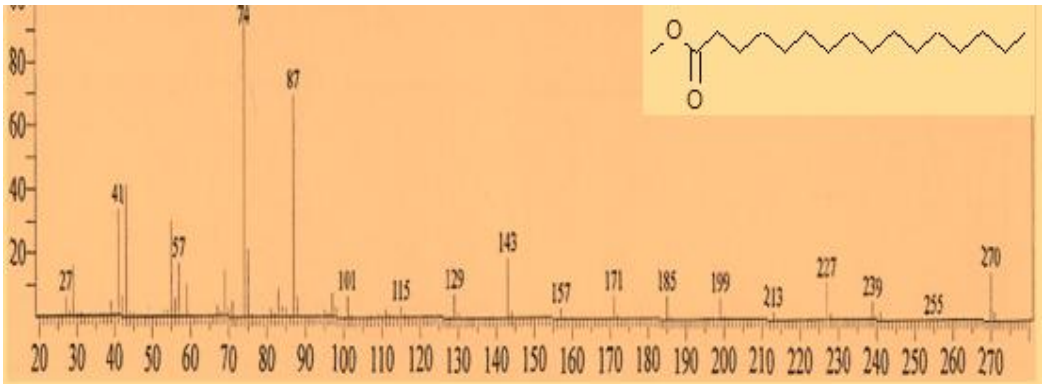

Figure 3: Mass spectrum of hexadecanoic methyl ester

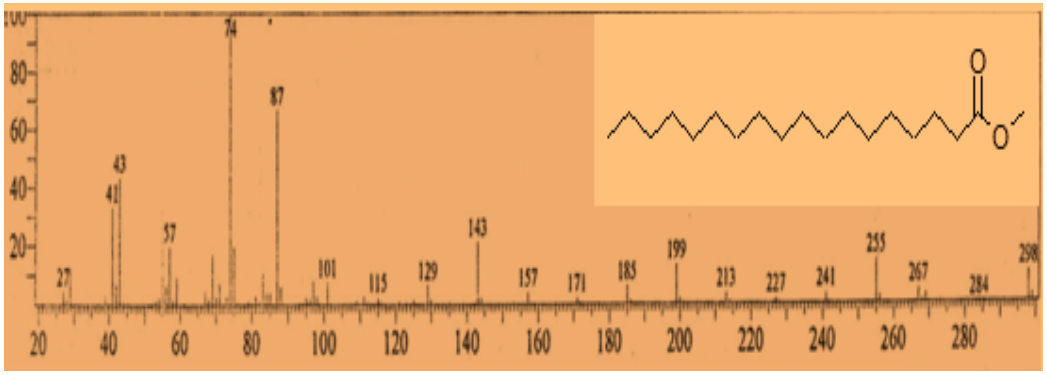

Figure 4: Mass spectrum of methyl stearate

Antimicrobial activity

The oil was screened for antimicrobial activity against five standard microorganisms. The average of the diameters of the growth inhibition zones are shown in Table (2). The results were interpreted in terms of the commonly used terms (<9mm: inative; 9-12mm: partially active; 13-18mm: active; >18mm: very active) .

Table 2: Antimicrobial activity of *Acacia nubica* oil

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	20	22	17	14	--
Ampicilin	40	30	15	--	--	--
Gentacycin	40	19	25	22	21	--
Clotrimazole	30	--	--	--	--	38

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

The oil showed significant activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and moderate inhibitory effect against *Pseudomonas aeruginosa*. However, it failed to exhibit anticandidal properties.

References

[1]. Seigler, D. S., Phytochemistry of Acacia-sensu lato, Biochemical Systematic and Ecology, 2003, 31: 845-873.
[2]. Sultana N, Akhter M and Khatoon Z, Nat Prod Res., 2010, 24(5), 407-415.
[3]. Gaara A. H., Nassar M. I., Younis, M., Elmegeed, G. A., Mabry, T. J., Pare, P. W. Biologically active polyphenolic compounds from Acacia ehrenbbergiana. Rev. Latinoamer. Quim., 2008, 36: 52-59.
[4]. Boulos, L. "Medicinal Plants of North Africa", 1983,. Algonac, Michigan. 115-117.
[5]. Almagboul A. Z., Bashir A. K., Saleh A. K., Farouk A., Khalid S. A. Antimicrobial Activity of Sudanese Plants used in Folkoloric medicine. Screening for antibacterial Activity. Fitoterapia, 59: 57 (1988).
[6]. Shetty, K. A. B., Indian Farming, 1977, 26(11), 82.
[7]. Joshi, P., Ethnomedicine of tribal Rajasthan - An over view; In: Pushpangadan et al. (Eds.), Gilmpses of Indian Ethnopharmacology, TBGRI, Thiruvunanthapuram, India, 1994; 147-162.
[8]. Jain, A., Katewa, S. S., Galav, P. K. and Sharma, P. Indian J Ethnopharmacol., 2005,102(2), 143-157.
[9]. Kubmarawa, D., Ajoku, G. A., Enwerem, N. M. and Okorie, D. A. Afr J Biotechnol., 2007, 6(14), 1690-1696.
[10]. Sahni, M. Important Trees of the Northern Sudan. Khartoum University Press, 1968, 40-63.
[11]. Enayat, A., Somia, A., Abdel Razig, H., Walid, M. Sayed, A., Phytomedicine, 2012, 19(12), 1059-1067.
[12]. Rasha, B., Tarig, H., Khalifa, E., Rehab, M., Florian, L. and Amal, M., Nutr. J., 2012, 11, 111.
[13]. WWW.drugs.com/npp/Acacia-gum.
[14]. Duduku, K., Rosalam, S., Rajesh, N., Food and Bioproducts Processing, 2011, 89(3), 217.
[15]. Meldini, M., Sosa, S., Montoro, P., Giangaspero, A., Balick, M.J., Pizza, C., Della, R., J. Ethnopharmacology, 2009, 122(3), 430-433.