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Phytochemical Composition and Biological Activities of Selected Essential Oils and Their Discrimination through Principal Component Analysis

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Abstract

The present study aimed to determine the phytochemical characterization, anticancer and antioxidant activities of essential oils (EOs) obtained from nine commonly used medicinal and aromatic plants (MAPs) including *Cymbopogon citratus* (DC) Stapf (Poaceae), *Cistus creticus* L. (Cistaceae), *Foeniculum vulgare* Mill. (Apiaceae), *Laurus nobilis* L. (Lauraceae), *Myrtus communis* L. (Myrtaceae), *Pinus cembra* L. (Pinaceae), *Echinacea purpurea* (L.) Moench, *Helichrysum arenarium* (L.) Moench, and *Inula viscosa* (L.) Aiton (Asteraceae) taxa from Türkiye. The chemical composition of the EOs was identified using gas chromatography-mass spectrometry (GC-MS), and their antioxidant activities were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical scavenging methods. In addition, the anticancer potential of the EOs were assessed using MTT assay against human cell lines, including hepatocellular carcinoma (HepG2), gastric carcinoma (NCI-N87), breast adenocarcinoma (MCF-7), and human prostate carcinoma (LNCaP clone FGC-Luc2) cancer cells, as well as non-cancerous human umbilical vein endothelial cells (HUVECs). The antioxidant activities of the EOs varied from 35.21 to 79.12%. Consistent with their antioxidant activities, the anticancer activities of the EOs of *C. citratus, L. nobilis* and, *C. creticus* exhibited significantly higher anticancer activities compared to the others. However, almost all EOs were found to inhibit cell viability and induce apoptosis in cancer cells. Among the tested concentrations, the highest anticancer activity was observed against against MCF-7 cells, followed by NCI-N87, LNCaP, and HepG2 cancer cells, with the IC_{50} values ranging from 15.10 ± 1.00 to 78.12 ± 0.32 µg/mL, respectively. The major components of the EOs distilled from studied MAPs were found as anethole, borneol, carvacrol, citral, α-pinene, eucalyptol and β-citral at different concentrations in the EOs. Overall, it can be clearly concluded that the EO samples distilled from studied MAPs contain valuable bioactive compounds and accordingly exhibit remarkable biological activities.

Key Words: Phytochemical, antiproliferative, antioxidant, natural component, chromatography

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1. Introduction

Cancer is a global health concern, characterized by the uncontrolled growth of cells in the human body, resulting in the formation of tumors composed of malignant cells. The exact causes of many forms of cancer are largely unknown, however genetic and environmental factors such as mutations, obesity, viral and bacterial infections, aging population, environmental pollution, sedentary lifestyle, alcohol abuse, and smoking cause to incidence of cancer is rapidly increasing worldwide[1,2]. According to the World Health Organization (WHO) cancer reports, approximatelly 18 million new cases of cancer and 10 million cancer-related deaths were recorded globally in 2018, with the number of new cncer cases projected to double to about 29–37 million by 2040[3]. Among various cancer types, hepatocellular carcinoma (HCC), breast cancer (BC), gastric cancer (GC) and human prostate carcinomas (PC) remain the most common and lethal cancers worldwide [4]. Among all other types of cancer, HCC is the second-leading mortality rate in the world and its incidence is increasing globally. HCC is responsible for almost 80% of primary liver cancers worldwide[5]. Prostate cancer exacts a heavy disease burden. In 2018, PC is the second most diagnosed cancer among men with an estimated

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1.3 million cases and approximately 365.000 deaths worldwide[3]. Gastric cancer is one of the deadliest cancers worldwide because recent studies demonstrate that GC is associated with 5.7% (1.03 million cases) of all new cases and 8.2% (787,200 deaths) of mortality from all cancer types[6]. Similarly, breast cancer (BC) remains the most frequently diagnosed malignancy in women, with 2.1 million new cases and 630.000 cancer-related deaths reported in 2018, particularly in less-developed and developing countries, including Türkiye. In Türkiye, the incidence of breast cancer is 52.5 cases per 100.000 women [7].

Although there have been important developments in traditional cancer treatments such as targeted therapies, gene therapies, surgery, hormones, radiotherapy, chemotherapy, immunotherapy, hemopoietic and peripheral blood stem cell transplantation in recent years, especially the anti-proliferative drugs have high costs, unexpected side effects, and toxicities. Therefore, a new biocompatible and cost-effective strategy for cancer therapy is needed. Natural products obtained from medicinal plants play a critical role in the discovery and development of anticancer agents, especially natural essential oils and their bioactive components have attracted increasing attention in the past 30 years since their potential as a novel cancer-preventive and therapeutic agent [4,8,9,10,11].

The East Mediterranean region has a rich plant biodiversity due to its unique geographical position, climate, and geological composition. Around 25.000 plant taxa are naturally distributed in this region, many of which have been used in traditional folk medicine for centuries [12,13]. While previous ethnobotanical studies and investigations into the biological activities of medicinal plants in this region have been conducted, comprehensive research combining phytochemical characterization with biological activity remains limited[2,14,15]. The present study is part of a comprehensive and long-term research into the biological activities of various MAPs. The main objective of this study was to determine the essential oil (EO) profiles, as well as their antioxidant and antiproliferative properties, in nine medicinal plant taxa. Furthermore, the EOs were tested against four cancer cell lines including hepatocellular carcinoma, gastric carcinoma, breast adenocarcinoma, and human prostate carcinoma cancer in order to provide a direction in the discovery of potential novel, safe, and efficacious natural cancer therapy in the future.

2. Material and methods

2.1. Plant materials

Fresh leaves of *Cistus creticus* (pink rock-rose), *Foeniculum vulgare* (Fennel), *Laurus nobilis* (laurel), *Myrtus communis* (common myrtle), *Helichrysum arenarium* (dwarf everlast),

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and *Inula viscosa* (false yellowhead) taxa were collected from their natural habitats between May and December 2024, while *Cymbopogon citratus* (lemongrass) and *Echinacea purpurea* (eastern purple coneflower) samples was collected from its cultivated field in Hatay Province in Turkiye during flowering periods. Also, essential oil of *Pinus cembra* (Swiss pine) was purchased from a herbalist in Hatay city. The studied plant samples were identified by co-author botanist Dr. Karahan. The collection of plant material was established in compliance with the national guidelines. Voucher specimens were also deposited at the Herbarium of Hatay Mustafa Kemal University (Hatay, Türkiye). Detailed information on their scientific and vernacular names, gathering localities, and collector numbers are also given (Table 1).

Table 1. The collection locality of the studied medicinal plants taxa.

No	Taxa	Common/ Vernacular name	Locality	Voucher specimens numbers
1	*Cymbopogon citratus* **Poaceae**	Lemon grass/limon otu	Experimental Fields of Hatay Mustafa Kemal University	MKU1507
2	*Helichrysum arenarium* **Asteraceae**	Sandy everlasting, Golden grass/Altın otu, ölmez çiçek	Dörtyol	MKU1420
3	*Laurus nobilis* **Lauraceae**	Laurel/Defne	Amanos mountain	MKU1378
4	*Myrtus communis* **Myrtaceae**	Common myrtle/Murt, mersin	Antakya	MKU1383
5	*Echinacea purpurea* **Asteraceae**	Echinacea/Ekinezya	Experimental Fields of Hatay Mustafa Kemal University	MKU469
6	*Foeniculum vulgare* **Apiaceae**	Fennel/Rezene	Arsuz	MKU1403
7	*Inula viscosa* **Asteraceae**	False yellowhead, sticky fleabane/Yapışkan andız otu	Belen	MKU1392
8	*Pinus cembra* **Pinaceae**	Swiss pine/	-	-
9	*Cistus creticus* **Cistaceae**	Pink rock-rose, hoary rock-rose/ laden	İskenderun	MKU1397

2.2. Isolation of plant essential oils

The essential oils were obtained from dried leaves using a hydro-distillation methos. A total of 25 g of each of the ground plant samples was used for each experiment. A weighed sample weight was individually and carefully placed into a 1 L flask. Distilled water was then added until it covered the sample completely. Essential oils were obtained by hydro-distillation method which was carried out in an all-glass Clevenger-type distillation. The trial was repeated three times for each sample. The EOs were dried over anhydrous sodium sulfate and stored in dark vial bottles at 4°C until analysis[16,17].

2.3. Characterization of essential oils by GC–MS

The essential oil components of the plants were analysed by gas chromatography–mass spectrometry (GC–MS) using a Thermo Scientific ISQ Single Quadrupole instrument. A TR-FAME MS column (5% phenyl methylpolysiloxane, 0.25 mm i.d. × 60 m, 0.25 μm film

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thickness) was used. Helium (99.9%) was employed as the carrier gas at a constant flow rate of 1 mL min^{-1}. The mass spectra were recorded at 70 eV over a mass range of 1.2–1200 m/z in scan mode. The MS transfer line temperature was set at 250°C, the ionization source temperature at 220°C, and the injection port temperature at 220°C. The column temperature was initially held at 50°C and then increased to 220°C at a rate of 3°C min^{-1}. Compound identification was achieved through comparison of their mass spectra with those in the Wiley and NIST libraries using the Xcalibur software. The relative amounts of individual components were calculated using the Xcalibur Report program. The retention indices (RI) of the compounds were determined relative to a homologous series of n-alkanes (C8–C40) under the same chromatographic conditions. The experimentally obtained RI values were compared with literature values reported for a dimethylsilicone stationary phase containing 5% phenyl groups (DIMS5P, DB-5 equivalent) as described by Babushok et al. (2011).

2.4. Free radical scavenging activity

The free radical scavenging activities of the EOs were analyzed using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) assays, conducted through spectrophotometric analysis with a UV–Vis spectrometer (Thermo Lab systems 408 Multiskan, Thermo Scientific Fisher). Antioxidant potentials of the EOs were determined as described in the previous[20,21]. Trolox was used as the standard for both assays. The EOs and Trolox were prepared in DMSO at various concentrations. For the DPPH assay, each EO was dissolved in DMSO, with concentrations ranging from 0 to 1000 µg/mL, followed by the preparation of a 0.1 mM solution of DPPH in methanol (MeOH, 98%). Each EO sample was combined with 1.0 mL DPPH solution. The mixture was gently shaken and allowed to incubate for 30 min in darkness at room temperature. In the case of the ABTS assay, a mixture of 10 mL of 2.6 mM potassium persulfate was added to 10 mL of 7.4 mM ABTS solution and it was left to stand for 12–15 hours in the dark at room temperature. This stock solution was subsequently diluted by adding 1 mL to 60 mL of MeOH (98%). The samples were mixed with this diluted stock solution and incubated for 2 hours in the dark. The antioxidant activities of the EOs against DPPH and ABTS free radicals were quantified spectrophotometrically at wavelengths of 517 nm and 734 nm, respectively. The results were reported as percentage of radical inhibition ± standard deviation (SD), calculated using a standard formula.

$$\left[\frac{(Absorbance\ of\ the\ blank\ sample - Absorbance\ of\ the\ \ EO}{Absorbance\ of\ the\ blank\ sample)\ \ x\ 100}\right]$$

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The antioxidant activity of the essential oils was evaluated at four different concentrations (0, 250, 500, and 1000 µg/mL). The half maximal inhibitory concentration (IC_{50}, µg/mL) was estimated by linear interpolation between the two experimental points that flanked 50% inhibition. IC_{50} values were reported for both DPPH and ABTS assays.

2.5. Cell lines and culture conditions

The examination of cancer prevention properties of various essential oils was performed on selected human carcinoma cell lines, such as hepatocellular carcinoma (HepG2, ATCC®-HB-8065), gastric carcinoma (NCI-N87, ATCC®-CRL-5822), breast adenocarcinoma (MCF-7, ATCC®-HTB-22), and metastatic prostate adenocarcinoma (LNCaP clone FGC-Luc2, ATCC®-CRL-1740-LUC2) cancer cells, as well as non-cancerous human umbilical vein endothelial cells (HUVECs, ATCC®-100010). These cell lines were procured from the American Type Culture Collection (ATCC). The culture medium for the LNCaP and NCI-N87 cell lines comprised Roswell Park Memorial Institute Medium 1640 (RPMI-1640, Gibco), enriched with 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 mg/L glucose, and 1500 mg/L sodium bicarbonate, and further enhanced with 10% (v/v) fetal bovine serum (FBS). Conversely, the remaining cancer cell lines, along with the non-tumorigenic control HUVEC, were cultured in Dulbecco's Modified Eagle Medium (DMEM, ThermoFisher Scientific) supplemented with 10% (v/v) fetal bovine serum (FBS), non-essential amino acids, 1% antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin), and 2 mM L-glutamine. Cultivation of these cells was carried out in flasks maintained at a constant temperature of 37°C in a 5% CO_2 incubator.

2.6. Anticancer activity

To evaluate the anticancer activities of the essential oils (EOs), the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was performed according to the method originally described [22], with slight modifications [10,23]. Briefly, cell densities of 5×10^3 were seeded in 200 µL of medium per well in 96-well plates and allowed to adhere for 24 h. Subsequently, the EOs at different concentrations were applied, and the plates were incubated at 37 °C for 48 h. After incubation, the medium was removed, and 50 µL of MTT solution (5 mg/mL) was added into each well. The optical density was then measured at 570 nm using a multi-spectrophotometer (Thermo Lab Systems 408 Multiskan, Thermo Fisher Scientific). For this assay, the EOs were first dissolved in dimethyl sulfoxide (DMSO) to obtain a stock

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concentration of 1000 µg/mL, from which serial dilutions were prepared. DMSO served as the negative control, while doxorubicin (99% pure) was used as the positive control. The half maximal inhibitory concentration (IC_{50}, µg/mL) values for each cell line were calculated using the following equation, and the results were expressed as mean ± standard deviation (SD).

$$\left[\frac{1 - mean\ OD\ with\ EOs}{mean\ OD\ with\ DMSO\ (negative\ control)\ x\ 100}\right]$$

2.7. Statistical Analysis

All the assays were conducted in triplicate, and the antiproliferative activity results were expressed as IC_{50} (µg/mL) values, presented as mean ± standard deviation (SD). Statistical differences between the reference and sample groups were evaluated by one-way analysis of variance (ANOVA) using SPSS statistics (version 28.0.1), and p values <0.05 was considered statistically significant. In addition, multivariate analysis was conducted through Pearson's correlation coefficients, the principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) using the XLSTAT. In PCA, the data were mainly examined through two principal axes: the first factor was axis 1 (Dim1) and these condone was axis 2 (Dim2).

3. Results and Discussion

3.1. Chemical composition of the EOs

The essential oil compositions of studied medicinal plant samples were determined in triplicate by GC/MS analyses. The compounds were listed according to their retention indices calculated retention time order on a GC-column. The retention indices and percentage composition of chemical compounds were demonstrated in Table 2. Comparison of the major chemical components and numbers of compounds differed considerably among some of the major components in the samples. Overall, fifty constituents were identified (35 in *H. arenarium*, 33 in *L. nobilis,* 30 in *M. communis* and *C. creticus* taxa, 30 in *E. purpurea*, 29 in *P. cembra*, 27 in *C. citratus* and *F. vulgare*, 26 in *I. viscosa*) representing 99.82%, 99.44%, 98.88%, 98.35%, 92.16%, 99.28%, 99.17%, 99.28%, and 96.03% of the total oils, respectively. However, terpenes were the main constituents in all EOs, except *F. vulgare*, with a ratio ranging from 69.71% (*I. viscosa*) to 98.19% (*C. citratus*).

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Table 2. Chemical composition of the EOs obtained from the studied *MAP*s taxa

	Compound Name	*RI*	RI_{lit}	*Cymbopogon citratus*	*Helichrysum arenarium*	*Laurus nobilis*	*Myrtus communis*	*Echinacea purpurea*	*Foeniculum vulgare*	*Inula viscosa*	*Pinus cembra*	*Cistus creticus*
1	α-Pinene	1025	1025	0.79	12.57	4.71	4.48	1.78	0.49	0.04	46.37	2.32
2	Camphene	1086	1086	0.55	nd	0.16	nd	nd	nd	1.17	2	0.19
3	β-Pinene	1110	1110	0.35	0.23	2.1	0.97	0.85	nd	0.09	6.03	0.21
4	Sabinene	1122	1122	0	nd	8.46	1.67	nd	0.09	nd	nd	nd
5	Myrcene	1160	1160	14.59	nd	0.18	nd	nd	0.04	nd	0.87	0.59
6	3-Carene	1165	1169	0.21	0.32	nd	0.4	nd	0.3	0.06	0.5	nd
7	α-Phellandrene	1186	1186	nd	nd	0.11	nd	0.95	nd	nd	16.25	0.23
8	α-Terpinene	1197	1197	nd	nd	0.24	nd	nd	nd	nd	nd	0.34
9	Limonene	1219	1219	0.14	1.69	1.4	4.7	0.62	4.6	nd	17.01	0.36
10	γ-Terpinene	1266	1266	nd	0.23	0.93	nd	nd	0.08	nd	nd	8.14
11	Eucalyptol	1231	1231	3.46	0.35	46.13	20.16	nd	0.33	nd	nd	1.21
12	p-Cymene	1310	1310	0.14	0.22	1.68	0.71	1.83	0.18	nd	0.25	24.41
13	α-Copaene	1360	1370	3.08	2.35	0.1	nd	nd	nd	nd	0.88	0.30
14	α-Bergamotene	1414	1414	1.04	1.22	nd	nd	nd	nd	nd	nd	nd
15	Fenchyl acetate	1480	1480	nd	0.14	0.58	nd	nd	0.02	25.58	1.27	nd
16	Linalool	1537	1543	1.94	0.48	1.67	0.52	0.69	2.46	0.15	0.05	nd
17	Linalyl acetate	1540	1544	nd	0.26	nd	nd	4.74	nd	0.36	nd	0.23
18	α-Citral	1600	1689	11.91	0.35	0.19	nd	0.56	nd	0.42	0.84	nd
19	β-Citral	1638	1685	35.72	0.21	0.1	nd	nd	1.35	0.1	nd	0.29
20	Aromadendrene	1648	1650	nd	nd	nd	0.83	1.01	nd	nd	nd	0.14
21	Verbenol	1654	1654	8.6	0.88	1.14	0.4	5.16	7.01	0.72	0.02	nd
22	trans-Caryophyllene	1683	1685	nd	5.63	4.34	2.57	4.87	0.11	1.21	0.54	0.54
23	α-Terpineol	1694	1694	nd	nd	4.5	1.29	0.7	0.12	0.2	0.5	0.11
24	Borneol	1699	1699	0.38	nd	nd	0.23	1.12	0.02	59.98	0.18	0.28
25	α-Terpinyl acetate	1718	1718	nd	nd	14.68	0.48	7.28	nd	nd	1.31	nd
26	β-Himachalene	1723	1723	nd	21.9	0.22	nd	1.55	0.06	nd	nd	0.4
27	α-Selinene	1725	1725	nd	6.35	nd	nd	nd	nd	nd	0.12	nd
28	Verbenone	1735	1735	3.91	0.29	nd	nd	2.69	0.03	0.09	0.07	nd
29	Neryl acetate	1738	1740	nd	17.41	0.17	nd	nd	0.06	0.07	0.13	nd
30	Cadinene	1769	1769	1.21	1.57	nd	nd	3.4	nd	nd	1.45	nd
31	Calarene	1775	nd	nd	8.89	nd	nd	0.59	nd	nd	0.87	nd
32	Homomyrtenol	1790	1790	4.94	nd	0.25	0.38	2.22	nd	0.37	0.08	nd
33	Nerol	1025	1025	0.76	0.15	0.35	0.8	nd	0.06	nd	nd	nd
34	trans-Carveol	1086	1086	nd	0.49	0.27	29.27	1.23	0.08	nd	nd	nd
35	Anethole	1110	1110	nd	1.52	0.18	0.24	nd	78.35	0.02	0.17	nd
36	Methyleugenol	1122	1122	3.39	8.02	1.88	0.82	3.38	nd	0.79	0.59	0.51
37	Globulol	1160	1160	0.17	0.43	nd	0.65	1.4	0.29	0.26	nd	0.21
38	Spathulenol	1165	1169	nd	0.74	0.13	3.57	10.86	0.04	0.01	nd	0.32
39	Eugenol	1186	1186	nd	0.71	0.89	0.67	nd	2.62	0.07	nd	0.37
40	α-Cadinol	1197	1197	0.24	nd	0.38	1.58	3.72	nd	0.69	0.55	1.05
41	Carvacrol	1219	1219	nd	0.44	nd	0.38	0.83	0.06	0.06	0.11	48.75
42	α-Eudesmol	1266	1266	1.31	1.8	0.75	1.86	2.66	nd	nd	nd	1.43
43	α-Levantenolide	1231	1231	1.07	0.93	nd	2.87	1.39	0.32	nd	nd	1.85
	Terpenes			98.92	71.76	81.14	94.81	76.76	18.23	69.71	95.81	97.24

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Phenolic compounds	0.98	10.25	2.87	1.73	3.38	80.97	0.31	0.76	0.51
Esters	nd	17.81	15.43	0.48	12.02	0.08	26.01	2.71	0.23
Acid	nd	nd	nd	1.86	nd	nd	nd	nd	0.37
Total	**99.90**	**99.82**	**99.44**	**98.88**	**92.16**	**99.28**	**96.03**	**99.28**	**98.35**

*Nd, not detected; ***components higher than 1% are given. RI—retention index

Briefly, in the essential oil (EO) of Cymbopogon citratus, known as 'Lemongrass' in Türkiye, α-Citral (11.91%) and β-Citral (35.72%) were the dominant components, followed by myrcene (4.22%) and verbenol (2.56%) as shown in Table 2. *Cymbopogon citratus* has been used in aromatherapy and was reported to possess antibacterial, antidiarrheal, antifilarial, antifungal and anti-inflammatory properties. In previous studies, both α- and β-Citral were detected as major components, with myrcene also reported as a dominant compound."[24,25,26].

Helichrysum arenarium named as 'Altın otu' in Anatolia belongs to the *Helichrysum* genus, in the Asteraceae family. It is used in pharmaceutical, cosmetic and perfume industries and has healing properties in medicine[27]. The main components were β-himachalene (21.90%), neryl acetate (17.41%) and α-pinene (12.57%) in the EO obtained from this medicinal plant (Table 2). Calarene (8.89%), methyl eugenol (8.02%), α-selinene (6.35%) and trans-caryophyllene (5.63%) was identified at high ratios in addition to these components. Similar to these findings, In the volatile compounds of *H. arenarium*, α-pinene and α-himachalene were identified as the major constituents [28]. Another study also reported α-pinene as a main component in the essential oil of *H. arenarium* leaves [27].

It was reported that the essential oil of laurel was used in food, perfumery, medicine and liquor industry[29]. In the present study, eucalyptol was detected as a main component at a ratio of 46.13% in the essential oils of *Laurus nobilis*. Eucalyptol (1,8-cineole) possessed disinfectant, insect repellent properties and was used in cosmetic and pharmaceutical industries [30]. It was followed by α-terpinyl acetate (14.68%), sabinene (8.46%), α-pinene (4.71%), α-terpineol (4.50%) and trans-caryophyllene (4.34%), respectively (Table 2). Similarly, previous studies reported that 1,8-cineole (60.4–64.57%), sabinene (10.1%), and α-pinene (6.2%) were identified as the major compounds of *L. nobilis* essential oil samples [31,32]. Additionally, 1,8-cineole and a-terpinyl acetate were also determined as the main components in laurel samples collected from different countries such as Greece (30.8 and 14.9%), Georgia (29.2 and 22.6%), Tunisia (24.55%), and Morocco (38.86%), respectively [33].

In *Myrtus communis*, trans-carveol (29.27%) and eucalyptol (20.16%) were found as the major compounds (Table 2). α-Pinene and eucalyptol were also identified as main constituents in the essential oil of *M. communis*[34]. Similarly, eucalyptol and α-pinene were reported as

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dominant compounds, and myrtle essential oil was described as having insecticidal, antioxidant, anti-inflammatory, and antimicrobial activities [35]. Researchers reported that *M. communis* was one of the important aromatics and medicinal plants and was used in the culinary, cosmetic, pharmaceutical, therapeutic, and industrial applications[34]. Eremanthin (6.50%), caryophyllene oxide (5.70%), limonene (4.70%), α-pinene (4.48%), spathulenol (3.57%), α-levantenolide (2.87) and trans-caryophyllene were determined as other compounds that were detected high ratios detected in *M. communis* essential oil.

Echinacea known as 'purple coneflower' belongs to the Asteraceae family and has antibacterial, antiviral and antifungal properties [36]. In the present study, the EO components of *Echinacea purpurea* were detected at lower ratios but higher in numbers (Table 2). Caryophyllene oxide (18.51%) and spathulenol (10.86%) were dominant components followed by α-terpinyl acetate (7.28%), verbenol (5.16%), trans-caryophyllene (4.87%), linalyl acetate (4.74%), α-cadinol (3.72%), cadinene (3.40%), methyleugenol (3.38%), widdrol (3.07%), verbenone (2.69%), α-eudesmol (2.66%), α-cyperone (2.50%), homomyrtenol (2.22%).

When the EO obtained from *Foeniculum vulgare* leaves was examined, anethole (78.35%) was detected as a main component (Table 2). Similar to the present study, anethole was determined as a dominant component in the essential oil of *F. vulgare* [37]. It was also reported that anethole can be found in fennel oils at levels ranging from about 30% to 90% [38]. Anethole was known to have anti-inflammatory, insecticidal and estrogen properties [39]. Verbenol (7.01%), limonene (4.60%), eugenol (2.62%) and linalool (2.46%) were the other dominant components in the essential oil obtained from *F. vulgare*. In another study[31], the amount of Trans-anethole as the main component and the DPPH activity of the taxa were calculated as 80.4% and 66.5%, respectively. It was also reported that fennel oil was used in the food and perfume industry and exhibited antifungal and antibacterial properties [40].

Inula viscosa which belonged to the Asteraceae family, was reported to have been used in treatment of wounds, muscle relaxant, cardiovascular and digestive in addition to antimicrobial, anti-inflammatory and antifungal properties [41]. In the present study, the main constituents were borneol (59.98%) and fenchyl acetate (25.58%) in the EO obtained from *I. viscosa*. It was reported that, in addition to its anti-inflammatory, antioxidant, anti-apoptotic, and anticoagulant properties, borneol was also used in the treatment of cardio-cerebrovascular diseases and for improving energy metabolism [42]. Contrary to the present study, in the EO of *I. viscosa*, polygodial, phytol, fokienol, isocostic acid and (E)-nerolidol were determined as major VCs in the previous studies [11,43,44]. For instance, a study focused on the chemical composition, antioxidant, and antiproliferative activity of *Inula viscosa* collected from South of Lebanon [45].

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Their comprehensive study demonstrated that *I. viscosa* extracts were rich in lupeol and its derivatives, and had high anticancer activity against MCF-7, HepG2, ovarian cancer (SK-OV-3), and colorectal cancer (HCT116) cell lines with the 54.0 ± 4.9 µg/mL, 59.9 ± 7.5 µg/mL, 80.0 ± 5.7 µg/mL, and 39.2 ± 6.1 µg/mL, respectively. In our results, caryophyllene oxide was also found at a ratio of 3.27% in the EO of *I. viscosa* (Table 2).

As shown in Table 2, α-pinene (46.37%), limonene (17.01%) and α-phellandrene (16.25%) were detected dominantly in the EO of *Pinus cembra*. Similar to these findings, α-pinene and limonene were also determined at the highest ratios in the *P. cembra* essential oil [46,47]. In the present study, β-pinene (6.03%) and camphene (2.00%) were identified as other major compounds. Moreover, α- and β-pinene, described as "a miracle gift of nature," were reported to possess various therapeutic effects such as anticoagulant, antitumor, antimicrobial, antimalarial, antioxidant, and anti-inflammatory properties[48]. In the EO of *Cistus creticus*, carvacrol (48.75%) and p-cymene (24.41%) were the major components followed by γ-terpinene (8.14%) and α-pinene (2.32%). In the previous studies about *C. creticus* essential oil, main components were determined as α-pinene, camphene, α-cubebene, α-cadinene, Δ-cadinene, viridiflorol, bulnesol, ledol, α-copaene, β-selinene, manoyl oxide[49,50]. Carvacrol tested in terms of antifungal effect in a previous study was determined at highest ratio in the essential oil of *Origanum dubium* plant [51]. It was reported that carvacrol exhibits antibacterial effects on both Gram-positive and Gram-negative bacteria [52].

When our obtained results were compared with previous related studies, it could be clearly seen that the phytochemical content of natural plants varies considerably[53,54,55]. This variability in chemical composition and stability was affected by many factors, including genetic traits, collection time, vegetative stage, extraction protocols, and environmental conditions such as photoperiod, altitude, temperature, biotic and abiotic stresses, soil characteristics, and seasonal influences, which thus affected the concentrations of the main constituents and their biological activities.

3.2. Free radical scavenging activity of the EOs

Free radical scavenging activities of the selected EOs at various concentrations were evaluated by measuring the percentage inhibition of DPPH and ABTS free radicals. The antioxidant activities were summarized in Table 3 as percent inhibition (%) of EOs at 1000 µg/mL concentration. In general, the EOs displayed considerable antioxidant properties compared to those of the Trolox. Among the tested EOs, *C. citratus, L. nobilis* and, *C. creticus* showed potent free radical scavenging activity, consistent with the results of anticancer

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capacity of the EOs. Furthermore, IC_{50} values were calculated using dose–response data obtained at four different concentrations (0, 250, 500, and 1000 µg/mL). As shown in Table 3, *C. citratus* exhibited the highest radical scavenging activity, with inhibition values of 79.12 ± 1.46% (DPPH, $p < 0.01$) and 73.10 ± 0.05% (ABTS, $p < 0.05$). In contrast, *F. vulgare* displayed the weakest antioxidant activity, yielding only 37.16 ± 1.10% (DPPH, $p < 0.01$) and 39.42 ± 0.30% (ABTS, $p < 0.01$), followed by *I. viscosa* with 39.78 ± 0.89% (DPPH) and 35.21 ± 0.94% (ABTS). Consistent with these inhibition profiles, the IC_{50} analysis confirmed that *C. citratus* possessed the strongest antioxidant capacity (IC_{50} = 530.32 µg/mL for DPPH; 696.05 µg/mL for ABTS). *L. nobilis* and *C. creticus* also demonstrated notable antioxidant potential, with IC_{50} values below 750–850 µg/mL. In contrast, *F. vulgare*, *I. viscosa*, and *P. cembra* exhibited markedly weaker activities, showing IC_{50} values exceeding 1000 µg/mL in both assays. These results corroborate the percentage inhibition findings and collectively highlight the superior antioxidant performance of *C. citratus*, *L. nobilis*, and *C. creticus* (Table 3).

Table 3. Free radical scavenging activities of the selected EOs

		DPPH		ABTS	
No	**Plant species** [a]	**Radical scavenging (%)** [b]	**IC_{50} values** [c] **(µg/mL)**	**Radical scavenging (%)** [b]	**IC_{50} values** [c] **(µg/mL)**
1	*C. citratus*	79.12 ± 1.46**	530.32 µg/mL	73.10 ± 0.05*	696.05 µg/mL
2	*H. arenarium*	46.15 ± 0.10**	>1000 µg/mL	54.14 ± 0.62*	923.33 µg/mL
3	*L. nobilis*	75.10 ± 0.08*	641.43 µg/mL	69.14 ± 1.12*	718.53 µg/mL
4	*M. communis*	59.16 ± 0.35*	830.37 µg/mL	53.25 ± 0.71**	932.29 µg/mL
5	*E. purpurea*	52.90 ± 0.66**	946.30 µg/mL	55.16 ± 1.03*	907.86 µg/mL
6	*F. vulgare*	37.16 ± 1.10**	>1000 µg/mL	39.42 ± 0.30**	>1000 µg/mL
7	*I. viscosa*	39.78 ± 0.89**	>1000 µg/mL	35.21 ± 0.94**	>1000 µg/mL
8	*P. cembra*	42.78 ± 0.55**	>1000 µg/mL	38.94 ± 0.17*	>1000 µg/mL
9	*C. creticus*	66.46 ± 0.18*	734.52 µg/mL	58.16 ± 0.50*	843.08 µg/mL
Trolox [d]		83.16 ± 1.26**	495.10 µg/mL	80.72 ± 0.50**	610.02 µg/mL

[a] *(1) Cymbopogon citratus (2) Helichrysum arenarium (3) Laurus nobilis (4) Myrtus communis, (5) Echinacea purpurea, (6) Foeniculum vulgare, (7) Inula viscosa, (8) Pinus cembra, (9) Cistus creticus*
[b] *The values were given as inhibition (%) ± standard deviation.*
[c] *Values were presented as half-maximal inhibitory concentration (IC_{50}, µg/mL).*
[d] *Trolox was used as a commercial standard for DPPH and ABTS assay.*
**p value of < 0.05; **p value of < 0.01.*

According to our results, the main components of medicinal plants with the highest antioxidant capacity were found as citral (48.75% in *C. citratus*), 1,8-cineole (48.75% in *L. nobilis*), and carvacrol (48.75% in *C. creticus*). Extensive studies were carried out to explore

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the various aspects related to these components such as extraction from different medicinal plants, characterization, biological activity, etc. Recent studies showed that citral, 1,8-cineole, and carvacrol extracted from various plants species had an inhibitory effect against oxidative damage induced by various human and rat cells, and delayed oxidative processes [56,57,58]. Therefore, it was estimated that the high antioxidant capacity of the studied samples in the present work was associated with these major components.

3.3. Anticancer activity of the EOs

Anticancer activities of the selected EOs were analyzed against HepG2, NCI-N87, MCF-7, and LNCaP clone FGC-Luc2 human cancer cells, in comparison with non-tumor HUVECs. The results revealed that the application of these EOs resulted in significant inhibition of cell growth and viability across the cancer cell lines, exhibiting both dose- and time-dependent characteristics. The anticancer effects of the EOs were presented in Table 4 as IC_{50} (µg/mL) values, determined after a 48-hour treatment period with an initial EO concentration of 1000 µg/mL (Table 4).

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Table 4. Anticancer activities of the selected EOs against human cancer cells

		IC_{50} values[a](µg/mL)				
No	**Plant species** [b]	**HepG2**	**LNCaP**	**MCF-7**	**NCI-N87**	**HUVEC**
1	*C. citratus*	32.05 ± 0.24**	21.16 ± 0.18**	15.10 ± 1.00**	17.40 ± 0.96**	98.17 ± 0.56*
2	*H. arenarium*	68.12 ± 1.06*	56.10 ± 0.41**	37.05 ± 0.34*	45.18 ± 0.23**	161.30 ± 1.28**
3	*L. nobilis*	36.48 ± 0.90**	27.48 ± 0.01**	19.86 ± 0.24**	21.34 ± 0.54*	105.78 ± 0.50**
4	*M. communis*	52.86 ± 0.12*	39.14 ± 1.45*	26.34 ± 0.89*	32.24 ± 1.22**	129.48 ± 0.98**
5	*E. purpurea*	58.15 ± 0.55**	45.22 ± 0.36**	29.16 ± 0.50**	38.00 ± 0.36**	142.31 ± 0.55**
6	*F. vulgare*	78.12 ± 0.32*	76.78 ± 0.21**	53.78 ± 0.42**	64.98 ± 0.18*	189.14 ± 1.39**
7	*I. viscosa*	76.04 ± 0.28**	69.50 ± 0.86*	47.14 ± 1.13*	59.15 ± 0.95**	183.40 ± 0.30*
8	*P. cembra*	69.98 ± 1.04**	61.00 ± 0.62*	41.25 ± 0.10**	52.40 ± 0.43**	170.16 ± 0.02**
9	*C. creticus*	40.10 ± 1.62**	36.19 ± 0.14**	23.45 ± 0.32*	27.12 ± 0.50*	116.00 ± 1.95**
Doxorubicin [c]		3.45 ± 0.04**	2.86 ± 0.10**	2.51 ± 0.14**	2.48 ± 0.76**	5.96 ± 0.48**

[a] *Values were presented as IC_{50} ± SD from three independent experiment (n=3).*
[b] *(1) Cymbopogon citratus (2) Helichrysum arenarium (3) Laurus nobilis (4) Myrtus communis, (5) Echinacea purpurea, (6) Foeniculum vulgare, (7) Inula viscosa, (8) Pinus cembra, (9) Cistus creticus*
[c] *Doxorubicin was used as a positive control.*
**p value of < 0.05; **p value of < 0.01, based on one-way ANOVA followed by Tukey's post hoc test.*

The selected EOs exerted the highest anticancer activity towards MCF-7 breast cancer cells, whilst the lowest anticancer activity was found against HepG2 hepatocellular carcinoma cells. Among the tested EOs, the EOs of *C. citratus* showed the most potent activity, closely

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followed by the EOs of *L. nobilis* and *C. creticus* against the tested cancer cells. In contrast, EOs of *F. vulgare, I. viscosa,* and *P. cembra* exhibited moderate cancer prevention potential against the tested human cancer cells. In another word, the highest anticancer activity was detected in the EOs of *C. creticus* towards MCF-7 cells (IC_{50} = 15.10±1.00, $p < 0.01$), while it was defined with the IC_{50} value of 17.40±0.96, 21.16±0.18, and 32.05±0.24 ($p < 0.01$) against NCI-N87, LNCaP, and HepG2 cells, respectively. The results revealed that the potential of these EOs as candidates for further research and development into anticancer therapies. The lowest anticancer efficiency was found to belong to the EOs of *F. vulgare* (IC_{50} = 78.12±0.32, $p < 0.05$), closely followed by the EOs of *I. viscosa* (IC_{50} = 76.04±0.28, $p < 0.01$) against HepG2 cancer cell lines as summarized in the Table 4. The differential sensitivity of the cancer cells to the EOs indicated the selective cytotoxicity of these natural compounds. In particular, the EOs were less toxic to HUVECs, supporting their potential use as effective and selective anticancer agents.

In anticancer test results, *C. citratus*, *L. nobilis*, and *C. creticus* species showed antiproliferative properties, as also observed in our antioxidant analyzez results. Considering lemongrass, pink rock-rose, and laurel leaf extracts major ingredient profiling, it was possible that antiproliferative efficacy observed was due to they rich in citral, eucalyptol (1,8-cineole), and carvacrol. Our obtained results were consistent with previous studies performed to reveal antioxidant and antitumoral potentials of our species in the present study. For instance, it was determined that the main component of Cymbopogon citratus samples collected from Vietnam was citral (61.20%–76.46%), and these samples exhibited the strongest cytotoxic effects against various lung cancer cell lines, including A549 and H1975 cells[59]. The anticancer effects of lemongrass extracts with rich bioactive components had been also reported in many other works. For instance, citral, geraniol, α-myrcene, and α-limonene were shown to exhibit high antiproliferative activity against different cancer cell lines including hepatocellular carcinoma, prostate cancer, pancreatic cancer, human colon cancer, breast cancer, liver cancer, intestinal mucosa cancer, cervical and ovarian cancer cell lines in rat models [60,61,62,63,64,65]. In one study, the volatile compounds and antiproliferative activity of four medicinal plants - *Laurus nobilis, Origanum syriacum, Origanum vulgare,* and *Salvia triloba*- collected from Amman were compared [66]. The researchers reported that laurel samples were rich in 1,8-cineole (40.91%) and exhibited strong antiproliferative activity against MCF-7 cell lines (24.49±8.17 µg/mL). Another in vitro study carried out by researchers[67] stated that laurel samples cultivated in Egypt had potent antiproliferative effect against HepG2 (1.83µg/mL), which was attributed to their main components including 1,8-cineole (27.41%), linalool (19.37%), and a-Terpinyl Acetate

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(14.65%). Likewise, Jaradat et al. (2023) investigated the phytochemical profile and biological activities of laurel samples collected from Palestine and identified 36 compounds, with 1,8-cineole (40.39%) and terpinyl acetate (15.07%) as the predominant constituents. Their experimental results demonstrated that laurel essential oil possessed strong antioxidant capacity (IC_{50}=2.2±1.38 µg/mL) and notable inhibitory activity against MCF-7 and cervical adenocarcinoma (HeLa) cell lines. Similarly, Mocan *et al.* (2022) evaluated the phytochemical composition and antioxidant, antimicrobial, anti-inflammatory, and cytotoxic properties of *Cistus creticus* naturally distributed on Rhodes Island, Greece. Their findings revealed that the samples were rich in myricetin and quercetin derivatives and exhibited high inhibitory potential against MCF-7 (IC_{50}=58 ± 5 µg/mL), HepG2 (IC_{50}= IC_{50}=81 ± 5 µg/mL), HeLa (IC_{50}=42 ± 2 µg/mL), and NCI-H460 lung cancer cell lines (46 ± 2 µg/mL), as well as notable DPPH radical scavenging activity (IC_{50} = 46.1 ± 0.4 µg/mL)[68].

3.4. Interrelationships between phytochemical components, antioxidant and anticancer capacities of the Eos

In recent years, the integration of data obtained from scientific studies with multivariate data analysis has been widely used to better visualize similarities and differences among complex datasets and to facilitate this complexity more comprehensibly. In this study, the strength of the relationship between the features evaluated for antioxidant capacity and anticancer activity was assessed using Pearson correlation coefficients and principal component analysis (PCA) (Figure 1-A and B). The phytochemical components of EOs were presented in Table 2, while the differences among plant species were illustrated in the PCA graph (Figure 1-C).

PCA analysis allowed for easier explanation of the impact of applications on the evaluated parameters. The PCA correlation scatter plot (Figure 1-A, B, and C) visualized the comparison of the efficacy of EOs obtained from plant species using analytical techniques: anticancer activity parameters (NCI-N87, HepG-2, LNCaP, and MCF-7), antioxidant capacity properties (DPPH and ABTS), and components of the EOs. While the anticancer activity parameters, antioxidant capacity properties, and EO components were explained by Dim1 (81.9%, 73.5%, and 22.5% respectively) in the PCA graph, Dim2 explains the variation in plant species (17.4%, 24.7%, and 17.0% respectively). Anticancer parameters (NCI-N87, HepG-2, LNCaP, and MCF-7) clustered on the right side of the PCA plot (Figure 1A), whereas antioxidant parameters (DPPH and ABTS) clustered on the left (Figure 1B), indicating that variables within each cluster exhibited interrelated characteristics.

When examining the relationship between anticancer activity parameters and plant species (Figure 1-A), it was observed that plant species numbered 2, 7, 6, and 8 were grouped together, indicating that these species exhibited similar anticancer profiles, characterized by relatively higher IC_{50} values and therefore lower anticancer potency compared to the others. In contrast, species outside this cluster demonstrated stronger anticancer activity. In the PCA graph associating antioxidant capacity with plant species (Figure 1-B), species numbered 1, 3, 5, and 9 were grouped together, suggesting that these plants exhibited higher antioxidant capacities relative to the remaining species. Additionally, the PCA analysis based on EO phytochemical composition (Figure 1-C) revealed that plant species with accession numbers 1, 3, 6, 7, and 9 clustered closely, indicating similar essential oil profiles, while the others formed distinct clusters due to their differing chemical compositions.

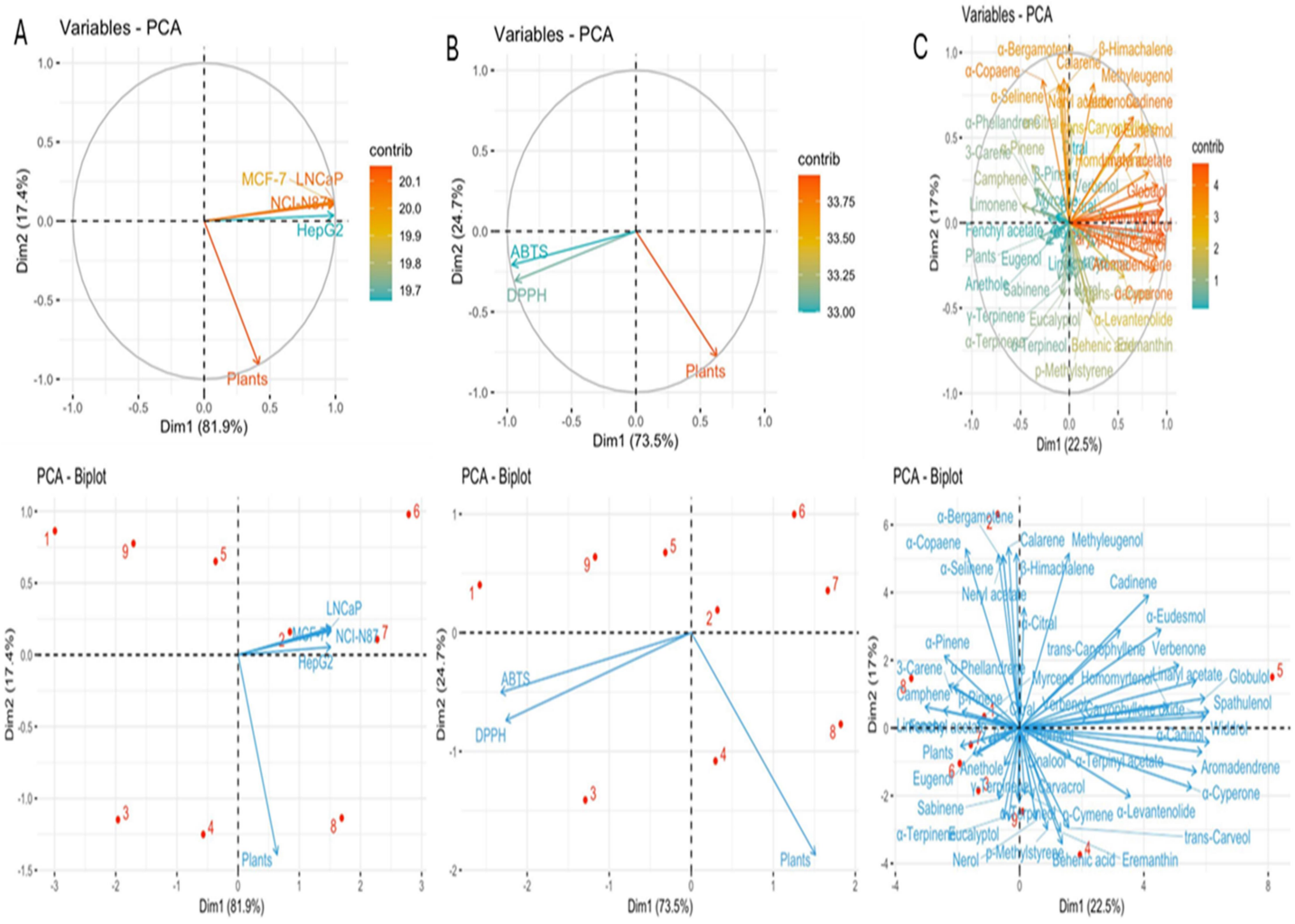


Figure 1. A. Principal component analysis (PCA) correlation scatter plot and PCA score scatter pilot of anticancer activities of EOs; B. Principal component analysis (PCA) correlation scatter plot and PCA score scatter pilot of antioxidant capacities of EOs; C. Principal component analysis (PCA) correlation scatter plot and PCA score scatter pilot of main phytochemical compounds of EOs; 1. *Cymbopogon citratus*, 2. *Helichrysum arenarium*, 3. *Laurus nobilis*, 4. *Myrtus communis*,

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5. *Echinacea purpurea*, 6. *Foeniculum vulgare*, 7. *Inula viscosa*, 8. *Pinus cembra*, 9. *Cistus creticus*

5. Conclusion

In conclusion, the present study focused on the phytochemical analyses of nine commonly used MAPs and their efficacy as anticancer and antioxidant agents. The GC-MS data obtained from phytochemical profiling showed that the studied MAPs extracts are rich sources of anethole, borneol, carvacrol, citral, α-pinene, eucalyptol, and β-citral, present at varying concentrations as major components. Compared with previous studies, it was observed that the type and quantity of chemical content of the samples can vary significantly depending on different environmental conditions. This result showed the importance of cultivating medicinal plants under controlled conditions and standardizing their chemical composition and biological activity levels. Furthermore, it was revealed that the EOs extracted from the studied samples exhibited significant antioxidant and antiproliferative activities against various cancers including HepG2, NCI-N87, MCF-7, and LNCaP cell lines. Among the tested species, *Cymbopogon citratus*, *Laurus nobilis*, and *Cistus creticus*, which exhibited the highest biological activity, could be considered potent anticancer agents. These species may hold promise for use in the chemotherapy of hepatocellular carcinoma, gastric carcinoma, breast adenocarcinoma, and prostate carcinoma in vitro models. However, further comprehensive scientific and clinical studies are necessary to characterize and validate the active compounds responsible for these biological activities in in vivo models. Overall, this study highlights those phytochemicals such as anethole, borneol, carvacrol, citral, α-pinene, eucalyptol, and β-citral represent valuable natural resources for drug design and development. Their preventive and therapeutic potential positions them as promising alternatives to synthetic drugs, offering a natural solution for addressing medical challenges.

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Data availability statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions: M.T.; GC-MS analyses and statistical analyses; S.G.; Anticancer and antioxidant activity assays, and statistical analyses, F.K.; Collection and identification of the plant materials, M.T., S.G., and F.K.; Write and review the manuscript. All authors have read and agreed to the published version of the manuscript.

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