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GCMS profiling and and Antioxidant activity of *Carum carvi* and *Pimpinella anisum* used in Sudanese Ethnomedicine

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Abstract: In this study, the chemical constituents and the antioxidant activity of the crude ethanolic extract of *C. carvi* and *P. anisum* seeds were revealed. Where the chemical detection using GC/MS of *C. carvi* ethanolic extract showed the presence of eighteen compounds, and the main compounds were Oleic acid, n-Hexadecanoic acid, Ethyl oleate, 9,12-Octadecadienoic acid; while it did not showed antioxidant activity. Whereas, the chemical detection by GC/MS of the ethanolic extract of *P. anisum* showed the presence of nine compounds, and the main compounds were Oleic acid, Ethyl oleate, n-Hexadecanoic acid, 9,12-Octadecadienoic acid, in addition to it was given a high activity as an antioxidant.

Keywords: GC/MS, Antioxidant activity, *Carum carvi*, *Pimpinella anisum*, Sudanese ethnomedicine

Introduction

In the Sudan, medicinal plants have played an important role in the treatment of diseases especially in rural areas (Musa, 2009). Traditional medicine together with use of medicinal plants is an important part of the cultural heritage of Sudan (Khider, 2018). *Carum carvi* L is belonging to family Apiaceae and is one of the oldest cultivated herbs

in Asia, Africa and Europe. This plant is used as a repellent for stomach disorders, diarrhea and stomachache. In addition, *C. carvi* used in traditional Sudanese medicine and other folk medicines as a carminative, since it is effective against spasmodic gastrointestinal complaints, flatulence, irritable stomach, indigestion, lack of appetite, and dyspepsia in adults (Abdalaziz *et al.*, 2017). *Pimpinella anisum* L. belong to family Umbelliferae origin is mediterranean region. The major production area in Sudan is Northern Sudan, while there is a very limit production in Khartoum state. This plant used as flavouring, digestive, carminative, and relief of gastrointestinal spasms. Consumption of aniseed in lactating women increases milk and reliefs their infants from gastrointestinal problems (Salim *et al.*, 2016). Also anise seeds are used as analgesic in migraine and also as carminative, aromatic, disinfectant, and diuretic in traditional medicine (Shojaii and Fard, 2012). The aim of this study is to identify chemical Constituents by GCMs and to identify the antioxidant activity of ethanolic extracts for *Carum carvi* and *Pimpinella anisum*.

Material and Methods

Plant Samples

The seeds of two plants (*C. carvi* and *P. anisum*) were purchased from the local market in Khartoum. Moreover, it was classified in the herbarium of the Faculty of Pure and Applied Sciences at the International University of Africa. The seeds of *Carum carvi* and *Pimpinella anisum* were air-dried, coarsely powdered and were then extracted.

Preparation of Crude Extracts

50g were macerated in 500 ml of ethanol at room temperature with occasional shaking for 24 h at room temperature, the supernatant was decanted and clarity field by filtration through a filter paper, after filtration, the solvent was then removed under reduced pressure by rotary evaporator at 55°C. Then stored at 4°C in tightly sealed glass vial ready for use.

Gas Chromatography–Mass Spectrometry (GC-MS) analysis

GC-MS technique was used in this study to identify the phytocomponents present in the most active fractions. The tested extracts were analyzed by GC-MS using Shimadzu Mass Spectrometer-2010 series. 1 µL of sample was injected in GC-MS equipped with a split injector. The MS was operated in the electron ionization (EI) mode (70 eV). Helium was employed as the carrier gas and its flow rate was adjusted to 1.2 mL/min. The analytical column connected to the system was an Rtx-5 capillary column (length-30 m × 0.25 mm i.d., 0.25 µm film thickness). The column head pressure was adjusted

to 93.9 kPa. Column temperature programmed from 110 °C (7 min) to 200 °C at 10 °C/min and from 200-280 °C at 5 °C/min withholds time 0 and 9 min respectively. A solvent delay of 4.50 min was selected. Theinjector temperature was set at 250 °C. The GC-MS interface was maintained at 280 °C. The MS was operated in the ACQ mode scanning from m/z 40 to 550.0. In the full scan mode, EI mass spectra in the range of 40–550 (m/z) were recorded at electron energy of 70 eV. Compounds were identified by comparing mass spectra with library of the National Institute of Standard and Technology (NIST), USA/Wiley.

Antioxidant Activity

DPPH radical scavenging assay

The DPPH free radical scavenging activity Principle: The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments were carried out according using the method of Alhassan *et al.* (2018), with slight modification. Active samples can reduce the stable radical DPPH to the yellow-colored diphenyl- picrylhydrazine.

Assay

Test samples were allowed to react with 2.2 di (4-tretoctylphenyl)-1-picrylhydrazyl stable free radical (DPPH) for half an hour at 37oc in 96-wells plate. The concentration of DPPH was kept at (300µM). The test sample was dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage of radical scavenging activity of the sample was determined in comparison with a DMSO treated control. All tests were conducted in triplicate.

DPPH radical scavenging (%) = 100-{(Ac-At)/Ac} × 100

Where, At= Absorbance value of test compound; Ac=Absorbance value of control.

Result and Discussion

The crude extracted of *C. carvi*, *P. anisum* were investigated chemical compounds using GC-MS instrument, and the results were presented in Table (1 and 2). Where the GCMS analysis of ethanolic extract of *C. carvi* seeds revealed the presence of eighteen compounds, and the main compounds were Oleic acid, n-Hexadecanoic acid, Ethyl oleate, 9,12-Octadecadienoic acid and many other compounds were identified as low-level**.** GCMS analysis of ethanolic extract of *P. anisum* seeds also showed the presence of nine compounds. The main compounds were Oleic acid, Ethyl oleate, n-

Hexadecanoic acid, 9,12-Octadecadienoic acid and other compounds that were identified as low level. The study mainly observed the presence of fatty acid compounds (Oleic acid, n-Hexadecanoic acid, Ethyl oleate, 9,12-Octadecadienoic) for the ethanolic extracts of this plants. The study carried out by (Abdalaziz *et al*., 2017) reported the analysis of caraway seed oil by GC and its results were different from this study. In addition, the study carried out by (Meshkatalsadat *et al*., 2012) mentioned that main compound of caraway oil is Carvone, and this study agreed with the study carried out by (Abou El-Soud *et al*., 2014), as it differed with the current study. This difference is because the oil was extracted and analyzed in previous studies, while the crude ethanolic extract was used in this study. Alrasheid *et al*., (2018) also reported that the ethanol and chloroform extracts of anise seeds showed that there were forty three compounds and the main compounds were Butanoic acid, 2-methyl-, 2-methoxy-(4-propenyl) phenyl ester and Anethole. These results are also different from the current study. The study by Albulushi *et al*., (2014) of anise seed oil and its analysis by GC MS also showed different results as well as the results of this study.

Table (1): Results major compounds of crud extract of *C. carvi*

Peak	R.Time	Area %	Name	Chemical Formula	MW
1	5.864	3.91	Glycerin	$C_3H_8O_3$	92.09
2	7.781	2.19	Dodecerin	$C_{19}H_{12}O_8$	368.294
3	9.906	1.98	Estragole	$C_{10}H_{12}O$	148.2
4	11.674	0.23	Anethole	$C_{10}H_{12}O$	148.2
5	11.981	0.39	Undecanal	$C_{11}H_{22}O$	170.29
6	12.221	0.09	2,4-Decadienal, (E,E)-	$C_{10}H_{16}O$	152.23
7	12.778	0.40	1,2-Cyclohexanediol,1-methl-4(1-methy	$C_{10}H_{18}O_2$	170.25
8	14.839	1.82	1,3-propanediol, 2-(hydroxymethyl)-2-nitr	$C_4H_9NO_5$	151.1180
9	16.727	4.09	8-Hexadecenal, 14-methyl-, (Z)-	$C_{17}H_{32}O$	252.4
10	19.803	0.95	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37
11	22.328	19.13	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
12	22.567	4.70	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.5
13	24.125	6.11	9,12-Octadecadienoic acid(Z,Z)-	$C_{18}H_{32}O_2$	280.4
14	24.278	39.70	Oleic Acid	$C_{18}H_{34}O_2$	282.5
15	24.358	7.26	Ethyl Oleate	$C_{20}H_{38}O_2$	310.5
16	24.582	2.27	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.5304
17	25.983	3.78	2H-pyran,2-(2-heptadecynyloxy)tetrahyd	$C_{22}H_{40}O_2$	336.6
18	26.892	0.99	N1-Isopropyl-2-methyl-1,2propanediamir	$C_7H_{18}N_2$	130.23

Table (2): GC-MS analysis major compounds of crud extract of *P. anisum*

Peak	R.Time	Area %	Name	Chemical Formula	MW
1	7.671	0.28	L-Fenchone	$C_{10}H_{16}O$	152.23
2	9.910	8.64	Estragole	$C_{10}H_{12}O$	148.2
3	11.701	0.50	Anethole	$C_{10}H_{12}O$	148.2
4	22.292	16.22	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
5	22.565	3.62	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.5
6	24.135	10.47	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280.4
7	24.204	35.55	Oleic Acid	$C_{18}H_{34}O_2$	282.5
8	24.300	5.26	Linoleic acid ethyl ester	$C_{20}H_{34}O_2$	306.5
9	24.346	19.47	Ethyl Oleate	$C_{20}H_{38}O_2$	310.5

The antioxidant activity results of *C. carvi* and *P. anisum* ethanolic extracts are shown in table (3). Where the highest activity of the ethanolic extract of P. *anisum* (70±0.04), while *C. carvi* ethanolic extract was lower activity (15±0.02), in compared to the standard propylgallate of 95±0.01. The study conducted by Trifan *et al*., (2016) showed that caraway seed oil has high potency against oxidation. The study conducted by Ahmed and Aly (2019) also showed that there is a high efficacy of the ethanol extracts of anise seeds at different concentrations, and these results are different from the results of this study. This difference is
attributed to the different extraction method and the different concentrations used in the two studies. There are compounds detected by GCMS in caraway seed ethanolic extract in large proportions that have antioxidant activity.

Table (3) Antioxidant Activity Results of *Carum carvi* and *Pimpinella anisum* ethanolic extracts

No.	Samples	%RSA±SD(DPPH)
1	*P. anisum*	70±0.04
2	*C. carvi*	15±0.02
Standard	Propyl Gallate	95±0.01

Conclusion:

This study revealed chemical contents of ethanolic extracts of *Carum carvi* and *Pimpinella anisum*, as well as that *Carum carvi* has high antioxidant activity.

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