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Research Article

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GC-MS Studies and Antioxidant Activity of *Pongamia pinnata* Grown in Sudan

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Abstract The fruits and sprouts of *Pongamia pinnata* have been used traditionally against cancer. Seeds are claimed to treat fever, bronchitis, whooping cough, rheumatism and skin diseases. Seed oil is used by local healers against piles, leprosy, ulcers and scabies. Plant juice and oil possess antiplasmodial, antiinflammatory, antinociceptive, antihyperglycemic, antiulcer, antidiarrhoeal and antihyperammonemic activity. In this study *Pongamia pinnata* oil was characterized by GC-MS. A total of 25 constituents were identified. Major constituents are: i) 9-octadecenoic acid methyl ester (53.58%) ii) methyl stearate (15.00%) iii) 9,12-octadecadienoic acid (Z,Z)-, methyl ester (13.31 %) and iv) hexadecanoic acid, methyl ester (12.39%). The antioxidant activity of *Pongamia pinnata* oil was conducted. The studied oil showed good free radical scavenging capacity in the DPPH assay.

Keywords *Pongamia pinnata*, Oil, GC-MS Analysis, Antioxidant Activity

Introduction

Pongamia pinnata is a medium-sized, evergreen tree in the family Luguminaceae [1]. The plant contains some bioactive molecules including flavonoids which are known antioxidants. Some flavones and chalcones have been isolated from the leaves and stem [2]. The fruits and sprouts have been used traditionally against cancer [2]. Seeds are claimed to treat fever, bronchitis, whooping cough, rheumatism and skin diseases [3]. Seed oil is used by local healers against piles, leprosy, ulcers and scabies [4]. Plant juice and oil possess antiplasmodial, anti-inflammatory [5], antinociceptive, antihyperglycemic, antiulcer, antidiarrhoeal and antihyperammonemic activity [6].

The antiulcer activity of root extract has been reported [7-9]. It has been shown that a decoction of *Pongamia pinnata* possesses selective antidiarrhoeal effect with efficacy against cholera [10]. Leave extract detoxified ammonia, urea and creatinine in ammonium chloride – induced hyperammonium models [11-13]. It has been shown that the flower extracts exhibited significant antihyperglycemic and antilipidperoxidative activity [13]. The extract also enhanced the antioxidant defense system in alloxan-induced diabetic models [13,14]. The ethanol extract of the leaves showed significant antiinflammatory effect [15,16]. The antiviral [17,18] and antibacterial activities of the leaves have been documented [19-21].

Material and Method

Collection of Plant Material

Pongamia pinnata seeds were collected from Damazin (Sudan). The plant material was taxonomically authenticated at herbarium of Medicinal and Aromatic plant and Traditional Medicine Research Institute, National Center for Research, Khartoum, Sudan.

Extraction of oil

Dry powdered *Pongamia pinnata* seeds (400g) were extracted with n-hexane at room temperature for 72h and filtrated. The solvent was removed under reduced pressure to yield the oil.

Instruments and chromatographic conditions:

Instrument: GC-MS –QP2010 Ultra (Japan)

Carrier gas: Helium.

Oven temperature program

Rate	Temperature (°C)	Hold time (min $^{-1}$)
--	150.0	1.00
4.00	300.0	0.00

Chromatographic conditions

Colunm Oven Temp.**:** 60.0 °C

Injectio Temp.: 300.00 °C

Injection Mode: Split

Flow Control Mode: Linear Velocity

Pressure: 100.0 kPa

Purge Flow: 3.0mL/min

Split Ratio:-1.0

GC Program (GC-MS –QP2010 Ultra)

Ion Source Temp.: 200.00°C

Interface Temp.: 250.00°C

Solvent Cut Time: 2.50min

Detector Gain Mode: Relative

Detector Gain: 0.86 kv +0.00kv

Threshold: 0

Free radical scavenging activity

The antioxidant activity was assessed by bleaching of the purple-colored solution of DPPH$^{\cdot}$ according to the technique reported by Shimada et al [22] with a slight modification. In this assay, the bleaching rate of a stable free radical (DPPH$^{\cdot}$**)** is monitored at a characteristic wavelength (517 nm) in the presence of samples. The test sample was mixed with DPPH$^{\cdot}$ (0.1 mM, 0.5 mL). The absorbance was recorded at λ_{max} 517 nm and compared with standards. The result of the reaction was the change of color from purple to yellow due to decrease of molar absorptivity of DPPH$^{\cdot}$ radical.

Results and Discussion

GC-MS analysis of oil

Gas chromatography - mass spectrometry has been used for the identification and quantification of the studied oil. Total ions chromatograms is presented in Fig. 1. The analysis revealed the presence of (15) components - Table 1.

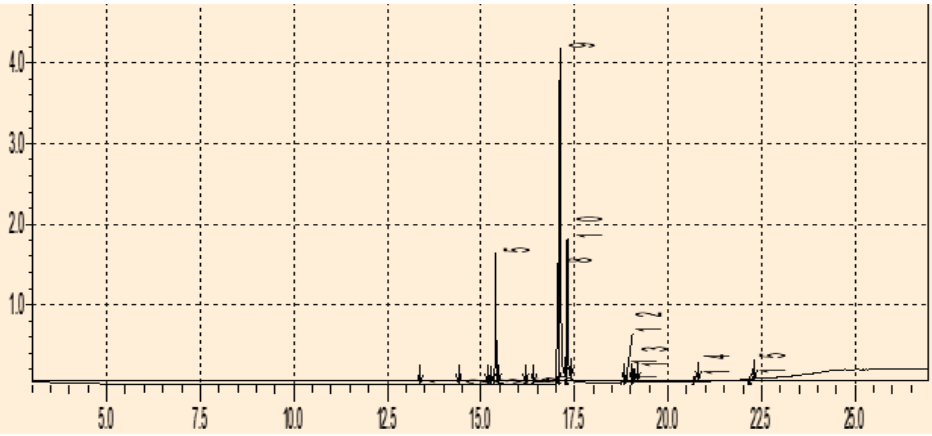


Figure 1: Total ions chromatograms

Table 1: Constituent of *Pongamia pinnata* L. seed oil

No.	Name	Ret. Time	Area%
1.	Methyl tetradecanoate	13.314	0.03
2.	Pentadecanoic acid, methyl ester	14.370	0.02
3.	7-Hexadecenoic acid, methyl ester, (Z)-	15.152	0.05
4.	9-Hexadecenoic acid, methyl ester, (Z)-	15.196	0.06
5.	Hexadecanoic acid, methyl ester	15.390	12.39
6.	cis-10-Heptadecenoic acid, methyl ester	16.157	0.06
7.	Heptadecanoic acid, methyl ester	16.365	0.14
8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.045	13.31
9.	9-Octadecenoic acid (Z)-, methyl ester	17.118	53.58
10.	Methyl stearate	17.308	15.00
11.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.798	0.41
12.	cis-11-Eicosenoic acid, methyl ester	18.884	1.01
13.	Eicosanoic acid, methyl ester	19.083	2.09
14.	Docosanoic acid, methyl ester	20.712	0.91
15.	Tetracosanoic acid, methyl ester	22.219	0.94

Major constituents of the oil are:

i) 9-Octadecenoic acid methyl ester (53.58%).

ii) Methyl stearate (15.00%).

iii) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (13.31 %).

iv) Hexadecanoic acid, methyl ester (12.39%)

The mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is shown in Figure 2. The peak at m/z 296 with retention time 17.118 accounts for the molecular ion M^+ $[C_{19}H_{36}O_2]^+$. Figure 3 shows the mass spectrum of methyl stearate. The signal at m/z 298 (retention time: 17.308) is due to the molecular ion M^+ $[C_{19}H_{38}O_2]^+$. The peak at m/z 267 is due to loss of a methoxyl. Fig. 4 illustrates the mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester. The signal at m/z 294 which appeared at RT.17.045 accounts for the molecular ion: M^+ $[C_{19}H_{34}O_2]$. The peak at m/z 263 is attributed to loss of a methoxyl. Fig. 5 shows the mass spectrum of hexadecanoic acid methyl ester. The signal at m/z 270 (RT. 15.390) is due to M^+ $[C_{17}H_{34}O_2]$, while the peak at m/z 239 is attributed to loss of a methoxyl function.

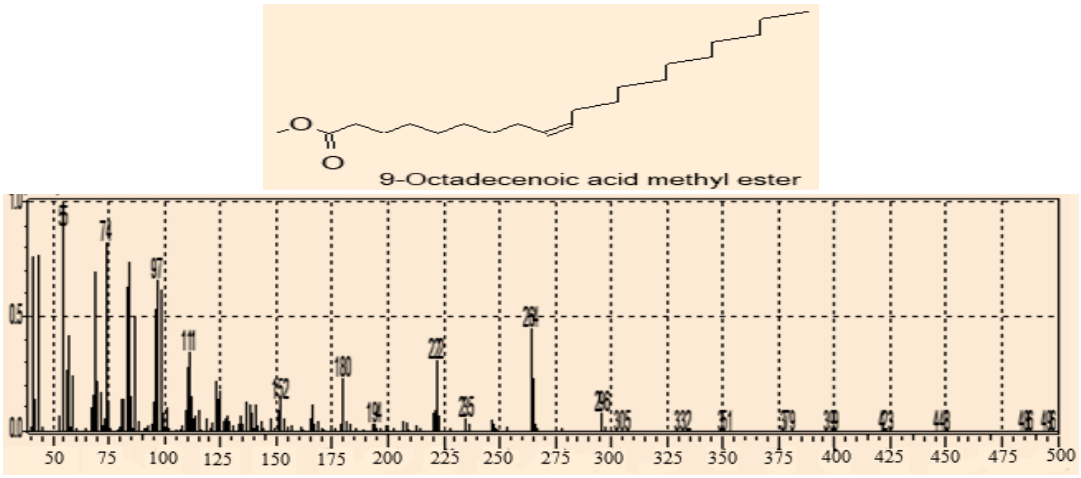
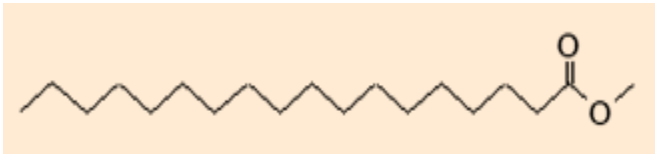


Figure 2: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester



Methyl stearate

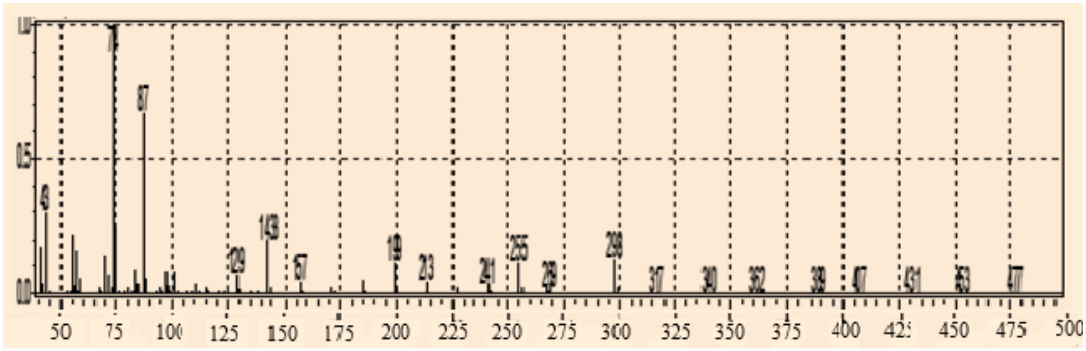


Figure 3: Mass spectrum of methyl stearate

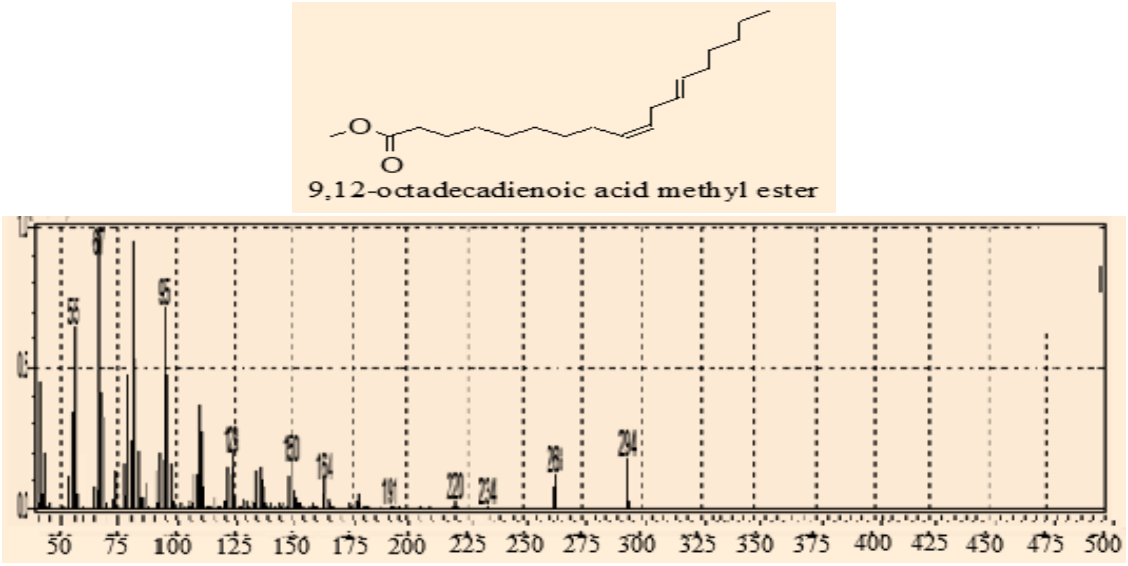


Figure 4: Mass spectrum of 9,12-octadecadienoic acid methyl ester

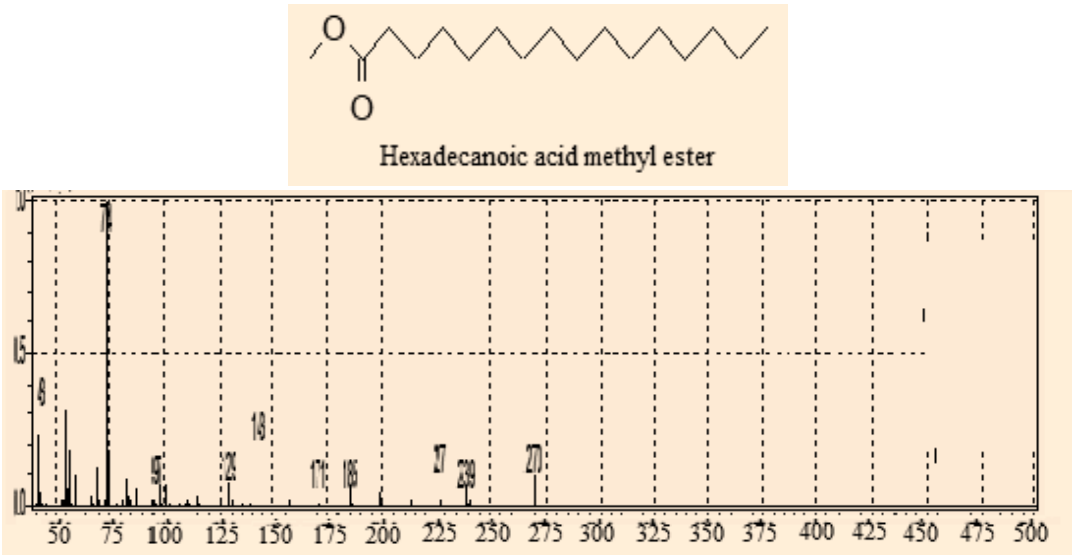


Figure 5: Mass spectrum of hexadecanoic acid, methyl ester

Antioxidant Assay

The DPPH – scavenging model is a widely used assay for assessment of the antioxidant activity. The DPPH ˙is a stable free radical and can accept one electron or hydrogen radical to form a stable diamagnetic molecule. The studied oil showed goof free radical scavenging capacity in the DPPH assay (Table 2).

Table 2: Antioxidant activity of the oil

Sample	%RSA± SD (DPPH)
Pongamia pinnata oil	46±0.08
Propyl gallate (control)	89±0.01

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