Phytochemicals and biological activities of caraway (*Carumcarvi* L.) essential oil

Mohamed F.A. El-Rady[a,b], Nagwa M.H. Rasmy[a], Nessrien M. Yasin[a], Hanea A. Fahmy[b], Ahmed Amer[c]

[a]Department of Food Science, Faculty of Agriculture, Ain Shams University, Cairo, [b]Department of Special Food and Nutrition Research, Food Technology Research Institute, Agriculture Research Center, Giza, [c]Department of Plant Biotechnology, Biotechnology Research Institute, National Research Center, Giza, Egypt

Correspondence to Mohamed F.A. El-Rady, MSc, 9 El-Gamaa Street, Giza, Egypt.
Tel: +20 114 865 8344;
e-mail: mfawzy1981m@yahoo.com

Received: 20 October 2022
Revised: 21 November 2022
Accepted: 21 November 2022
Published: 27 June 2023

Egyptian Pharmaceutical Journal 2023, 22:285–293

Background and aim
Caraway is a famous medicinal plant in various pharmaceutical, food, and cosmetic industries. This study aimed to investigate the chemical composition, antioxidant, antimicrobial, and anticancer activities of this plant's essential oil (EO).
Materials and methods
Caraway EO was obtained from dried caraway seeds using the hydrodistillation process. The composition of caraway EO was inspected by gas chromatography-mass spectrometry (GC–MS) analyses. The antioxidant activity of caraway EO was determined by three different *in vitro* antioxidant assays: 2,2-diphenylpicrylhydrazyl ($DPPH^{\bullet}$), 2,2'-azino-bis3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) scavenging activity and reducing power. The agar well diffusion method was used to assess the antimicrobial action. The cytotoxic activity was evaluated using the MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay, and the data were expressed as the half-maximal inhibitory concentration (IC_{50}).
Results and conclusion
Carvone was the major compound of caraway EO, followed by limonene. Estimation of the antioxidant activity using $DPPH^{\bullet}$ scavenging activity, $ABTS^{\bullet+}$ scavenging activity, and reducing power assays revealed effective efficacy [IC_{50}=32.46±0.75, 2.44±0.44, and 17.65±0.70 μg/ml, respectively, compared with 11.55±0.53, 1.50±0.29, and 23.19±0.78 μg/ml for standard control (butylated hydroxyanisole), respectively]. Strong anticancer activity was detected against all types of cancer cells, especially the colon cell line (HCT-116) and liver cell line (HepG-2). These results suggest that caraway EO can be used as a preservative food agent in food industries as well as in the field of pharmacy, as it presents promising anticancer properties.

Keywords:
antimicrobial, antioxidant, caraway, carvone, cytotoxicity, preservative

Egypt Pharmaceut J 22:285–293

1687-4315

Introduction

Plant-derived molecules have long been recognized in medicine and pharmacy [1] for their wide range of biological activities, including antioxidant [2], antimicrobial [3,4], cytotoxicity [5,45], anti-acetylcholinesterase [6,7], and anti-inflammatory [5]. The number and types of these compounds vary among species and individuals within the same plant group [8]. They shield plants from biotic (such as bacteria, fungi, nematodes, insects, or animal grazing) and abiotic (such as heat, salinity, drought, and heavy metal) stresses. Owing to their high economic worth, humans employ them mostly as chemicals for medications, flavors, perfumes, insecticides, and colors [9]. Essential oils (EOs) are potent plant-based medicines that have long been used to both prevent and treat a wide range of illnesses [10]. In terms of chemistry, herbs' secondary metabolites, including EOs, serve various functions, such as defense against herbivores, pests, and bacteria that interact with other plants of the same species and signal inside the plant in response to external stimuli [11]. To protect itself from a certain predator or set of predators, every plant species or subspecies creates its own 'signature' blend of EO chemical components [12]. In various fields, including the pharmaceutical, cosmetic, and food sectors, plant EOs and the bioactive elements they contain that are derived from spices and herbs are gaining more and more attention [13,14]. EOs with a high potential for scavenging free radicals may help prevent diseases, including cancer, cardiovascular disease, cognitive dysfunction, and ageing of the immune system, along with the dramatically increased consumer interest in using natural remedies to cure a variety of conditions [2,15]. Many studies have shown the

benefits of using EOs from culinary herbs, which are frequently regarded as an important part of health care, with exceptional safety and no adverse effects [16].

Carum carvi L., also known as caraway, is a biennial plant species in the Apiaceae family with fine, ribbed stems. The umbels have oval-shaped leaves and white or occasionally pink blooms. Its height is scarcely more than 60–75 cm, and its seeds have a diameter of 5 ml. *C. carvi L* contains D-carvone-rich EO, fatty oils, and polysaccharides [6]. This plant has a long history of use in traditional medicine for various conditions, including digestive distress, gas, loss of appetite, bloating, heartburn, and moderate stomach and intestine spasms [17]. Caraway seeds have been shown to have various medicinal benefits: antibacterial properties, analgesic, anti-inflammatory, anti-anxiety, anti-hyperglycemic, and anti-spasmodic. It has also been used to treat hysteria, flatulent indigestion, diarrhea, and dyspepsia [18]. There are 25 species in the genus *Carum*, but only the annual and biannual caraway is used commercially as a spice, appetizer, and carminative in the food and medicinal industries. According to Olennikov and Kashchenko [19], EOs (3–7%), fatty acids (10–18%), proteins (20%), carbohydrates (15%), phenols, and flavonoid chemicals are all present in *C. carvi* seeds [20]. *C. carvi* extracts contained terpenoids, alkaloids, and tannins [21]. Caraway oils are present in every part of the plant, but when extracted from the seeds using a hydrodistillation technology, the seeds contain the maximum oil concentration. Approximately 95% of an EO's components comprise the two primary ingredients, carvone and limonene. D-carvone (50–65%) and (þ)-limonene (up to 45%) are the two main EOs found in caraway seeds, which have an overall EO content of 3%. Caraway's primary chemical, D-carvone, gives it its distinctive aroma [22]. Additionally, caraway EO has been demonstrated to have cancer-chemopreventive properties against dimethylhydrazine-induced colon premalignant damage [23]. Caraway EO's antimicrobial, antioxidant, anti-acetylcholinesterase, and antidiabetic properties have also been reported by Dadkhah *et al.* [24]. According to Laribi *et al.* [25], carvone, limonene, b-myrcene, and a-selinene made up most of the Egyptian chemotype's chemical components (61.6, 29.1, 3.9, and 10.9%, respectively). Owing to their active antioxidant components, such as phenolic compounds, which are substantially more significant than the typical antioxidant compounds, and their antibacterial properties, caraway seeds have antioxidant properties [26]. Therefore, this work aimed to study the phytochemicals of Egyptian caraway (*C. carvi L.*) EO extracted by a hydrodistillation method. The antioxidant activity of caraway EO has been evaluated *in vitro* using different assays. The antimicrobial activity of caraway EO on some pathogenic and spoilage microorganisms and anticancer effect against three cells [colon cell line (HCT-116), liver cell line (HepG-2), and Caucasian breast adenocarcinoma (MCF-7)] were also studied.

Materials and methods

Overall, 5 kg of caraway seeds (*C. carvi L.)* was purchased from the Medicinal and Aromatic Plants Research Department of Horticulture Institute, Agriculture Research Center, Dokki, Giza, Egypt. The media were purchased from Hi-Media and Difco. All chemicals were of analytical grade and were purchased from Sigma-Aldrich.

Extraction of caraway essential oil

The mature and healthy caraway seeds were carefully cleaned in tap water and then drained in an oven set to a specific temperature (40°C). The dried seeds were ground into a fine powder to extract the EO. Following Maisonneuve's [27] recommended procedure, the EO was extracted using a hydrodistillation method and a Clevenger's type apparatus. A light yellow oil with a pleasant aroma was dried using the least amount of anhydrous sodium sulfate to remove any moisture. To be used later, the obtained EO was stored in 250-ml dark bottles in a freezer at 4°C.

Chemical analysis of caraway essential oil

An Agilent type 7890 A gas chromatograph (GC) outfitted with an Agilent 5975C mass spectrometer (MS) and a DB-5MS capillary column was used to analyze caraway EO (30 m0.25 mm, 0.25 μm film thickness). From 35 to 280°C, the oven temperature was increased at a rate of 10 °C/min (kept isothermally for 7.5 min). Other characteristics of the analysis system were as follows: inlet temperature of 250°C, a split ratio of 50 : 1, carrier gas of helium (flow rate of 1 ml/min), and EO injection volume of 0.2 μl. Caraway EO was analyzed by GC using an Agilent 6890 GC fitted with a flame ionization detector. The column and GC conditions were identical to those described previously. Individual EO components were identified by comparing their retention indices (RI) relative to (C_8–C_{20}) n-alkanes with those published in the literature and by comparing their recorded mass spectra with those in the Wiley 275 mass spectral library from the GC–MS database. The electronic integration of the FID peak areas yielded quantifiable data [28].

In vitro antioxidant activity analysis

Three methods assessed the antioxidant activity of caraway EO: DPPH radical and ABTS radical cation scavenging assays and reducing power test.

Caraway EO was diluted with methanol, and each assay's concentration range is mentioned. Butylated hydroxyanisole (BHA) was used as a positive control. All spectrophotometric measurements were conducted using an ultraviolet spectrophotometer.

DPPH radical scavenging assay

According to the approach proposed by Mighri *et al.* [29], DPPH radical scavenging activity was evaluated. Overall, 1 ml of each EO dilution in methanol (290–4640 μg/ml) was combined with 1 ml of DPPH methanolic solution (0.04%, wt/vol). The mixtures were vortexed and left in the dark at room temperature for 30 min. The absorbance was then determined at 517 nm. The following equation was used to determine the percentage of DPPH radical scavenging:

$$100 \times [(A_0 - A_t)/A_0]$$

Where A_0 represents the absorbance of the blank and A_t represents the absorbance when an EO or positive control is present.

ABTS radical cation scavenging assay

The test was conducted as previously described by Re *et al.* [30]. Equal amounts of ABTS aqueous solution (7 mM) and potassium persulfate were combined to produce ABTS radical cation ($ABTS^+$) (2.45 mM). Before use, the mixture was stored in the dark at room temperature for 12–16 h. $ABTS^{\cdot+}$ solution was diluted with methanol to an absorbance of 0.7, 0.02 at 734 nm before testing. Overall, 0.02 ml of each EO dilution in methanol (concentrations ranging from 29 to 464 mg/ml) was combined with 1.98 ml of $ABTS^+$ solution. After 6 min of treatment, the 734-nm absorbance was measured. $ABTS^+$ scavenging activity (percent) was computed using the following formula:

$$100 \times \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}}\right],$$

A_{control} is the absorbance of the control, and A_{sample} is the absorbance with EO/positive control present.

Reducing power assay

Oyaizu's method [31] was used to conduct the assay. Overall, 500 μl of each EO methanolic dilution (0.52–4.16 mg/ml), 1.2 ml of 0.2 M sodium phosphate buffer (pH 6.6), and 1% potassium ferricyanide were combined (1.5 ml), and then incubated for 20 min at 50°C. Thereafter, 10% trichloroacetic acid (1.25 ml) was added to the mixture after cooling, and the mixture was then centrifuged for 10 min at 3000 rpm. Ultrapure water and 0.1% (w/v) ferric chloride were added to the solution's upper layer (1.25 ml) (0.25 ml). The absorbance at 700 nm was determined after 10 min. A combination with a high absorption rate has a potent lowering capacity. BHA was used as a positive control in all antioxidant experiments. Results were expressed as IC_{50} values (μg/ml), which were calculated by linear interpolation between values above and below 50% activity, with the exception of the reducing power assay, in which the IC_{50} value represents the concentration of EO/positive control that results in an absorbance of 0.5.

Antimicrobial activity of caraway essential oil

Microorganism strains

The antimicrobial activity of caraway EO was studied against five bacterial cultures (*Escherichia coli ATCC10536*, *Staphylococcus aureus ATCC 9027*, *Salmonella* sp. *ATCC14028.*, *Bacillus cereus ATCC14579*, and *Listeria monocytogenes ATCC4957)* and two fungal culture (*Aspergillus niger NRRL595*, and *Candida sp. MTCC183*). Several microorganisms were collected from the microbiology department of the NCRRT, Egyptian Atomic Energy Authority, Cairo, Egypt.

Determination of antimicrobial activity by the agar-well diffusion method

The emulsifying agent Tween 80 was used to create an emulsion of 10% w/w caraway EOs. The resulting emulsion was sterilized by filtration via 0.45-μm hydrophilic membrane filters and kept at 4°C in a tightly closed sterile container.

Antibacterial activity was determined using the agar-well diffusion method [32]. Overall, 50-ml pieces of molten sterile nutrient agar (NA) and potato dextrose agar maintained at 50°C were inoculated with 100 μl of adequately diluted and thoroughly mixed with active microbial inoculum. The inoculated mixture was put into a 15-cm sterile Petri dish and left to solidify. Using a sterile cork borer, 6-mm wells were cut through the agar, and the agar was removed, leaving wells filled with 10% EO emulsion. After ~2 h at room temperature, the plates were incubated at 30–35°C for 24 h for bacteria and 48 h for yeast. The resulting inhibition zones have been measured, and the average values have been calculated. In the same settings as the tested oil, the standard medications gentamicin (10 μg/well) for bacteria and nystatin (100 IU) for mold and

yeast were employed as positive controls as standard drugs.

Determination of minimum inhibitory and bactericidal concentrations

Hasika *et al.* [33] used the broth dilution method to assess the least inhibitory concentration [minimum inhibitory concentration (MIC)] and the minimum bactericidal and fungicidal concentrations (MBC and MFC) of caraway EO. For establishing the MIC, the EOs with the highest antibacterial activity in the agar-well diffusion assay were chosen. A loop was used to collect one colony of each microbial strain, which was subsequently inoculated into 25 ml of broth medium. After 18–24 h of incubation at 37°C, 10^9 CFU/ml of bacterial suspension was obtained. Each stock solution was diluted with buffered peptone water (Oxoid) to yield suspended bacterial cultures at a concentration of 10^5 CFU/ml. In a test tube, 0.5–5 µl/ml dilutions of EOs in broth medium were combined with bacterial suspensions to yield a volume of 4 ml and a final concentration of around 5×10^4 CFU/ml. Earlier specified temperatures were used to incubate the final solutions. The MIC is the lowest concentration of EO that inhibits observable microbial growth. The MBC and MFC were measured by subculturing 100 µl from each negative test tube onto PCA plates. MBC or MFC was defined as the lowest concentration yielding a negative subculture or a single colony following incubation. The experiments were conducted in duplicate four times.

Determination of in vitro cytotoxicity by MTT assay

MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) was used to investigate the cytotoxicity of caraway EO on colon cell line (HCT-116), liver cell line (HepG-2), Caucasian breast adenocarcinoma (MCF-7), and normal kidney cells (VERO) [34]. In 96-well plates, 1×10^5 cells were seeded in 0.2 ml of medium in each well. Following incubation, the media from the wells was removed carefully for the MTT experiment. Each well was rinsed two to three times with MEM (without FCS), and then 200 µl of MTT (5 mg/ml) was added. In a 5% CO_2 incubator, the plates were incubated for 6–7 h to test for cytotoxicity. After incubation, 1 ml of DMSO (a solubilizing agent) to each well was added, micropipette-mixed, and left for 45 s. Owing to forming of formazan crystals, the presence of live cells was shown by developing a purple hue. The suspension was placed in a spectrophotometer cuvette, and the OD (optical density) readings were measured at 595 nm using DMSO as a blank. The concentration required for a 50% inhibition of viability (IC_{50}) was visually estimated after measurements were conducted. Using the concentration of the EO on the X-axis and relative cell viability on the Y-axis, a Standard Graph was constructed.

$$\text{Cell viability}(\%) = \text{Mean OD}/\text{Control OD} \times 100$$

Where mean OD is the mean of optical density for different concentrations of EO on treated cells and control OD is the mean of optical density for different concentrations of EO on control cells.

Statistical analysis

Analysis was done using the statistical analysis system software for Windows (Statistical Analysis System, Version 9.1.3, SAS Institute Inc. Cary, NC, USA) [9]. The data were given as mean±SD. Analysis of variance was used for statistical analysis, and Duncan's multiple range tests were used to compare the experimental findings ($P\geq0.05$) [35].

Results and discussion

Chemical composition of caraway essential oil

The GC analysis of caraway EO revealed the presence of 25 compounds, representing 99.33% of the EO's total composition (Table 1). Carvone (56.52%) was the major compound of caraway EO, followed by limonene (39.49%). The chemical class characterization showed that caraway EO is mainly composed of monoterpene ketones (56.71%), represented by carvone and camphor, and monocyclic terpenes (39.63%), with limonene as the main constituent, followed by small proportions of groups of bicyclic terpenes, aliphatic hydrocarbon, aromatic hydrocarbon, oxides, alcohols, esters, aldehydes, sesquiterpene, and unknown compounds. These findings are consistent with those of Siwar *et al.* [36], who discovered that oxygenated monoterpenes (59.6%), monoterpenehydrocarbons (39%), and phenylpropanoids (0.1%) were the main components of caraway EO. The most prevalent chemicals were carvone (58.2%) and limonene (38.5%).

In vitro antioxidant activity of caraway essential oil

Antioxidant activity is an extraordinarily complex mechanism, encompassing a variety of processes, including free radical scavenging, inhibition of hydrogen abstraction, reducing capacity, and binding of transition metal ions [37]. EOs are complex combinations of bioactive molecules with multifunctional capabilities as their functional groups and chemical activity vary [33]. Therefore, the antioxidant activity of caraway EO was using three different *in vitro* antioxidant assays to evaluate

Table 1 Gas chromatography-mass spectrometry analysis of caraway essential oil

Serial no.	Chemical classes	Compound	Percentage %
1	Monocyclic terpenes		
		d-limonene	39.49
		γ-terpinene	0.09
		α-terpinolene	0.05
	Total		39.63
2	Bicyclic terpenes		
		α-thujene	0.05
		α-pinene	0.21
		camphene	0.15
		β-pinene	0.19
		8,3-carene	0.10
	Total		0.7
3	Aliphatic hydrocarbon	β-myrcene	0.09
	Total		0.09
4	Aromatic hydrocarbon		
		Trans cymene	0.10
	Total		0.10
5	Oxides		
		Trans-limonene oxide	0.06
	Total		0.06
6	Alcohols	p- Mentha-6,8-dien-2-ol, acetate, cis	0.27
		Linalool	0.04
		Terpinene-4-ol	0.09
		α-Terpineol	0.03
		Trans-D-dihydrocarveol	0.09
		Citronellol	0.05
	Total		0.57
7	Esters		
		(–)-Carvyllactate	0.09
	Total		0.09
8	Aldehydes		
		Z-citral	0.07
		Cuminaldehyde	0.02
	Total		0.09
9	Monoterpene ketones		
		(–)- carvone	56.52
		Camphor	0.09
	Total		56.71
10	Sesquiterpene		
		β-elemene	0.09
		β-caryophyllene	0.05
		α-cadinene	0.28
	Total		0.42
11	Unknown		0.41
			0.29
			0.17

(DPPH$^{\bullet}$ scavenging assay, ABTS$^{\bullet+}$ scavenging assay, and reducing power test) and compared to a widely used synthetic antioxidant, BHA (Table 2).

The DPPH values of EO caraway (IC_{50}=32.46 ±0.75 μg/ml) were marginally lower than those of the antioxidant BHA (IC_{50}=11.55±0.53 μg/ml), and the ABTS results followed a similar pattern, although with better efficiency (IC_{50}=2.44±0.44 μg/ml) compared with the antioxidant BHA (IC_{50}=1.50 ±0.29 μg/ml). However, in the reducing power assay, caraway EO could convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), and its IC_{50} value (17.65±0.70 μg/ml) was only 1.31 fold lower than that of the positive control, BHA (23.19±0.78 μg/ml). In all antioxidant assays, caraway EO showed significant antioxidant activities. These results are consistent with those reported by Hajlaoui *et al.* [4]. The high antioxidant

Table 2 Antioxidant activity of caraway essential oil

Essential oil/control	IC_{50} (μg/ml)		
	DPPH•scavenging assay	$ABTS^{\bullet+}$scavenging assay	Reducing power assay
Caraway essential oil	32.46±0.75	2.44±0.44	17.65±0.70
BHA	11.55±0.53	1.50±0.29	23.19±0.78

Values are mean±SD. BHA, butylated hydroxyanisole.

Table 3 Antimicrobial activity of caraway essential oils determined by agar well diffusion method.

	Inhibition zone (mm) Including 6 mm of well		
		Antibiotics	
Test organisms	Caraway essential oil	Gentamicin	Nystatin
Staphylococcus aureus	25.23±0.17[b]	31±0.00[a]	
Escherichia Coli	24.66±0.08[b]	29±0.02[b]	
Salmonella sp.	23.60±0.11[c]	25±0.01[d]	
Bacillus cereus	13.73±0.26[f]	27±0.02[c]	
Listeria monocytogenes	26.40±0.25[a]	28±0.02[b]	
Aspergillus niger	21.63±0.18[e]		27±0.00[b]
Candida sp.	22.80±0.17[d]		29±0.01[a]

Values are mean±SD, *n*=3. Different letters in columns represent statistical differences for inhibition zones (mm) when *P* value more than or equal to 0.05.

capacity of caraway EO may be attributable to its high terpene concentration. As a pre-aromatic monoterpene hydrocarbon, the non-phenolic molecule terpinene has exhibited antioxidant action and is also capable of inhibiting lipid peroxidation [38]. Edible lipids could benefit significantly from significant increases in their oxidative stability and shelf-life by adding EO rich in terpinene. Similar to past studies, the presence of carvonein, especially at high concentrations, was found to be responsible for *C. carvi*'s potent free radical scavenging abilities. Carvone comprises conjugated double bonding and has significant antioxidant activity [39]. In addition, carvone has been proved to be a lipid peroxidation inhibitor [40].

In vitro antimicrobial activity of caraway essential oil

The antimicrobial activity of caraway EO was tested against *S. aureus*, *E. coli*, *Salmonella sp.*, *B. cereus*, *L. monocytogenes*, *A. niger*, and *Candida sp*. using the agar well diffusion method (Table 3).

Caraway EO showed effective inhibitory activity against all tested cultures with an inhibition zone ranging from13.73±0.26 mm for *B. cereus* to 26.40 ±0.25 mm for *L. monocytogenes*. The MIC values of the caraway EO varied from 1.0 and 3.0 μl/ml for the five tested bacterial strains (Table 4). The lowest MIC value was recorded for both *S. aureus* and *L. monocytogenes*, whereas *B. cereus* showed the highest MIC value. The MIC values of caraway EO for *A. niger* and *Candida sp* were 2.00 and 1.50, respectively.

Table 4 Minimum inhibitory concentration and minimum bactericidal concentration and minimum fungicidal concentration (μl/ml) of caraway essential oil

Microorganisms	MIC	MBC	MFC
Staphylococcus aureus	1.00	1.50	
Escherichia coli	1.50	2.00	
Salmonella sp.	1.50	2.00	
Bacillus cereus	3.00	4.00	
Listeria monocytogenes	1.00	1.00	
Aspergillus niger	2.00		2.50
Candida sp.	1.50		2.50

MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration; MIC, minimum inhibitory concentration.

These results are in harmony with the results reported by many authors. Katarzyna *et al*. [41] discovered that caraway EO had moderate antibacterial properties, with carvone identified as an active component. Simic *et al.* [42] showed that caraway oil's MIC inhibited fungal growth at 2.5 mg/ml, but they did not compare the observed results to oil composition. Jaripa *et al.* [43] discovered that caraway EO was tested for antibacterial effectiveness against 10 pathogenic bacteria and six phytopathogenic fungi. Even at 2 μl/ disc, the EO exhibited a good inhibitory effect against all test microorganisms. The EO's MIC (100–300 ppm) and MBC (200–400 ppm) were determined. The EO's antifungal screening revealed that at 100 ppm, it inhibited 100% of the test fungi's radial mycelial growth. The MIC and MFC values range between 50 and 300 ppm and 200 and 400 ppm, respectively.

In vitro cytotoxicity

Understanding the biological and pharmacological characteristics of caraway EOs requires determining the effective concentration (IC_{50}) that reduces the cell growth inhibitory concentration. The proliferation rates of the colon cell line (HCT-116), liver cell line (HepG-2), and Caucasian breast adenocarcinoma (MCF-7) were compared with normal kidney cells (VERO) by studying the IC_{50} value of eight different concentrations (78.1, 156.2, 312.5, 625, 1250, 2500, 5000, and 1000 μg/ml) of caraway EO (Table 5).

The results indicated that caraway EO has a toxic effect on the cancer cells under study, as the increase in the concentration used increased the properties corresponding to the rise in the toxic effect on cancer cells, as the effect of the oil was greater on the colon cell line (HCT-116, IC_{50}=390.12±0.41 μg/ml), followed by liver cell line (HepG-2 IC_{50}=589.75 ±0.60 μg/ml) and Caucasian breast cancer (MCF-7 IC_{50}=843.52±0.75 μg/ml), compared with untreated cells from each of the above cell types (% viability=100%, %toxicity=0%) Additionally, caraway EO has been tested on normal cells to ensure the safety of using caraway. These results show the potential of caraway EO on cancer treatment because it contains biologically active compounds such as carvone and limonene. This opinion is consistent with Kamaleeswari and Nalini [44], who stated that oils recovered from caraway seeds might help to reduce tumor volume and occurrence. It made resistant tumor cells exposed to free radical attack, which resulted in a decrease in cancerous cell propagation. The activation of antioxidant enzymes scavenges the free radicals in colon cancer rats. Caraway EO may have anticancer benefits owing to the presence of carvone, a monoterpene with cancer-preventive and anthelmintic qualities [22]. Owing to its ability to trigger apoptosis by upregulating pro-apoptotic factors and downregulating anti-apoptotic issues, limonene has also been discovered to be an anticancer drug [19].

These results indicate that caraway EO can be used safely in the prevention and treatment of colon, liver, and breast cancers.

Table 5 Anticancer activity (IC_{50}) of the caraway essential oil against three human cancer cell lines and one normal cell

	Cell lines											
	VERO				HCT-116			MCF-7			HepG-2			
Conc. (μg/ml)	Mean OD	Viability %	Toxicity %	IC50	Mean OD	Viability %	Toxicity %	IC50	Mean OD	Viability %	Toxicity %	IC50	Mean OD	Viability %	Toxicity %	IC50
Control	0.755	100	0		0.986	100	0		0.822	100	0	843.52	0.914	100	0	
10 000	0.0207	2.737	97.263		0.0410	4.158	95.842		0.0350	4.258	95.742		0.0540	5.908	94.092	
5000	0.0917	12.141	87.859		0.1213	12.306	87.694		0.0983	11.963	88.037		0.1553	16.995	83.005	
2500	0.1373	18.190	81.810		0.2003	20.318	79.682		0.1903	23.155	76.845		0.2207	24.143	75.857	
1250	0.2223	29.448	70.552	785.93	0.2823	28.634	71.366	390.12	0.3163	38.483	61.517		0.3717	40.664	59.336	589.75
625	0.4620	61.192	38.808		0.3383	34.314	65.686		0.5557	67.599	32.401		0.4217	46.134	53.866	
312.5	0.6120	81.060	18.940		0.5177	52.502	47.498		0.6977	84.874	15.126		0.6080	66.521	33.479	
156.2	0.7277	96.380	3.620		0.7653	77.620	22.380		0.8103	98.581	1.419		0.7727	84.537	15.463	
78.1	0.7433	98.455	1.545		0.8370	84.888	15.112		0.8077	98.256	1.744		0.9013	98.614	1.386	

Conclusions

In this work, carvone and limonene were identified as the main compounds in caraway EO. This EO demonstrates significant antimicrobial action against a variety of pathogenic and food spoilage pathogens,

with large widths of growth inhibition zones and low minimal inhibitory doses for nearly all tested strains. The anticancer activity of caraway EO was investigated. Using a Vero and three tumor cell lines of human hepatocarcinoma (HepG-2), human breast carcinoma (MCF-7), and colon cell line, the most interesting biological effect of EO on the cell proliferation stimulating activity at extremely low doses was demonstrated (HCT-116). At low doses, 50% of cancer cells were found to be killed by the EO, demonstrating its potency.

Acknowledgements

The authors would like to thank the Food Science Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, for their help completing this work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Amer AM, Aly UI. Antioxidant and anti-bacterial properties of anise (*Pimpinella anisum L)*. Egypt Pharmaceut J 2019; 18:68–73.

2 Farag R, Swaby S. Antimicrobial effects of wasp (*Vespa orientalis*) venom. Egypt Pharmaceut. J 2018; 17:218–222.

3 Amer AM. Antimicrobial effects of Egyptian local chicory, *cichorium endivia sub sp*. *Pumilum*. Int J Microbiol 2018; 2018:6475072.

4 Hajlaoui H.H., Arraouadi S, Noumi E, Aouni K, Adnan M, Khan MA, *et al.* Antimicrobial, antioxidant, anti-acetylcholinesterase, antidiabetic, and pharmacokinetic properties of *Carum carvi L.* and *coriandrum sativum L*. Essential oils alone and in combination. Molecules 2021; 26:3625.

5 Aye MM, Aung HT, Sein MM, Armijos CA. Review on the phytochemistry, medicinal properties and pharmacological activities of 15 selected Myanmar medicinal plants. Molecules 2019; 24:293.

6 Goyal M, Gupta VK, Singh N, Mrinal A. *Carum carvi* an updated review. Indian J Pharm Biol Res 2018; 6:14–24.

7 Hajlaoui H, Arraouadi S, Mighri H, Chaaibia M, Gharsallah N, Ros G, *et al.* Phytochemical constituents and antioxidant activity of Oudneya Africana L. leaves extracts: evaluation effects on fatty acids and proteins oxidation of beef burger during refrigerated storage. Antioxidants 2019; 8:442.

8 Mohamed GM, Amer AM, Osman NH, Sedikc MZ, Hussein MH. Effects of different gelling agents on the different stages of rice regeneration in two rice cultivars. Saudi J Biol Sci 2021; 28:5738–5744.

9 SAS/STAT Software (Version 9.1.3) (SAS Institute, Cary, NC, 2007).

10 Asbahani AE, Miladi K, Badri W, Sala M, Addi EHA, Casabianca H, *et al.* Essential oils: from extraction to encapsulation. Int J Pharm 2015; 483:220–243.

11 War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S, Sharma HC. Mechanisms of plant defense against insect herbivores. Plant Signal Behav 2012; 7:1306–1320.

12 Blowman K, Magalhaes M, Lemos MFL, Cabra C, Pires IM. Anticancer properties of essential oils and other natural products. Evid Based Complement Alternat Med 2018; 2018:3149362.

13 Elshafie HS, Camele I. An overview of the biological effects of some mediterranean essential oils on human health. BioMed Res Int 2017; 2017:9268468.

14 Liang JY, Xu J, Yang YY, Shao YZ, Zhou F, Wang JL. Toxicity and synergistic effect of elsholtziaciliata essential oil and its main components against the adult and larval stages of triboliumcastaneum. Foods 2020; 9:345.

15 Elswaby S, Sadik M, Azouz A, Emam N, Ali M. *In vitro* evaluation of antimicrobial and antioxidant activities of honeybee venom and propolis collected from various regions in Egypt. Egypt Pharmaceut J 2022; 21:207–213.

16 Taiwo MO, Adebayo OS. Plant essential oil: an alternative to emerging multi drug resistant pathogens. J Microbiol Exp 2017; 5:00163.

17 Keshavarz A, Minaiyan M, Ghannadi A, Mahzouni P. Effects of *Carum carvi L.* (Caraway) extract and essential oil on TNBS-induced colitis in rats. Res Pharm Sci 2013; 8:1–8.

18 Miraj S, Kiani S. Pharmacological activities of *Carum carvi L*. Der Pharm Lett 2016; 8:135–138.

19 Olennikov DN, Kashchenko NI. Polysaccharides. Current state of knowledge: an experimental and scientometric investigation. Chem Plant Raw Mater 2014; 1:5–26. (In Russ.). DOI: https://doi.org/10.14258/jcprm.1401005

20 Sachan AK, Das DR, Kumar M. *Carumcarvi*-an important medicinal plant. J Chem Phar Res 2016; 8:529–533.

21 Senapati S, Mahanta AK, Kumar S. Controlled drug delivery vehicles for cancer treatment and their performance. Sig Transduct Target Ther 2018; 3:7.

22 Malhotra SK. Handbook of herbs and spices. In: Peter KV, editor. Woodhead Publishing Series in Food Science, Technology and Nutrition. 2nd ed. Cambridge, UK: Woodhead Publishing 2012. 2.

23 Bakari S, Ncir M, Felhi S, Hajlaoui H, Saoudi M, Gharsallah N, Kadri A. Chemical composition and in vitro evaluation of total phenolic, flavonoid, and antioxidant properties of essential oil and solvent extract from the aerial parts of Teucriumpolium grown in Tunisia. Food Sci Biotechnol 2015; 24:1943–1949.

24 Dadkhah A, Allameh A, Khalafi H, Ashrafihelan J. Inhibitory effects of dietary caraway essential oils on 1,2-dimethylhydrazine-induced colon carcinogenesis is mediated by liver xenobiotic metabolizing enzymes. Nutr Cancer 2010; 63:46–54.

25 Laribi B, Kouki K, Bettaieb T, Mougou A, Marzouk B. Essential oils and fatty acids composition of Tunisian, German and Egyptian caraway (*Carum carvi L*.) seed ecotypes: a comparative study. Ind Crop Prod 2013; 41:312–318.

26 Rasooli I, Allameh A. Caraway (*Carum carvi L*.) essential oils. In: Preedy VR, editor. Essential oils in food preservation, flavor and safety. San Diego, CA: Academic Press 2016. 287–293

27 Maisonneuve SA. Sainte Ruffine European Pharmacopoeia, vol 1. Canada: Maisonneuve; 1983.

28 Snoussi M, Noumi E, Punchappady-Devasya R, Trabelsi N, Kanekar S. Antioxidant properties and anti-quorum sensing potential of *Carum copticum* essential oil and phenolics against *Chromobacterium violaceum*. J Food Sci Technol 2018; 55:2824–2832.

29 Mighri H, Hajlaoui H, Akrout A, Najjaa H, Neffati M. Antimicrobial and antioxidant activities of Artemisia herba-alba essential oil cultivated in Tunisian arid zone. CRC Chimie 2010; 13:380–386.

30 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio Med 1999; 26:1231–1237.

31 Iordache TA, Vlase L, Istudor V, Gîrd CE. Researches regarding obtaining selective extracts with hypoglycemiant properties from vegetal indigenous products (*Cichoriiherba* and *Fraxini folium*). Note III. Phenolic compounds analysis from Fraxini folium. Farmacia 2013; 61:625–631.

32 Deans S, Ritchie G. Antibacterial properties of plant essential oils. J. Food Microbiol 1978; 5:165–180.

33 Hasika M, Rémi D, Véronique D, Abdesselam Z, Georges D, Antoine C. Antimicrobial activities of commercial essential oils and their components against food-borne pathogens and food spoilage bacteria. Food Sci Nutr 2014; 2:403–416.

34 Horiuchi N, Nakagava K, Sasaki Y, Minato K, Fujiwara Y, Nezu K. In vitro antitumor activity of mitomycin C derivative (RM-49) and a new anticancer antibiotic (FK973) against lung cancer cell lines determined by tetrazolium dye (MTT) assay. Cancer Chemother Pharmacol 1988; 22:246–250.

35 Duncan DB. Multiple range and multiple 'F' tests. Biometrics 1955; 11:1–42.

36 Siwar G, Kaïss A, Adel K, Mejdi S. GC-MS profiling, vibriocidal, antioxidant, antibiofilm, and anti-quorum sensing properties of *Carum carvi L*. essential oil: in vitro and in silico approaches. Plants 2022; 11:1072.

37 Miguel MG. Antioxidant and anti-inflammatory activities of essential oils: a short review. Molecules 2010; 15:9252–9287.

38 De Oliveira TM, Carvalho RBF, Costa IHF, Oliveira GAL, Souza AA, Lima SG, Freitas RM. Evaluation of p-cymene, a natural antioxidant. Pharm Biol 2015; 53:423–428.

39 Wojtunik KA, Ciesla LM, Waksmundzka-Hajnos M. Model studies on the antioxidant activity of common terpenoid constituents of essential oils by means of the 2.2-diphenyl-1-picrylhydrazyl method. J Agric Food Chem 2014; 63:9088–9094.

40 Vinothkumar R, Sudha P, Viswanathan M, Kabalimoorthy J, Balasubramanian T, Nalini N. Modulating effect of d-carvoneon 1.2-dimethylhydrazine-induced pre-neoplastic lesions, oxidative stress and biotransforming enzymes in an experimental model of rat colon carcinogenesis. Cell Prolif 2013; 46:705–720.

41 Katarzyna S, Bogdan K, El□bieta K, Jan B. Microbiological activity of caraway (*Carum carvi L.*) essential oil obtained from different origin. Maringá 2013; 35:495–500.

42 Simic A, Rancic A, Sokovic M.D., Ristic M, Grujic-Jovanovic S, Vukojevic J, Marin MD. Essential oil composition of *Cymbopogon winterianus* and *Carum carvi* and their antimicrobial activities. Pharma Biol 2008; 46:437–441.

43 Jaripa B, Nazru IB, Jasim UC, Nuzmul H, Nural A. Antimicrobial activity of essential oil from seeds of *carum carvi* and its composition. Bangladesh J Microbiol 2008; 25:85–89.

44 Kamaleeswari M, Nalini N. Dose-response efficacy of caraway (Carumcarvi L.) ontissue lipid peroxidation and antioxidant profile in ratcolon carcinogenesis. J Pharma Pharmacol 2010; 58I:1121–1130.

45 Bakari S, Daoud A, Felhi S, Smaoui S, Gharsallah N, Kadri A. Proximate analysis, mineral composition, phytochemical contents antioxidant and antimicrobial activities and GC-MS investigation of various solvent extracts of Cactus cladode. Food Sci Technol 2017; 27:286–293.