Research Article

Phytochemical Analysis of Volatile and Non-volatile Fractions, Antioxidant, and Anti-Cancer Activities of *Dracocephalum polychaetum* and *Dracocephalum kotschyi*

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Abstract

The present study was conducted to determine the volatile and non-volatile fractions and the antioxidant and anti-cancer activities of ethanolic extracts of *Dracocephalum polychaetum* and *D. kotschyi*. The volatile and non-volatile fractions were investigated by gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC). The cytotoxicity effect of two ethanol extracts and the major phenolic components has been evaluated on breast and colon cancer cells by the MMT assay. GC-MS of the essential oils identified about 50 compounds, and perillylaldehyde and D-limonene were the main constituents in the essential oils of the two species. Moreover, high-performance liquid chromatography- Diode array detector analysis demonstrated that the ethanolic extract of *D. polychaetum* and *D. kotschyi* were the source of phenolic compounds such as rosmarinic acid, protocatechuic acid, naringin, apigenin, syringic acid, epicatechin, chlorogenic acid, thymol, carvacrol, rutin, p-coumaric acid, gallic acid, benzoic acid, cinnamic acid, resorcinol, quercetin, salicylic acid, 4-hydroxybenzoic acid, and ferulic acid. Rosmarinic acid and thymol were the main predominant phenolic constituents in *D. kotschyi* and *D. polychaetum* ethanolic extracts. The cytotoxicity effect of *D. kotschyi* and *D. polychaetum* ethanol extracts and the major phenolic components including rosmarinic acid, thymol, apigenin, quercetin, and nariginin has been evaluated on breast and colon cancer cells by MMT assay and results indicated IC_{50} values in the range of 90 to 140 ($\mu g.ml^{-1}$) after 48 hours of treatment with ethanol extracts. Among phenolic components, thymol caused the lowest cell viability and Narengin showed the lowest anti-proliferative activity. Both extracts also showed antioxidant activity using DPPH assay. The findings of this research suggest that the *Dracocephalum* have precious bioactive and natural compounds with significant antioxidant and *in vitro* anti-cancer activities.

***Keywords*:** Anti-cancer activity, Antioxidant activity, Essential oil, Phenolic compounds

Introduction

Medicinal plants have natural products that can be used as a source for potential drugs. Today, finding new bioactive compounds with medicinal properties has been the subject of many studies and some researchers have focused on replacing natural plant-based compounds with chemical compounds in the medical, food, and pharmaceutical industries (Kchaou et al., 2016). Essential oils (EOs) are unique and valuable resources of secondary metabolites due to their medicinal properties, such as anti-cancer, antiviral, and anti-bactericidal activities (Golkar and Moattar, 2019). In addition, phenolic compounds are another main class of secondary metabolites in plants derived from the shikimic acid pathway. They are composed of phenolic acids, flavonoids, and colored anthocyanins. The anti-inflammatory, antimicrobial, antioxidant, and antiproliferative activities of these compounds have also been reported in the literature (Apostolouet al., 2013).

Dracocephalum is a genus of flowering plants belonging to the Labiate family. *Dracocephalum* varieties are used in traditional medicine to heal many diseases. The chemical composition of *Dracocephalum* species consists of phenolic compounds, flavonoids, flavonols, terpenoids, and alkaloids. *D. polychaetum*, known as Zaroo or Mofaroo in Kerman folk medicine, is exclusively grown in a few geographical areas of Kerman province in Iran (Fallah and Hosseini Cici, 2020). *D. kotschyi*, locally known as 'Zarrin-giah' or 'Badrandjboie-Dennaie', is an aromatic and perennial herb found at an altitude of 2000-3000 metres above sea level in the central-western parts of Iran (Fattahi et al., 2013). Despite substantial information about different species in the Labiate family, research considering the phytochemical

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composition and biological activities of *D. kotschyi* and *D. polychaetum* is limited so far.

The present study aimed to determine the EOs composition, phytochemical components, total phenolic content (TFC), total flavonoids (TFD), total flavonols (TFL), and anthocyanin (Ant) of *D. polychaetum* and *D. kotschyi* by gas chromatography-mass spectrophotometry (GC-MS), and high-performance liquid chromatography-diode array detector (HPLC-DAD). Furthermore, the antioxidant activity of *D. polychaetum* and *D. kotschyi* ethanol extracts was assayed by DPPH, and the cytotoxicity activities of ethanol extracts and major phenolic components of these two plants were assayed on breast and colon cancer cells.

Materials and Methods

Plant materials

The *D. polychaetum* plants were obtained from wild-growing populations of Kerman Province (Hezar altitude of about 4000 m) in the Southeast of Iran. *D. kotschyi plants were* gathered from wild-growing populations in Isfahan Province (Fereydunshahr altitude of about 2700 m).

Volatile fraction analysis

Gas Chromatography-Mass Spectrophotometry analysis

The essential oils were extracted by the hydro-distillation method. The crushed air-dried biomass (50 g) of *D. polychaetum* and *D. kotschyi* aerial parts were hydrodistilled for 4 hours using a clevenger-type apparatus (yield 0.7 v/w %). The yields were estimated after extraction with diethyl ether. Essential oil constituents were identified by an Agilent GC7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA). The GC system is equipped with a HP-5MS capillary column (HP-5MS 5% Phenyl Methyl Silox: Agilent, USA, 30 m in length × 0.25 mm in diameter, flm thickness of 0.25 µm), and coupled with a mass-selective detector from the same company. The carrier gas was helium, at a flow rate of 1 mL min^{-1}. The temperature of the detector and injector was 240 °C. At first, the temperature of the oven was set at 40 °C for 3 min. Then, it was extended to 290 °C at a rate of 5 °C min^{-1}. Finally, the temperature was kept at 280°C for 15 min. The samples were injected in a pulsed split mode (the flow was 3 mL min^{-1}, split ratio 20:1). The mass-selective detector was operated at a ionization energy of 70 eV using a spectral range of 25-1000 amu. Identification of the compounds was achieved by comparing their retention indices to *n*-alkanes and their mass spectra with those recorded in the National Institute of Standard and Technology and those reported in the studies (Adams, 2007).

Nonvolatile fraction analysis

Leaf extraction procedure

Dried and powdered aerial parts of plants (300 mg DW) were homogenized with 2 mL of ethanol (Merck, Germany) and centrifuged at 10,000 rpm for 25 min. Then, the supernatant was collected and held at -20°C for further analysis.

Total Phenolic content

Total phenolic contents were mesured using the Folin-Ciocalteau method as discussed in Chua et al. (2011). The concentration of total phenol content was expressed in terms of mg equal gallic acid in 1 g of dry weight (mgGA g^{-1}DW).

Total Flavonoid and Flavonol content

Total flavonol and flavonoid contents were determined according to Miliauskas and Venskutonis (2004). The TDF and TFL were then calculated by a calibration curve method using rutin as a standard. The contents of TDF and TFL were expressed as mg rutin equivalents per gram of dry mass (mg RU g^{-1}DW).

Anthocyanin content

Total anthocyanin was estimated according to the protocol of Hara (2003). The anthocyanin content was estimated by measuring the absorbance at 511 nm using the spectrophotometer and calculated by the extinction coefficient of 33,000 M^{-1} cm^{-1}.

DPPH free radical scavenging activity

Free radical scavenging activities were measured by DPPH (1, 1-di- phenyl-2-picrylhydrazyl) assay according to Burits and Bucar protocol (2000). The reduction of DPPH absorption was read at 517 nm. Butylated hydroxytoluene (BHT) was used as the standard drug. The inhibition percentage was calculated as follows: $I\% = (A_{con} - A_{sam}) / A_{sam} \times 100$, where A_{con} and A_{sam} are the absorbance of control and sample, respectively. Radical scavenging activity was expressed as the inhibition concentration (IC_{50}), i.e., the concentration of extract necessary to decrease the initial concentration of $DPPH^{\bullet}$ by 50% (IC_{50}) under the specified experimental conditions.

Phytochemical products by HPLC analysis

Phytochemical products of plants were extracted according to Taghizadeh et al. (2019). For quantitative and qualitative measurements of phytochemicals in ethanol, an HPLC program equipped with a UV-Vis photodiode-array detector (DAD-HPLC Waters e 2695, Alliance, Milford, MA, 2489 UV-Vis detector) was applied. To confirm the peak identity, their absorption spectra and retention times were compared with those of pure (>99%) standards (syringic acid, gallic acid, protocatechuic acid, chlorogenic acid, 4-hydroxybenzoic acid, epicatechin, benzoic acid, naringin, ferulic acid, salicylic acid, rosmarinic acid, rutin, quercetin, p-coumaric acid, carvacrol and apigenin were bought from Sigma (USA), thymol and cinnamic acid were bought from Merck (Germany).

Anti-cancer analysis

Cell culture and reagent

Human breast (MCF-7) and colon (HT-29) cancer cell lines (from the Pasteur Institute of Iran, Tehran) were grown in DMED medium (Gibco, Grand Island, USA) containing 10% fetal bovine serum (FBS; Biowest, France) and 100 U mL^{-1} penicillin and streptomycin (Biowest, France) and cultured at 37°C in a humidified atmosphere with 5 % CO_2.

The rosmarinic acid, apigenin, quercetin, and naringin were purchased from Sigma-Aldrich (USA), and thymol was obtained from Merck (Germany). All phytochemical components were dissolved in ethanol.

Cytotoxic activity assay

The cytotoxic activities of rosmarinic acid, thymol, apigenin, quercetin, naringin, and ethanol extracts of *D. polychaetum* and *D. kotschyi* on MCF-7 breast and HT-29 colon cancer cell lines were measured by 3- (4,5-dimethyl thiazol-2yl) -2,5-diphenyltetrazolium bromide (MTT) assay. The cells were subcultured by trypsinization. The 5×10^3 cells were seeded in 96-well plates. After 24 h, the culture medium was changed with a medium containing increasing concentrations of samples ranging from 10 to 1280 µg mL^{-1} in each well for 48 h.

Since the solvents (DMSO and ethanol) have cytotoxic effects, the same proportion of DMSO/ethanol was added as controls. Later, 20 µL of the MTT solution (5 $mgmL^{-1}$) was added to each well, and the plate was reincubated for 3 h. Finally, the medium was removed, and 100 µL of DMSO was added to dissolve formazan crystals; absorbance was measured at 490 nm using a multi-plate reader (ELISA reader; BioTek-ELx800, USA). The ratio of the absorbance of treated cells to the absorbance of control cells was determined as cell viability (%). Half-maximal inhibitory concentration (IC_{50}) of each component was calculated using Prism 6.0 (GraphPad Software, Inc., San Diego, California, USA).

Data Presentation and Statistical Analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version No 19.0, Chicago, IL, USA, with three replications. The experiments followed a completely randomized design, and the values were presented as mean ± SD (standard deviation). The significant differences among treatments were evaluated using the LSD test at $P \leq 0.05$.

Results and Discussion

GC/MS of essential oil

The oxidation of fatty acids produces mainly EOs through an intracellular biogenic pathway (Golkar and Moattar, 2019). The GC-MS analysis of the EOs of *D. polychaetum* and *D. kotschyi* showed 54 compounds of the total oils. These compounds and their relative proportions in the EOs are presented in Table S1 (Supplementary file) According to the data, perillyl aldehyde and D-limonene were the main constituents of the EOs in both *Dracocephalum* species. The major components in EOs of *D. kotschyi* were perillyl aldehyde, D-limonene, p-mentha-1(7), and 8(10) -dien-9-ol. Similar to *D. kotschyi*, perillyl aldehyde, D-limonene, p-mentha-1(7), and 8(10)-dien-9-ol were identified as the main components in EOs of *D. polychaetum*. According to these data, o-cymene, ethanone, 1-(2-methylphenyl), (-)-myrtenol, trans-carveole, 2,6-octadienal, 3,7-dimethyl-, (Z)-,l-carvone, cis-geraniol,3cyclohexene1-ethanol, β,4-dimethyl, terpineol acetate, caryophyllene, cadinene, caryophyllene oxide, and carotol existed in *D. kotschyi*, but they did not exist in *D. polychaetum*. The variation in the composition of EOs of *Dracocephalum* species (type and proportion) depends on plants' genetic and environmental conditions such as humidity, temperature, altitudes, and light intensity in areas where the *Dracocephalum* species grow (Fallah et al. 2020). In another study, α-pinene, α-terpinene, p-cymene,

limonene, linalool, p-mentha-1,5-dien-8- ol, α-terpineol, trans-carveol, and caryophyllene were reported as the main components in *D. kotschyi* (Fallah et al., 2020). In a previous investigation into the EOs composition of *D. polychaetum*, the authors identified perilla aldehyde, terpinene-7-al, and limonene (Khodaei et al., 2018). Thus, the results of the EOs analysis in the present research might be used as supplementary and confirmatory data to the previous literature on *D. kotschyi* and *D. polychaetum*.

Total phenolics

Phenolic compounds are considered important secondary metabolites, possessing various roles in plants. One of these roles is the antioxidant activity in the defensive response of plants to biotic and abiotic stresses. These compounds eliminate reactive oxygen species (ROS) without causing any oxidative stress damage (Dias et al., 2016). According to this research, *D. poychaetum* exhibited a higher level of TPC as compared to *D.kotschyi* (Table 1). Previous studies have reported various contents for TPC depending on *Dracocephalum* species. Our results are consistent with other studies that reported TPC in the methanolic extract of *D. kotschyi* was 175.6 mg $GAEg^{-1}$ DW (Sharifi et al., 2017) and 188.08 mg $GAEg^{-1}$ DW (Mirzania and Farimani, 2018). Also, plant genetic and environmental conditions such as plants' geographic location and climatic factors (i.e., light intensity, photoperiod, and temperature) significantly affected the TPC. These factors also influence the biosynthesis of many secondary metabolites in many plant species (Jaakola and Hohtola, 2010).

Total Flavonoid and Flavonol

Flavonoids belong to a class of low molecular weight phenolic compounds with various functions and can be subdivided into different subgroups including flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins, and chalcones. As the main compounds of the non-antioxidant system, they act as chemical messengers and physiological regulators (Falcone Ferreyra, 2012). Flavonols are an important subgroup of flavonoids and, by their antioxidant activity, play a very significant role in causing plants to respond to environmental stresses (Golkar et al., 2019). A previous study reported the presence of many flavonoids and flavonols in different *Dracocephalum* species (Kamali et al., 2016). The findings of this research show that the TFD level is higher in *D. polychaetum* than in *D. kotschyi* Furthermore, *D. poychaetum* exhibited a higher level of TFL as compared to *D. kotschyi* (Table 1). The TFD and TFL are highly affected by circumstances like the plant's geographic location, environmental condition, and dominant climatic factors (Jaakola & Hohtola, 2010). Furthermore, different extraction methods can significantly affect the type and amount of TDF and TFL. To the best of our knowledge, this is the first report on the TFL and TDF values in *D. polychaetum.*

Anthocyanin content

Anthocyanins are a subgroup of flavonoids, a class of phytochemical compounds with antioxidant, antibacterial, antiviral, anti-cancer, and anti-inflammatory effects (Golkar et al., 2019). As presented in Table 1, the Ant contents in *D. polychaetum* and *D. kotschyi* were measured at 13.24 µmol g^{-1} DW and 12.07 µmol g^{-1} DW, respectively. A review of the previous literature revealed a lack of any studies on Ant content in the two species. In this regard, it is useful to increase Ant content because of its medicinal value and colorant properties, which make it a valuable biochemical for the food industry.

HPLC analysis

Phytochemicals are a powerful group of compounds belonging to secondary metabolites of plants that have antioxidant and anti-radical roles in the defense response of the plant to environmental stress and also have valuable pharmaceutical and medical properties.

According to the HPLC analysis in this study, chromatographic separation plants identified 18 phytochemical compounds in each species. Table 2 presents the quantitative determination of each phytochemical compound (µgg^{-1} dry weight). Our findings indicated the phytochemical compounds of both species consisted of naringin, rosmarinic acid, apigenin, epicatechin, carvacrol, rutin, thymol, 4-hydroxybenzoic acid, p-coumaric acid, benzoic acid, gallic acid, cinnamic acid, resorcinolchlorogenic acid, salicylic acid, syringic acid, quercetin, protocatechuic acid, and ferulic acid. The main differences between the two species were the quantitative amounts of each compound. Genetic and environmental factors can significantly affect the metabolism and accumulation of the phytochemicals (Dong et al., 2011). Here, different plant species can employ diverse mechanisms to distribute phytochemicals in their organs by various means. These results are in agreement with previous studies as they reported that *D. kotschyi* phytochemicals consisted of lcosmosiin, luteolin 3′-

O-β-d-glucuronide, luteolin, apigenin, cirsimaritin, isokaempferide, penduletin, xanthomicrol, calycopterin, epicatechin, chlorogenic acid, and the polyphenol rosmarinic acid (Fattahi et al., 2016, Jahaniani et al., 2005). The present results indicated that rosmarinic acid, apigenin, quercetin, naringin, thymol, and carvacrol were the predominant phytochemical compounds in *D. polychaetum* and *D. kotschyi* ethanolic extracts. In this respect, the highest amount of rosmarinic acid was observed in *D. kotschyi*. Earlier studies showed that rosmarinic acid was also the most abundant polyphenol in Iranian *D. moldavica* and *D. kotschyi* (Fattahi et al., 2013).

Moreover, monoterpenoids such as carvacrol and thymol were found for the first time in ethanolic extracts of *D. polychaetum* and *D. kotschyi*. Hence, the highest amounts of carvacrol and thymol existed in *D. polychaetum*. According to our results, the highest content of quercetin, as a flavonol compound, was found in *D. kotschyi*. This study showed no significant differences between apigenin (as a flavone compound) and naringin content of the studied species. To the best of our knowledge, there is no report in the literature demonstrating the phytochemical constituents of *D. polychaetum*. Furthermore, according to this study, resorcinol, protocatechuic acid, 4-hydroxybenzoic acid, syringic acid, ferulic acid, and naringin were identified as new phytochemical compounds in *D. kotschyi*.

Cytotoxic activity against breast and colon cancer cell proliferation

As shown in Figure. 1, the cytotoxicity assay after 48 h treatment revealed that ethanol extracts from *D. polychaetum* and *D. kotschyi* had significant cytotoxic activity on MCF-7 cancer cells with IC_{50} values of 140 μgmL^{-1} and 133 μgmL^{-1}, respectively, and significant cytotoxicity on HT29 cell line with IC_{50} values of 90 μgmL^{-1} and 126 μgmL^{-1} respectively. Among the active constituents of these extracts, thymol exhibited the highest anti-proliferative effect with IC_{50} values of 10 μgmL^{-1} for HT-29 and 23 μgmL^{-1} for MCF-7, and Naringin showed the lowest anti-proliferative activity with IC_{50} values 270 μgmL^{-1} for HT-29 and 399 μgmL^{-1} for MCF-7. Also, rosmarinic acid, apigenin, and quercetin exhibited considerable inhibitory activity against MCF-7 and HT-29 cells with IC_{50} values ranging from 35- 40 μgmL^{-1}.

In vitro cytotoxicity assay using MTT showed that both ethanol extracts and their constituents have cytotoxic activities against colon and breast cancer cell lines. These data propose that the anti-cancer potential of *D. kotschyi* and *D. polychaetum* ethanol extracts could be attributed to their main contents, including thymol, rosmarinic acid, apigenin, and quercetin. The anti-proliferative activities of thymol, rosmarinic acid, apigenin, and quercetin on the growth of various cancer cells have been reported in many studies (Zhang et al., 2018).

Despite the antioxidant, antidiabetic, anti-hyperlipidemic, and anti-lipid peroxidative properties of ethanol extract of *D. polychaetum* aerial parts (Pouraboli et al., 2016), there is no report on the anti-cancer potential of this plant. On the contrary, the anti-proliferative activity of *D. kotschyi* against several cancer cells has been indicated in several studies. For instance, it was shown that leaf extract of *D. kotschyi* could inhibit the proliferation of a number of human tumor cell lines and tumor proliferation in mice. In the reported study, xanthomicrol was recognized as an active flavone in the leaf extract of *D. kotschyi*, which is responsible for inhibiting the proliferation of malignant cells (Jahaniani et al., 2005). Furthermore, *in vitro*, cytotoxic, anti-proliferative effects of different fractions of *D. kotschyi* extract against lung cancer cell lines showed that the dichloromethane fraction and essential oil are the most effective fractions (Sani et al., 2017) for this purpose. The role of *D. kotschyi* as a promising anti-liver cancer agent was demonstrated by inducing reactive oxygen species production, cytochrome c release, and mitochondrial membrane permeabilization. These compounds start apoptosis signaling through the formation of the caspase-3 activation complex. In another research on the cytotoxicity of hexane, aqueous, chloroform, and ethanolic extracts of *D. kotschyi* aerial parts on the MDA-MB-231 breast cell line, the ethanolic extract showed the highest cytotoxicity effect (Faghihinia et al., 2015). This study confirmed the anti-cancer effect of *D. kotschyi* on breast and colon cancer cells.

DPPH scavenging activity

Bleaching the purple ethanol solution of DPPH was used to measure the DPPH scavenging activity of the ethanolic extracts of *D. polychaetum* and *D. kotschyi* and BHT as a standard compound. The concentration of the sample required to reduce the primary concentration of DPPH by 50% (IC_{50}) under the experimental situation is presented in Table 3. According to our results, the DPPH scavenging activity of *D. kotschyi* and *D. polychaetum* increased in a dose-dependent manner (Figure 2). The IC_{50} values showed the higher antioxidant activity of the *D. kotschyi* than *D. polychaetum extract*. Both

extracts showed less antioxidant activity than BHT as the standard. Other species of *Dracocephalum*, such as *D. moldavica*, also showed remarkable scavenging effects against DPPH (Fattahi et al., 2013).

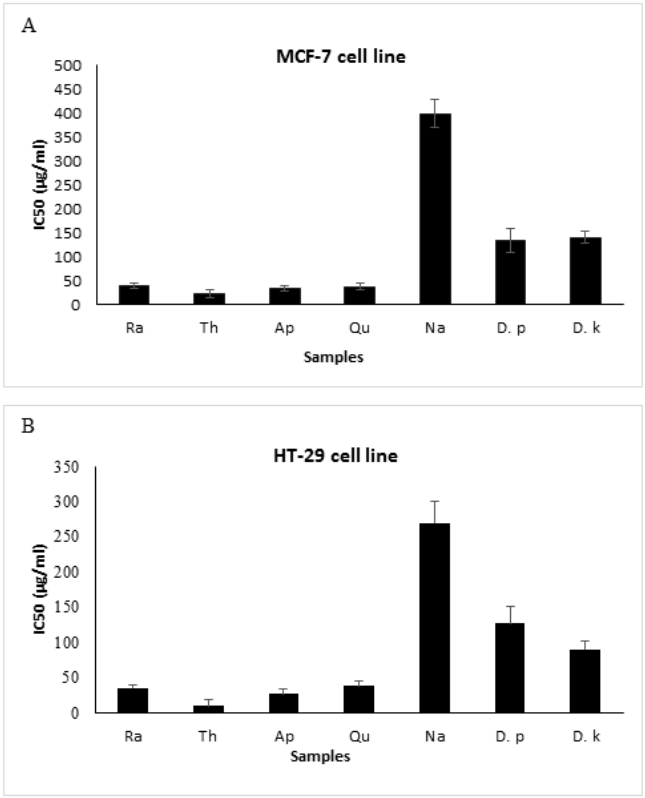

Figure 1. Anti-proliferative effects of Rosmarinic acid (Ra), Thymol (Th), Apigenin (Ap), Quercetin (Qu), Naringin (Na), *D. polychaetum* (*D. p*) and *D. kotschyi* (*D. k*) ethanolic extracts against human breast MCF-7 (A) and colon HT-29 (B) cancer cell lines. Anti-proliferative effects were determined by MTT assay. IC50 values are expressed as the mean ± SD of three independent experiments. IC50 is the drug concentration that causes a 50% decrement of cell viability.

Table 1. Contents of total phenolics, total flavonoids, total flavonols and anthocyanin in *D. kotschyi* and *D. polychaetum.*

	Studied traits			
Species	TPC[a] (mg GAE/g DW)	TFD (mg QE/g DW)	TFL (mg QE/g DW)	Ant (µg/ g DW)
D. polychaetum	209.15±1.78[a]	72.83±0.91 [a]	58.93±1.03[a]	13.24±5.3[a]
D. kotschyi	188.73±1.12 [b]	65.91±0.62 [b]	50.16±1.2[b]	12.07±3.7[b]

Data are means ± SD of three replicates. Means followed by the same letter are not significantly different at P≤0.05 according to the LSD test. [a]: TPC: Total phenolic content; TFD: Total flavonoids; TFL: Total flavonols, Ant: anthocyanin.

Conclusion

Overall, the present study was conducted to identify and quantify volatile and non-volatile fractions and evaluate the antioxidant and anti-cancer potentials of *D. kotschyi* and *D. polychaetum*, two endemic species of Iran. In this study, the antioxidant activities of the TPC, TDF, TFL, and Ant in *D. polychaetum* plants were found to be stronger than those of *D. kotschyi*. Moreover, the ethanolic extracts of *D. polychaetum* and *D. kotschyi* and the major phytochemical components such as rosmarinic acid, thymol, apigenin, quercetin, and naringin showed high cytotoxic effects on breast and colon cancer cells, as determined by MMT assay. However, further research is required to find other new natural compounds in *D. kotschyi* and *D. polychaetum*. The findings of this research suggest that the *Dracocephalum* has precious bioactive and natural compounds with significant antioxidant and *in vitro* anti-cancer activities that have promising applications in the food, pharmacological, and medicinal industries, especially in cancer therapy.

Table 2. Phytochemical compounds (µg. g^{-1} DW) in the *D. polychaetum* and *D. kotschyi* plants, identified by HPLC.

Phytochemical Compounds (µg. g^{-1} DW)	[a]RT (min)	*D. polychaetum*	*D. kotschyi*
Gallic acid	9.8	153.99±23.4[b]	189.74±36.95[a]
Resorcinol	12.3	40.05±7.8[a]	21.35±8.04[b]
Protocatechuic acid	15.5	4.25±0.89[a]	4.27±0.47[a]
4-hydroxybenzoic acid	18.5	13.85±2.23[a]	8.6±0.44[b]
Chlorogenic acid	32.5	22.44±7.5	42.3±5.7
Epicatechin	34.6	91.76±12.7	83.16±6.96
Syringic acid	36.8	14.17±2.4	8.99±1.98
Benzoic acid	40.95	19.74±2.37	9.43±0.94
p-coumaric acid	42.89	6.73±0.36	5.73±0.45
Ferulic acid	46.57	8.12±0.54	8.06±0.76
Salicylic acid	50.18	11.43±1.9	40.33±5.95
Naringin	55.2	96.48±10.53	96.54±8.64
Rosmarinic acid	57.9	2267.73±137.65	7883.06±165.17
Rutin	59.11	98.75±6.56	33.58±4.86
Cinnamic acid	61.18	42.41±4.76	53.57±5.38
Quercetin	68.15	160.02±9.54	189.65±6.52
Apigenin	76.03	3507.38±109.03	3073.39±89.76
Thymol	76.25	7771.04±197.86	1753.77±93.05

Data are means ± standard deviation (SD) of three replicates. Means followed by the same letter are not significantly different at P≤0.05 according to LSD test. [a]: RT: retention time.

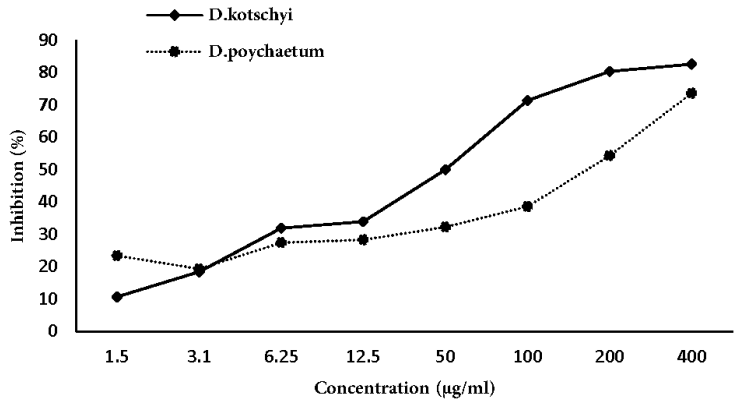

Figure 2. Graph comparing the DPPH's radical scavenging activity of different concentrations of *D. polychaetum* (*D. p*) and *D. kotschyi* (*D. k*) ethanolic extracts.

Table 3. The inhibitory concentration 50% (IC50) (µg.ml^{-1}) of ethanol extract of *D. polychaetum* and *D. kotschyi*in DPPH test.

	D. polychaetum	*D. kotschyi*	BHT
IC50	175±5.3	50±3.7	7.45 ± 1.08

Data are means ± SD of three replicates. Means followed by the same letter are not significantly different at P≤0.05 according to the LSD test.

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Conflict of Interest
The authors declare that they have no conflicts of interest.

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Supplementary File:

Table S1. The GC-MS analysis indicating constituents of essential oils of *D. kotschyi* and *D. polychaetum*

Compound name [a]	**RT**	**RI**	***D. k* %** [b]	***D. p* %** [b]
α-Pinene	9.026	939	1.25	2.57
3-p-Menthene	10.148	978	0.04	-
2,3-Dehydro-1,8-cineole	10.63	995	-	0.04
Ocimene	11.04	1009	-	0.04
Terpinolene	11.407	1021	0.03	0.04
o-Cymene	11.643	1029	0.35	-
D-Limonene	11.815	1035	16.45	13.13
3-Carene	12.639	1062	0.09	0.07
cis-β-Terpineol	12.883	1070	-	0.012
cis-Linalool Oxide	13.019	1075	0.06	0.017
Linalool	13.779	1101	0.71	0.56
trans-p-Mentha-2,8-dienol	14.422	1123	0.45	0.23
±-4-Acetyl-1-methylcyclohexene	14.721	1133	0.17	0.15
cis-p-Mentha-2,8-dien-1-ol,	14.83	1137	0.46	0.26
L-Pinocarveol	14.993	1143	0.18	-
cis-Verbenol	15.138	1148	0.24	-
Ethanone, 1-(2-methylphenyl)-,	16.279	1187	0.67	-
Menthofuran	16.396	1191	1.47	0.82
Terpineol	16.487	1194	0.86	0.67
(-)-Myrtenol,	16.65	1200	0.81	-
Perillyl aldehyde	16.976	1211	27.9	56.7
Trans-Carveole	17.546	1232	1.28	-
2,6-Octadienal, 3,7-dimethyl-, (Z)-	17.809	1241	1.14	
l-carvone	17.936	1246	2.4	
cis-Geraniol	18.126	1253	1.51	
P-Menth-1-en-9-al	19.013	1285	1.5	0.4
6-Propenylbicyclo [3.1.0]hexan-2-one	19.04	1286	-	2.63
p-Mentha-1(7),8(10)-dien-9-ol	19.203	1292	11.3	10.9
3-Cyclohexene-1-ethanol, β,4-dimethyl	19.375	1298	3.43	-
Terpineol acetate	20.688	1349	0.41	-
Eugenol	20.888	1357	1.6	0.1
Copaene	21.476	1380	-	0.23
1,5,5-Trimethyl-6-methylene-cyclohexene	22.047	1402	-	0.22
Perillyl acetate	22.228	1409	0.84	2.96
Caryophyllene	22.599	1424	0.17	-
β-Ionene	24.102	1486	-	0.1
δ-Cadinene	25.026	1525	0.54	-
α-Calacorene	25.488	1545	0.03	0.03
3-Hexen-1-ol, benzoate, (Z)-	26.049	1569	0.28	-
2,4,4,6-Tetramethyl-6-phenyl-1-heptene	26.221	1576	0.03	-
Caryophyllene oxide	26.484	1587	0.89	-
Carotol	26.782	1600	0.38	-
Calarene epoxide	27.063	1613	0.23	-
Lanceol, cis	27.425	1629	-	0.49
Cyclohexene-1-acetaldehyde, α,4-dimethyl	27.778	1645	0.86	
2-Pentadecanone, 6,10,14-trimethyl	31.781	1835	0.35	
Androstan-17-one, 3-ethyl-3-hydroxy-,(5α)-	41.352	2369	-	3.1
Heptacosane	45.97	2654	0.14	-
Nonacosane	48.686	2822	0.24	-

[a] Compounds are listed in order of their retention index from a HP-5 column. RT: Retention Time, RI: Retention Index.
[b] Relative proportions of the EO components expressed as percentages obtained by GC-MS responses.