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Original Article

GC/MS Analysis and Antioxidant Activity of Fixed Oil from Sudanese Safflower *(Carthamus tinctorius* L*)* Seeds

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ABSTRACT

Background: The aim of the present study was to investigate the chemical constituents of the Fixed oil from Sudanese Safflower (*Carthamus tinctorius* L.) seeds and to evaluate the potential antioxidant activity.
Methods: Soxhlet method was used to extract the essential oil from Sudanese Safflower Seeds. The chemical constituents of Sudanese Safflower *(Carthamus tinctorius* L*)* were identified and quantified by GC-MS and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used determined the capacity antioxidant activity.
Results: Nineteen components were identified, seven of which are namely 9,12-Octadecadienoic acid (Z,Z)-, methyl ester 1.61%) , 9-Octadecenoic acid (Z)-, methyl ester (10.50%), Hexadecanoic acid, methyl ester (8.93%), Methyl stearate (8.41%), Eicosanoid acid, methyl ester (1.39%), cis-11-Eicosenoic acid, and methyl ester (1.01%). The DPPH assay showed moderate antioxidant potential by 23.05±0.01 compared with that of standard by 91±0.01.
Conclusion: The results of this study showed that the *Sudanese Safflower (Carthamus tinctorius* L*.)* seeds has oil rich of bioactive chemical compounds and has a significant anti-oxidant effect. It can be used to deal with in flavoring agent, food industry and medicinal purposes.
Keywords: Safflower, Antioxidant Activity, GC -MS Analysis

1. Introduction

Carthamus tinctorius L is a famous traditional herbal medicinal plant, belonging to the (Asteraceae) family; it has been used across North Africa including Sudan [1-3]. Safflower is a multipurpose oil seed crop that can be used for the paint industry and its edible oil is used for cooking, making margarine

and salad [4, 5]. Safflower is also grown for its flowers which are used as cut flowers, coloring and flavoring foods, making dyes for the textile industry, livestock forage, vegetable, and making herbal tea [2]. In China, Safflower is grown for medicinal purposes to treat cardiovascular diseases, male and female sterility, lowering blood cholesterol, release of retained placenta and still birth, induction of labour in expectant women, delayed, heavy and painful various types of rheumatism (sciatica, thorax, arthritis), respiratory diseases (whooping cough, chronic bronchitis), gastritis. In addition to it is medicinal uses, is also highly nutritious [6, 7].

Safflower seeds contain 30% oil, 20% protein, and crude fiber 35% [9]. Also Safflower seeds are rich in minerals (Zn, Cu, Mn, and Fe), vitamins, and the tocopherols (alpha, beta, and gamma) [8][8]. Safflower leaves, petals, and seeds have tremendous medicinal and therapeutic significance, and the petals are also used for extracting dye for coloring textiles and foodstuffs [8]. Safflower oil contains 70% polyunsaturated fatty acid (linoleic acid) 10% mono-unsaturated (oleic acid) with small amounts of stearic acid [9]. The flowers of *C. tinctorius* are an important medicinal material in prescriptions used for cardiovascular, cerebrovascular and gynecological diseases. In China, the water extract of *C. tinctorius* L has been developed as an intravenous injection, which is extensively applied to treat cardiovascular diseases clinically. Its dye is mainly used as a coloring agent [10, 11]. Remarkable biological activities of safflower has made this plant both as a food and a medicine in many parts of the world [12], *C. tinctorius* L has recently been shown to have anticoagulant, vasodilator antioxidant, anti-inflammatory, anticoagulant, vasodilator and antidiabetic activities [12, 13]. So far, the Sudanese *C. Tinctorius* L seed oils are not being sufficiently investigated to determine their properties and subsequently classified into classes in order to suit world market requirements, especially if it is to be commercially produced in Sudan as potential sources for export. Therefore, this study aimed to investigate the chemical constituents and antioxidant activity of *C. tinctorius* L seed oil to increase the economic feasibility of future commercial cultivation and products of this plant.

2. Material and Methods

2.1. Plant material

Carthamus tinctorius L**,** seeds were purchased in august 2018 from the local market of Bahri, Sudan, and it was identified at the herbarium of the Aromatic and Medicinal Plants Research Institute. Voucher specimens, (No. 20181011) have been deposited in the herbarium of the author's laboratory.

Figure1. The flower and seeds of Safflower (*Carthamus tinctorius* L)

2.2. Extraction of oil

The *C. tinctorius* seeds were ground into powder using mortar and pestle. 100 g of seeds powder was extracted with n-hexane using Soxhlet extractor apparatus for six hours. The solvent was evaporated under reduced pressure using rotary evaporator apparatus, and the oil was kept in a refrigerator for further manipulation

2.3. Method of GC/MS analysis

The qualitative and quantitative analysis of the sample was carried out by using GM/MS technique model (GC/MS-QP2010-Ultra) from Japanese Simadzu Company, with serial number 020525101565SA and capillary column (Rtx-5 ms-30 m×0.25 mm×0.25 µm). 1 µL of sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 mL/min, the temperature program was started from 60 ^{o}C with rate 10 ^{o}C /min to 300 ^{o}C as final temperature degree with 3 minutes hold time, the injection port temperature was 300 ^{o}C, the ion source temperature was 200 ^{o}C and the interface temperature was 250 ^{o}C. The sample was analyzed by using scan mode in the range of m/z 10-600 charges to ratio and the total run time was 30 minutes. Identification of the components of the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library of the National Institute of Standards and Technology (NIST) and the results were recorded.

2.4. DPPH radical scavenging assay

Free radical scavenging ability of the extracts was tested by DPPH radical. Scavenging assay was determined as described [14, 15] with some modification. In 96–well plate (Sigma Aldrich), they were allowed to react with DPPH for half an hour at 37 ^{o}C. The concentration of DPPH was kept as (300 μLM). The test sample was dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance of each solution was measured at 517 nm using a mutilate reader spectrophotometer. The control was done in the same way by adding methanol and DPPH without the sample extract. Percentage of radical scavenging activity was determined in comparison with DMSO treated control group. Every measurement was replicated three times.

3. Results

3.1. Chemical constituents of oil

The chemical constituents of Sudanese Safflower seed oil were identified and quantified by GC-MS running time was 30 minutes, revealing the presence of nineteen components. The GC-MS chromatogram is presented in Figure 2. The name of active compounds with their Retention Time (RT), base peak and some Key fragment ions are presented in Table 1. The spectra of the compounds (Figure 3-8) are matched with Wiley, 9.0 and NIST libraries.

3.2. Antioxidant Activity

According to DPPH scavenging activity, the seed oil showed moderately antioxidant potential 23±0.01 µg/mL activity comparable to that of Propyl Gallate (Standard) 89±0.01 µg/m against DPPH (Table 2).

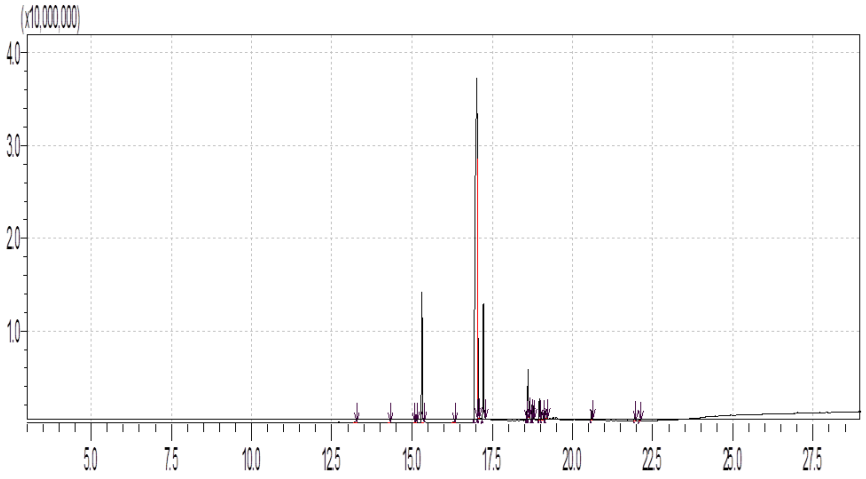

Figure 2. The typical GC chromatogram of *(Carthamus tinctorius.* L*)*

Table 1. Chemical Constituents of *(Carthamus tinctorius* L.*)* Seeds oil

Peak report TIC					
ID	**RT**	**Name of compound**	**M+**	**BP**	**Key fragment ions**
1	13.212	Methyl tetradecanoate	242	74	143 129 87 74 57 41
2	14.289	Pentadecanoic acid, methyl ester	256	74	143 115 101 87
3	15.073	7-Hexadecenoic acid, methyl ester, (Z)-	268	55	152 123 98 87 74 69
4	15.113	9-Hexadecenoic acid, methyl ester, (Z)-	268	55	236 194 123 96 74
5	15.310	Hexadecanoic acid, methyl ester	270	74	239 227 143 87 57
6	16.283	Heptadecanoic acid, methyl ester	284	74	284 143 87 57 43 41
7	17.023	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	294	67	263 109 95 81 55
8	17.043	9-Octadecenoic acid (Z)-, methyl ester	296	67	263 150 95 81 55
9	17.223	Methyl stearate	298	74	255 143 87 57 43 41
10	18.576	7,10-Octadecadienoic acid, methyl ester	294	67	263 150 109 81 55
11	18.614	11,14-Eicosadienoic acid, methyl ester	322	74	291 109 81 67 41
12	18.741	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	312	55	171 155 87 74 69 41
13	18.767	cis-11-Eicosenoic acid, methyl ester	324	55	292 208 97 83 69 41
14	18.970	Eicosanoic acid, methyl ester	326	74	295 283 143 87 57
15	19.052	9,12,15-Octadecatrienoic acid, methyl ester, Z,Z,Z)-	292	99	226 108 93 79 67 55
16	19.164	8,11,14-Eicosatrienoic acid, methyl ester	320	55	289 222 95 83 6741
17	20.590	Docosanoic acid, methyl ester	354	74	323 143 87 57 43 41
18	21.938	15-Tetracosenoic acid, methyl ester, (Z)-	380	55	350 348 125 97 83 69
19	22.093	Tetracosanoic acid, methyl ester	382	74	339 143 87 57 43

Table 2. Antioxidant activity of *(Carthamus tinctorius* L.) *Seeds,*oil

Sample Code	%RSA±SD (DPPH) µg/mL
Hibiscus sabdariffa seeds oil	23.05±0.01
Propyl Gallate (Standard)	91±0.01

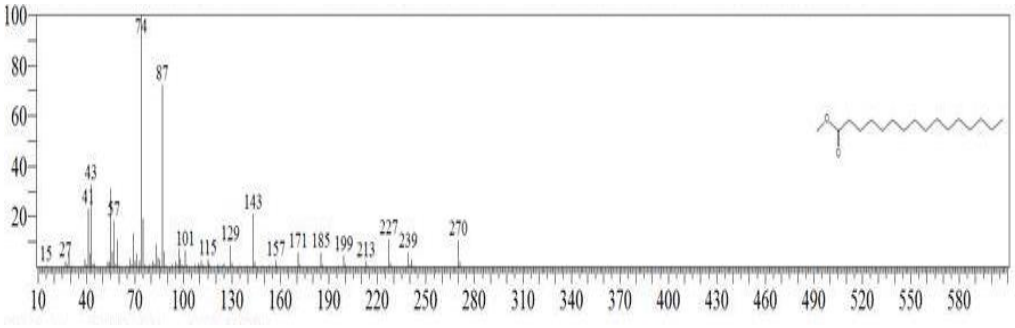

Figure 3. The Mass spectrum of Hexadecanoic acid, methyl ester

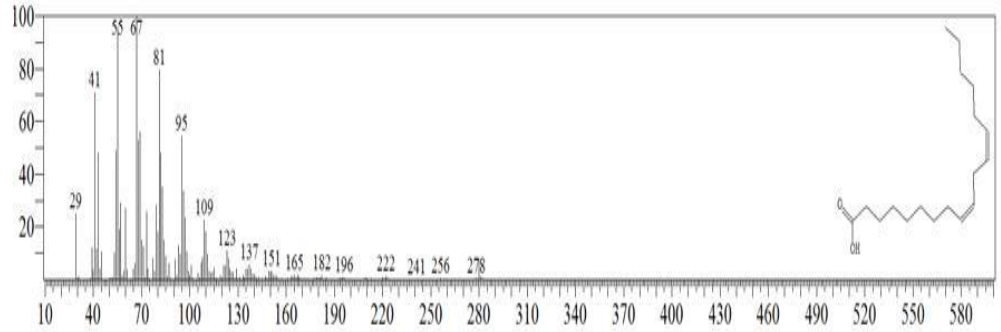

Figure 4. The Mass spectrum of 9,12-Octadecadienoic acid (Z,Z)-, methyl ester

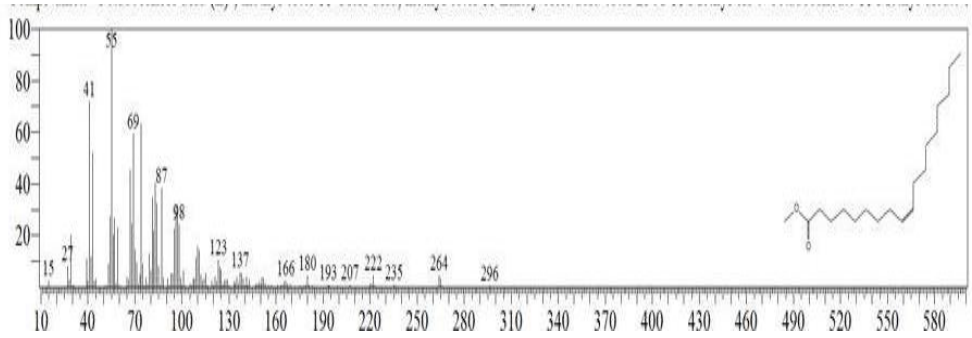

Figure 5. The Mass spectrum of 9-Octadecenoic acid (Z)- methyl ester

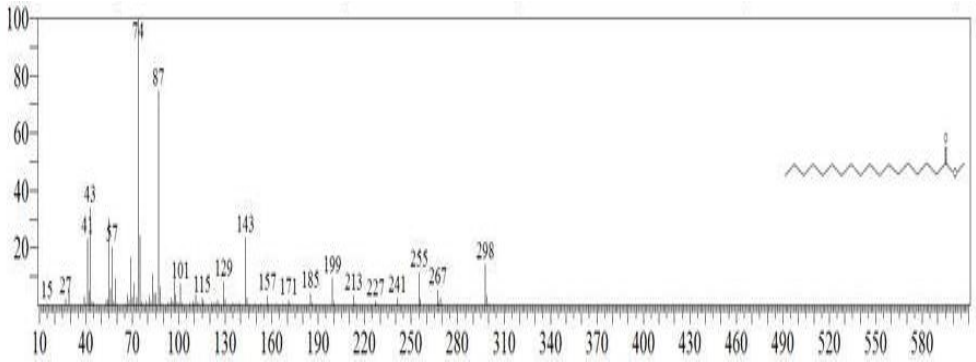

Figure 6. The Mass spectrum of methyl stearate

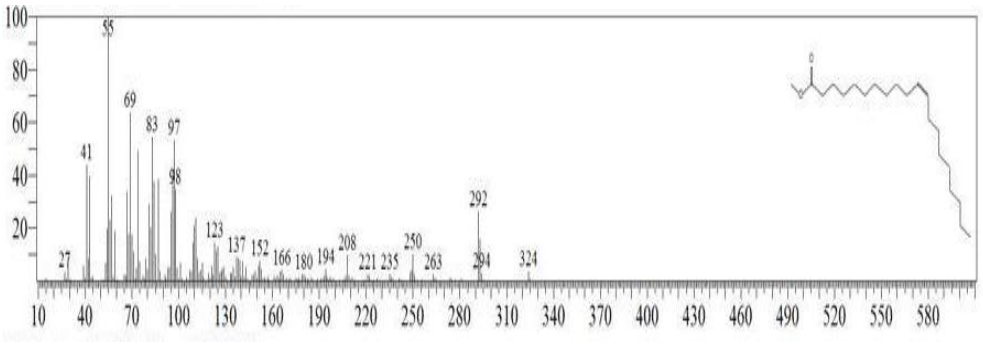

Figure 7. The Mass spectrum of cis-11-Eicosenoic acid, methyl ester

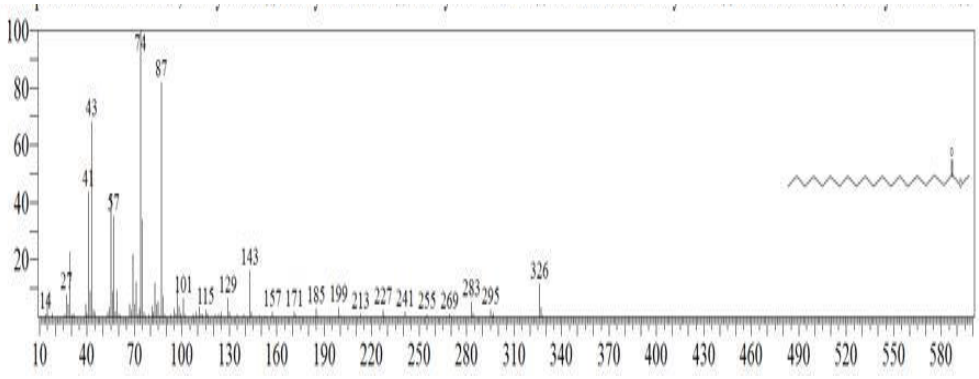

Figure 8. The Mass spectrum of Eicosanoic acid, methyl ester

4. Discussion

The most abundant of component in *(Carthamus tinctorius* L.*)* seed oils were 9,12-Octadecadienoic acid (Z,Z)-, methyl ester 1.61%) , 9-Octadecenoic acid (Z)-, methyl ester (10.50%), Hexadecanoic acid, methyl ester (8.93%), Methyl stearate (8.41%), 11,14-Eicosadienoic acid, methyl ester (3.41%), Eicosanoic acid, methyl ester (1.39%) and cis-11-Eicosenoic acid, methyl ester (1.01%). As the main constituent, 7-Hexadecanoic acid, methyl ester appeared at 15.310 min with area 8.93%, and has Ms ions *m/z* 270 [M] + corresponding to formula $C_{17}H_{34}O_2$ in its mass spectra (Figure 3) as well as the following fragment ions: 239, 227, 143, 87, 57, 41 and 74 (base peak) which are in agreement with Hexadecanoic acid, methyl ester [16, 17]. This compound has been reported to cause autolysis of membranous structures, induce significant aortic dilation, and inhibit phagocytic activity and nitric oxide production of certain cells [18, 19].

The peak at 17.023 min with Area (61.61%) on GC chromatogram produced molecular ion peaks *m/z* at 294 [M]+corresponding to formula $C_{19}H_{34}O_2$ in it MS spectra (Figure 4) in addition of fragment ions: 263, 109, 95, 81, 55, 41, 27and 67 (base peak) by direct comparison with the standard Mass Library spectral data and those reported in [20]. This compound was identified to be 9, 12 octadecadienoic acid (Z,Z), methyl ester which is known to possess antifungal potential. This fatty acid ester is implicated in human physiology and pathology; deficiency of linolate caused hair loss and poor wound healing in model animals [21].

There is another peak which appeared at 17.043 min with area (10.50%), its mass spectra produced molecular ion m/z at 296 [M]+ corresponding to molecular formula $C_{19}H_{36}O_2$ (Figure 5) as well as the following fragment ions: 263, 150, 95, 81, 55, 41, 27and 67 (base peak)

which are in agreement with 9-octadecenioc acid, methyl ester [22] which possess Antioxidant, anti-cancer. The GC chromatogram showed a peak at 17.223 min with area (8.41%); its Mass spectrum showed ions *m/z* 298 [M] + corresponding to formula $C_{19}H3_8O_2$ (Figure 6)**,** as well as the following fragment ions: 255, 143, 87, 57, 43, 41 and 74 (base peak) which are in good agreement with methyl stearate [23]. The chromatogram also showed two peaks at 18.767 and 18.970 min and their masses spectrum (Figure 7 and 8) produced molecular ion peaks *m/z* at 322 $[M]^+$, *m/z* at 324 $[M]^+$ and *m/z* at 326 $[M]^+$ respectively, corresponding to formula $C_{21}H_{40}O_2$ and $C_{21}H_{42}O_2$ which were in good agreement with cis-11-Eicosenoic acid, methyl ester and Eicosanoic acid, methyl ester, respectively.

According to DPPH scavenging activity, the seed oil showed moderately antioxidant potential 23±0.01 µg/mL activity comparable to that of Propyl Gallate (Standard) 89±0.01 µg/m against DPPH.

5. Conclusion

In the present study, nineteen chemical constituents in the *Sudanese Safflower (Carthamus tinctorius* L.*)* seeds oil were identified using GC-MS analysis. Among the identified compounds, seven were major; moreover, the anti-oxidant capacity gave a moderately activity than DPPH radical scavenging assay, suggesting its use in flavoring agent, food industry and medicinal purposes.

Abbreviation:

GC/MS: Gas Chromatography/Mass Spectrometry

DPPH: 2,2-diphenyl-1-picrylhydrazyl

TIC: entatively Identified Compounds

Conflict of Interest Statement:

The authors declare that they have no conflict of interest

Consent for publications:

All the authors read and approved the manuscript for publication.

Availability of data and material:

We declare that all the data is embedded in the manuscript

Authors' contributions:

All authors contributed in designed the idea, analyzed the data and wrote the manuscript.

Ethics approval and consent to participate:

No human or animals were used in the present study.

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