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Antioxidant and Antimicrobial activity of *Argemone mexicana* (Papaveraceae) grown in Sudan

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Abstract
Argemone mexicana (Papaveraceae) is a herb with various traditional applications around the world. It has several pharmacological activities, among these the anticancer activity. In this study, the GC-MS analysis of *Argemone Mexicana* oil revealed the presence of 9 components. Major constituents are: oleic Acid, 9, 12-octadecadienoic acid (Z, Z) -, isopropyl linoleate and 9, 12-octadecadienoic acid (Z, Z)-, methyl ester. The oil was evaluated for antimicrobial activity against five standard pathogenic microbes. The antioxidant activity was also assessed. At a concentration of 100mg/ml. the oil showed significant activity against *Pseudomonas aeruginosa* and moderate activity against *Staphylococcus aureus*. In the DPPH assay, *Argemone mexicana* oil showed significant radical scavenging capacity compared to the positive control (propyl gallate).

Keywords: *Argemone mexicana*, oil, GC-MS analysis, antimicrobial activity, antioxidant activity

Introduction
Argemone mexicana (Papaveraceae) is a herb with various traditional applications around the world [1, 3]. Majority of seeds normally do not germinate during their first season after shedding [4]. but instead enter into the seed bank, thus producing seedlings, even in a well- maintained field, probably for several years [5]. *Argemone Mexicana* is one of the important medicinal plant which naturally occurs in various countries like India, Australia, South Africa, Tanzania and other parts of the world [8]. The alkaloids sanguinarine and dihydrosanguinarine are the toxic principles present in oil [6, 7]. The plant is adapted to a wide variety of habitat and tends to grow even in soil of low fertility. It has different chemical constituents and several pharmacological activities [9].
Medicinal properties have been attributed to the sap and oil from the seed. *Argemone mexicana* is used as an infusion against asthma. The root is taken for stomach pain [10]. Sap from the cut end of the stem is applied to cavities as a treatment for toothache. Children having difficulty with urination are given infusions of petals [10]. In India it is reported to be a homeopathic drug. In West Africa it is used as a cosmetic. The plant is also used traditionally in management of cancer [11], it also contribute to the development of successful immune therapies of some carcinomas due to its apoptotic potential [12]. *Argemone mexicana* has very good peripheral activity and significant analgesic activity in comparison to aspirin [13]. *Argemone mexicana* demonstrated wound healing activity in experimental animals [14].
Root could be a potential source of natural antioxidant, that could have greater importance as therapeutic agent in preventing or slowing oxidative stress related to degenerative diseases [15]. The plant possesses antihistaminic activity which could be due to its polar constituents [16].

Materials and Methods
Plant material
Argemone mexicana seeds were collected from Khartoum, Sudan. The plant was identified and authenticated by the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan

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

Microorganisms
The antimicrobial assay was performed using the following standard microorganisms:
*Bacillus subtilis (*G+ve), *Staphylococcus aureus (*G+ve), *Pseudomonas aeroginosa* (G-ve), *Escherichia coli* (G-ve) and *Candida albicans* (fungus).

Extraction of oil
Argemone Mexicana seeds (300g) were exhaustively extracted with n-hexane by maceration. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further work.

GC-MS analysis
Chromatographic conditions are as follows: column oven temperature: 150.0^{o}C; injection temperature: 300.0oC; injection mode: split; flow mode: linear velocity; pressure: 139KPa; total flow: 50.0ml/min; column flow: 1.54ml/sec; linear velocity: 47.2cm/sec.; purge flow: 3.0 ml/min.; split ratio: -1.0. Oven temperature program is presented Table 1.

Table 1: Oven temperature program

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

Antimicrobial assay

The cup-plate agar diffusion bioassay was used, with some minor modifications, to assess the antimicrobial activity of the oil. Standardized bacterial stock suspension (2ml) was mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes.

Cups (6 mm in diameter) were cut using sterile cork borer (No 4). The agar discs were removed and the cups were filled with 0.1 ml of test solution and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured and recorded as average of two replicates.

Results and Discussion

GC-MS analysis of *Argemone mexicana* oil was conducted and the identification of the constituents was based on retention times and computer matching of the MS data with the (NIST) mass spectrum library. The GC-MS analysis of the studied oil revealed the presence of 9 components (Table 2).The typical total ion chromatograms (TIC) is depicted in Fig.1.

Table 2: Constituents of *Argemone Mexicana* oil

No	Name	Ret. Time	Formula	Area%
1	Hexadecanoic acid, methyl ester	15.621	$C_{17}H_{34}O_2$	2.66
2	n-Hexadecanoic acid	16.015	$C_{16}H_{32}O_2$	4.97
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.272	$C_{19}H_{34}O$	14.23
4	9-Octadecenoic acid (Z)-, methyl ester	17.316	$C_{19}H_{36}O_2$	5.99
5	Methyl stearate	17.535	$C_{19}H_{38}O_2$	1.32
6	9,12-Octadecadienoic acid (Z,Z)-	17.705	$C_{18}H_{32}O_2$	22.97
7	Oleic Acid	17.731	$C_{18}H_{34}O_2$	35.76
8	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	19.050	$C_{19}H_{36}O_3$	3.61
9	Isopropyl linoleate	20.528	$C_{21}H_{38}O_2$	8.49

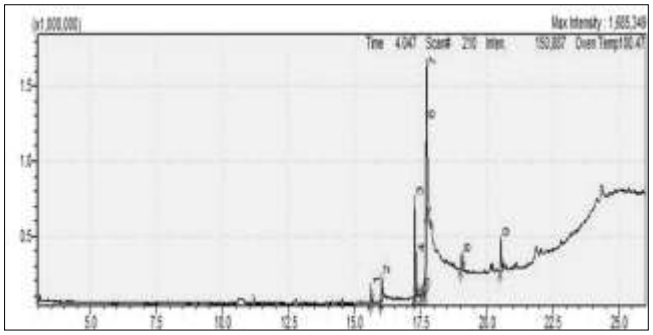
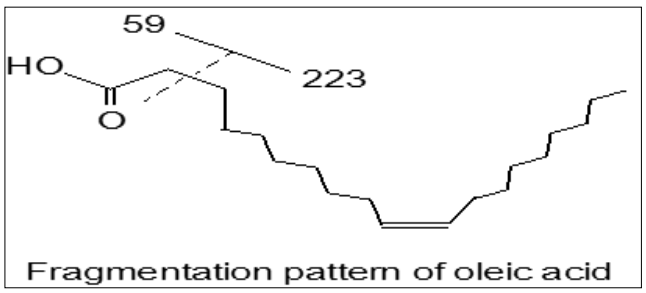


Fig 1: Total ions chromatograms

The mass spectra of the major constituents of the oil are discussed below:

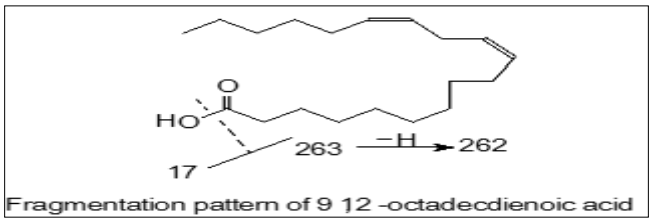
Oleic Acid (35 .76 %)



The mass spectrum of oleic acid is shown in Fig. 2.The peak at m/z 282, which appeared at R.T. 17.731 in total ion chromatogram, corresponds M+[$C_{18}H_{34}O_2$]+. The signal at m/z 223 is due to loss of - CH_2CO_2H group.

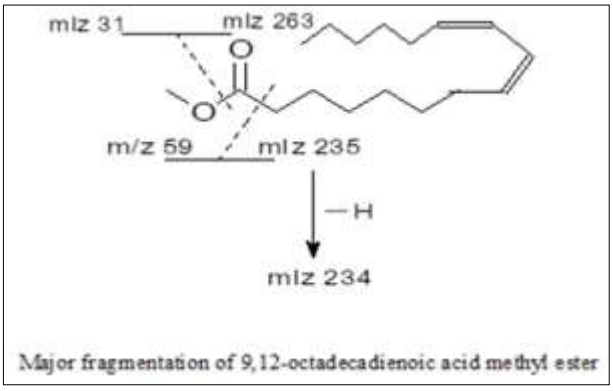
9, 12-Octadecadienoic acid (Z, Z)-(22.97%)

Figure 3 displays the mass spectrum of 9, 12-octadecadienoic acid (Z, Z). The peak at m/z



280(R.T 17.705) is due to M^+ [C18H32O2] $^+$, the signal at m/z 262 is due to loss of a hydroxyl function.

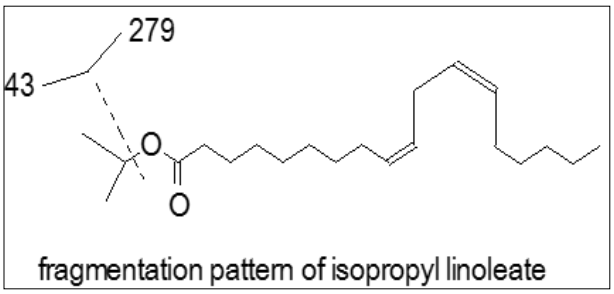
9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (14.23%)



The mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester is shown in Fig. 4.The peak at m/z 294(R.T. 17.272) corresponds M^+ [$C_{19}H_{34}O_2$] $^+$

Isopropyl linoleate (8.49%)

In Fig. 5 (mass spectrum of isopropyl linoleate), the molecular ion: M^+ [C19H36O3] $^+$ appeared at m/z 322 (R.T. 20.528).



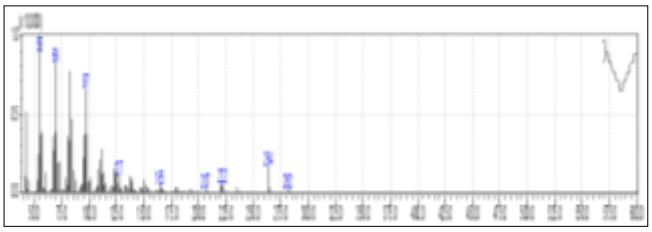


Fig 2: Mass spectrum of oleic acid

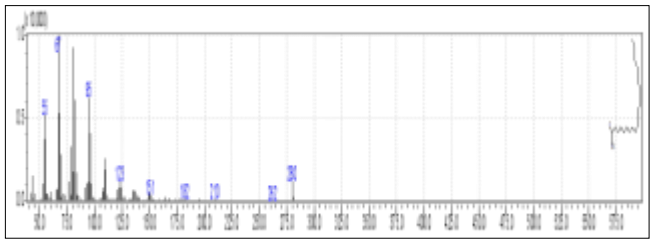


Fig 3: Mass spectrum of 9, 12-Octadecadienoic acid (Z, Z)-

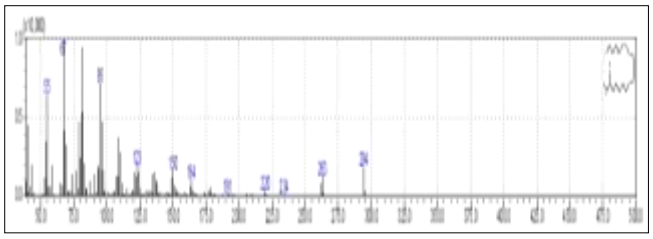


Fig 4: Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester

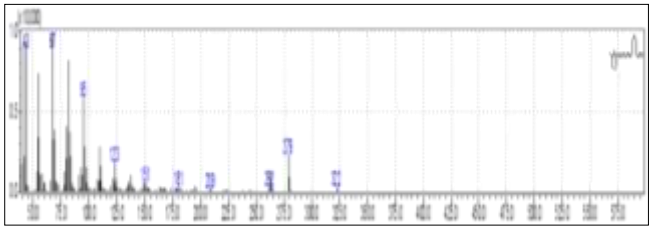


Fig 5: Mass spectrum of Isopropyl linoleate

Antimicrobial activity

In cup plate agar diffusion assay, the oil was assayed for antimicrobial activity. The averages of the diameters of the growth inhibition zones are listed in Table (3) Ampicilin, gentamycin and clotrimazole were used as positive controls. At a concentration of 100mg/ml. the oil showed significant activity against *Pseudomonas aeruginosa* and moderate activity against *Staphylococcus aureus*.

Table 3: Inhibition diameters (mm) of the oil

Drug	Conc.(mg/ml)	Ec	Pa	Sa	Bs	Ca
corochorus olitorius oil	100	--	22	14	--	--

Table 4: Antibacterial activity of standard chemotherapeutic agents: M.D.I.Z (mm)

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

Table 5: Antifungal activity of clotrimazole: MDIZ (mm)

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*
- *Bs.*: *Bacillus subtilis*
- Ca.: *Candida albicans*

Antioxidant activity

In the DPPH assay, Argemone mexicana oil showed significant radical scavenging activity (Table 6) compared to the positive control (propyl gallate).

Table 6: Antioxidant activity of *Argemone mexicana* oil

sample	%RSA± SD (DPPH)	IC_{50}± SDmg/ml (DPPH)
A. Mexicana oil	85.5 ± 0.06	0.131±0.01
Standard (propyl gallate)	92.2 ±0.01	0.077 µg ±0.01

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