Research Article

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Ducrosia ismaelis Asch. essential oil: chemical composition profile and anticancer, antimicrobial and antioxidant potential assessment

<https://doi.org/10.1515/chem-2020-0019>

received December 19, 2019; accepted January 23, 2020.

Abstract: The essential oil of *Ducrosia ismaelis* Asch. (Apiaceae) that grows wild in Saudi Arabia was investigated utilizing gas chromatography (GC), and gas chromatography-mass spectrometry. Fifty constituents were characterized, representing 96.1% of the total oil. The *D. ismaelis* essential oil (DIEO) was distinguished by a high composition of oxygenated monoterpenes (51.6%). Decanal (40.6%), α -pinene (15.1%) and dodecanal (13.7%) were the fundamental components. Additionally, DIEO was evaluated for its cytotoxic, antibacterial, antifungal and antioxidant activities. DIEO revealed a great cytotoxic effectiveness against the tested cancer cell lines with IC_{50} values between 66.2 and 137.3 μ g/mL particularly against MCF-7 cancer cells. Furthermore, the induction of apoptosis against MCF-7 cells has been asserted using staining assay (annexin V-FITC and/or propidium iodide (PI) dyes) and flow cytometry technique. The DIEO possessed a strong antimicrobial activity against Gram-positive bacterial and fungal strains with MIC-values between 0.07 and 0.31 mg/ml. The values of MBC or MFC were almost once higher than those of MIC's. Moreover, the β -carotene-bleaching

and DPPH free radical-scavenging tests showed that DIEO had a moderate activity (68%) as an antioxidant agent in decolouring of the β -carotene at 1.0 mg/mL and a moderate radical scavenging for DPPH (66 and 72%) at 0.50 and 1.0 mg/mL.

Keywords: *Ducrosia ismaelis*; essential oil; GC, GC/MS; cytotoxic; antimicrobial; antioxidant.

1 Introduction

The genus *Ducrosia* belongs to the family Apiaceae and comprises only six species: *D. anethifolia* (DC.) Boiss., *D. flabellifolia* Boiss., *D. ismaelis* Asch., *D. assadii* Alava., *D. areysiana* Pimenov & Kljuykov, and *D. inaccessa* Pimenov and Kljuykov, which grow wild in Africa and Asia particularly in Iran, Iraq, Syria, Pakistan, Afghanistan and countries along the Arabian Gulf [1-3]. The genus of *Ducrosia* is represented in Saudi Arabia by three species namely *D. anethifolia*, *D. flabellifolia* and *D. ismaelis*. *D. ismaelis* is indigenous to the central province of Saudi Arabia [4, 5]. This species is a small, glabrous, perennial plant with herbaceous branches from a woody base [5].

The aerial parts of this plant have a distinctive aroma and have been applied in the traditional folk medicine in Saudi Arabia to treat skin infections and as a repellent agent for insects and reptilians [5]. Generally, different species of *Ducrosia* are utilized as pain killers and in common cold treatments in traditional medicine [6]. Moreover, various pharmacological activities e.g. antioxidant, antimycobacterial, antianxiety, central nervous system depressant, antibacterial and antifungal have been accounted for several *Ducrosia* species, including *D. anethifolia*, *D. assadii*, *D. flabellifolia* and *D. ismaelis* [7-10].

Only a single report was found on the essential oil of *D. ismaelis* which showed myrcene, n-undecane, α -pinene,

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n-butylbenzene, γ -cadinene, 3,5-dimethylstyrene, 5-methylindan, *p*-menthadiene, cymene, γ -terpinene, sabinene, decanol, and fenchone as the principal ingredients [11]. Moreover, a phytochemical investigation was performed on the extract obtained from *D. ismaelis* and indicated the presence of isoflavonoids, chalcones, flavonoids, lignans, and phenolic compounds [12]. Previous reports on other *Ducrosia* species e.g. *D. anethifolia* demonstrated the presence of coumarin derivatives e.g. pangolin [10]. Many scientific reports described the chemical composition of the volatile oils of other species belonged to the genus *Ducrosia* particularly *D. anethifolia*, *D. assadii* and *D. flabellifolia* [9,10,13-17]. The phytochemical analysis of *D. anethifolia* essential oil revealed the presence of α -pinene (59.2%), myrcene, (11.6%) and limonene (7.5%) as the major compounds [13], whereas Shahabipour et al., 2013 confirmed that the main compounds of *D. anethifolia* and *D. flabellifolia* oils were dodecanal and decanal [14]. In addition, another study of the essential oil of *D. anethifolia*, demonstrated that α -pinene, terpineolene and ocimene are the major constituents [15]. While n-decanol, n-decanal, and dodecanal were the major constituents in *D. flabellifolia* [16], Mostafavi et al. indicated similar results for the essential oil of *D. assadii* where n-decanal, n-decanol, and α -pinene predominated as well [17].

In spite of the utilization of *D. ismaelis* conventionally in herbal medicine in Saudi Arabia, there is a lack of knowledge on the pharmacological activities and biochemical compounds of the essential oil of *D. ismaelis*. Analysis of previous studies available shows that only a single scientific study has been done regarding the chemical compositions of the essential oil of *D. ismaelis* growing in Saudi Arabia. Continuing our investigations of aromatic plants that widely grow in our region, we illustrate here the cytotoxic, antibacterial, antifungal and antioxidant potentials, and chemical content of the essential oil of *D. ismaelis* (DIEO).

2 Experimental

2.1 Plant material

The aerial part of *D. ismaelis* was collected from the Riyadh region in Saudi Arabia in January 2016. The authentication of *D. ismaelis* was performed at the department of Pharmacognosy, College of Pharmacy, King Saud University (KSU) and a voucher specimen (KSU 16240) was stored in the herbarium of the college.

2.2 Isolation of the volatile oil

The dried aerial part of *D. ismaelis* (300 g) was coarsely ground, placed in a round flask containing 500 ml of water, and distilled utilizing a Clevenger apparatus for 4 h. A desiccation of the acquired essential oil was done over anhydrous sodium sulphate. After filtration, the oil was stored at +4°C until investigation.

2.3 Gas Chromatography/Mass spectrometry investigation

Gas chromatographic analysis was conducted with a Gas Chromatograph (GC 5975) connected with a mass spectrometer (Agilent, USA; SEM Ltd., Istanbul, Turkey). Innowax FSC column (60 m x 0.25 mm, 0.25 μ m film thickness) was utilized as stationary phase. The mobile phase in this experiment was helium with a flow rate of 0.8 mL/min. The injection volume was 0.1 μ L with a split ratio of 40:1. The temperature of the oven was set at 60°C for 10 min, thereafter raised constantly (4°C/min) to reach 220°C, maintained constant for 10 min and then raised slowly (1°C/min) to reach 240°C. The detection of mass spectra was done at 70 eV with scan mass extent of *m/z* 35-450.

2.4 Gas Chromatography/FID investigation

The analysis was conducted with the gas chromatograph system with FID detector (an Agilent Technologies 6890 N GC). The same stationary and mobile phases used in GC-MS experiment and same operating conditions were executed in triplicate. The FID temperature was adjusted to 300°C. Auto injection was implemented simultaneously to acquire equivalent retention times. Quantitative data was calculated from the FID peak area percent normalization.

2.5 Chemical content

The identification of the chemical compounds of the DIEO was achieved by the comparison of the MS with those of similar components based on their indexes of retention using Adams library [18], Mass Finder terpenoid library [19], Wiley GC/MS Library [20] and our own Baser Library of Volatile Oil Constituents. The DIEO compounds were specified by comparison of the retention times with genuine reference compounds and by comparing the

Table 1: Chemical content of the essential oil of *Ducrosia ismaelis*.

No.	RRI	Compounds	%	Identification	No.	RRI	Compounds	%	Identification
1	1032	α -Pinene	15.1	t_R , MS	30	1763	Decanol	3.4	t_R , MS
2	1035	α -Thujene	0.1	MS	31	1772	Citronellol	3.1	t_R , MS
3	1048	2-Methyl-3-buten-2-ol	tr	MS	32	1856	Geraniol	0.2	t_R , MS
4	1076	Camphene	0.2	t_R , MS	33	1864	<i>p</i> -Cymen-8-ol	0.2	t_R , MS
5	1118	β -Pinene	0.6	t_R , MS	34	1871	1-Undecanol	0.3	MS
6	1132	Sabinene	0.8	t_R , MS	35	1875	(E)-2-Dodecenal	0.3	MS
7	1138	Thuja-2,4 (10)-dien	0.1	MS	36	1925	2,3,4-Trimethyl benzaldehyde	0.4	MS
8	1174	Myrcene	3.7	t_R , MS	37	1937	Tetradecanal	0.4	MS
9	1195	Dehydro-1,8-cineole	0.3	t_R , MS	38	1965	1-Dodecanol	0.1	MS
10	1203	Limonene	1.8	t_R , MS	39	2096	(E)-2-Tetradecenal	0.5	MS
11	1213	β -Phellandrene	0.8	t_R , MS	40	2127	10- <i>epi</i> -g-Eudesmol	0.2	MS
12	1232	(E)-2-Hexenal	0.1	t_R	41	2157	5- <i>epi</i> -7- <i>epi</i> -a-Eudesmol	tr	MS
13	1255	γ -Terpinene	0.1	t_R , MS	42	2174	Nonanoic acid	tr	MS
14	1280	<i>p</i> -Cymene	0.1	t_R , MS	43	2187	g-Eudesmol	tr	MS
15	1290	Terpinolene	0.6	t_R , MS	44	2200	<i>trans</i> -Methyl isoeugenol	0.8	MS
16	1296	Octanal	0.1	t_R , MS	45	2219	4-Vinyl guaiacol	tr	t_R , MS
17	1384	α -Pinene oxide	0.1	MS	46	2246	a-Eudesmol	tr	MS
18	1400	Nonanal	0.4	MS	47	2255	b-Eudesmol	0.2	MS
19	1477	6-Camphenone	0.6	MS	48	2286	Decanoic acid	3.4	MS
20	1487	Citronellal	0.7	MS	49	2296	Myristicine	0.5	MS
21	1506	Decanal	40.6	MS	50	2503	Dodecanoic acid	0.3	t_R , MS
22	1553	Linalool	0.7	t_R , MS			Monoterpene hydrocarbons	23.4	
23	1562	Octanol	tr	t_R , MS			Oxygenated monoterpenes	51.6	
24	1571	<i>trans</i> - <i>p</i> -Menth-2-en-1-ol	tr	MS			Oxygenated sesquiterpenes	0.4	
25	1611	Terpinen-4-ol	0.3	t_R , MS			Others	20.7	
26	1638	<i>cis</i> - <i>p</i> -Menth-2-en-1-ol	0.1	MS			Total %	96.1	
27	1664	Nonanol	0.7	MS					
28	1706	α -Terpineol	0.1	t_R , MS					
29	1722	Dodecanal	13.7	t_R , MS					

RRI; Relative retention indices calculated against n-alkanes. %; calculated from the FID chromatograms; tr; Trace (<0.1 %); Identification method: t_R , identification based on the retention times (t_R) of genuine compounds on the HP Innowax column; MS, detected by software matching of the mass spectrum with those of the Wiley and MassFinder libraries and comparison with literature results.

retention index (RRI) relative to C8-C30 of n-alkanes under the operational conditions described above [21]. The results obtained from three replicates were statistically analysed and arranged in Table 1 (mean $\pm$ standard deviation).

2.6 Determination of cytotoxic potential (MTT assay)

The cytotoxic potential of DIEO was investigated utilizing MTT assay [22] against three diverse human cancer cell

lines included HepG2 (liver-cancer), LoVo (colon-cancer) and MCF-7 (breast-cancer). The cells were incubated using DMEM enhanced with 10% FBS at 37°C with 5% CO₂. Shortly, one mL of cell suspension (5 X 10⁴ cells/mL) was seeded in 24-well plate. The treatment with different concentrations of DIEO was performed after the cultivation of the cells for 24 hours. Then, each well of the plates received 100 µL of MTT solution (5 mg/ml) and incubated at 37°C for 2-4 hours. The measurement of the diminished MTT was achieved at 540 nm with microplate ELISA reader (Thermo Fisher Scientific, USA); wells containing cells treated with DMSO (1%) were considered as controls. Vinblastine was utilized as a positive control. The IC₅₀ of DIEO was measured from the dose-response curves using the following formula:

$$\% \text{ Cell Viability} = \frac{\text{Mean absorbance of DIEO}}{\text{Mean absorbance of control}} \times 100$$

2.7 Light microscopy study

MCF-7 cells have been cultivated using 12-well plates, followed by the incubation for 24 h and the treatment with and without DIEO at the IC₅₀ concentration. The observation of the morphological alterations of the apoptotic cells were achieved utilizing phase contrast inverted microscope (Evos, USA).

2.8 Apoptosis assessment by DAPI staining and acridine orange/ethidium bromide assays

MCF-7 cells were handled with IC₅₀ of DIEO for 24 h, then washed with phosphate buffered saline (PBS) and fixed with ethanol for 15 min at 25°C. After that cells were stained with DAPI (2 µg/mL) and incubated again in the dark for 30 min. Finally, MCF-7 cells were investigated and imaged utilizing a fluorescence microscope (Evos, USA). In order to perform the acridine orange/ethidium bromide staining test, 2 µL of acridine orange/ethidium bromide (one part each of 3 mg/mL acridine orange and 3 mg/mL ethidium bromide in PBS) was mixed with 1 mL suspension of MCF-7 cell in a 12-well plate. The examination of the cells was achieved on an EVOS® imaging connected to a digital imaging system.

2.9 Flow cytometry analysis of cell apoptosis

The apoptosis was detected using the procedure depicted in the manufacturer's instructions of Alexa Fluor 488

Annexin V/Dead Cell Apoptosis kit (Thermo Fisher Scientific, Inc.). In short, MCF-7 cells were seeded for adherence in a tissue culture plate (6-well) at a density of 1x10⁶ cells/well at 37°C for 24 hours, and then were treated with DIEO IC₅₀. After a 24 h treatment with DIEO, the cells were gathered, washed twice in cold PBS and resuspended in kit specific binding buffer. Then, the cells were incubated in the dark with 5 µL of Annexin V-FITC and 5 µL of PI for 15 min. Finally, 400 µL of annexin-binding buffer was given to the cells and promptly analysed using flow cytometry.

2.10 Determination of antimicrobial activity (Determination of MIC, MBC, MFC)

The effectiveness of DIEO as an antimicrobial agent was determined using two-fold micro-dilution assay following by cultivation method for measuring MIC, MBC and MFC. The microorganisms used in the present work included *Staphylococcus aureus* TCC 25923, *Staphylococcus epidermidis* ATCC 12228), *Escherichia coli* ATCC 25922, *Acinetobacter* sp. ATCC 49139, *Aspergillus ochraceus* AUMC 9478, *Penicillium chrysogenum* AUMC 9476, *Candida albicans* ATCC 60193 and *Rhodotorula* sp. Wild type [23]. The MIC (minimal inhibition concentration) of DIEO was assigned as the lowest concentration exhibiting no tangible microbial growth. Gentamycin and nystatin (125 to 0.97 µg/mL) were applied as positive antibacterial and antifungal controls. The minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) were assessed among the concentrations that had ability to kill all microbial cells in wells using the sub-cultivation method.

2.11 Determination of antioxidant activity

2.11.1 DPPH free radical-scavenging assay

The antioxidative activity of DIEO was assessed in terms of hydrogen donating capability utilizing 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging test as described previously by Brand-Williams et al. [24]. The absorbance was measured at λ = 517 nm utilizing a UV-spectrophotometer (UV mini-1240, Shimadzu, Japan) and calculated stratifying (utilizing?) the formula:

$$\% \text{ of anti-radical activity} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100.$$

Figure 1: Major constituents of DIEO.

2.11.2 β-Carotene bleaching assay

The potential of DIEO as antioxidant agent has been investigated with the β-carotene bleaching test as reported previously by Mothana and co-workers [25]. The prepared solution of β-carotene (1 mL of 200 µg dissolved in 1 mL chloroform) was added to a solution of 200 µL of Tween-20 and 20 µL of linoleic acid in a flask, followed by evaporation of the chloroform. Then, distilled water (100 mL) was added into the flask and mixed for 2 min. 200 µL of the DIEO (1000 µg/mL) was mixed with 2 mL of the β-carotene-linoleic acid emulsion and incubated at 40°C for 2 hours. Finally, the absorbance was read at intervals of 30 min at 470 nm. Rutin (1000 µg/mL) was the positive control. The following formula determined the antioxidant activity:

$$\text{Antioxidant activity (\%)} = 1 - (\text{Abs}_0 - \text{Abs}_t) / (\text{Abs}_0 - \text{Abs}^0_t) \times 100$$

where, Abs₀ and Abs⁰₀ are the optical densities of DIEO and blank samples respectively, that recorded at zero time of incubation. Abs_t and Abs⁰_t are the optical densities determined for DIEO and blank samples at 120 min, respectively.

2.12 Experimental design and statistical analysis

The present experiment was carried out in a completely randomized design (CRD) and the mean and standard deviations (SD) (N=3) were calculated. The significant differences were evaluated using the Tukey test in one-way ANOVA at P < 0.05 (IBM, SPSS, statistics 25).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

The aerial part of *D. ismaelis* afforded a light yellow oil (0.28% w/w) with a strong aromatic odour.

3.1 Chemical content of DIEO

The results of the GC/FID and GC/MS analysis are shown in Table 1. The components of the oil, their retention indices, percentage composition and identification methods are summarized in Table 1. The GC/FID analyses of the *D. ismaelis* essential oil (DIEO) led to the identification of fifty compounds, representing 96.1% of the total oil. The oil (DIEO) was characterized by a high content of oxygenated monoterpenes (51.6%) followed by monoterpene hydrocarbons (23.4%). Decanal (40.6%), α-pinene (15.1%) and dodecanal (13.7%) were the major constituents found in DIEO (Table 1, Figure 1).

3.2 Cytotoxicity and apoptosis staining

The essential oil of *D. ismaelis* (DIEO) exhibited a noteworthy cytotoxic potential against the tested cancer cell lines with IC₅₀ values ranging between 66.24 to 137.32 µg/mL (Table 2 and Figure 2). The greatest activity was demonstrated against MCF-7 cancer cells with IC₅₀ value of 66.24 µg/mL. All tested cells showed a dose-dependent decrease in cell proliferation (Figure 2). The morphology of MCF7 cells was examined after DIEO treatment to determine if any changes occurred in the appearance of cell morphology. The treatment of MCF7 cells with the IC₅₀ concentration of the DIEO for 24 h had significant effect on the appearance and morphology of the MCF-7 cells including decreased densities of the cells, shrinkage detachment and loss of cell integrity compared to cells cultivated with DMSO vehicle control (Figure 3). Furthermore, there was a significant decrease in cell number of the treated cells. Two cell staining assays (DAPI

Table 2: Cytotoxic activity of *D. ismaelis* essential oil against different cancer cells.

Sample	Cell lines and IC ₅₀ (µg/ mL)		
	MCF-7	HepG2	LoVo
DIEO	66.24 ± 1.26	137.32 ± 2.48	102.53 ± 1.0
Vinblastine	1.8 ± 0.26	2.5 ± 0.38	2.4 ± 0.8

Figure 2: Effect of DIEO on growth inhibition of MCF7, HepG2 and LoVo cell lines. The cells were incubated with increasing concentrations of DIEO, the cell growth was measured using the MTT assay after 48h. The data shows mean values ± standard deviation (SD) of at least 3 independent experiments. The data were analyzed using Origin 8 Pro and significant differences confirmed by student T-test * (P < 0.05).

and acridine orange/ethidium bromide) were employed to examine the effect of DIEO on the nuclear morphological changes in treated MCF7 cells after treatment with IC₅₀. MCF-7 cells treated with a DMSO-vehicle control showed primarily nuclear staining with acridine orange and no ethidium bromide staining, while treatment with the DIEO for 24 h exhibited a change in cell morphology including changes in nuclear staining corresponding to condensation and presence of some staining with ethidium bromide (Figure 3). The representative signs of apoptosis in the treated cells were also obvious (Figure 3). The induction of apoptosis was affirmed quantitatively utilizing flow cytometer analysis. The labelling of the cells was achieved with Annexin VFITC and/or propidium iodide (PI) dyes (Figure 4). DIEO mediated apoptosis in treated MCF-7 cell lines at 24 hours of handling. There

Figure 3: Morphological changes and apoptosis detection in MCF 7 cells treated with DIEO. MCF7 cells were cultured and treated with the IC₅₀ of DIEO for 24h. (A) Morphological changes seen under light phase contrast microscope, (B) Apoptotic cells were investigated by fluorescence DAPI staining, (C) Cells were stained with a mixture of (AO: Acridine Orange, EtBr: Ethidium Bromide). Viable cells excluded EtBr and their nuclei were bright green, while apoptotic cells were orange to red color.

was no incidence of apoptosis (0 percent) in untreated cells suggesting that the DIEO is the source of apoptosis induction (Figure 4). As the treatment time increased to 24 hours, the apoptotic cells percentage (early and late) was also raised in proportion to the exposure time.

3.3 Antimicrobial activity

The antimicrobial potential against bacterial and fungal strains is shown in Table 3. Minimal inhibitory concentrations (MICs), minimal bactericidal concentration (MBCs) and minimal fungicidal concentration (MFCs) of DIEO are shown in Table 3. The DIEO exhibited different growth inhibitions of the microbial strains with MIC-values ranging from 0.07 to 5.0 mg/mL. The values of MBC or MFC were, once, almost higher than those of MIC's (Table 3). While the Gram-negative bacterial strains e.g. *E. coli* showed less sensitivity with MIC-value of 2.5 mg/mL, the most prone microbial strain (MIC: 0.07 mg/mL) was the Gram-positive *S. aureus* followed by the fungal strains (0.15 to 0.31 mg/mL). The Gram-negative bacterial strains e.g. *E. coli* showed less sensitivity with MIC-value of 2.5 mg/mL.

Figure 4: Apoptosis detection using flow cytometry after annexin V-FITC/propidium iodide (PI) staining for (a) Control and (b) DIEO. Viable cells are in the lower left quadrant (AA3); early apoptotic cells are in the lower right quadrant (AA4); late apoptotic cells are in the upper right quadrant (AA2); and non-viable necrotic cells are in the upper left quadrant (AA1).

Table 3: Antimicrobial activity of the *D. ismaelis* essential oil (DIEO) (mg/mL).

Microorganisms		Activity	DIEO	Gentamycin	Nystatin
Bacteria	<i>S. aureus</i>	MIC	0.07	7.8	NT
		MBC	0.15	15.6	NT
	<i>S. epidermidis</i>	MIC	0.07	7.8	NT
		MBC	0.15	15.6	NT
	<i>E. coli</i>	MIC	2.5	3.9	NT
		MBC	5.0	7.8	NT
Fungi	<i>Acintobacter</i> sp.	MIC	2.5	3.9	NT
		MBC	5.0	7.8	NT
	<i>C. albicans</i>	MIC	0.31	NT	3.5
		MFC	0.62	NT	7.0
	<i>Rhodotorula</i> sp.	MIC	0.15	NT	3.5
		MFC	0.62	NT	7.0
	<i>A. ochraceus</i>	MIC	0.15	NT	3.5
		MFC	0.31	NT	7.0
	<i>P. chrysogenum</i>	MIC	0.15	NT	3.5
		MFC	0.31	NT	7.0

S. aureus: *Staphylococcus aureus* ATCC 25923, *S. epidermidis*: *Staphylococcus epidermidis* ATCC 12228, *E. coli*: *Escherichia coli* ATCC 25922, *Acintobacter* sp. ATCC 49139, *C. albicans*: *Candida albicans* ATCC 60193, *Rhodotorula* sp. Wild type, *A. ochraceus*: *Aspergillus ochraceus* AUMC 9478 and *P. chrysogenum*: *Penicillium chrysogenum* AUMC 9476, Values are presented as mg/ml for DIEO and µg/mL for positive controls, NT: not tested. MIC: Minimal inhibitory concentrations, MBC: minimal bactericidal concentration, and MFC: minimal fungicidal concentration.

3.4 Antioxidant activity

The results of the radical scavenging and antioxidative potential of DIEO are displayed in Table 4. DIEO exhibited a marked power in the β -carotene-bleaching assay to diminish the β -carotene bleaching at of 1.0 mg/mL with a total antioxidant value of 68% (Table 4). In addition, DIEO had a notable free radical scavenging (66 and 72%) at the concentrations 0.5 and 1.0 mg/mL (Table 4).

4 Discussion

Volatile oils are commonly used in the traditional medicine throughout human history. The use of this type of natural products include a wide range of curative and hygienic applications. Several investigations in various cell and animal models have shown the efficacy of the volatile oils as antimicrobials, antioxidants, anti-inflammatory and anti-cancer agents [26]. Investigations on aromatic medicinal plants therefore need to be pursued in order to explore novel and more effective remedies. Consequently, this study has been conducted in our search for substantial and hopeful natural products from medicinal plants grown in the Middle East. In the current work, we evaluated the chemical content of the essential oil of *D. ismaelis* by GC/FID and GC/MS. Furthermore, the existing investigation has been expanded to assess the cytotoxic, antimicrobial and antioxidant potentials and to report the conceivable apoptotic passageway with Annexin VFITC and/or propidium iodide (PI) dyes and flow cytometer analyses.

The obtainable information on the species *D. ismaelis* is generally limited. Only one single report was found

Table 4: Antioxidant and free radical scavenging activities of the *D. ismaelis* essential oil (DIEO).

Samples	Total antioxidant activity in % (1000 µg/mL)	Free radical scavenging activity in % (DPPH) assay				
		10	50	100 (µg/mL)	500	1000
DIEO	68.5 ± 2.2	17.9 ± 1.8	28.8 ± 3.0	40.2 ± 2.5	66.9 ± 1.9	72.1 ± 2.8
Ascorbic acid	NT	71.8 ± 2.1	80.2 ± 3.1	87.5 ± 2.4	92.2 ± 3.0	94.2 ± 2.8
Rutin	91.2 ± 2.9	NT	NT	NT	NT	NT

NT: not tested.

on the chemical composition of the essential oil of *D. ismaelis* which was carried out in 1985 and demonstrated the presence of myrcene, n-undecane, α-pinene, n-butylbenzene, γ-cadinene, 3,5-dimethylstyrene, 5-methylindan, p-menthadiene, cymene, γ-terpinene, sabinene, decanol, and fenchone as the fundamental compounds [6].

Our data showed that DIEO was characterized by a high oxygenated monoterpene content (51.6%) followed by monoterpene hydrocarbons (23.4%), of which decanal (40.6%), α-pinene (15.1%) and dodecanal (13.7%) were the dominant constituents. The comparison of our data with the former information revealed in the chemical content of the volatile oil of *D. ismaelis* [11], a significant difference in quantity and quality among them. On the other hand, our results were, to some extent, in agreement with a previous report on *D. assadii* [27] which indicated the presence of 46.68% and 42.21% of n-decanal in flowers and fruits of *D. assadii*, respectively. In addition, Rustaiyan and co-workers reported in 2006 the presence of n-decanal (36.4%) as the predominant compound in the essential oil of the aerial part of *D. assadii* [28]. Moreover, Mazloomifar and Valian (2013) investigated the essential oil of *D. anethifolia* and reported that the major components were n-decanal (30.3%), dodecanal (14.3%) and n-decanol (11.0%) [29]. Our findings were also to some extent in agreement with the previous study by Shahabipour and co-workers (2013) [14], who identified dodecanal (28.8%), decanal (21.1%), (2E)-tridecen-1-al (15.8%) and (2E)-dodecenal (13.4%) in the oil of *D. anethifolia* and decanal (32.8%), dodecanal (32.6%), decanol (4.3%) and (2E)-tridecen-1-al (3.3%) in the oil of *D. flabellifolia* as the major components.

In the existing investigation, we observed auspicious cytotoxic, antimicrobial and antioxidative activities for DIEO. DIEO's cytotoxic effect was evaluated against three different cancer cell lines (MCF-7, HepG2 and LoVo) utilizing MTT assay. DIEO showed remarkable cytotoxic activity against the three cancers investigated, in particular MCF-7 cancer cells. Overall, data on cytotoxicity

of DIEO are still rare however, there are few reports on the cytotoxicity of other *Ducrosia* species e.g. *D. flabellifolia*, *D. anethifolia* which indicated good to moderate cytotoxic activity of the isolated essential oils [14]. Additionally, Talib and co-workers [7] reported a promising activity against MCF-7 cell line with IC₅₀ value of 25.3 µg/mL for the ethanolic extract of *D. flabellifolia* [7]. They also observed that the anticancer impact was exerted by inducing programmed cell death (apoptosis) as demonstrated by the fragmentation of DNA and apoptotic bodies formation in treated cancer cells [7].

Apoptosis (programmed cell death) is a physiological process in which harmed or abnormal cells can be discarded. Consequently, the induction of apoptosis appears to be an appealing aim to kill tumour cells and is recognized, therefore, as a useful strategy in both treatment as well as prevention of cancers [30-33]. In the existing study, the morphological alterations of apoptotic cells have been monitored utilizing fluorescence microscopy after double staining with acridine orange and ethidium bromide (AO/EtBr) and DAPI fluorescence. Furthermore, the apoptosis was proven utilizing flow cytometry after staining with annexin V-FITC/PI. While untreated cells, on the contrary, exhibited normal morphology DIEO-treated cells showed morphological changes. The main characteristics observed in our investigation, involve cell shrinkage, chromatin condensation and DNA fragmentation. An earlier study [34] carried out by Liu and co-workers (2012) indicated a strong cytotoxic effect against Hela cell line for the sweet orange oil and the isolated decanal with IC₅₀ values of 5.5 and 4.5 µg/mL, respectively. Moreover, compounds such as carvacrol, α-pinene, thymol and eugenol showed a cytotoxic activity against several cancer cells e.g. HepG2, MCF-7 and MDA-MB-468 [35-37]. Accordingly, the observed cytotoxic activity of DIEO could probably be attributed to decanal and α-pinene which were the fundamental constituents of DIEO. In our experiments, observed antimicrobial activity for DIEO was also in agreement with the results by Al-meshal who

reported antimicrobial activity for the essential oil of *D. ismaelis* [6]. Liu and co-workers [34] reported interesting antimicrobial and antioxidant activities for decanal isolated from sweet orange oil. The liposolubility of DIEO and the major compounds may affect membrane fluidity and cause degradation or destruction of cell membrane, and this in turn, can lead to antimicrobial and cytotoxic activity [34,38].

5 Conclusion

The analyses with GC and GC/MS concluded that the chemical composition of *D. ismaelis* essential oil (DIEO) was high in oxygenated monoterpenoids where the fundamental constituents were decanal (40.6%), α -pinene (15.1%) and dodecanal (13.7%). The results obviously revealed that DIEO has interesting cytotoxic, antimicrobial and mild antioxidant activities. The acquired results proposed that DIEO induced apoptosis in MCF-7 cells. Our findings, therefore, support the assumption that DIEO may be a hopeful cytotoxic and antimicrobial agent. Additional experiments are required to isolate the active principles and to reveal the mechanism of actions.

Author Contributions: R.A.M. performed study design, supervised the experimental work, carried out collection and interpretation of the data, literature search and wrote the manuscript; F.A.N. and N.A. performed the cytotoxicity and apoptosis experiments; JMK performed the antimicrobial assays; O.M.N. and O.M.A. carried out the antioxidant assays; AJA collected the medicinal plant; M.K. performed the GC and GC/MS experiments. All authors read and approved the final manuscript.

Conflict of interests: The authors of this MS have declared no conflict of interests.

Acknowledgments: The authors extend their appreciation to Researchers Supporting Project number (RSP-2019/119), King Saud University, Riyadh, Saudi Arabia for funding this work.

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