TRADITIONAL AND INTEGRATIVE MEDICINE

Trad Integr Med, Volume 6, Issue 4, Autumn 2021

Original Research

Antioxidant and Anticancer Activities of *Anethum graveolens* L., *Citrus limon* (L.) Osbeck and *Zingiber officinale* Roscoe Essential Oils

Mahmoud Osanloo[1,2], Ali Ghanbariasad[2,3]*, Ali Taghinezhad[2]

[1]*Department of Medical Nanotechnology, Schools of Advanced Medicine in Technologies, Fasa University of Medical Sciences, Fasa, Iran*
[2]*Noncommunicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran*
[3]*Department of Medical Biotechnology, Schools of Medicine, Fasa University of Medical Sciences, Fasa, Iran*

Received: 15 Apr 2021 **Revised:** 22 Aug 2021 **Accepted:** 25 Aug 2021

Abstract

Since synthetic chemotherapeutic drugs produce a certain degree of drug resistance and due to their common side effects, such as damage to hematopoietic cells and hair loss, it is necessary to use herbal medicine as a substrate to develop new anticancer drugs. The ingredients of three essential oils (EO) were identified using gas chromatography–mass spectrometry (GC-MS) analysis. Their anticancer activities have been investigated on four human breast cancer cell lines, including MCF-7, MDA-MB-175, MDA-MB-231, and MDA-MB-468. In addition, their antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The three plants were investigated for identifications of the ingredients of their EOs, and major ingredients were identified in each plant as alpha-phellandrene (26.75 %) in *Anethum graveolens* L., limonene (61.83 %) in *Citrus limon* (L.) Osbeck, and zingiberene (30.28 %) in *Zingiber officinale* Roscoe. Among the EOs, *C. limon* was significantly more effective than others; its half-maximal inhibitory concentration (IC50) on MCF-7 was obtained at 201 $\mu g.mL^{-1}$. Furthermore, *Z. officinale* EO showed a higher antioxidant activities in comparison to the two other EOs. Considering the antioxidant and anticancer effects of the EOs, they could be further investigated as a possible complementary medicine in cancer.

Keywords: *Citrus limon*; *Anethum graveolens*; *Zingiber officinale*; Anticancer activity; Antioxidant effects

Citation: Osanloo M, Ghanbariasad A, Taghinezhad A. **Antioxidant and Anticancer Activities of *Anethum graveolens* L., *Citrus limon* (L.) Osbeck and *Zingiber officinale* Roscoe Essential Oils.** Trad Integr Med 2021;6(4):333-347.

*Corresponding Author: Ali Ghanbariasad
Department of Medical Biotechnology, Schools of Medicine, Fasa University of Medical Sciences, Fasa, Iran
Tel: +9871 53357091
Email: a.ghanbari@fums.ac.ir

Introduction

Cancer is one of the most important global health problems and is currently the second leading cause of death after heart disease [1]. It is characterized by uncontrolled growth, caused by too many changes in multiple genes and the ability to spread (metastasis) from the origin to other organs [2]. With 14 % of all cancer-related deaths among women, breast cancer is one of the most common cancers worldwide [3,4]. Common methods of treating cancer include surgery, radiation, and chemotherapy [5, 6]. Among the aforementioned methods, synthetic chemotherapy drugs have shown encouraging results in treating various cancers [7]. However, some anticancer drugs have high toxicity or side effects and resistance rates [8,9]. Therefore, it is necessary to research and develop new medicine resources, especially herbal substrates. Essential oils (EO)s are oily liquids secreted as secondary metabolites in different parts of aromatic plants such as rhizomes, bark, and fruits [10,11]. EOs have been widely used in sterilization, cosmetics, and food applications and has insecticidal, fungicidal, and antioxidant properties [12,13]. In addition, some EOs have also shown excellent anticancer properties [14,15]. For instance, the half-maximal inhibitory concentration (IC_{50}) of *Myrcia splendens* (Sw.) DC. and *Thymus alternans* Klokov EOs against MCF-7 and MDA-MB-231 were reported as 5.53 and 5.96 $\mu g.mL^{-1}$, respectively [16,17].

Citrus limon (L.) Osbeck*, Anethum graveolens* L., and Zingiber officinale Roscoe are common medicinal plants that have been widely used in traditional medicine. EO of C. limon (CLEO) has antioxidant, antidepressant, and antibacterial effects [18,19]. In addition, its protective effect on hepatotoxicity and nephrotoxicity have also been proven [20]. *A. graveolens* EO (AGEO) is commonly used for indigestion, flatulence, and management of diabetes [21,22]. Various studies have shown that it also has anticancer, anti-inflammatory, antimicrobial, antioxidant, and analgesic effects [23]. Moreover, *Z. officinale* EO (ZOEO) possesses strong antimicrobial, antifungal, and antioxidant activities [24,25]. It is useful for managing obesity, metabolic syndrome, and Alzheimer's disease, and it can also prevent nausea and vomiting [26].

In the current study, a comprehensive *in vitro* comparison was performed on anticancer activities of AGEO, CLEO, and ZOEO on four types of human breast cancer cell lines, including MCF-7, MDA-MB-175, MDA-MB-231, and MDA-MB-468. Additionally, their antioxidant activities were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

Materials and Methods

Materials

Powders of 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), DPPH, and phosphate-buffered saline (PBS) tablets were purchased from Sigma-Aldrich (USA). Cell culture grades of dimethyl sulfoxide (DMSO), penicillin-streptomycin (Pen / Strep), RPMI cell culture medium, and trypsin were purchased from Shellmax (China). Fetal Bovine Serum (FBS) was provided by Gibco (USA). AGEO and CLEO, and ZOEO were purchased

by Barij Essence Pharmaceutical Co (Iran) and Zardband Pharmaceuticals Co (Iran). Breast cancer cell lines including MCF-7 (ATCC: HTB-22), MDA-MB-175 (ATCC: HTB-25), MDA-MB-231 (ATCC: HTB-26) and MDA-MB-468 (ATCC: HTB-132) were provided by the Pasteur Institute of Iran. The distilled water was provided by the Central Laboratory of the Fasa Medical University (Iran).

Identification of ingredients of EOs

In order to identify the components of EO, Gas chromatography–mass spectrometry (GC-MS) analysis was used. In short, a 6890 networked GC system and a 5975 network mass selective detector (Agilent Technologies, Santa Clara, California, USA) were used for GC-MS analysis. The separation of EOs components was performed on an HP-5MS silica fusion chromatography column (length 30 m, inner diameter 0.25 mm, film thickness 0.25 µM). The programming of the GC-MS column temperature is as follows: the initial temperature was set at 40°C and fixed for 1 min, then it increased to the final temperature of 250°C at a rate of 3°C/min and kept for 20 min. Other instrument parameters were set as split flow, 25 mL/min, septum purge (6 mL/min), and column flow rate (1 mL/min). Helium gas with a purity of 99.99% is used as the carrier gas. The EOs components were identified using the method described in our previous report [27].

Cell culture

The cell lines were cultured in a complete medium, i.e., RPMI containing FBS (10%) and Pen/Strep (1%). The cell lines were incubated with air (95%) and CO2 (5%) at 37°C. When the cells reached confluence, they were harvested and seeded in 96-well plates (~7000 cells/well) using an 8-channel pipette. Then plates were incubated for another 24 h at the mentioned condition for attaching the cells to the plates and reach confluence to at least 80%. Before treating the cells with EOs, wells content was discarded, and a complete fresh medium was added to each well (100 µL/well).

All the EOs were dissolved in RPMI at a concentration of 2560 $\mu g.mL^{-1}$ as a stock solution. Noted that EOs at a higher amount of 2560 $\mu g.mL^{-1}$ was not dissolved in RPMI. Then, a serial dilution from each EO was prepared with a two-fold dilution of the stock solution in a concentration range of (10 - 2560 $\mu g.mL^{-1}$). After adding those concentrations to the wells separately, the concentration of each EO was fixed at 5, 10, 20, 40, 80, 160, 320, 640, and 1280 $\mu g.mL^{-1}$. Then plates were incubated for 48 h; for determining the anticancer activities of the EOs, the MTT assay was performed.

Evaluation of anticancer activities of EOs

The metabolic activity of each treated well was determined by the MTT assay and compared with the metabolic activity of untreated cells (control). In this regard, MTT powder (5 $mg.mL^{-1}$) was dissolved in sterile PBS. Then, the MTT solution was diluted (10 times) in a complete medium (the MTT concentration reached 0.5 $mg.mL^{-1}$). The contents of the plates (48 h incubated) were discarded, and MTT solution was added (100 µL/well), and the plates

were incubated for another 4 h. Finally, 100 µL of DMSO was added to each well and mixed thoroughly to dissolve the dye crystals. Absorbance was measured using a plate reader (Synergy HTX Multi-Mode Reader, USA) at 570 nm. The MTT assay was repeated in triplicate, and for each replicate, 12 wells were considered control groups (no-treated with EOs) [28]. High optical density corresponded to the high intensity of dye color, which is related to control cells. The cell viability was calculated by equation 1.

Equation1.

Cell viability (%) = Mean absorbance of sample test / Mean absorbance in control wells × 100

Evaluation of antioxidant activities of EOs

EOs were dissolved in ethanol in the same concentration range of the MTT assay (5-1280 $\mu g.mL^{-1}$). The DPPH assay was performed to evaluate the antioxidant activity of EOs. Briefly, 11.83 mg of DPPH powder (MW: 394.32 $g.mole^{-1}$) was dissolved in 10 mL of ethanol, avoid light. The prepared mixture was used as a stock solution (3 mM); it was diluted ten times to prepare a 0.3 mM standard solution.

To prepare the reaction mixture, 40 and 160 µL from each concentration of EO and DPPH standard solution were added to each well, respectively. The treated plates were incubated 30 min, away from light, for performing the reaction and then subjected to the plat reader. The absorbance of the wells was read at 517 nm. Using equation 2, the antioxidant activity of EO was calculated at different concentrations [29,30]. The decreased absorbance value of the reaction mixture indicated an increased percentage of free radical scavenging activity. Higher absorbance was related to the control groups, filled with ethanol (40 µL) and DPPH standard solution (160 µL). The test was repeated three times, and in each replicate, 12 wells were considered as control groups

Equation2.

Antioxidant activity (%): (Mean absorbance of control -Mean absorbance of sample test)/(Mean absorbance of control) × 100

Statistical Analyses

All the tests were repeated three times, and final values were reported as mean ± standard deviation. Calculation of means, standard deviations, and drawing charts were done using Excel software (Version 2010, Microsoft Corporation, USA). CalcuSyn software (Free version, BIOSOFT, UK) was used for the calculation of IC50s. For comparison of anticancer/oxidant activities of EOs together, one-way analysis of variances (ANOVA) or independent sample t-test was used; $p < 0.05$ was considered a significant difference. Those analyses were performed using statistical package for the social sciences (SPSS) software (Version 22, SPSS Inc, USA).

Results

Identified ingredients the EOs

Identified compounds in AGEO using GC-MS analysis are listed in Table 1. Compounds of alpha-Phellandrene, p-Cymene, Dill ether, cis-Sabinol, and Carvone with a portion (%) of 26.75, 24.81, 9.78, 3.61, and 10.77 were identified as major components.

Table 1. Identified ingredients in A. graveolens using GC-MS analysis

No	Ret Time	Compound	%	RI
1	6.72	alpha-Thujene	0.38	446
2	8.88	beta-Myrcene	0.76	600
3	6.97	alpha-pinene	1.76	612
4	7.42	Comphene	0.10	623
5	9.73	alpha-Phellandrene	26.75	634
6	8.38	Sabinene	0.67	646
7	10.80	p-Cymene	24.81	675
8	11.77	gamma-Terpinene	0.13	709
9	12.91	alpha-Terpinolene	0.53	737
10	12.97	Dehydro-p-cymene	0.29	739
11	14.45	Delta-3-carene	0.25	776
12	14.81	cis-Limonene oxide	0.10	785
13	15.64	Prehnitene	1.73	805
14	16.65	Borneol	0.91	825
15	17.38	Dill ether	9.78	839
16	17.82	cis-Dehydrocarvone	1.44	848
17	18.21	cis-Sabinol	3.61	856
18	19.57	Pulegone	1.24	884
19	20.25	Carvone	10.77	897
20	20.50	Propellane	2.88	902
21	26.12	Piperitenone	0.98	1008
22	26.85	Prehnitene	1.51	1022
23	27.04	1,3-Adamantanediol	1.59	1025
24	27.61	1(2H)-Naphthalenone, octahydro-8a-hydroxy	2.50	1036

25	28.13	2-Cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methylethyl), trans	1.82	1046
26	28.84	Prehnitole	1.11	1060
27	35.18	Dillapiole	0.29	1184
28	35.73	delta-Cadinene	0.17	1195
29	42.49	Allylchlorodimethylsilane	0.79	1331
30	48.81	Hexadecanoic acid	0.29	1485

Ret Time: Retention Time, RI: Refractive index

Thirty-eight components were identified in CLEO with five major constituents, including alpha-pinene (3.46%), sabinene (16.99%), limonene (61.83%), Limonene oxide, cis- Limonene oxide (2.27%), and trans- Limonene oxide (3.08%) (see table 2).

Table 2. Identified ingredients in C. limon using GC-MS analysis

No	Ret Time	Compound	%	RI
1	9.45	alpha-Pinene	3.46	613
2	10.03	Camphene	0.07	623
3	11.35	Sabinene	16.99	646
4	11.57	cis-p-Menth-8-ene	0.03	704
5	11.98	beta-Myrcene	0.73	714
6	12.56	Octanal	0.09	729
7	12.76	3-Carene	0.04	734
8	13.98	Limonene	61.83	765
9	18.01	p-mentha-E-2,8(9)-dien-1-ol	0.47	853
10	18.57	cis- Limonene oxide	2.27	864
11	18.80	trans- Limonene oxide	3.08	869
12	21.07	1-Methyladamantane	0.12	913
13	21.47	6-Methyl-1,4-cyclooctadiene	0.66	921
14	21.57	Myrtenol	0.29	923

15	21.62	(4R,8R)-8,9-epoxy-p-menth-1-ene	0.39	924
16	21.72	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl), cis	0.14	926
17	22.64	trans-Carveol	0.91	943
18	23.17	cis-Carveol	0.64	953
19	23.59	Z-Citral	0.32	961
20	23.70	Carvone	0.67	963
21	24.93	E-Citral	0.61	986
22	26.11	Verbenone	0.19	1008
23	26.21	Perilla aldehyde	0.26	1010
24	26.59	trans-Pinocarveol	0.20	1017
25	26.67	1,3,8-p-Menthatriene	0.48	1019
26	27.25	cis-p-Mentha-2,8-dien-1-ol	0.33	1030
27	27.33	Myrtenal	0.31	1031
28	27.70	2,6-Octadienal, 3,7-dimethyl, (Z)	0.13	1038
29	28.15	1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)	1.51	1047
30	28.82	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis	0.13	1060
31	29.01	Neryl acetate	0.48	1063
32	29.12	trans-Carveol	0.53	1066
33	29.77	Carveol2	0.30	1078
34	29.82	Nerol	0.27	1079
35	30.36	Carveol	0.07	1089
36	30.83	4,5-epoxy-1-isopropyl-4-methyl-1-cyclohexene	0.45	1098
37	32.39	trans-2-Decalone	0.28	1129

In table 3, identified ingredients in ZOEO are listed. Among the 70 determined compounds, five of them had a significant portion (%) compared to others. They were including Camphene (6.73), alpha-Curcumene (11.61), Zingiberene (30.28), beta-Bisabolene (10.69), and beta-Sesquiphellandrene (12.37).

Table 3. Identified ingredients in *Z. officinale* using GC-MS analysis

No	Ret Time	Compound	%	RI
1	8.95	Tricyclene	0.17	576
2	9.19	alpha-Thujene	0.01	604
3	9.46	alpha-Pinene	2.41	613
4	10.11	Camphene	6.73	624
5	10.82	1,3,8-p-Menthatriene	0.02	649
6	11.14	Sabinene	0.08	677
7	11.24	beta-Pinene	0.30	689
8	11.86	6-Methyl-5hepten-2-one	0.69	711
9	11.98	beta.-Myrcene	0.79	714
10	12.50	alpha-Phellandrene	0.37	727
11	12.75	3-Carene	0.05	734
12	13.06	alpha-Terpinene	0.02	741
13	13.46	o-Cymene	0.07	751
14	13.69	beta-Phellandrene	5.51	757
15	13.78	1,8-Cineole	3.05	760
16	15.03	gamma-Terpinene	0.03	791
17	16.40	alpha-Terpinolene	0.21	820
18	16.67	2-Nonanone	0.05	826
19	16.89	Rosefuran	0.24	830
20	17.04	Linalool	0.25	833
21	17.54	Methyl bornyl ether	0.04	843
22	17.76	(E)-4,8-Dimethyl-1,3,7-nonatriene	0.03	848
23	19.01	Camphor	0.10	873
24	19.21	exo-methyl-camphenilol	0.05	877

25	20.12	Borneol	1.19	895
26	20.58	Rose furan epoxide	0.27	904
27	21.27	alpha-Terpineol	0.57	917
28	21.93	Decanal	0.11	930
29	23.05	beta-Citronellol	0.13	951
30	23.56	Z-Citral	0.06	960
31	24.24	Geraniol	0.21	973
32	24.92	E-Citral	0.09	986
33	25.53	Borneol, acetate	0.13	997
34	25.91	2-Undecanone	0.20	1004
35	27.74	alpha-Terpinene	0.15	1039
36	28.50	cis-2,6-Dimethyl-2,6-octadiene	0.05	1054
37	28.76	Eugenol	0.05	1059
38	28.90	(+)-Cycloisosativene	0.25	1061
39	29.39	alpha-Cubebene	0.82	1071
40	29.82	Geranyl acetate	0.43	1079
41	30.01	beta-Cubebene	0.11	1083
42	30.11	beta-Elemene	1.08	1084
43	30.67	Zingiberene	0.36	1095
44	31.21	trans-Caryophyllene	0.14	1106
45	31.62	Germacrene D	0.06	1114
46	31.82	gamma-Elemene	0.48	1118
47	31.90	alpha-Bergamotene	0.15	1119
48	31.91	alpha-Bergamotene	0.15	1120
49	32.44	alpha-Gurjunene	0.11	1130

50	32.79	trans-beta-Farnesene	0.76	1137
51	32.93	Aromadendrene	0.33	1140
52	33.61	alpha-Amorphene	0.24	1153
53	34.00	alpha-Curcumene	11.61	1161
54	34.70	Zingiberene	30.28	1175
55	35.07	beta-Bisabolene	10.69	1183
56	35.38	(-)-alpha-Panasinsen	0.63	1189
57	35.73	beta-Sesquiphellandrene	12.37	1196
58	35.89	trans-gamma-Bisabolene	0.51	1199
59	36.56	Elmol	0.52	1212
60	36.69	Sesquisabinene	0.25	1215
61	37.06	Farnesol	0.51	1222
62	38.96	Unknown from lime oil	0.60	1261
63	39.47	alpha-Costol	0.27	1271
64	40.32	beta-Eudesmol	0.19	1288
65	41.72	gamma-Curcume	0.44	1318
66	41.96	Farnesol	0.21	1324
67	43.40	Mintsulfide	0.09	1357

Anticancer activities of the EOs

Anticancer activity of AGEO against the cancer cell lines is illustrated in figure 1. By increasing the concentration of the EO, cell viabilities were decreased, e.g., cell viabilities of MDA-MB-175, MDA-MB-468, and MDA-MB-231 decreased to ~ 10, 20, and 25%, respectively, at a concentration of 1280 µg.mL^{-1}. However, the cell viability of MCF-7 was higher than others: its viability at 1280 µg.mL^{-1} was around 55%. The effect of CLEO on targeted cell lines is demonstrated in figure 2. Like AGEO, anticancer activities improved by increasing the concentration of CLEO. Interestingly, at a concentration of 1280 µg.mL^{-1}, viabilities of three cell lines, including MCF-7, MDA-MB-175, and MDA-MB-468, decreased to around 10%. Furthermore, viability of MCF-7 at concentra-

tion of 320 µg.mL^{-1} was around 40%; while this amount did not occur even at 4-fold higher concentration of AGEO (i.e., 1280 µg.mL^{-1} = ~ 50%). It can be implied that MCF-7 is more sensitive to CLEO than AGEO.

The anticancer activity of ZOEO on four human breast cancer cell lines is shown in figure 3. In detail, the EO is invalid for MCF-7. When the concentration is as high as 640 µg.mL^{-1}, the survival rate is about 100%. Only at the concentration of 1280 µg.mL^{-1} its survival rate was reduced to 68%. At the same time, the survival rate of other cell lines (MDA-MB-175, MDA-MB-231, and MDA-MB-468) is about 35%.

Obtained IC50s of the EOs against targeted cell lines are listed in Table 4. CLEO with IC50 of 201 µg.mL^{-1} was significantly more potent than AGEO and ZOEO against MCF-7 (one-way ANOVA, $p < 0.05$). In addition, observed IC50s of CLEO against MDA-MB-231 (243 µg.mL^{-1}) and MDA-MB-468 (210 µg.mL^{-1}) was significantly more potent than ZOEO (sample t-test $p < 0.05$). However, no significant difference was observed compared to AGEO (Independent sample t-test, $p > 0.05$). Moreover, the potency of the EOs on MDA-MB-175 was not significantly different from each other (one-way ANOVA, $p > 0.05$).

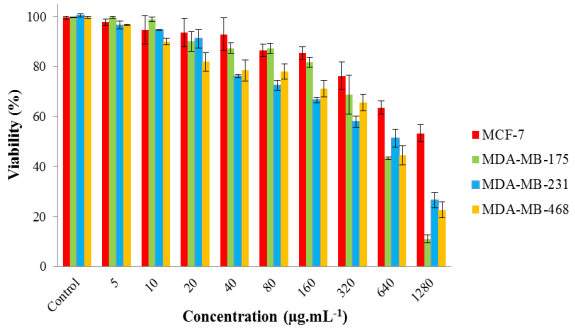

Figure 1. Anticancer activity of EO of *A. graveolens*

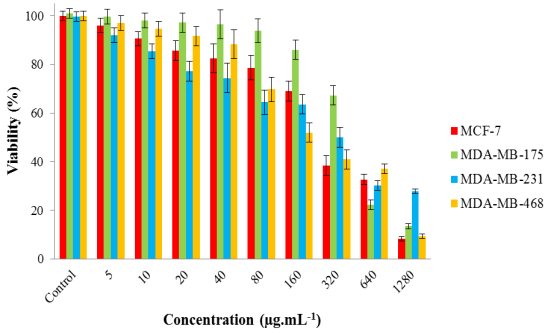

Figure 2. Anticancer activity of EO of *C. limon*

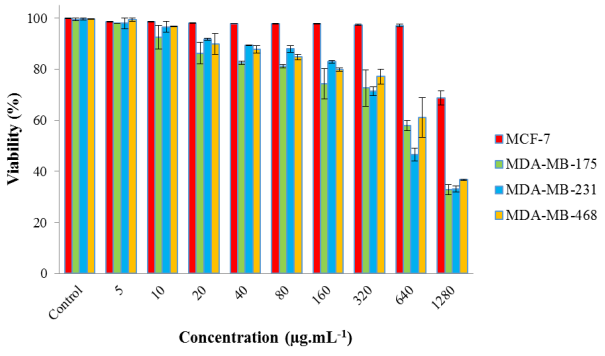

Figure 3. Anticancer activity of EO of *Z. officinale*

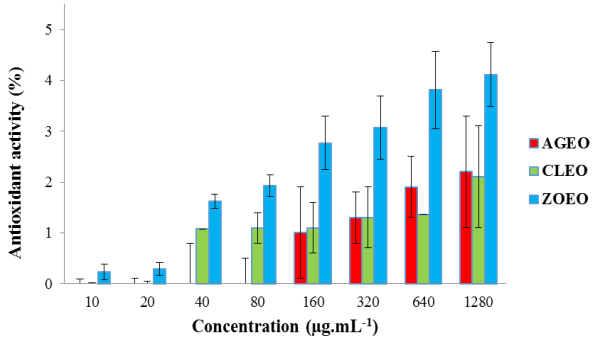

Figure 4. Antioxidant activity of *Anethum graveolens* (AGEO), *Citrus limon* (CLEO), and Zing*iber officinale* (ZOEO) EOs

Table 4. Obtained IC50s (with related parameters) of EOs against four human breast cancer cell lines

Cell lines	MCF-7	MDA-MB-175	MDA-MB-231	MDA-MB-468
Parameter	IC_{50} ($\mu g.mL^{-1}$) LCL-UCL	IC_{50} ($\mu g.mL^{-1}$) LCL-UCL	IC_{50} ($\mu g.mL^{-1}$) LCL-UCL	IC_{50} ($\mu g.mL^{-1}$) LCL-UCL
AGEOa	1908 1238-2941	370 217-633	408 286-580	403 236-689
CLEOb	201 137-296	406 272-606	243 185-319	210 162-273
ZOEOc	NA	719 370-1397	723 490-1067	775 361-1665

[a]*A. graveolens* EO, [b]*C. limon EO*, [c] *Z. officinale* EO

Antioxidant activities of the EOs

As shown in figure 4, the antioxidant activity of the EOs was not acceptable; the highest values in the EOs were ~ 5%, which occurred at a concentration of 1280 $\mu g.mL^{-1}$ of AGEO. The antioxidant activity of ZOEO was significantly potent than two other EOs at concentrations of 40, 80, 160, 320, 640, and 1280 $\mu g.mL^{-1}$ (one-way ANOVA, $p < 0.05$).

Discussion

Limonene, alpha-phellandrene, and zingiberene were identified as major ingredients of the used EOs in the current study; their various biological effects have been reported in the literature. For instance, limonene has remarkable anticancer activities connected to the inhibition of tumor initiation, growth, and angiogenesis [31]. Furthermore, the mechanism of action is potentially connected to inducing apoptosis [32]. The antiproliferative activity of limonene on BW5147 cells, colon, gastric, melanoma, and mammary gland tumors has been investigated positively [33]. Antitumor activities of limonene on A549 cells and human melanoma A375-S2 cells are also confirmed [34]. Moreover, alpha-phellandrene (5-isopropyl-2-methyl-1,3-cyclohexadiene) is a cyclic monoterpene with a wide range of biological activities, e.g., showed inhibitory effects against bacteria at concentrations of < 4 mg/mL [35,36]. It also showed a dose-dependent antifungal effect on *Penicillium cyclopean*; The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) are 1.7 and 1.8 mL/L, respectively [37]. Interstingly, it has a necrosis effect in human liver tumor cells [38]. Besides, zingiberene (2-Methylcyclohexa-1,3-diene) is a sesquiterpene hydrocarbon present in ginger rhizomes [39]. It has anti-inflammatory and antioxidant effects [40,41]. It has also possessed anticancer effects on the human colon and gingival cells [42,43]. No report was found about the anticancer activity of AGEO on targeted cell lines (MCF-7, MDA-MB-175, MDA-MB -231, and MDA-MB-468) in the literature. However, many oth-

er uses for AGEO in health and medicine were reported. For instance, reducing hyperlipidemia [44], antibacterial effect [45], antiglycation, and antioxidant properties [46]. A few reports were found on the anticancer activity of CLEO and ZOEO against targeted cell lines. For example, IC50 of CLEO against MCF-7 was determined as 1430 $\mu g.mL^{-1}$, but ZOEO was considered not active [47]. While, in other reports, the effect of ZOEO on MCF-7 was reported as 50.6 and 82.6 $\mu g.mL^{-1}$ [48,49], these values on MDA-MB-231 were 54.7 and 57.5 $\mu g.mL^{-1}$ [48,50]. The mentioned results were significantly different together and compared with the results of this study. Unfortunately, ingredients of used EOs in those researches were not reported in contrast to this research; comparing results is thus impossible. Reviewing the anticancer activities of other EOs showed that some of the obtained IC50 in this research were better than many other reports. For instance, the anticancer activity of CLEO on MCF-7 (IC50: 201 $\mu g.mL^{-1}$) was better than EOs of Mentha spicata L. 284 $\mu g.mL^{-1}$ [51], *Pimpinella anisum* L. 300 $\mu g.mL^{-1}$ [51], and *Pinus peuce* Griseb. 600 $\mu g.mL^{-1}$ [52]. Also, IC50 of CLEO on MDA-MB-231 was better than Pistacia lentiscus L. EO (243 and 616 $\mu g.mL^{-1}$, respectively) [53]. Two articles were also found about MDA-MB-468: IC50 of EOs of *Kelussia odoratissima* Mozaff., *Peristrophe bicalyculata* (Retz.) Nees, and *Borreria verticillata* (L.) G.Mey. were reported as 85, 66.6, and 20.4 $\mu g.mL^{-1}$ respectively [54,55]. AGEO, CLEO, ZOEO could be used in supplementary/complementary medicine, considering the obtained results in the current study. However, their anticancer effects should be investigated *in vivo*, and their potency could be compared with their major ingredients.

Conclusion

Ingredients of EOs of *C. limon, A. graveolens,* and *Z. officinale* were first identified using GC-MS analysis, and their antioxidant activities were then evaluated. Furthermore, their anticancer activities on four human breast cancer cell lines, including MCF-7, MDA-MB-175, MDA-MB-231, and MDA-MB-468, were investigated. Considering the antioxidant and anticancer effects of the EOs, they could be further investigated as a possible complementary medicine in cancer.

Funding

Fasa University of Medical Sciences, with grant number 97011, supported this research. Also, that is ethically approved IR.FUMS.REC.1397.037.

Conflict of Interest

There is no conflict of interest to the authors.

Acknowledgments

None.

References

[1] Bagheri SM, Abdian Asl A, Shams A, Mirghazanfari Bafghi SA, Hafizibarjin Z. Evaluation of cytotoxicity effects of oleo-gum-resin and its essential oil of ferula assa-foetida and ferulic acid on 4T1 breast cancer cells. Indian J Med Paediatr Oncol 2017;38:116-120.

[2] Cao Y, DePinho RA, Ernst M, Vousden K. Cancer research: past, present and future. Nat Rev Cancer 2011;11:749-754.

[3] Sharma GN, Dave R, Sanadya J, Sharma P, Sharma KK. Various types and management of breast cancer: an overview. J

Adv Pharm Technol Res 2010;1:109-126.
[4] Howlader N, et al. SEER Cancer Statistics Review, 1975-2018, National Cancer Institute 2021 [updated April 19, 2021]. Available from: https://seer.cancer.gov/csr/1975_2018.
[5] Rahman SA, Abdul wahab N, Abd Malek SN. In vitro morphological assessment of apoptosis induced by antiproliferative constituents from the rhizomes of *Curcuma zedoaria*. Evid Based Complement Alternat Med 2013;2013:257108.
[6] Asif M, Yehya AHS, Dahham SS, Kithur Mohamed S, Shafaei A, et al. Establishment of in vitro and in vivo anti-colon cancer efficacy of essential oils containing oleogum resin extract of Mesua ferrea. Biomed Pharmacother 2019;109:1620-1629.
[7] Masselink IH, van der Mijden TL, Litvak N, Vanberkel PT. Preparation of chemotherapy drugs: Planning policy for reduced waiting times. Omega 2012;40:181-187.
[8] Liu K, Deng W, Hu W, Cao S, Zhong B, et al. Extraction of 'gannanzao'orange peel essential oil by response surface methodology and its effect on cancer cell proliferation and migration. Molecules 2019;24:499.
[9] Zubair H, Azim S, Ahmad A, Aslam Khan M, Patel GK, et al. Cancer chemoprevention by phytochemicals: Nature's healing touch. Molecules 2017;22:395.
[10] Guo Q, Liu K, Deng W, Zhong B, Yang W, et al. Chemical composition and antimicrobial activity of Gannan navel orange (Citrus sinensis Osbeck cv. Newhall) peel essential oils. Food Sci Nutr 2018;6:1431-1437.
[11] Yeap SK, Abu N, Mohamad NE, Beh BK, Ho WY, et al. Chemopreventive and immunomodulatory effects of Murraya koenigii aqueous extract on 4T1 breast cancer cell-challenged mice. BMC Complement Altern Med 2015;15:306.
[12] Ghadimi SN, Sharifi N, Osanloo M. The leishmanicidal activity of essential oils: A systematic review. J HerbMed Pharmacol 2020;9:300-308.
[13] Osanloo M, Ghaznavi G, Abdollahi A. Surveying the chemical composition and antibacterial activity of essential oils from selected medicinal plants against human pathogens. Iran J Microbiol 2020;12:577-583.
[14] Andrade MA, Braga MA, Souza Cesar PH, Cardoso Trento MV, Esposito MA, et al. Anticancer properties of essential oils: an overview. Curr Cancer Drug Targets 2018;18:957-966.
[15] Bayala B, Bassole I, Scifo R, Gnoula C, Morel L, et al. Anticancer activity of essential oils and their chemical components-a review. Am J Cancer Res 2014;4:591-607.
[16] Scalvenzi L, Grandini A, Spagnoletti A, Tacchini M, Neill D, et al. Myrcia splendens (Sw.) DC. (syn. M. fallax (Rich.) DC.) (Myrtaceae) Essential Oil from Amazonian Ecuador: A Chemical Characterization and Bioactivity Profile. Molecules 2017;22:1163.
[17] Dall'Acqua S, Peron G, Ferrari S, Gandin V, Bramucci M, et al. Phytochemical investigations and antiproliferative secondary metabolites from Thymus alternans growing in Slovakia. Pharm Biol 2017;55:1162-1170.
[18] Lopes CLM, e Sa CG, de Almeida AAC, de Costa JP, Marques THC, et al. Sedative, anxiolytic and antidepressant activities of Citrus limon (Burn) essential oil in mice. Pharmazie 2011;66:623-627.
[19] Campêlo LM, Gonçalves FC, Feitosa CM, de Freitas RM. Antioxidant activity of citrus limon essential oil in mouse hippocampus. Pharm Biol 2011;49:709-715.
[20] Bouzenna H, Dhibi S, Samout N, Rjeibi I, Talarmin H, et al. The protective effect of citrus limon essential oil on hepatotoxicity and nephrotoxicity induced by aspirin in rats. Biomed Pharmacother 2016;83:1327-1334.
[21] Manzuoerh R, Farahpour MR, Oryan A, Sonboli A. Effectiveness of topical administration of Anethum graveolens essential oil on MRSA-infected wounds. Biomed Pharmacother 2019;109:1650-1658.
[22] Goodarzi MT, Khodadadi I, Tavilani H, Abbasi Oshaghi E. The Role of Anethum graveolens L. (Dill) in the Management of Diabetes. J Trop Med 2016;2016:1098916.
[23] Jana S, Shekhawat GS. Anethum graveolens: An Indian traditional medicinal herb and spice. Pharmacogn Rev 2010;4:179-184.
[24] Lima DAN, Pelegrini BB, Alves Uechi FA, Varago RC, Pimenta BB, et al. Evaluation of Antineoplasic Activity of Zingiber Officinale Essential Oil in the Colorectal Region of Wistar Rats. Asian Pac J Cancer Prev 2020;21:2141-2147.
[25] de Silva D, de Cunha KF, Fonseca LM, Antunes MD, Mello El Halal SL, et al. Action of ginger essential oil (Zingiber officinale) encapsulated in proteins ultrafine fibers on the antimicrobial control in situ. Int J Biol Macromol 2018;118:107-115.
[26] Wang J, Ke W, Bao R, Hu X, Chen F. Beneficial effects of ginger Zingiber officinale Roscoe on obesity and metabolic syndrome: a review. Ann N Y Acad Sci 2017;1398:83-98.
[27] Zarenezhad E, Agholi M, Ghanbariasad A, Ranjbar A, Osanloo M. A nanoemulsion-based nanogel of Citrus limon essential oil with leishmanicidal activity against Leishmania tropica and Leishmania major. J Parasit Dis 2020;45:441-448.
[28] Abedinpour N, Ghanbariasad A, Taghinezhad A, Osanloo M. Preparation of Nanoemulsions of Mentha piperita Essential Oil and Investigation of Their Cytotoxic Effect on Human Breast Cancer Lines. BioNanoScience 2021;11:428-436.
[29] Patil M, Patil K, Ngabira D, Bae Seo Y, Kim GD. Phytochemical, antioxidant and antibacterial activity of black tea (Camellia sinensis). Int J Pharmacogn Phytochem Res 2016;8:341-346.
[30] Garcia EJ, Cadorin Oldoni TL, de Alencar SM, Reis A, Loguerico AD, et al. Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. Braz Dent J 2012;23:22-27.
[31] de Araújo□Filho HG, Dos Santos JF, Carvalho MTB, Picot L, Fruitier-Arnaudin I, et al. Anticancer activity of limonene: A systematic review of target signaling pathways. Phytother Res 2021;35:4957-4970.

[32] de Vasconcelos Cerqueira Braz J, de Carvalho FO, Meneses D, Calixto FAF, Santana HSR, et al. Mechanism of action of limonene in tumor cells: a systematic review and metanalysis. Curr Pharm Des 202;27:2956-2965.
[33] Manuele MG, Barreiro Arcos ML, Davicino R, Ferraro G, Cremaschi G, et al. Limonene exerts antiproliferative effects and increases nitric oxide levels on a lymphoma cell line by dual mechanism of the ERK pathway: relationship with oxidative stress. Cancer Invest 2010;28:135-145.
[34] Mukhtar YM, Adu-Frimpong M, Xu X, Yu J. Biochemical significance of limonene and its metabolites: future prospects for designing and developing highly potent anticancer drugs. Biosci Rep 2018;38:BSR20181253.
[35] Demirci F, Kirimer N, Demirci B, Noma Y, Basre KH. Screening of biotransformation products of carvone enantiomers by headspace-SPME/GC-MS. Z Naturforsch C J Biosci 2001;56:58-64.
[36] İşcan G, Kirimer N, Demirci F, Demirci B, Noma Y, et al. Biotransformation of (-)-(R)-α-phellandrene: antimicrobial activity of its major metabolite. Chem Biodivers 2012;9:1525-1532.
[37] Zhang J-H, Sun H-L, Chen S-Y, Zeng L, Wang T-T. Anti-fungal activity, mechanism studies on α-Phellandrene and Nonanal against Penicillium cyclopium. Bot Stud 2017;58:13.
[38] Hsieh S-L, Li Y-C, Chang W-C,Chung J-G, Hsieb L-C, et al. Induction of necrosis in human liver tumor cells by α-phellandrene. Nutr Cancer 2014;66:970-979.
[39] Jeena K, Liju VB, Kuttan R. A preliminary 13-week oral toxicity study of ginger oil in male and female Wistar rats. Int J Toxicol 2011;30:662-670.
[40] Li J, Thangaiyan R, Govindasamy K, Wei J. Anti-inflammatory and anti-apoptotic effect of zingiberene on isoproterenol-induced myocardial infarction in experimental animals. Hum Exp Toxicol 2021;40:915-927.
[41] Türkez H, Toğar B, Çelik K. In vitro study of human lymphocytes cytological and biochemical effects by zingiberene. J Essent Oil Res 2014;26:367-371.
[42] Chen H, Tang X, Liu T, Jing L, Wu J. Zingiberene inhibits in vitro and in vivo human colon cancer cell growth via autophagy induction, suppression of PI3K/AKT/mTOR pathway and caspase 2 deactivation. J BUON 2019;24:1470-1475.
[43] Chopra A, Gayathri R, Vishnu Priya V. Cytotoxic activity of zingiberene on human gingival fibroblast cell lines. Drug Invent Today 2019;12:488-490.
[44] Mirhosseini M, Baradaran A, Rafieian-Kopaei M. Anethum graveolens and hyperlipidemia: A randomized clinical trial. J Res Med Sci 2014;19:758-761.
[45] Kaur GJ, Arora DS. Antibacterial and phytochemical screening of Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi. BMC Complement Altern Med 2009;9:30.
[46] Oshaghi EA, Khodadadi I, Tavilani H, Goodarzi MT. Aqueous extract of Anethum Graveolens L. has potential antioxidant and antiglycation effects. Iran J Med Sci 2016;41:328-333.
[47] Zu Y, Yu H, Liang L, Fu Y, Efferth T, et al. Activities of ten essential oils towards Propionibacterium acnes and PC-3, A-549 and MCF-7 cancer cells. Molecules 2010;15:3200-3210.
[48] Ghasemzadeh A, Jaafar HZ, Karimi E. Involvement of salicylic acid on antioxidant and anticancer properties, anthocyanin production and chalcone synthase activity in ginger (Zingiber officinale Roscoe) varieties. Int J Mol Sci 2012;13:14828-14844.
[49] Lee Y. Cytotoxicity Evaluation of Essential Oil and its Component from Zingiber officinale Roscoe. Toxicol Res 2016;32:225-230.
[50] Ansari JA, Ahmad MK, Khan AR, Fatima N, Khan HJ, et al. Anticancer and antioxidant activity of zingiber officinale roscoe rhizome. Indian J Exp Biol 2016;54:767-773.
[51] Fitsiou E, Mitropoulou G, Spyridopoulou K, Tiptiri-Kourpeti A, Vamvakias M, et al. Phytochemical profile and evaluation of the biological activities of essential oils derived from the greek aromatic plant species ocimum basilicum, mentha spicata, pimpinella anisum and fortunella margarita. Molecules 2016;21:1069.
[52] Basholli-Salihu M, Schuster R, Hajdari A, Mulla D, Viernatein H, et al. Phytochemical composition, anti-inflammatory activity and cytotoxic effects of essential oils from three Pinus spp. Pharm Biol 2017;55:1553-1560.
[53] Catalani S, Palma F, Battistelli S, Benedetti S. Oxidative stress and apoptosis induction in human thyroid carcinoma cells exposed to the essential oil from Pistacia lentiscus aerial parts. PLoS One 2017;12:e0172138.
[54] Momtazi AA, Askari-Khorasgani O, Abdollahi E, Sadeghi-Aliabadi H, Mortazaeinezhad F, et al. Phytochemical analysis and cytotoxicity evaluation of kelussia odoratissima mozaff. J Acupunct Meridian Stud 2017;10:180-186.
[55] Ogunwande IA, Walker TM, Bansal A, Setzer WN, Essien EE. Essential oil constituents and biological activities of Peristrophe bicalyculata and Borreria verticillata. Nat Prod Commun 2010;5:1815-1818.