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Phytochemical screening, chemical composition and antioxidant activity of seeds essential oil of *Coriandrum sativum* L. from the Sudan

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Abstract
In this research, the *Coriandrum sativum* L. seeds were extracted using petroleum ether, *n*-hexane, ethanol, methanol and aqueous solvents through maceration or soxhlet extraction method. The preliminary phytochemical screening of the polar extracts of ethanol, methanol and aqueous of *C. sativum* seeds revealed the presence of saponins, tannins and cardenolides in all three extracts and the absence of triterpenes, anthraquinones and leucoanthocynins in all three extracts. The essential oil of the seeds of *Coriandrum sativum* was extracted using steam distillation and analyzed by gas chromatography-mass spectrometry (GC-MS). It contained forty nine compounds including linalool (66.7%) as a major constituent. The other important components identified were; γ-terpinene (4.45%), decanal (4.44%), geranyl acetate (2.14%), α-pinene (1.04%) and limonene (0.86%). The antioxidant activity using DPPH radical-scavenging method was applied for essential oil of *C. sativum* seeds. Our results showed that the oil of *C. sativum* has an effective antioxidant activity.

Keywords: *Coriandrum sativum*, Sudan, essential oil, antioxidant activity

1. Introduction
C. sativum is an annual herb, originating from the Mediterranean countries, belongs to the Apiaceae family. This herb is cultivated commercially in Europe, Asia and Africa. It is one of miraculous herb that functions as both spice and herbal medicine [1]. The fruit of *C. sativum* is a diuretic, antipyretic, stomachic, aphrodisiac stimulant, laxative, anathematic and cures biliousness, bronchitis and vomiting. The leaves are hypotonic, analgesic, and useful in hiccough, suppuration, piles, inflammation, toothache, jaundice, scabies and gland tuberculosis. *C. sativum* seeds showed a significant hypoglycemic action on rats [2].
All parts of the plant are edible but the fresh leaf and the dried seeds are the most common parts used in cooking. In the Indian traditional medicine, *C. sativum* is used in treating the disorders of digestive, respiratory and urinary systems, as it has diaphoretic, diuretic, carminative and stimulant activity. In Iranian traditional medicine, *C. sativum* has been indicated for a number of medical problems such as dyspeptic complaints, loss of appetite, convulsion, anxiety and insomnia [3]. Major active constituents of *C. sativum* are essential oils and fatty oil. The weight of essential oil content of ripe and dried fruits of *C. sativum* vary between 0.03% and 2.6% whereas the content of fatty oil varies between 9.9% and 27.7% respectively. The previous studies showed that the chemical composition of *C. sativum* varies considerably with variety, region and age of the product. The content of volatile oil of the seeds may be as high as 8%.
The volatile oil contains about α-pinene (1.5%), β-pinene (0.2%), sabinene (2.8%), myrcene (1.6%), α-phellandrene (0.2%), limonene (11.6%), 1,8-cineole (36.3%), γ-terpinene (0.7%), terpinolene (0.5%), linalool (3%), linalyl acetate (2.5%), terpinene (0.9%), α-terpineol (2.6%), α-terpinyl acetate (31.3%), citronellol (0.3%), nerd (0.5%), geraniol (0.5%), methyl eugenol (0.2%) and *trans*-nerolidol (2.7%) [4].
The basic *C. sativum* aroma produced by a combination of the major components, 1,8-cineole and α-terpinyl acetate[4]. *C. sativum* oil is used in food, perfumery, flavor and carminative. In medicine, it is used as a powerful aromatic, antiseptic, stimulant, carminative, stomachic, expectorant, antispasmodic and diuretic [4]. The physiochemical properties of *C. sativum* essential oil such as density (25 ^{0}C), acid value, ester value, and optical rotation (25 ^{0}C) were found to be 0.8310, 1.4592, 4.0, 23.7 and +11.5 g/cm^3 respectively [5].

1.1 Pharmacological properties and traditional uses

C. sativum has been reported to possess many pharmacological activities like antioxidant [6], antidiabetic [7], anti-mutagenic [8], anti-lipidemic [9], anti-spasmodic[10]. The aqueous extract of *C. sativum* seed possesses diuretic and saluretic activity [3]. Bhat and his co-workers reported that *C. sativum* has high antioxidant activity since it is a good source of polyphenols and phytochemicals [1]. The leaf of *C. sativum* contains high concentrations of antioxidants than seeds.

The antioxidant ability of Coriander is attributed to the high content of pigments specially carotenoids. In recent years, essential oils have been qualified as natural antioxidants [11]. The anticonvulsant effects of aqueous and ethanolic extracts of *C. sativum* seeds were studied in order to evaluate the folkloric use of this plant species [3]. The aqueous and hydroalcoholic extracts and essential oil of *C. sativum* seeds possess sedative and hypnotic activity [3]. The well-diffusion technique was employed. The aqueous infusion and decoction of *C. sativum* were examined against 186 gram positive bacterial isolates and two isolates of *Candida albicans* didn't show any antimicrobial activity [3].

The anti-mutagenic activity of *C. sativum* juice was investigated using the Ames reversion mutagenicity assay (his- to his+) with the *S. typhimurium* TA strain as indicator organism. *In vitro* anthelmintic activities of crude aqueous and hydro-alcoholic extracts of the seeds of *C. sativum* (Apiaceae) were investigated on the egg and adult nematode parasite *Haemonchus contortus*. Both extract types of *C. sativum* inhibited hatching of eggs completely [3].

C. sativum plays a protective role against the deleterious effect in lipid metabolism in experimental colon cancer. Some of the acids present in *C. sativum* such as linoleic, oleic, palmitic, stearic and ascorbic acid are very effective in reducing the cholesterol level in the blood. They also reduce the cholesterol deposition along the inner walls of the arteries and veins [12].

C. sativum has metal detoxification ability. It can be used as a natural cleansing agent as it has potential to remove toxic metals from body. This effect was due to the binding effect of carboxylic group to mercury. The chemical compounds present in *C. sativum* attached to toxic metals and removed them from cells. Bhat and his co-workers observed that this plant is very effective to remove mercury ion (Hg^{2+}) and methyl mercury ($HgCH_3$) from aqueous solutions [1]. These results clearly showed that sorbent can be used to remove inorganic and methyl mercury from contaminated water [13].

1.2 Objectives of this study

The objective of this study was to determine *C. sativum* essential oil composition by GC-MS and to determine the physiochemical properties of *C. sativum* oil that extracted using petroleum ether and *n*-hexane solvents. The phytochemical screenings of the polar solvent extracts were evaluated. Also, to evaluate the antioxidant activity of the *C. Sativum* seed extract.

2. Materials and methods

2.1 Plant materials

The seeds of *C. sativum* were purchased from the local market at Al-Nuba city, Gezira state, Sudan.

2.2 Instrumentation

The GC-MS Spectra were recorded on Mass Spectrometer Shimadzu, GCMS-QP2010 Ultra (Japan).

2.3 GC-MS analyses of essential oils

2.3.1 GC-MS analysis

The essential oils from seeds of *C. sativum* were analyzed by GC-MS electron impact ionization method on GC-17A gas chromatograph (Shimadzu) coupled to a GC-MS QP 5050A mass spectrometer (Shimadzu); fused silica capillary column (30mm x 0.25mm; 0.25 mm film thickness), coated with DB-5 (J&W); column temperature 100 ^{0}C (2 min) to 250 ^{0}C at the rate of 3 ^{0}C/min; carrier gas, helium at constant pressure of 90 Kpa. Acquisition parameters full scan; scan range 40-350 amu.

2.3.2 Compounds identification

The compounds identification was done by comparing the NIST library data of the peaks with those reported in literature. The percentage composition was computed from GC-MS peak areas on DB-5 column without applying correction factors.

2.4 DPPH free radical scavenging assay

The antioxidant activity of the seed plant extracts was assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by adopting modified method of Braca[14]. The diluted working solutions of the extracts were prepared in dimethyl sulfoxide (DMSO). A solution of 0.004% of DPPH was prepared in methanol, and 1.0 mL of this solution was mixed with 1.0 ml of both sample and standard solutions separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using spectrophotometer. The mixture of DMSO (1.0 mL) and DPPH solution (1.0 mL) was used as a blank. The optical density was recorded and the inhibition percentage was calculated using the formula given below:

Inhibition percentage of DPPH activity (%) = [(A-B)/A)] *100

Where A= Absorbance of the blank solution and B= Absorbance of the tested solution.

3. Results and discussion

3.1 Extraction and phytochemical screening of *C. sativum* seeds

The extraction of *C. sativum* seed was performed using solvents of different polarity as well as different extraction methods. Two major extraction techniques were used to obtain essential oil from *C. sativum* seeds namely steam distillation and organic solvent extraction (soxhlet). Each technique has its benefits and drawbacks as far as operating cost, yield, quality and quantity of the extracts. The powder of *C. sativum* seed was extracted separately and successively with petroleum-ether, chloroform and methanol using continuous Soxhlet technique in order to fractionate the crude extract into different groups made it possible to determine which fraction is responsible for specific biological activity. The petroleum-ether extract gave the highest yield (8.82% w/w) followed by chloroform (7.07% w/w) and finally methanol (6.72% w/w).

The preliminary phytochemical screening of *C. sativum* seeds crude extracts (ethanol, methanol and aqueous) were carefully carried out using the prescribed methods [16]. The results presented in (Table 1) summarize the classes of natural compounds present.

Table 1: Preliminary phytochemical screening of *Coriandrum sativum* seed extracts

Class of compounds	Aqueous extract	Ethanol extract	Methanol extract
Triterpenes	_	_	_
Unsaturated sterols	_	++	+
Saturated sterols	_	_	++
Saponin (Forth)	+	+	+
2-Deoxy sugars	_	+++	+++
Cardenolides (Balajact)	+	+	+
Flavonols	_	+	_
Flavones	_	++	++
Cyanidin	_	+	_
Leucoanthocyanins	_	_	_
Tanins (gelatin solution)	–	–	–
Tanins ($FeCl_3$ solution)	+++	+++	+
Anthraquinones	_	_	_
Alkaloids (Mayer's method) method)	+	–	–
Alkaloids (Wagner's method)	++	–	–
Coumarins	_	+	+
Carbohydrate	_	+++	+++
Reducing compound	_	++	+++

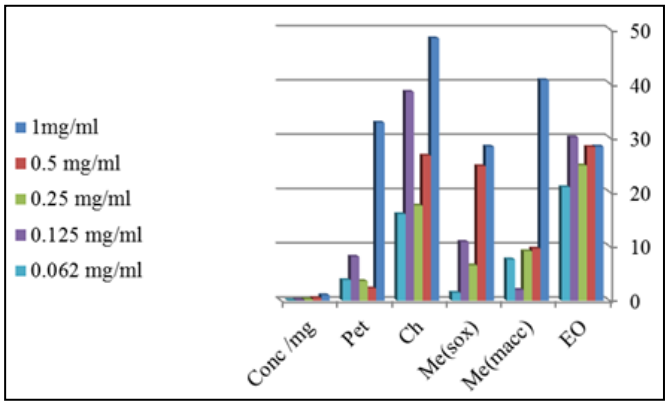
The current preliminary phytochemical screening showed the presence of saponins, cardenolides and tannins in all three crude extracts (methanol, ethanol and water) while triterpenes, anthraquinones and leucoanthocyanin were absent in all crude extracts. Other classes of natural products such as unsaturated sterols, flavones, flavonoids, coumarins, reducing compounds and carbohydrates were found in both methanol and ethanol extracts. Alkaloids were detected in the aqueous extract only. In general, the preliminary phytochemical screening of the crude extracts of *C. sativum* showed that the seeds were rich in saturated and unsaturated sterols, sugars, flavones, tannins and carbohydrates.

3.2. DPPH radical-scavenging activity of *C. sativum* seed extracts

Different concentrations of the various *C. sativum* extracts were carefully assessed for their antioxidant activity and the results have been shown in (Table 2).

Table 2: Evaluation of antioxidant activity of *C. sativum* seed extracts

EO	Me(mac)	Me(sox)	Chl	Pet-ether	Conc. mg/mL
28.4	40.6	28.36	48.3	32.8	1.0
28.3	9.57	24.76	26.7	2.25	0.5
24.9	9.1	6.49	17.53	3.59	0.25
30.1	1.98	10.82	38.45	8.1	0.125
20.9	7.59	1.44	15.94	3.8	0.0625



Abbreviations: pet =petroleum ether chl =chloroform Me (sox) =Methanol soxhlet Me (mac) = Methanol maceration EO = Essential oil.

The petroleum-ether, chloroform and methanol soxhlet extracts were evaluated for their *in vitro* antioxidant activity using DPPH radical-scavenging assay. The DPPH radical-scavenging activity of seeds crude extracts were concentration dependent. The maximum antioxidant effect was observed in the highest concentration (1.0mg/mL) of all tested samples except essential oil whereas the maximum antioxidant effect observed at low concentration (0.125mg/mL). The concentration (0.125mg/mL) has the highest inhibition percentage than concentration (0.0625mg/mL). Accordingly, the chloroform extract of seed exhibited high antioxidant activity with inhibition percentage of 48.3% followed by methanol (mac) extract with inhibition percentage of 40.6% then the petroleum-ether extract exhibited medium antioxidant activity with inhibition percentage of 32.8% and methanol (sox) extract shown as a weak antioxidant activity with inhibition percentage of 28.36%.

The essential oil exhibited medium antioxidant activity with inhibition percentage of 30.1%. The results showed that these extracts are effective as antioxidants. The highest activity percentage (about 48%) was shown by the chloroform (sox) extract of *C. sativum* seeds followed by methanol (mac) (40.6%), petroleum ether (32%), essential oil (30.1%) and methanol (sox) extracts (28%).

In present study, the chemical composition of *C. sativum* essential oil afforded forty nine compounds with linalool (66.7%) as a major constituent. The other important components identified were; γ-terpinene (4.45%), decanal (4.44%), geranyl acetate (2.14%), α-pinene (1.04%) and limonene (0.86%). The physiochemical properties (iodine value, IV, saponification value, SV, and acid value, AV) of *C. sativum* oil extracted by using soxhlet with two solvents namely: *n*-hexane and petroleum-ether were evaluated. The results were showed in (Table 3).

Table 3: Physicochemical properties of pet-ether (60-80 ^{0}C) and *n*-hexane extracts (soxhlet)

Physiochemical property	petroleum ether extract	*n*-hexane extract
Oil yield %	8.82%	10.65%
Odour	Pleasant	Pleasant
Color	Spicy	Spicy
Taste	Greenish brown	Greenish brown
Acid value	9.9896	8.1699
Iodine value	44.0	85.9
saponification value	297.33	259.72
Ester value	287.3404	251.5501

The *C. sativum* seed (petroleum-ether 60-80 ^{0}C, *n*-hexane) extracts were analyzed for physicochemical characteristics namely color, odor, acid value, iodine value, free fatty acid, saponification value and ester value. The results showed that there are simple different between petroleum ether and *n*-hexane extracts in all physicochemical properties except iodine value, the difference is large between petroleum ether and *n*-hexane extract and this is may be attributed to solvent used in extraction or extraction method. The slightly variations in physicochemical properties may be attributed to some factors such as type and origin of cultivar, seed maturity and harvest, storage conditions and method of extraction can influence such properties [5].

4. Conclusions

The essential oil obtained by hydro-distillation was studied using GC-MS to know its constituents and antioxidant activity. Essential oil and especially its main component, linalool is an extremely important raw material in the perfume and cosmetic products [15]. The present literature supports the potential of *C. sativum* as a medicinal tree. In view of the nature of the plant, more research can be done to investigate the unexplored and unexploited potential of this plant [16]. The antioxidant activity of each extracts was determined. It was clear from the antioxidant tests, the chloroform solvent of choice increases of the antioxidant activity; due to its ability to remove all the active antioxidant compounds.

The preliminary phytochemical screening of the active morphological sample is extremely valuable in giving information about the nature of constituents found in each plant sample. Serious attempts have been made to find out the best organic solvent that can extract the antioxidant active components from the (petroleum-ether, chloroform and ethanol) were tried and bioassayed. Forty nine compounds in *C. sativum* essential oil were separated and identified by GC-MS. Phytochemical studies on *C. sativum* seeds extracts illustrated the presence of sterols and flavonoids, tannins, coumarins, cardenolides and saponins. Chloroform as a solvent of choice in this study (made good candidates of natural antioxidant). The physicochemical properties of hexane and petroleum ether extracts were determined and compared with physicochemical properties of essential oil. The results of this study are promising because; petroleum ether, chloroform and methanolic extracts of seed have a considerable antioxidant activity.

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