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Chemical composition of *Pluchea dioscoridis* (L.) DC. essential oils from different natural habitats with their anticancer and antimicrobial potential

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Abstract

Plants of the Asteraceae family are widely used all over the world as traditional medicinal taxa for the therapy of various ailments. This study aims to investigate the chemical profiles of *Pluchea dioscoridis* (L.) DC. essential oils (EOs) from four different natural habitats; canal habitat (CH), urban habitat (UH), agricultural habitat (AH), and wadi habitat (WH), in addition to evaluating their antimicrobial and cytotoxic effects. The EOs were extracted by hydrodistillation method and were analyzed by gas chromatography-mass spectroscopy (GC-MS) technique. Fifty-six compounds have been identified, sesquiterpenes being the major components of the EOs with 69.62% (CH), 60.68% (UH), 78.27% (AH), and 94.87% (WH). The predominant sesquiterpenes were 6-epi-shyobunol (26.38%, WH), aromadendrene (16.72%, CH), Ç-muurolene (14.51%, CH), himachalol (13.48%, UH), tau-muurolol (12.5%, WH), cubebol (9.78%, WH), cubenol (9.39%, WH), and caryophyllene oxide (8.27%, WH). Moreover, the antimicrobial effects of the investigated EOs were studied against ten pathogens, including three Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus mutants*), three Gram-negative (*Escherichia coli*, *Enterobacter cloacae*, *Proteus vulgaris*), and four fungi (*Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Syncephalastrum racemosum*) using standard agar disc-diffusion technique. Among the studied organisms, *C. neoformans* showed significant antimicrobial potency for the EOs from the four habitats. Furthermore, EOs of the four habitats exhibited potential cytotoxicity against the colon carcinoma cell line (HCT116) with LC_{50} of 29.4 (CH), 23.6 (UH), 18.0 (AH), and 16.1 (WH) µg/ml, respectively. No effects were observed for breast adenocarcinoma (MCF-7), and hepatocellular carcinoma (HepG2) cell lines. EOs extracted from *P. dioscoridis*, especially for (WH) could be used as alternatives to synthetic antifungal and anticancer agents.

Keywords: Asteraceae, *Conyza*, *Cryptococcus neoformans*, HCT116, hydrodistillation, sesquiterpenes

1. Introduction

Medicinal and aromatic plants include a wide range of bioactive pharmacological chemicals that have inclusive varieties of therapeutic effects and contribute to both ecological and economic benefits [1, 2]. EOs are aromatic liquids created by a number of complicated metabolic pathways in plants with the aim of protecting them against a variety of pathogens [3, 4]. The soil conditions, genotype, species, harvest seasons, climatic factors, fertilizers, extraction techniques, and mode of production are some of the variables affecting the chemical contents of EOs. Monoterpenes and sesquiterpenes are the most significant components of essential oils and are responsible for their unique aroma and different therapeutic actions [5, 6]. Many of the health benefits of EOs include anti-inflammatory, antibiotic, antibacterial, antiviral, and anticancer activities [7-9]. They have been widely used in various therapeutic diseases like sleep disorders, stress, cardiovascular problems, Alzheimer's disease, and pregnant labor pain [7, 8, 10]. In the past decade, various EOs have been examined for their *in vitro* and *in vivo* cytotoxic effect, attributed to numerous mechanisms, including loss of vital organ function,

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induction of apoptosis and/or necrosis, and cell cycle arrest [11].

There are over 150 plant species in the genus *Conyza* (family Asteraceae) [12], which were identified as traditional medicinal herbs for the treatment of diarrhea, rheumatism, hemorrhoids, wounds bleeding, skin diseases as well as toothaches [13-14]. *Conyza dioscoridis* (L.) Desf is one of the *Conyza* species, its name has been previously modified due to taxonomic criteria, and now displays as *Pluchea dioscoridis* (L.) DC. [15, 16]. *P. dioscoridis* is a widely dispersed wild plant that grows naturally in the Mediterranean coast, Sinai Peninsula, Western Desert, Eastern Desert, and Nile Delta [15]. The plant was mentioned in folk medicines for the treatment of a number of diseases, including colds, ulcers, colic, carminative, paediatric epilepsy, and rheumatoid pains. [17]. Numerous studies have shown that the various extracts of this plant have powerful biological effects, including diuretic, anti-inflammatory, antipyretic, anti-ulcer, antinociceptive, anti-diarrheal, antidiabetic, antibacterial, and antifungal potentials [17, 18]. From the phytochemical view, *P. dioscoridis* produced a wide range of bioactive secondary metabolites, such as triterpenes, steroids, phenolic acids, and flavonoids [14, 17, 19]. The objective of the current research was to examine the impact of habitat change on the chemical profiles of EOs from *P. dioscoridis*, in addition to evaluating antimicrobial and cytotoxic effects.

2. Experimental

2.1. Plant material

The stem branches of *P. dioscoridis* were collected from various habitats including a canal (CH), urban (UH), agricultural (AH), and wadi (WH) in January 2019 (**Table 1**). The collected samples were authenticated by Dr. Hend A. Saleh (co-author) according to Boulos [15]. Voucher specimens (No. WSM/1- WSM/4) were deposited by Prof. Dr. Mona M. Marzouk in the herbarium of the National Research Centre (CAIRC), Department of Phytochemistry and Plant systematics, Cairo, Egypt.

2.2. Hydrodistillation extraction of EOs

The extraction of *P. dioscoridis* EO was carried out with 200 gm, for each habitat. Separately, each sample was subjected to Clevenger-type apparatuses applying a round flask (2.5 L) containing 1.5 L of distilled water for hydrodistillation (5 h). Individually, the fatty layer of each sample was isolated and then dehydrated over anhydrous Na_2SO_4 (0.5 g). Finally, each oil sample was stored in glass vials and kept in the freezer till GC-MS analysis.

Table 1. Studied area of *P. dioscoridis*

Studied area	Locality (Egypt)	*Altitude*
CH	Ismailia canal, Qalyubia government	30 29 °*N* 31 38° *E*
UH	Belbeis, Al-Sharqiyah government	30 33°*N* 31 43° *E*
AH	Al Khankah agricultural land, Al-Qalyubia government	30 23 °*N* 31 38 °*E*
WH	The midstream of wadi Hagul, the Northern portion of the Eastern Desert, Cairo-Suez district	29 58 °*N* 32 08 °*E*

CH; Canal habitat, UH; Urban habitat, AH; Agricultural habitat, WH; Wadi habitat

2.3. GC-MS analysis

The four EOs samples were separately analyzed as stated by El Shamy et al. [20] using a GC-MS device: Trace GC Ultra Chromatographs (Thermo Scientific™ Corporate, Waltham, MA, USA) connected with EC single quadrupole mass spectrometer (Thermo Scientific ISQ™) at National Research Centre, Egypt.

2.4 Antimicrobial evaluation

2.4.1. Pathogen Strains

Ten tested microorganisms including three Gram-positive strains [*Bacillus subtilis* RCMB 015(1) NRRI B-543, *Staphylococcus aureus* RCMB 010010, and *Streptococcus mutants* (RCMB017(1) ATCC 25175], three Gram-negative strains [*Enterobacter cloacae* RCMB 001(1) ATCC23355, *Escherichia coli* RCMB 01005(2) ATCC25955, and *Proteus vulgaris* RCMB 004(1) ATCC 13315], as well as four fungal strains [*Aspergillus fumigatus* RCMB 002008, *Candida albicans* RCMB 005003(1) ATCC10231, *Cryptococcus neoformans* RCMB 0049001, and *Syncephalastrum racemosum* RCMB 016001(1)]. The tested strains were provided from Al–Azhar University, the Regional Centre for Mycology and Biotechnology, Egypt.

2.4.2. Antimicrobial Assays

The antimicrobial activities of the EOs of *P. dioscoridis* were evaluated using the Agar disc-diffusion method. Agar dishes are incubated with a standard inoculum of the tested microorganisms, filter paper discs containing the EOs (50 μL, 6 mm in diameter) are placed on the surface of the agar, and then Petri dishes are incubated under appropriate conditions. Commonly, an antimicrobial agent spreads into the agar layer and inhibits the germination of the tested pathogens. Finally, the diameters of each inhibition zone are measured [21]. Ketoconazole and Gentamicin (each, 50 μL) were used as the positive controls.

2.5. Cytotoxic Activity

2.5.1. Cell Lines

Human tumor cell lines: breast adenocarcinoma (MCF-7), colon carcinoma (HCT116), and hepatocellular carcinoma (HepG2) were examined. The cell lines were maintained at the Bioassay-Cell Culture Laboratory, National Research Centre, Egypt.

2.5.2. Cell Viability Assay

In the current study, the cytotoxic effects of *P. dioscoridis* EOs were carried out using the MTT assay according to the method of Khan et al., 2018 [22].

2.6. Statistical analysis

Experiments for soil and antimicrobial analysis were carried out in triplicates. Data were statistically analyzed using the ANOVA one-way analysis ($p<0.05$) to compare significant differences. The computations were done by using the SPSS software version (25). Values presented are means ± SD (standard deviation) of three replicates.

3. Results and Discussion

3.1. Soil Analysis

The soil analysis for the different habitats were shown in **Table 2**. The values of cations and anions were substantially distinct between the four habitats samples. Mainly, the greatest values were demonstrated in the AH region and the lowest ones were at WH.

Table 2. Mean values of soluble ions content in soil collected from different habitats

Analysis of soil					
Soluble ions		**CH**	**UH**	**AH**	**WH**
Cations	**Ca^{++}**	158.8 ±0.1[a]	858.1 ±0.1[c]	2452.5 ±0.5[d]	775.0 ±0.21[b]
	Mg^{++}	62.4 ±0.5[c]	43.7 ±0.4[b]	174.3 ±0.3[d]	25.0 ±0.005[a]
	Na^{+}	1307.8 ±0.1[d]	249.5 ±0.5[b]	1008.3 ±0.3[c]	147.7 ±0.7[a]
	K^{+}	311.5 ±0.5[c]	78.0 ±0.1[b]	460.6 ±0.09[d]	12.0 ±0.24[a]
Anions	**Cl^{-}**	1.6 ±0.1[c]	0.4 ±0.01[a]	0.79 ±0.1[b]	7.55 ±1.7[c]
	SO_4^{--}	1.28 ±0.01[b]	3.1 ±0.1[c]	12.5 ±0.1[d]	0.9 ±0.1[a]
	HCO_3^{-}	2887.1 ±0.1[c]	1370.4 ±0.1[a]	1501.8 ±0.1[b]	2997 ±10.1[d]

Values are the mean of three replicates ± standard deviation, the values in the same row with the same letter are not significant. CH; Canal habitat, UH; Urban habitat, AH; Agricultural habitat, WH; Wadi habitat

3.2. Chemical compositions of EOs of P. dioscoridis from four different habitats

The EOs of *P. dioscoridis* were extracted by hydrodistillation and given golden yellow colour. The chemical composition of the extracted EOs was characterized through the GC-MS assessment. Fifty-six compounds were identified from the EOs of *P. dioscoridis* representing 90.2, 91.64, 95.39, and 98.91 (area %) for CH, UH, AH, and WH, respectively. Peak areas were used for the measurements of quantitative determinations. All compounds were identified based on their retention times, mass values, chemical formula, and fragmentation pattern described by Adams [23] and confirmed with the spectral matches in Wiley and NSIT libraries (**Table 3**).

The EOs components of *P. dioscoridis* were described by the presence of six classes of compounds containing monoterpenes, diterpenes, sesquiterpenes, carotenoids derived compounds, hydrocarbon, and fatty alcohols. Among them, thirteen compounds are common in the EOs of the four habitats, they are represented as *P*-cymene, neoisolongifolene, á-Gurjunene, aromadendrene, Ç-muurolene, valencene, cubedol, caryophyllene oxide, cubenol, khusinol, pentacosane, heptacosane, and nonacosane. Terpenoids were found as abundant compounds in addition to traces of acyclic compounds with the profusion of sesquiterpenes detected in perfect agreement with previous data on the EOs of *P. dioscoridis* [19]. Sesquiterpenoids were noticed as the key contents of the identified EOs with mixtures of oxygenated and non-oxygenated compounds with 69.62% (CH), 60.68% (UH), 78.27% (AH), and 94.87% (WH). For the *P. dioscoridis* specific EOs, 6-epi-shyobunol, aromadendrene, Ç-muurolene, tau-muurolol, and trans caryophyllene were known previously for the prominent sesquiterpenes found in the *P. dioscoridis* leaves [19] while in the present results, the prevalent compounds were 6-epi-shyobunol (26.38%, WH), aromadendrene (16.72%, CH), Ç-muurolene (14.51%, CH), himachalol (13.48%, UH), tau-muurolol (12.5%, WH), cubebol (9.78%, WH), cubenol (9.39%, WH), and caryophyllene oxide (8.27%, WH) (**Table 3**).

Table 3. Components of essential oils of *P. dioscoridis* from four different habitats

No.	Rt (min.)	Compound name	MF	Area %				M.WT
				CH	UH	AH	WH	
			Monoterpenes					
1	34.25	p-cymene*[a]	$C_{10}H_{14}$	1.03	0.89	1.06[b]	0.20	134
2	41.7	à-terpinene	$C_{20}H_{32}$	-	-	0.38	-	272
			Diterpenes					
3	37.31	Phytol, acetate	$C_{22}H_{42}O_2$	-	-	4.65	1.78	338
4	38.23	geranylgeraniol	$C_{20}H_{34}O$	1.04	0.95	-	-	290
5	38.24	Cembrene	$C_{20}H_{32}$	-	-	0.85	-	272
6	38.65	Phorbol	$C_{20}H_{28}O_6$	0.29	-	-	-	364
7	39.29	Phytol [a]	$C_{20}H_{40}O$	6.14	-	5.49	-	296
			Sesquiterpenes					
8	20.57	Clovene	$C_{15}H_{24}$	-	0.83	5.52	1.12	204
9	20.61	á-Guaiene [a]	$C_{15}H_{24}$	0.80	-	2.25	1.18	204
10	21.58	Neoisolongifolene*	$C_{15}H_{24}$	0.35	4.21[b]	3.41	1.60	204
11	21.7	á-Gurjunene*[a]	$C_{15}H_{24}$	0.80	1.64	7.44[b]	4.38	204
12	21.76	Berkheyaradulene [a]	$C_{15}H_{24}$	-	3.66	6.91	3.87	204
13	22.56	á Koraiene	$C_{15}H_{24}$	-	2.01	-	-	204
14	22.65	Di-epi-à- Cedrene	$C_{15}H_{24}$	4.87	1.78	4.78	-	204
15	22.67	Trans caryophyllene [a]	$C_{15}H_{24}$	-	2.01	3.47	2.21	204
16	23.44	à-Humulene [a]	$C_{15}H_{24}$	0.87	-	1.67	1.49	204
17	23.59	Aromadendrene*[a]	$C_{15}H_{24}$	16.72[bc]	1.01	0.65	1.62	204
18	23.97	Ç-Muurolene*[a]	$C_{15}H_{24}$	14.51[b]	0.52	0.50	0.17	204
19	24.2	Valencene*	$C_{15}H_{24}$	5.15[b]	0.74	0.58	0.35	204
20	24.63	ë-Cadinene [a]	$C_{15}H_{24}$	8.33	-	0.61	-	204
21	25.02	á-copaene [a]	$C_{15}H_{24}$	0.41	-	4.83	1.14	204
22	25.03	á-ylangene	$C_{15}H_{24}$	-	5.40	5.28	1.30	204
23	25.46	Cubebol*	$C_{15}H_{26}O$	4.58	2.84	2.79	9.78[b]	222
24	25.78	Caryophyllene oxide*[a]	$C_{15}H_{24}O$	0.48	2.42	0.49	8.27[b]	220
25	25.83	Elemol [a]	$C_{15}H_{26}O$	1.46	2.21	-	0.20	222
26	25.97	Ç-Elemene [a]	$C_{15}H_{24}$	1.87	-	-	3.01	204
27	26.2	Ledene [a]	$C_{15}H_{24}$	0.35	-	2.31	-	204
28	26.5	Epizonarene	$C_{15}H_{24}$	0.41	-	-	-	204
29	26.66	Aromadendrene oxide [a]	$C_{15}H_{24}O$	-	0.64	0.65	0.22	220
30	27.38	Cubenol*[a]	$C_{15}H_{26}O$	1.26	2.10	2.84	9.39[b]	222
31	27.67	Khusinol*	$C_{15}H_{24}O$	1.19	1.30	1.29	1.96[b]	220
32	27.93	Ç-Eudesmol [a]	$C_{15}H_{26}O$	0.91	0.52	0.82	-	222
33	28.08	Vellerdiol	$C_{15}H_{24}O_2$	-	-	-	1.57	236
34	28.42	tau-Muurolol [a]	$C_{15}H_{26}O$	-	8.49	-	12.5	222
35	28.56	á-Acorenol	$C_{15}H_{26}O$	-	-	7.32	-	222
36	29.3	Himachalol	$C_{15}H_{26}O$	-	13.48[c]	-	-	222
37	29.49	6-epi-shyobunol [a]	$C_{15}H_{26}O$	-	-	10.03[c]	26.38[c]	222
38	30.6	9,10-dehydroiso-longifolene	$C_{15}H_{22}$	0.40	1.53	-	-	202
39	31.79	Longifolenaldehyde	$C_{15}H_{24}O$	0.96	0.56	-	0.40	220
40	31.82	Isospathulenol [a]	$C_{15}H_{24}O$	-	0.78	-	0.59	220
41	34.08	Furoscrobiculin B	$C_{15}H_{20}O_2$	0.38	-	-	0.17	232
42	35.98	Heliannuol	$C_{15}H_{20}O_4$	1.38	-	-	-	264
			Carotenoids derived compound					
43	27.15	á-Ionone [a]	$C_{13}H_{20}O$	-	0.69	-	-	192
			Hydrocarbon					
44	35	Eicosane	$C_{20}H_{42}$	-	1.16	-	-	282
45	36.85	Heneicosane	$C_{21}H_{44}$	1.9	1.18	-	-	296
46	38.58	Docosane [a]	$C_{22}H_{46}$	0.31	1.83	0.33	-	310
47	40.26	Tetracosane	$C_{24}H_{50}$	-	1.74	0.39	-	338
48	42.26	Tricosane	$C_{23}H_{48}$	0.35	-	-	-	324
49	43.44	Pentacosane*[a]	$C_{25}H_{52}$	1.96	2.40[b]	1.32	0.35	352
50	44.94	Hexacosane	$C_{26}H_{54}$	0.27	1.14	-	-	366
51	46.38	Heptacosane*[a]	$C_{27}H_{56}$	1.76	3.38[b]	1.07	0.71	380
52	47.75	Octadecane [a]	$C_{28}H_{58}$	1.26	2.14	-	-	394
53	49.11	Nonacosane*[a]	$C_{29}H_{60}$	1.70	2.49[b]	0.65	0.81	408
		Fatty alcohol						
54	37.33	1-Nonadecanol	$C_{19}H_{40}O$	2.28	8.62	0.50	-	284
55	40.13	1-Docosanol	$C_{22}H_{46}O$	-	1.73	0.43	-	326
56	40.15	1-Eicosanol	$C_{20}H_{42}O$	0.29	0.62	-	0.28	298
		Total of identified compounds		90.2	91.64	95.39	98.91	
		Monoterpenes		1.03	0.89	1.44	0.20	
		Diterpenes		7.47	0.95	10.99	1.78	
		Sesquiterpenes		69.62	60.68	78.27	94.87	
		Carotenoids derived compound		-	0.69	-	-	
		Hydrocarbon		9.51	17.46	3.76	1.87	
		Fatty alcohol		2.57	10.97	0.93	0.28	

Rt; retention time, MF; molecular formula, CH; Canal habitat, UH; Urban habitat, AH; Agricultural habitat, WH; Wadi habitat, *; Compounds were significantly affected by different habitats, [a:] Compounds reported before from *P. dioscoridis*, [b] the major compounds compare with samples, [c]; the major compounds of each distinct sample

The variability in EOs between the different habitats may be explained according to various environmental factors such as growth location, soil, light, and precipitation, which may change the quantitative and qualitative amount of the volatile constituents in the EOs in our study. The soil study indicates a significant difference in the soil's cations and anions, and variations in climatic conditions may also be the reason for EOs varied [24]. Among all identified sesquiterpenes, aromadendrene (16.72 %) is the major compound in (CH), himachalol (13.48 %) in (UH), while 6-epi-shyobunol (10.03%) in (AH) and (26.38 %) in (WH).

Because of the reactivity of oxygen, oxygenated terpenoids normally play a larger role in biological activity than non-oxygenated ones [25]. The richness of oxygenated terpenoid structures such as 6-epi-shyobunol could operate as individual or synergistic inhibitors of plant germination and growth.

The EOs profile of *P. dioscoridis* revealed that monoterpenes were trace components which is consistent with the results of Elshamy, et al. [19]. Only two compounds (*p*-cymene and à-terpinene) were detected, while five monoterpenes were reported in other published data [19].

Five diterpenes were distinguished, phytol which is the abundant component in (CH) and (AH) is the only diterpene structure found previously in the EO of *P. dioscoridis* leaves [26]. Moreover, α-ionone was the only described carotenoid-derived structure from *P. dioscoridis* EO in (UH) that was previously reported from the investigated species [27]. The rest compounds including hydrocarbons were traced in the investigated EOs and agreed with the previous data [19-28] except for (UH) which represented 17.46 % of total oil.

The differences in the secondary metabolites comprising EOs may be assigned to the plant organs, age, and development, as well as some environmental factors such as temperature, seasonality, altitude, atmospheric composition, and water availability [29, 30, 31].

The composition of soil in different ecosystems may have an impact on EO yield and properties. The soil that produces high EO has several characteristics that increased production, such as a high percentage of particles (less stony soil), and a high presence of salts (lower significant electrical conductivity) (**Table 2)**. The EO composition could potentially be influenced by edaphic factors. Different degrees of K^+ application, on the other hand, influenced both the EO production and components [32].

Furthermore, the production of EO is influenced by environmental factors such as light, ambient temperature, and soil nutrient accessibility [33]. Other research has found that altitude has an impact on the chemical makeup of essential oils from a variety of plants [34]. As a result, geographic location in terms of latitude and longitude could influence EOs biosynthesis. **Table 1** shows that there are only minor changes in EOs yield between populations according to their latitudinal location. With a lower latitude, the populations (AH) and (WH) produce much more EO.

3.3. Antimicrobial Activity of EOs of P. dioscoridis

The antimicrobial potentials of the EOs were screened against ten selected fungi and bacteria strains After being incubated in conditions that allowed for the growth of the tested pathogens, the activity was measured in terms of the diameter of the inhibition zone (mm) that formed around the discs. The positive antimicrobial effects were revealed using a fungal strain (*C. neoformans*), three gram-positive bacterial strains (*B. subtilis, S. aureus, S. mutants*), and one gram-negative bacteria (*E. cloacae*) **Table 4**. No effects were observed for the rest microbes.

Also, other bioactive terpenes, aldehydes, alcohols, and esters may impact the antimicrobial potential of EOs. The C10 and C15 terpenes with aromatic rings and phenolic hydroxyl groups that can create hydrogen bonds with the active sites of the target enzymes have mainly explained the antibacterial properties [35]. Additionally, these bonds reduced membrane potentials, proton pump stoppage, and ATP exhaustion, all may be playing a role in the observed antibacterial activity [36]. The recognized antibacterial activity of EOs is demonstrated by cell coagulation, cytoplasm leakage, potassium ion efflux, and cell apoptosis or necrosis, which causes the death of cells. It has been found that EOs abundant with *p*-cymene can effortlessly penetrate the fungal cell membrane that is present in the four *P. dioscoridis* habitats [37].

Moreover, the fungicidal effect which operates as a natural agent is assumed to be due to direct cell membrane destruction rather than metabolic dysfunction, resulting in fungal death [38]. Herein, the significant antifungal effects were observed against *C. neoformans*, where the EO of WH showed the highest potency (35 ±0.49 mm), followed by CH (26.7 ±2.22 mm), UH (24.17 ±0.59 mm), then AH (22.07 ±0.33 mm), compared with the reference compound; ketoconazole (25.03 ±0.71 mm).

Table 4. Antimicrobial activity of *P. dioscoridis* EOs from different habitats

Tested pathogens	Inhibition zone diameter (mm)				
	CH	UH	AH	WH	Control
Fungus					
C. neoformans	26.7 ±2.22*	24.17 ±0.59	22.07 ±0.33	35 ±0.49 *	25.03 ±0.71
Gram +ve bacteria					
S. aureus	-	-	12.9 ±0.56	14.07 ±0.16	24.03 ±0.43
B. subtilis	8.27 ±0.48	8.93 ±0.43	8.13 ±0.15	-	26.14 ±0.86
S. mutants	-	11.9 ±0.56	9.13 ±0.43	-	21.9 ±0.49
Gram -ve bacteria					
E. cloacae	8.93 ±0.43	8.07 ±0.71	8.03±0.33	-	30.8±0.53

Values are the mean of three replicates ± standard deviation, *; Statistically significant difference from the control ($P \leq 0.05$). CH; Canal habitat, UH; Urban habitat, AH; Agricultural habitat, WH; Wadi habitat

Caryophyllene oxide is an antimicrobial agent that has been demonstrated to be effective against several pathogens [39]. Other studies have demonstrated that caryophyllene and caryophyllene oxide are absorbed by the cell membrane of fungi and act as antifungal agents by releasing lipophilic drugs [40]. This is confirmed by the presence of caryophyllene oxide as the major compound in WH (3.27 %). Furthermore, the influence of EOs of (AH) and (WH) gives moderate effectiveness on *S. aureus* (13 and 14 mm), compared with the reference: gentamycin (24 mm). However, weak efficiency was observed for (AH) against *B. subtilis, S. mutants,* and *E. cloacae*, while there was no effect for (WH). The EO of (CH) and (UH) exhibited weak effectiveness on *B. subtilis*, and *E. cloacae* but that of (UH) displayed a moderate potency against *S. mutants.*

The existing data showed that increased concentration of EOs, especially sesquiterpenes increase the antimicrobial potential. This result comes to an agreement that these activities depend on the concentration of antimicrobial components [39, 41]. Thus, EOs have the potential to be used as natural antifungal products that are eco-friendly and should be recognized when developing crop protection methods. Furthermore, because of their beneficial effects as antibacterial agents, bio-stimulants, antifeedant activities, and fertilizers, plants containing EOs have long been employed as green manure [42, 43]. Moreover, various studies have shown that the volatile chemicals in EO have favorable effects on plants growing under stressed settings [44, 45]. As a result, the importance of EO's volatile components for crop stress control is highlighted.

3.4. Cytotoxic Activity of P. dioscoridis EOs

Amongst all examined cell lines, the EOs of *P. dioscoridis* exhibited significant cytotoxicity against HCT116 for all habitats in ascending order: WH>AH> UH >CH, with LC_{50} (µg/ml) 16.0> 18.1>23.6 >29.4 and LC_{90} (µg/ml) 29.1> 30.2>44.8 >55.6, respectively.

Cell viability assays have been used to clarify the processes underlying the inhibitory action of EOs on cancer cell proliferation. According to the literature, EO structures directly inhibit proteins and enzymes, impede the transcription of genes [46, 47], decrease the potential of the mitochondrial membrane, and cause apoptosis [48]. As a result, it has been suggested that EOs directly block proteins and enzymes, suppressing gene transcription [46,47], reducing the potential of the mitochondrial membrane as well as causing apoptosis [48].

Sesquiterpenes, in both oxygenated and non-oxygenated forms, were extremely potent antitumor leads [50]. Additionally, several states reported that the anticancer action of EOs was increased by the presence of more sesquiterpene content. which agrees with our results in AH and WH where the highest sesquiterpene contents were 78.27% and 94.87% [51,52].

4. Conclusions

The analysis of the EOs by GC-MS revealed the identification of 56 compounds. Sesquiterpenes were described as abundant constituents of EOs of *P. dioscoridis* obtained from the four different habitats. Additionally, the differences in EO composition among the investigated habitats revealed a weak antibacterial effect with varied values against *S. aureus*, *B. subtilis*, *S. mutants*, and *E. cloacae* and indicated considerable antifungal activity against *C. neoformans* in all habitats. Furthermore, *P. dioscoridis* EOs showed anticancer activity against a colon carcinoma cell line (HCT116). In order to obtain health benefits, the investigated EOs of these plants, particularly for (WH), may be employed as natural, eco-friendly antifungal, and anticancer drugs.

5. References

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