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Constituents and Biological Activity of Sudanese *Beta vulgaris* (Amaranthaceae) Seed Oil

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Abstract
Beta vulgaris is widely distributed in tropical and sub-tropical regions of Asia and Africa. Root and leave are edible. The root contains some pigments known as betalains. This plant is rich in vitamin C, folic acid, flavonoids and carotenoids. *Beta vulgaris* is used traditionally as hepatoprotective, hypotensive, hypoglycemic, vasodilating and anticancer. In this study, the GC-MS analysis revealed the following major constituents: (i) 9,12-octadecadienoic acid methyl ester (34.10%); hexadecanoic acid methyl ester (19.05%) (iii) 9-octadecenoic acid methyl ester (17.57%) and (iv) methyl stearate (8.68%). The oil was screened for antimicrobial activity. It showed significant activity against *Bacillus subtilis* and moderate anticandidal activity. it exhibited partial activity against other test organisms.

Keywords: *Beta vulgaris*, Oil, GC-MS Analysis, Antimicrobial Activity

1. Introduction
Beet (*Beta vulgaris*) is a plant in the family Amaranthaceae. The plant is widely distributed in tropical and sub-tropical regions of Asia and Africa [1]. Root, leave are edible. The root contains some pigments known as betalains [2]. This plant is rich in vitamin C, folic acid, flavonoids and carotenoids [3]. Some pharmacological effects of beet have been reported including: hepatoprotective [4], hypotensive [5, 6], hypoglycemic [7], vasodilating [8] and anticancer[9] activities.
Beta vulgare root is used traditionally as antifungal, antiviral, antiprotozoal and anticancer [10]. Leave contains some amino acids including threonine, valine, cystine, methionine and leucine [11, 12]. Pyridine and 4-picolene were reported as major volatiles of root [13]. A Preliminary pharmacognostic standardization has been reported [14].

Materials and Methods
Plant material
Beta vulgaris seeds were collected from, Folla, western Sudan. The plant was authenticated by the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan.

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

Test organisms
Test organisms used for antimicrobial assay are: *Bacillus subtilis* (Gram +ve), *Staphylococcus aureus* (Gram +ve), *Pseudomonas aeroginosa* (Gram –ve), *Escherichia coli* (Gram –ve) and the fungal species *Candida albicans.*

Methods
Extraction of oil
Powdered shade –dried seeds of *Beta vulgaris* (350g) were extracted-by maceration- with n-hexane. The solvent was removed under reduced pressure yielding the oil.

Antimicrobial assay
The oil was assessed for antimicrobial activity against five standard pathogenic bacteria (*Escherichia coli, Bacillus subtilis, Staphylococcus aureu, Pseudomonas aeruginosa and Candida albicans*).
The paper disc diffusion method was used to screen the antimicrobial activity of the oil and performed by using Mueller Hinton agar (MHA)-for bacterial culture- and Sabouraud dextrose agar for fungal culture. Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 100mg/ml of oil solution. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured. For fungus incubation continued for 72h at 25ºC.

Results and Discussion
GC-MS analysis
The GC-MS analysis of the oil showed the presence of 35 components. Total ions chromatograms is depicted in Fig.1, while the different constituents of the oil are presented in Table 1. Fatty acids constituted major bulk of the oil(99.67%). Terpenes (0.03%) and hydrocarbons (0.03%) appeared as minor constituents. The GC-MS analysis revealed the following major components:
- 9,12-Octadecdienoic acid methyl ester (34.10%)
- Hexadecanoic acid methyl ester (19.05%)
- 9-Octadecenoic acid methyl ester (17.57%)
- Methyl stearate (8.68%).

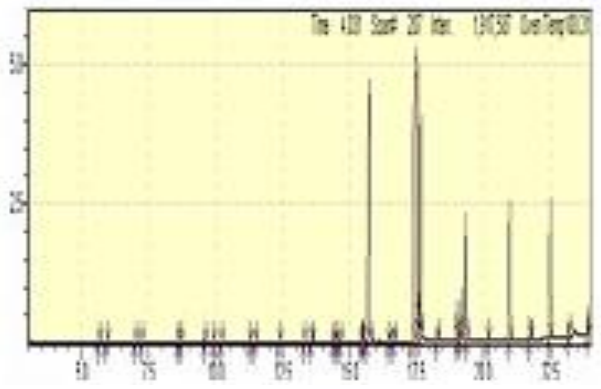

Fig 1: Total ions chromatgrams

The EI mass spectrum of 9, 12-octadecadienoic acid methyl ester is shown in Fig. 2.The peak at m/z 294, which appeared at R.T. 17.480 in total ion chromatogram, corresponds: $M^+[C_{19}H_{34}O_2]^{+\cdot}$ The peak at m/z263 corresponds to loss of a methoxyl function.

The mass spectrum of hexadecanoic acid methyl ester is displayed in Fig. 3. the peak at m/z 270(R.T. 15.749) is due to the molecular ion: $M^+[C_{17}H_{34}O_2]^{+\cdot}$ The peak at m/z239 accounts for loss of a methoxyl.

Fig. 4 shows the mass spectrum of 9-octadecenoic acid methyl ester**.**The peak at m/z 296, with R.T. 17.522, corresponds the molecular ion: $M^+[C_{19}H_{36}O_2]^{+\cdot}$ while the signal at m/z265 is due to loss of a methoxyl. The mass spectrum of methyl stearate is presented in Fig. 5.The signal at m/z 298, which appeared at R.T. 17.653 is attributed to $M^+[C_{19}H_{38}O_2]^{+\cdot}$The peak at m/z267 accounts for loss of a methoxyl.

Table 1: Constituents of Beta vulgaris oil

ID#	Name	Ret.Time	Area%
1.	Benzoic acid, methyl ester	5.641	0.02
2.	Hexanoic acid, 3-ethyl-, methyl ester	5.941	0.03
3.	L-.alpha.-Terpineol	6.993	0.03
4.	8-Nonenoic acid, methyl ester	7.231	0.01
5.	4-Decenoic acid, methyl ester	8.604	0.01
6.	Decanoic acid methyl ester	8.710	0.00
7.	1-Pentadecene	9.582	0.03
8.	10-Undecenoic acid, methyl ester	9.926	0.00
9.	Nonanoic acid, 9-oxo-, methyl ester	10.204	0.01
10.	Dodecanoic acid, methyl ester	11.265	0.01
11	10-Oxodecanoic acid. methvl ester	11.464	0.01
12.	Methyl myristoleate	12.364	0.01
13.	cis-5-Dodecenoic acid, methyl ester	13.308	0.01
14.	Methyl tetradecanoate	13.583	0.45
15.	6-Octadecenoic acid, methyl ester, (Z)-	14.391	0.04
16	5-Octadecenoic acid. methvl ester	14.496	0.02
17.	Pentadecanoic acid, methyl ester	14.657	0.10
18.	7-Hexadecenoic acid, methyl ester, (Z)-	15.449	0.09
19	9-Hexadecenoic acid. methvl ester. (Z)-	15.492	0.44
20.	Hexadecanoic acid, methyl ester	15.749	19.05
21.	cis-10-Heptadecenoic acid, methyl ester	16.455	0.21
22.	Heptadecanoic acid. methvl ester	16.664	0.44
23.	9.12-Octadecadienoic acid (Z.Z)-. methvl ester	17.480	34.10
24.	9-Octadecenoic acid (Z)-, methyl ester	17.522	17.57
25.	Methyl stearate	17.653	8.68
26.	trans-Geranylgeraniol	18.271	0.27
27.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	19.014	1.09
28	cis-11-Eicosenoic acid. methvl ester	19.162	1.67
29.	Eicosanoic acid. methvl ester	19.366	4.02
30.	Heneicosanoic acid, methyl ester	20.179	0.16
31.	Docosanoic acid, methyl ester	20.986	4.75
32.	Tricosanoic acid. methvl ester	21.738	0.63
33.	Tetracosanoic acid, methyl ester	22.489	4.90
34	Pentacosanoic acid. methvl ester	23.187	0.31
35.	Hexacosanoic acid, methyl ester	23.875	0.83

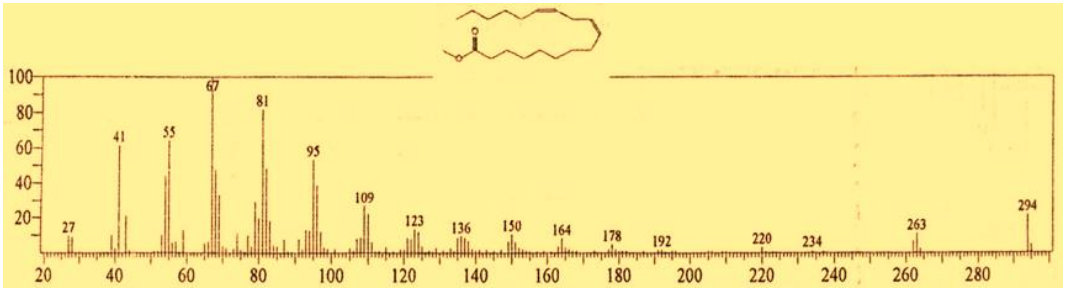

Fig 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester

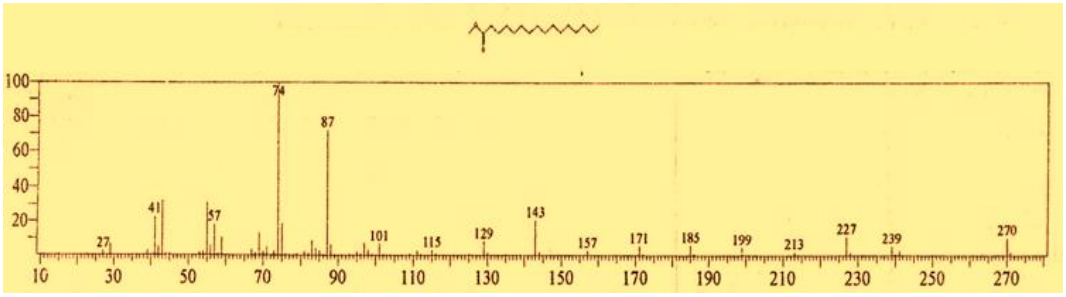

Fig 3: Mass spectrum of hexadecanoic methyl ester

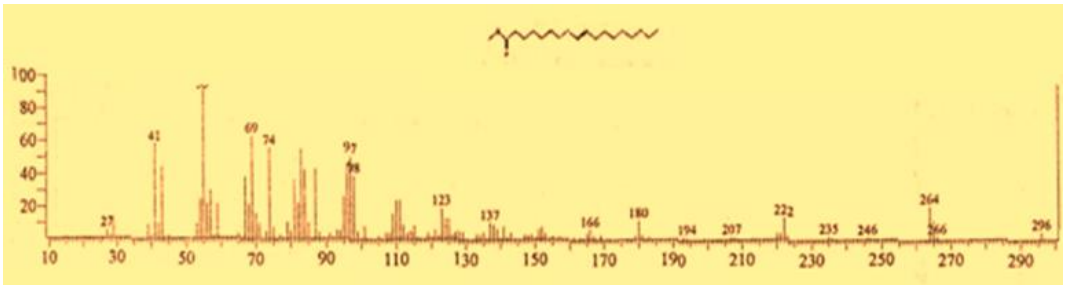

Fig 4: Mass spectrum of 9-octadecenoic acid methyl ester

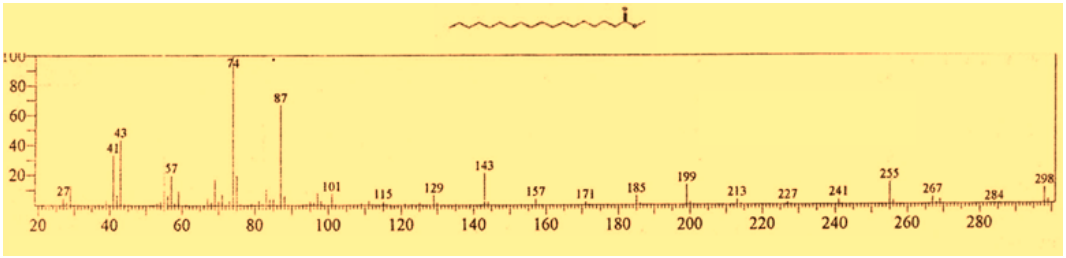

Fig 5: Mass spectrum of methyl stearate

Antimicrobial assay

Beta vulgaris oil was evaluated for antimicrobial activity against standard microorganisms using disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table (2). Results were interpreted in conventional terms: (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active). Ampicilin, gentamicin and clotrimazole were used as positive controls. The studied oil showed significant activity against *Bacillus subtilis* and moderate anticandidal activity.It exhibited partial activity against other test organisms.

Table 2: Inhibitory effect of *Beta vulgaris* oil

Sample	Sa	Bs	Ec	Ps	Ca
Oil(100mg/ml)	10	18	10	12	15
Ampicilin(40mg/ml)	30	15	--	--	--
Gentamicin(40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

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

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