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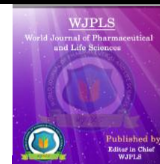

GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *ARACHIS HYPOGOEA* L. (FABACEAE) OIL

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ABSTRACT

Arachis hypogoea **L**. (peanut or groundnut)is an important crop in the pea family(Fabaceae). Peanut originated in South America and dissemination to Africa, Europe Asia occurred in the sixteenth century. The plant is widely grown in tropics and subtropics mainly for its edible oil. Peanut oil is used in cosmetics and as vehicle in many pharmaceutical preparations**.** Peanut oil was studied by GC-MS. The oil was also evaluated for antimicrobial activity. The GC-MS analysis showed 25 components. Major constituents are: 9-octadecenoic acid methyl ester(33.44%),9,12-octadecadienoic acid methyl ester(24.29%), hexadecanoic acid methyl ester(13.33%) and methyl stearate(6.28%). The antimicrobial assay showed significant activity against *Pseudomonas aeruginosa*, moderate activity against *Escherichia coli* beside moderate anticandidal effect.

KEYWORDS: *Arachis hypogoea*, Oil, GC-MS analysis, Antimicrobial Activity.

INTRODUCTION

Arachis hypogoea **L**. (peanut or groundnut)is an important crop in the pea family(Fabaceae). Peanut originated in South America and dissemination to Africa, Europe Asia occurred in the sixteenth century. The plant is widely grown in tropics and subtropics mainly for its edible oil. Peanut oil is used in cosmetics and as vehicle in many pharmaceutical preparations.[1]

Peanut is a vital source of protein and vegetative oil. Though being deficient in essential amino acids, the plant is rich in acidic amino acids.[2-7] Peanut contains sterols, poly- and mono-unsaturated fats beside polyphenols and dietary fiber.[8] It also contains magnesium, potassium, phosphorus and sulfur, vitamins, minerals and bioactive flavonoids.[9-16] The free radical scavenging capacity and protein glycation inhibitory effect of peanut falvonoids have been reported.[5,15] *In vivo* studies demonstrated that the aqueous extract of peanut skin significantly reduced epididymal fat. The extract reduced liver triglycerides and cholesterol level.[17] Also the polyphenol fraction of the kernel exhibited hypocholesterolemic properties.[18]

It has been reported that peanut peptides showed antioxidant activity in the DPPH assay.[5] The *in vivo* inhibitory effect of peanut stilbenoids on intracellular generation of reactive oxygen species has been documented. Some of these stilbenoids exhibited moderate cytotoxity against against HL-60 cells.[14,19]

Peanut proanthocyanidines reduced dermatological conditions such as melanogenesis and inflammation.[2,16] The *in vitro* cysticidal activity of peanut extract has been demonstrated.[14] The hypotensive properties of some peanut bioactive peptides has been documented.[20]

Peanut butter contains a phytochemical called resveratrol which is associated with increased antiviral immunity.[21] The anticancer activity of resveratrol has been reported in experimental models and in humans.[22] A Constituent of peanut shell-5,7-dihydroxychromane – exerted inhibitory effect on some pathogenic fungi.[23] Due to improper storage or severe draught during pod formation, peanut may become contaminated with *Aspergillus flavus* to produce carcinogenic aflatoxins.[24]

Materials

Plant material

Peanut was collected from Gezira, central Sudan. The plant was identified and authenticated by direct comparison with herbarium sample. The plant material was shade - dried at room temperature and powdered.

Materials for biological screening

Bacterial strains

Bacillus subtilis : NCTC 8236 (Gram + ve).
Staphylococcus aureus : ATCC 25923(Gram +ve).
Escherichia coli : ATCC 25922(Gram -ve)
Pseudomonas aeruginosa : ATCC 27853 (Gram -ve)

- Fungal strain
Candida albicans : ATCC7596.

- Positive controls
i. Ampicilin: antibacterial standard for G+ve bacteria.
ii. Gentamycin : antibacterial standard for G-ve bacteria.
iii. Clotrimazole: antifungal standard.

Methods
GC-MS analysis
Peanut seed oil was extracted by maceration. Volatiles of the oil were determined via Gillent Technologies 7890A GC-MS instrument. A Blank analysis was performed. Following esterification, the test sample was directly injected into the GC vial. The chromatographic conditions are; injection source: GC auto sampler and thermal separation probe (TSP); injection volume: 1 µL (test sample); injection mode: splitless and split ratio 1:5; oven temperature: initial 35 °C, increased to 180 °C (6 °C/min), held 5 min, increased to 230 °C (1°C/min) and held 20 min. Other settings; column: non-polar capillary DB-1 of 100% dimethyl-polysiloxane (30 m x 0.53 mm id, film thickness 0.25 µm); carrier gas: helium (1 ml/min); ionization energy: 70 eV; front inlet pressure: 6.78 psi, oven equilibrium time: 3 min; maximum oven, post- run, front inlet, MS source and MS quad temperature: 350^0, 290^0, 250^0, 230^0 and 150^0. Characterization of oil constituents was accomplished via the National Institute of Standards and Technology (NIST) Library- Chem-Station software.

Antimicrobial assay
One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette 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. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

The paper disc diffusion method was used to screen the antibacterial activity. The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines.[25] Agar plates were inoculated with a standardized inoculum of the test microorganism. Filter paper discs (6 mm in diameter), containing the test compound at 100mg/ml were placed on the agar surface. For bacteria, the inoculated plates were incubated at 37 °C for 24 h. Incubation continued for four days at 25^0 for fungi. Experiments were performed in duplicates and the diameters (mm) of the inhibition zones were measured and averaged.

RESULTS AND DISCSSION
The constituents of *Arachis hypogoea* oil were characterized by GC-MS. Twenty five components have been detected(Table 1). The ions chromatograms is displayed in Fig. 1.

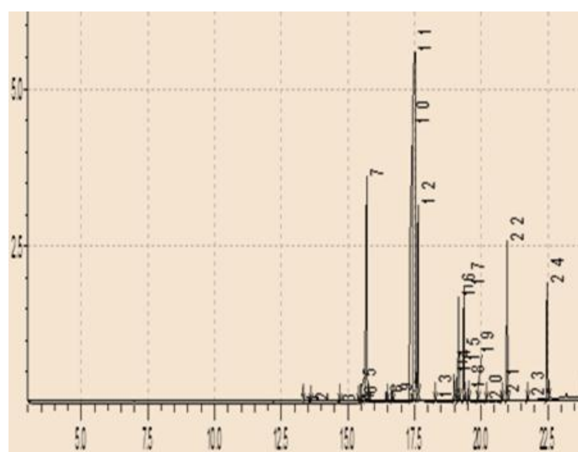

Fig. 1: Total ions chromatograms.

Main constituents of the oil are shown below:
9-Octadecenoic acid methyl ester(33.44%)
The EI mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig. 2. The molecular ion $M^+[C_{19}H_{36}O_2]^+$ appeared at m/z 296 (R.T.17.517).

9,12-Octadecadienoic acid methyl ester(24.29%)
Fig. 3 presents the mass spectrum of 9,12-octadecadienoic acid methyl. The signal at m/z 294, which appeared at R.T. 17.425, corresponds M^+ $[C_{19}H_{46}O_2]^+$. The peaks at m/z 263 is due to loss of a methoxyl.

Hexadecanoic acid methyl ester(13.33%)
The mass spectrum of hexadecanoic acid methyl ester is displayed in Fig. 4. The peak at m/z 270 (R.T. 15.714) is attributed to the molecular ion : $M^+[C_{17}H_{34}O_2]^+$, while the peak at m/z239 accounts for loss of a methoxyl function.

Methyl stearate(6.28%)
Fig. 5 shows the EI mass spectrum of methyl stearate. The molecular ion $M^+[C_{19}H_{38}O_2]^+$ appeared at m/z 298

(R.T. 17.631). The signal at m/z267 is due to loss of a methoxyl.

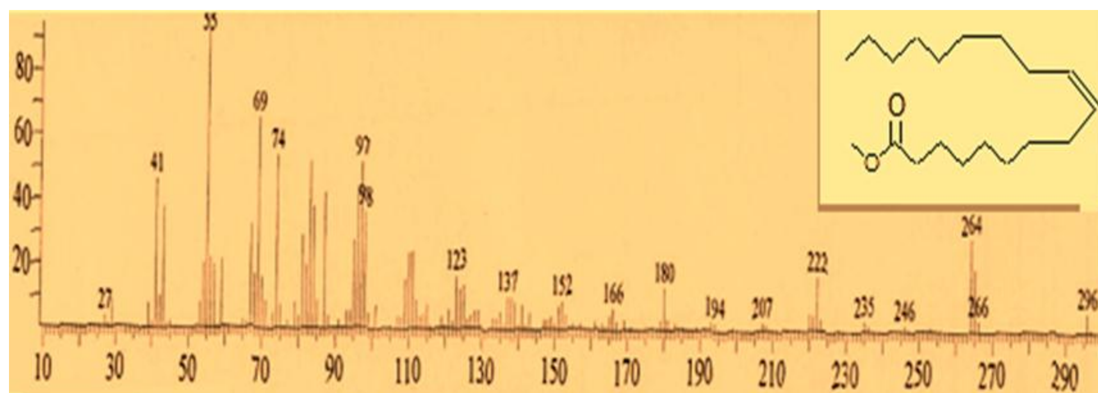

Fig. 2: Mass spectrum of 9-octadecenoic acid methyl ester.

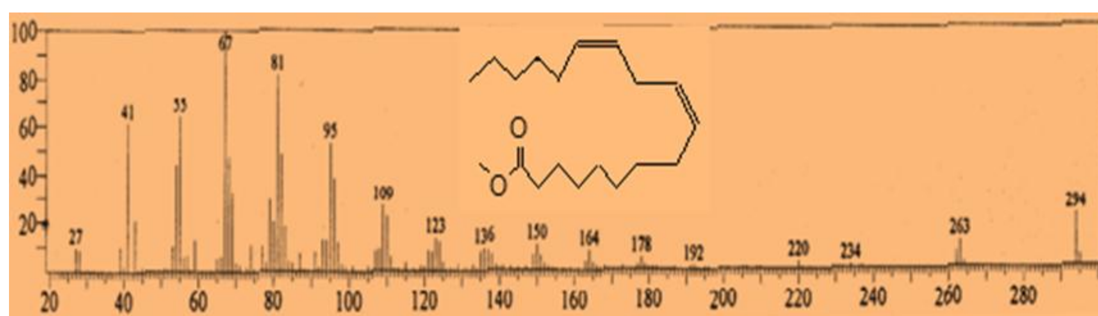

Fig. 3: Mass spectrum of 9,12-octadecadienoic acid methyl ester.

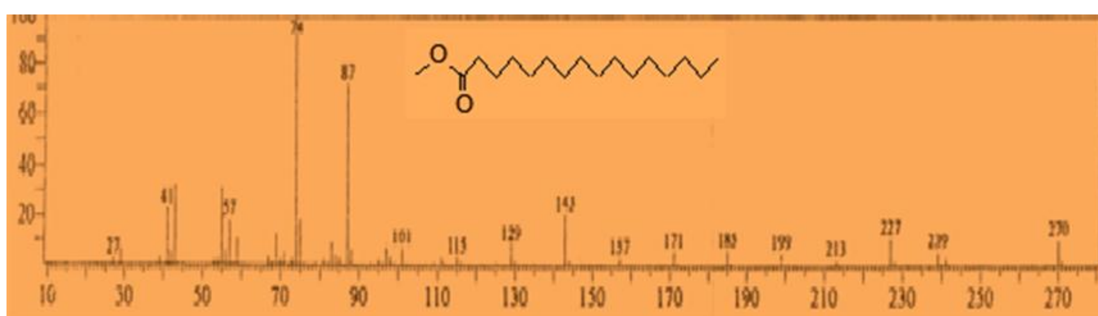

Fig. 4: Mass spectrum of hexadecanoic methyl ester.

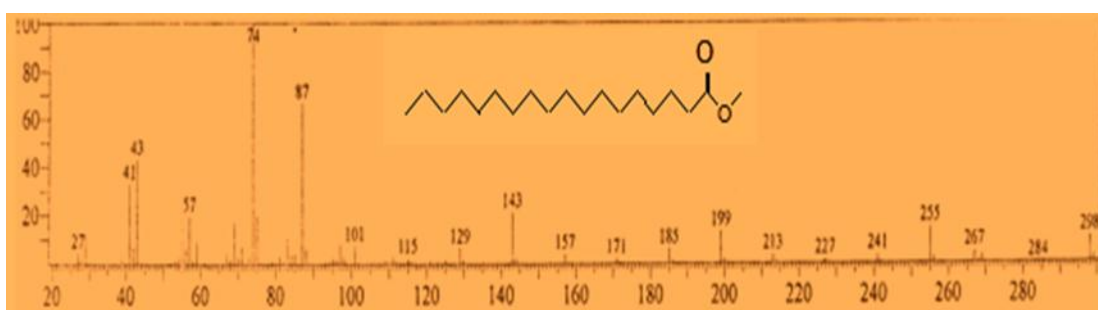

Fig. 5: Mass spectrum of methyl stearate.

Table 1: Constituents of *Arachis hypogoea* oil.

No	Compound name	R-time	Formula	Area%
1	5-Octadecenoic acid, methyl ester	13.298	C19H36O2	0.00
2	Methyl tetradecanoate	13.573	C15H30O2	0.10
3	Pentadecanoic acid, methyl ester	14.647	C16H32O2	0.03
4	7,10-Hexadecadienoic acid, methyl ester	15.377	C17H30O2	0.00
5	7-Hexadecenoic acid, methyl ester, (Z)-	15.434	C17H32O2	0.14
6	9-Hexadecenoic acid, methyl ester, (Z)-	15.479	C17H32O2	0.23
7	Hexadecanoic acid, methyl ester	15.714	C17H34O2	13.33
8	cis-10-Heptadecenoic acid, methyl ester	16.444	C18H34O2	0.30
9	Heptadecanoic acid, methyl ester	16.653	C18H36O2	0.41
10	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.425	C19H34O2	24.29
11	9-Octadecenoic acid (Z)-, methyl ester	17.517	C19H36O2	33.44
12	Methyl stearate	17.631	C19H38O2	6.28
13	cis-10-Nonadecenoic acid, methyl ester	18.266	C20H38O2	0.12
14	9,12-Octadecadienoyl chloride, (Z,Z)-	18.984	C18H31ClO	0.76
15	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	19.110	C19H36O3	1.26

Table 1: Contd.

No	Compound name	R-time	Formula	Area%
16	cis-11-Eicosenoic acid, methyl ester	19.153	C21H40O2	3.19
17	Eicosanoic acid, methyl ester	19.353	C21H42O2	3.91
18	8,11,14-Docosatrienoic acid, methyl ester	19.510	C23H40O2	0.33
19	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	19.849	C19H32O2	0.18
20	Heneicosanoic acid, methyl ester	20.168	C22H44O2	0.06
21	13-Docosenoic acid, methyl ester, (Z)-	20.786	C23H44O2	0.25
22	Docosanoic acid, methyl ester	20.980	C23H46O2	6.15
23	Tricosanoic acid, methyl ester	21.728	C24H48O2	0.14
24	Tetracosanoic acid, methyl ester	22.475	C25H50O2	4.24
25	Hexacosanoic acid, methyl ester	23.868	C27H54O2	0.86

Antimicrobial assay

The disc diffusion bioassay was used to screen the antimicrobial activity of the oil against five standard human pathogens. The results showed(Table 2) significant activity against *Pseudomonas aeruginosa*, moderate activity against *Escherichia coli* beside moderate anticandidal effect.

Table 2: Antimicrobial ctivity of *Arachis hypogoea* oil.

Type	Sa	Bs	Ec	Ps	Ca
Oil 100mg/ml	-	-	15	18	15
Ampicilin 40mg/ml	30	15	--	--	--
Gentacycin 40mg/ml	19	25	22	21	--
Clotrimazole 30mg/ml	--	--	--	--	38

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

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