

CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF *HELIANTHUS ANNUUM* (ASTERACEAE) OIL

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ABSTRACT

Helianthus annuum is considered as a significant source of vegetable oil and vegetable protein. Seeds are rich in polyunsaturated and monounsaturated fatty acids. They also contain tocopherols, folates, vitamin B, copper and zinc. This plant contains some biologically interesting molecules including flavonoids, alkaloids, saponins and tannins. Seed oil and herb tincture are used traditionally as antitumor, antiasthmatic, antioxidant, antipyretic and antimicrobial. GC-MS analysis of *Helianthus annuum* oil revealed the presence of the following major components: i) 9-octadecenoic acid (Z)-, methyl ester (53.92 %) .ii) 9,12-octadecadienoic acid (Z,Z)-, methyl ester (32.70 %).iii) methyl stearate (6.15%). iv) hexadecanoic acid, methyl ester (5.23 %). The oil was evaluated for its antimicrobial activity against five standard human pathogens. The oil showed moderate activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and the fungal species *Candida albicans*.

KEYWORD: *Helianthus annuum*, Oil, Constituents, Antimicrobial activity.

INTRODUCTION

Helianthus annuum is a plump, stiff annual plant (1-3m high) in the subfamily Helianthoideae and the family Asteraceae. The plant is native to north America.^[1,2] *Helianthus annuum* is considered as a significant source of vegetable oil and vegetable protein.^[3] Seeds are rich in polyunsaturated and monounsaturated fatty acids. They also contain tocopherols, folates, vitamin B, copper and zinc.^[4-7] This plant contains some biologically interesting molecules including flavonoids, alkaloids, saponins and tannins.^[8-11] Seed oil and herb tincture are used traditionally as antitumor, antiasthmatic, antioxidant, antipyretic, antihyperglycaemic and antimicrobial. Seed oil is diuretic, astringent, vermifuge, cathartic and stimulant.^[2] Seeds are also used traditionally in the treatment of pulmonary infections, laryngeal, cough, wounds and fever.^[12-14] Seed oil may prevent cancer and other oxidative stress – related diseases through its potent antioxidant properties.^[15,16]

The pharmacodynamic basis for the ethnobotanical uses of *Helianthus annuum* has been established. It has been reported that the plant possesses diverse pharmacological activities including: anti-inflammatory,^[17] antiasthmatic,^[18] antimicrobial,^[9,19,20] and antiviral,^[21] activities

MATERIAL AND METHODS

Plant material

The seeds of *Helianthus annuum* were collected from Kordofan-Sudan. The plant was authenticated by The Medicinal and Aromatic Plants Research Institute-Sudan.

Test organisms

Helianthus annuum oil was investigated for antimicrobial activity using the standard microorganisms shown in Table 1.

Table 1: Test organisms.

No.	Microorganism	Type
1	<i>Bacillus subtilis</i>	G+ve
2	<i>Staphylococcus aureus</i>	G+ve
3	<i>Pseudomonas aeruginosa</i>	G-ve
4	<i>Escherichia coli</i>	G-ve
5	<i>Candida albicans</i>	Fungi

Methods

Extraction of oil

Helianthus annuum seeds (250g) were macerated with hexane at room temperature. Removal of the solvent under reduced pressure gave the oil.

GC-MS analysis

Helianthus annuum oil was analyzed by gas chromatography- mass spectrometry. A Shimadzo GC-

MS-QP2010 ultra instrument was used. Helium was used as carrier gas. Oven temperature program is given in Table 2, while other chromatography conditions are displayed in Table 3.

Table 2: Oven temperature program.

Rate	Temperature	Hold time (min ⁻¹)
-	60	0.00
10	300	3.00

Table 3: Chromatographic conditions.

Column oven temperature	50.0c°
Injection temperature	300.00
Injection mode	Split
Flow control mode	Pressure
Pressure	100.00KPa
Total flow	50.0 ml/min
Column flow	1.61 ml/min
Linear velocity	46.3cm/sec
Purge flow	3.0 ml/min
Split ratio	-1.0

Testing of antimicrobial susceptibility

The paper disc diffusion was used to screen the antibacterial activity of the targeted oil and performed by using Mueller Hinton Agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines.^[22] Bacterial suspension was diluted with sterile physiological solution to 10⁸ cfu/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, 6mm in diameter) were placed on the surface of the MHA and soaked with 20µl of a solution of test sample. The inoculated plates were incubated at 37°C for 24 hours in the inverted

position. The diameters (mm) of the inhibition zones were measured.

RESULTS AND DISCUSSION

The GC-MS analysis of *Helianthus annuus* oil showed 12 components (Table 4) . Total ions chromatograms is depicted in Fig.1. The analysis of the oil revealed the following major components:

- i) 9-Octadecenoic acid (Z)-, methyl ester (53.92 %).
- ii) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (32.70 %).
- iii) Methyl stearate (6.15%).
- iv) Hexadecanoic acid, methyl ester (5.23 %)

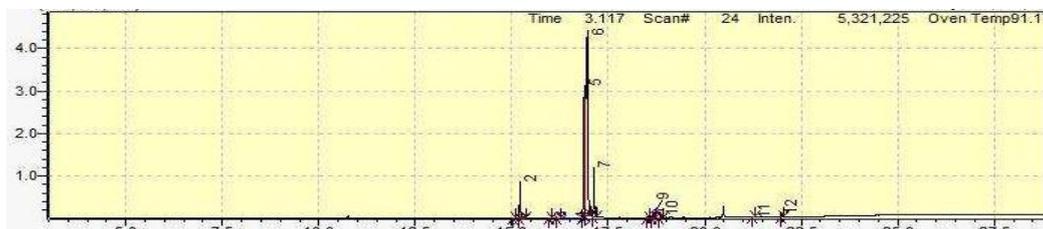


Fig. 1: Total ions chromatograms.

The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig.2. The signal at m/z 296 (RT.16.968) corresponds M⁺ [C₁₉H₃₆O₂]⁺. Fig.3 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294 (RT. 16.892) corresponds M⁺ [C₁₉H₃₄O₂]⁺. Fig. 4 shows the mass spectrum of methyl stearate. The signal at m/z 298 (R.T.17.129) corresponds

M⁺[C₁₉H₃₈O₂]⁺, while the peak at m/z 267 accounts for loss of a methoxyl. The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 5. The peak at m/z 270 (RT.15.213) is due to M⁺ [C₁₇H₃₂O₂]⁺.

Table 4: Constituents of *Helianthus annuus* oil.

No.	Name	Ret.Time	Area%
1.	9-Hexadecenoic acid, methyl ester, (Z)-	15.033	0.04
2.	Hexadecanoic acid, methyl ester	15.213	5.23
3.	cis-10-Heptadecenoic acid, methyl ester	15.999	0.05
4.	Heptadecanoic acid, methyl ester	16.199	0.07
5.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.892	32.70
6.	9-Octadecenoic acid (Z)-, methyl ester	16.968	53.92
7.	Methyl stearate	17.129	6.15
8.	Cyclopropaneoctanoic acid, 2-[(2-pentylcyclopropyl)methyl]-, methyl ester	18.524	0.26
9.	cis-11-Eicosenoic acid, methyl ester	18.670	0.59
10.	Eicosanoic acid, methyl ester	18.873	0.46
11.	Tricosanoic acid, methyl ester	21.255	0.06
12.	Tetracosanoic acid, methyl ester	21.986	0.47

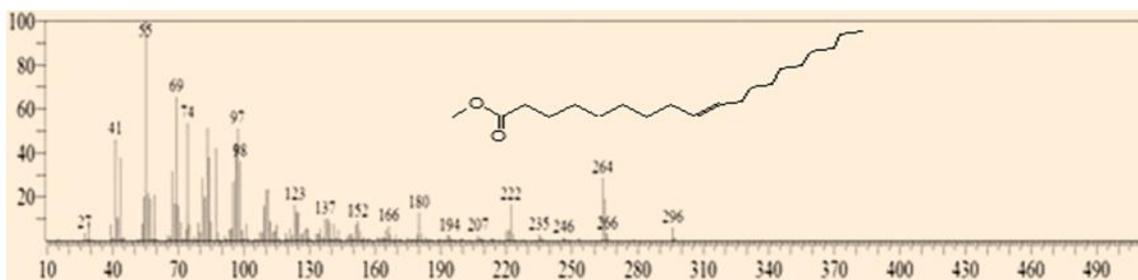


Fig. 2: Mass spectrum for 9-octadecenoic acid[z]-,methyl ester.

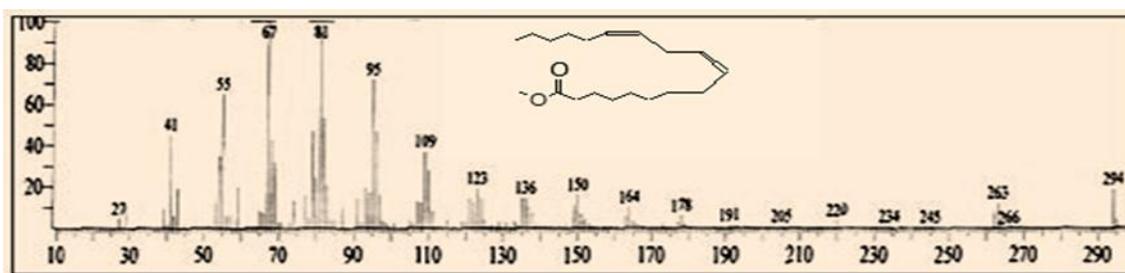


Fig. 3: Mass spectrum of 9,12-octadecadienoic acid.

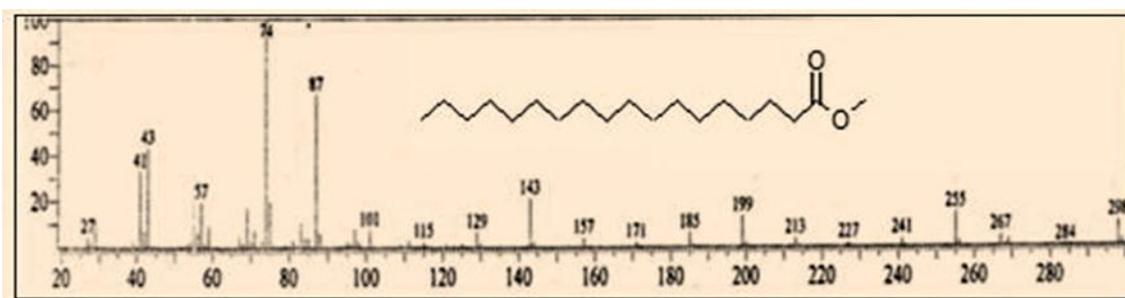


Fig. 4: Mass spectrum of methyl stearate.

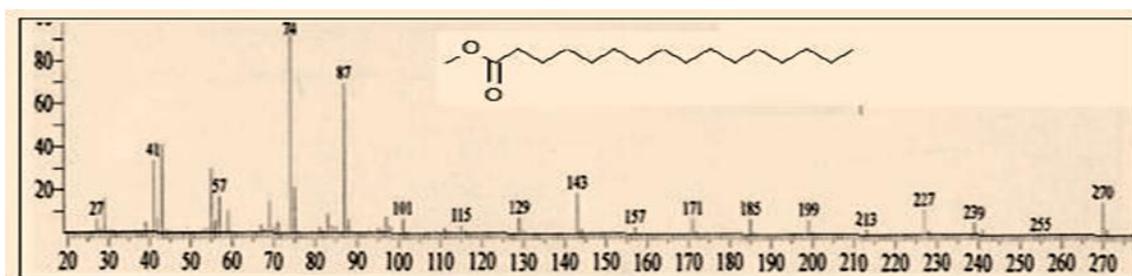


Fig. 5: Mass spectrum of hexadecanoic acid methyl ester.

Antimicrobial activity

Helianthus annuus oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth inhibition zones are presented in Table (5). Results were interpreted in the following terms: (<9mm: inactive; 9-12mm: partially

active; 13-18mm: active; >18mm: very active). Ampicilin, gentamicin and clotrimazole were used as positive controls. The oil showed moderate activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and the fungal species *Candida albicans*.

Table 5: Inhibition zones (mm/mg sample).

Type	Sa	Bs	Ec	Ps	Ca
Oil(100mg/ml)	15	13	13	15	14
Ampicilin(40mg/ml)	30	15	--	--	--
Gentamicin(40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

Sa.: *Staphylococcus aureus*

Bs.: *Bacillus subtilis*

Ec.: *Escherichia coli*

Pa.: *Pseudomonas aeruginosa*

Ca.: *Candida albicans*

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