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Original Research Article

Biochemical evaluation of antioxidant activity, phenol and flavonoid contents of *Dracocephalum Kotschyi Boiss* extracts obtained with different solvents

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ABSTRACT

Dracocephalum Kotschyi Boiss, is one of *Dracocephalum* species which has been used in traditional Iranian medicine with pharmacological and biological activities. The antioxidant properties and total flavonoid and phenolic contents of three different extracts of *Dracocephalum Kotschyi Boiss* were investigated. The antioxidant activity of the extracts was calculated using three different assay including DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing ability of plasma) and ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)). Among these extracts, the methanol extracts showed the highest radical scavenging activity 6.87±0.01 μmoL Trolox/g ferric reducing activity and the best activity in ABTS test (7.47±0.03 μmoL Trolox/g). The methanol extract of *Dracocephalum Kotschyi* showed also the highest total phenolic content 188.08±0.01 mg GAE/g, followed by ethyl acetate extract 95.58±0.01 mg GAE/g and also greatest total flavonoid content with 1037.00±0.01 mg quercetine equivalents/g. The results of this study showed that the potent antioxidant activity of *Dracocephalum Kotschyi* make use it as a natural antioxidant in food industries and other pharmaceutical purposes.

Keywords: *Dracocephalum Kotschyi Boiss*, FRAP, ABTS, DPPH, total phenol, flavonoid content

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INTRODUCTION

The task of free radicals in the extension of disease has engrossed attention from both scientists and professionals involved in human health care [1]. Free radicals are permanently produced in biological systems as byproducts of metabolism during many reactions. Although human and other organisms bear antioxidant vindication and repair mechanisms that have evolved to support them against oxidative damage. Incompetence of antioxidant defense may lead to oxidative stress, which might be affiliated with a diversity of irregularities including coronary heart disease, diabetes, arthritis and cancer [2]. When natural defenses are suppressed by extreme production of prooxidants, utilization of antioxidants, both conventional as well as derived from some novel origins, has become significant in the proper management of oxidative stress. Antioxidants are nutritive and non-nutritive factors that can postpone biologically wrecking chemical reactions in foods and in living systems. Antioxidants are widely utilized components in dietary supplements for maintaining health, as well as the treatment of several diseases. Indeed, antioxidant substances can act versus oxidants and free radicals by limiting the molecular destruction that could compromise the function of essential lipids, protein and nucleic acids [3,4]. Natural products with antioxidant properties can be a good alternative for synthetic antioxidant. Lately, traditional medicine has gained scientist attention for finding new origins for different treatments and efficacious biomaterials. The importance of the antioxidant constituents of herbal materials in the health and protection maintenance against heart diseases and cancer is also raising interest among scientists, food manufacturers and consumers as the trend for the future toward functional food with specific health effects [5]. Phenolic compounds have a usual structure composed of an aromatic hydroxyl nucleus and nearly 8000 known in nature [6]. Furthermore, the phenolic compounds are plant secondary metabolites widely propagated all over the plant kingdom [7]. The last focus of attention and interest in phenolic compounds stems from their potential preservative role, through ingestion of fruits and vegetables, against oxidative harm illnesses like coronary heart disease, stroke and cancers [8]. Phenolic compounds can be considered as simple phenols and phenolic acids (gallic and benzoic acid) and polyphenols

(flavonoids). Tannins are water-soluble polyphenols that are generally displayed in higher herbal and woody plants that have shown antimicrobial, antiparasitic properties and anticancer activity [9,10]. Polyphenols and carotenoid pigments are the great alimental antioxidants in food. Besides, flavonoids were reported to have health beneficial confidants, containing free radical scavenging. The flavonoids are the most usual group of polyphenolic components in the human diet and are generally found in fruits and vegetables. They can prevent coronary heart illness and have antioxidant capacities. Plant flavonoids and polyphenols are multifunctional which can be worked as reducing compounds, hydrogen atom donors, and singlet oxygen scavengers. They are furthermore efficient as antioxidants, capable of chelating transition metal ions, which may induce Fenton type oxidation reactions in their free states. In addition, flavonoids also are the most common type of polyphenolic compound in the plants that possess antioxidant activity. So, it was reasonable to determine their total amounts in the selected vegetables, fruits and also medicinal plants. The genus *Dracocephalumm*, commonly called Badraj or Varangbu in Persian, has eight species in Iran, (*D. Kotschyi*, *D. motdavica*, *D. aucheri*, *D. multicaute*, *D. subcapitatum*, *D. surmandinum*, *D. polychaetum* and *D. thymifolrum*) and whole these species are consumed as folk medicine for liver disturbance, migraine and congestion. They are also carminative and bracing and chewing the leaves gives an aromatic savory taste. This genus is extensively distributed in Iran. One of the important endemic species, *Dracocephalum Kotschyi Boiss*, is found in abundant parts of the Alborz mountains. This plant is utilized in traditional medicine as a treatment of stomach disorders. Its effective for headache, congestion and liver disease has also been described in folk medicine evidences [11,12]. This plant used in various forms such as essential oils, ointments, compresses and infusion or as carminative medicinal raw material as a cure for people who suffered from stomach bloating. It also used as a flavoring in some foods in Iran. In the present study, we attempted to assess antioxidant activities of *D. Kotschyi* extracts. A rapid and susceptible way for the antioxidant experiments of herbal extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the attendance of an antioxidant compound, DPPH radical contain one more electron and the

absorbance could be reduced [13,14]. Other ways for investigation of antioxidant activity are (ferric reducing antioxidant power) FRAP and ABTS. FRAP assay uses antioxidants as reductants in a redox colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. In this study hexane, ethyl acetate and methanol extracts of *D. Kotschyi* were studied for antioxidant activity.

MATERIALS AND METHODS

Collection of plants

The aerial parts of *D. Kotschyi* were collected at the time of flowering stage from Dizin area in the north of Tehran in Iran. A voucher specimen (MPH-1414) has been deposited in the herbarium of the Medicinal Plants and Drugs Research Institute in Shahid Beheshti University in Tehran.

Preparation of plant extracts

The plant material was cleaned to eliminate any residuals and then dried at room temperature for 14 days. The 20 g of dried and crushed plant specimen was extracted for 48 h using 250 mL n-hexane, ethyl acetate and methanol, successively by maceration. Extraction manner was accomplished at room temperature for 48 h along with shaking. The extracts were filtered through Whatman filter paper and the solvents were evaporated using a rotary evaporator at 35 °C to obtain condensed extracts.

Chemicals

2,2′-azin-obis(3-ethylbenothiazoline-6-sulphonic acid) diammonium salt (ABTS), butylated hydroxy toluene (BHT), gallic acid, folin-ciocalteu, 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium persulphate, ethanol, sodium acetate, quercetin, n-hexane, ethyl acetate and methanol were obtained from Merck (Germany).

Determination of antioxidant activity using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The antioxidant capacity was measured in terms of hydrogen donating or radical scavenging potency using the stable DPPH radical. The antioxidant activity of the juices was determined using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH [15]. 50 µL of diverse concentrations (5, 10, 20, 40, 80 µg/mL) of the extract solutions in methanol were added to 200 µL of 100 µM DPPH solution in methanol. The reaction solution was incubated for 30 min at room temperature and then absorbance were

determined at 517 nm with a microplate reader spectrophotometer (BioTek XS2 model). BHT was applied as the standard antioxidant. The controls included 50 µL of methanol replaceed the test samples, and the blank included absolute methanol in lieu of DPPH solution. Tests were carried out three times. For each concentration, inhibition was accounted according to the following equation:

$$\% \text{ inhibition} = [1 - (A_s - A_b)/A_c] \times 100$$

Where A_s is the absorbance of the mixture in the presence of the specimens, A_b is relevant to the blank and A_c is the absorbance of control. A less absorbance of the admixture demonstrated a higher DPPH radical scavenging ability. IC_{50} value (µg/mL) is the concentration at which 50% of DPPH radicals are scavenged and is received by interpolation from linear regression counting.

Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC test is a common way used for assessment of the antioxidant susceptibility of extracts to quench ABTS radicals. It could distribute antioxidant activity of hydrophilic and lipophilic components. The ABTS radical cation was prepared by mixing a solution of 7 mM ABTS in ethanol and 2.45 mM potassium persulphate. The blend was incubated for 12 h at room temperature until the reaction was used during 1 day [16]. For assessments, the ABTS radical solution was diluted. ABTS dilution (3.8 mL) and 100 µL of the herb samples were mixed and the absorbance were recorded at 734 nm after 1 min. Amount of inhibition at 734 nm was measured. Trolox was utilized as the standard, and the outcomes were represented as µmol of Trolox equivalents per gram anhydrous weight of plants.

Ferric reducing antioxidant power (FRAP) assay

The antioxidant ability of each sample was approximated by FRAP assay, following the procedure described by benzie and strains with some modifications. Briefly, 0.2 ml of the extracts were mixed with 1.8 ml of the fresh FRAP agent, which consisted of 2.5 ml of 10 mM TPTZ solution in 40 mM HCl also 2.5 ml of 20 mM $FeCl_3$ and 25 ml of 0.3 M acetate buffer (pH 3.6). The absorption of the mixture was determined at 595 nm. Ethanolic solutions of Fe (II) concentrations were utilized to obtain the calibration curve. The FRAP values have been calculated by contrasting the

absorbance conversion at 593 nm in test samples with those comprising ferrous ions.

Assay for total phenolic contents

The total phenolic contents of different extracts were determined using the folin-ciocalteu method with some modification [17]. 2.5 mL of sample was combined with 2.5 mL of folin-ciocalteu reagent. Then 50 µL of sodium carbonate was added to the mixture and the content was adjusted to 250 mL by increasing distilled water. The mixture was blended thoroughly for 30 minutes at room temperature in the dark space. Absorbance of the sample mixture versus a blank was specificed at 765 nm utilizing a micro plate reader. Results were declared as mg of gallic acid equivalent per gram of dry extract (mg GAE/g). Multiple concentrations of gallic acid as a standard (12.5, 25, 50, 100, 200 µg/mL) were utilized to construct a calibration curve. All assessments were carried out in triplicate.

Flavonoid contents

Total flavonoid content was evaluated using a colorimetric assay with aluminium chloride according to a formerly described method [18]. The absorbance was calculated against a blank at 510 nm. Results were presented as mg of quercetine equivalents per gram of anhydrous juice. Different concentrations of quercetine as standard (12.5, 25, 50, 100, 200 µg/mL) were utilized to draw a calibration curve. All calculations were carried out three times.

RESULTS

The DPPH radical scavenging activity of different extracts of *D. Kotschyi* is showed in Table 1. With regard to IC_{50} values among the examined samples, the methanol extract of *D. Kotschyi* with the lowest IC_{50} of 9.55±0.11µg/mL, exhibited considerable DPPH radical scavenging activity compared with BHT (14.50±1.20 µg/mL), followed by the ethyl acetate extract of this species (IC_{50} = 20.27±0.05 µg/mL) and the Hexane extract (IC_{50} value of 46.20 ±0.01 µg/mL). Table 1 also shows the ABTS radical scavenging activity of the extracts via TEAC values. The TEAC values varied from 7.47±0.03 to 51.14±0.05 µmol Trolox/g dry weight of the plant. Among the tested samples, the methanol extract was the strongest ABTS radical scavenger with TEAC value about 7.47±0.03 µmol Trolox/g of dry weight of the plant. Also, high ferric reducing antioxidant was observed for methanol extract 6.87±0.01 µmol Trolox /g. As shown in Table 2, the total phenolic contents varied from 56.41±0.02 to 188.08±0.01 mg GAE/g. The methanol extract of *D. Kotschyi* showed the highest total phenolic

content (188.08±0.01 GAE/g), followed by ethyl acetate extract of (95.58±0.01 mg GAE/g). Using the standard curve of quercetine, the flavonoid contents of the extracts were found ranging from 1037.00±0.01 to 30.00±0.03 mg quercetine equivalents/g of dry extract.

Table. 1. DPPH, ABTS and FRAP radical scavenging activity of *Dracocephalum Kotschyi* Boiss extracts

Samples	Extract	DPPH IC_{50} (µg/mL)	ABTS (µmoL Trolox/g)	FRAP (µmol Trolox /g)
***Dracocephalum Kotschyi* Boiss.**	Ethyl acetate	20.27±0.05	23.45±0.06	12.93±0.01
	Methanol	9.55 ±0.11	7.47±0.03	6.87±0.01
	Hexane	46.20±0.01	51.14±0.05	106.35±0.07
BHT	-	14.50±1.20	-	-

Table. 2. Total phenol and flavonoid content of *Dracocephalum Kotschyi Boiss* extracts.

Samples	Total phenol content (mg GAE/g)	Flavonoid content (mg QE/g)
Ethyl acetate	95.58±0.01	206.60±0.01
Methanol	188.08±0.01	1037.00±0.01
Hexane	56.41±0.02	30.00±0.03

DISCUSSION

In our study, the results showed the greatest total flavonoid content of methanol extract (1037.00±0.01 mg quercetine equivalents/g). The present investigation has provided useful information about antioxidant properties and flavonoid and phenolic contents of *D. Kotschyi* methanol extract exhibited considerable DPPH scavenging activity. A good correlation between ABTS, DPPH and FRAP values and total phenolic contents of the examined samples indicated that phenolic compounds could be responsible for ability of reducing oxidants in these specimens. In this study, it was confirmed that higher phenolic quantity contributes to lower IC_{50} amounts, which means higher antioxidant activity. The methanol extract was the most enriched with phenols (188.08±0.01 mg GAE/g of dry extract), followed by ethyl acetate (95.58±0.01 mg GAE/g of dry extract), which could explain the highest antioxidant activity of these extracts. The largest amount of flavonoids was found in the methanol extract (1037.00±0.01 mg QE/g of dry extract) and the lowest in the hexane extract (30.00±0.03 mg QE/g of dry extract). These results suggest that the high antioxidant activity of the methanol extract is probably due to some phenolic and flavonoid compounds. Thus phenolic compounds are main contributors to the antioxidant properties of *D. Kotschyi* aerial parts. The present investigation has provided useful information about antioxidant properties, and flavonoid and phenolic contents of *D. Kotschyi* different extracts. The three extracts that have been evaluated in the current study exhibited moderate to high antioxidant activity with IC_{50} values ranging between 9.55 ±0.11 and 46.20 ±0.01 µg/mL. All of the extracts exhibited considerable FRAP and ABTS scavenging activities. Strong correlations were observed between ABTS values and total flavonoid contents, ABTS and total phenolic contents as well as DPPH values and total flavonoid contents; however, a good correlation between DPPH values and total phenolic contents of the examined plants indicated that phenolic and flavonoid compounds could be responsible for ability of reducing oxidants in these extracts. There are several publications on biological property of polyphenolic compounds from *Dracocephalum* genus. The findings of our investigation are in agreement with previous phytochemical studies on some *Dracocephalum* species. Recently, Shi and coworkers evaluated the antihepatics and antioxidant activities of four *D. heterophyllum* extracts. The results

showed that the ethyl acetate juice of *D. heterophyllum* had the highest antihepatitis and antioxidant properties, followed by a petroleum ether juice [19]. Also, investigation into the biological properties of *D. palmatum* under *in vitro* conditions displayed that its extracts have a strong antioxidant activity due to the presence of high concentrations of phenolic components [20]. Aslanipour also investigated the phenolic ingredients and antioxidant activity of three different alcoholic extracts of *D. moldavica* L. The results of this study showed that the methanol extract had the highest phenolic and flavonoid content, anthocyanin, DPPH and H_2O_2 radical scavenging activity. The ethanol juice showed the lowest content of all. The methanol/ethanol extract demonstrated the highest amount in two oxides containing nitric and superoxide radical scavenging properties; it also displayed the highest ferric reducing ability power [21]. In other paper the antioxidant activity, total phenolic content of the ethanol, methanol and aqueous extract of *D. polychaetum* in flowering stage were evaluated. In this study, the highest antioxidant property was evaluated to the extract of methanol, ethanol and aqueous, respectively. Positive correlation has showed between the value of phenol and antioxidant activity which is in accordance with our research. The results show the different extracts of *D. polychaetum* have an antioxidant activity and could be considered as a potential origin of natural antioxidants for the therapy of some diseases [22]. Aprotosoaie et al. investigated the antigenotoxic and antioxidant capacities of the crude hydromethanolic extract from the aerial parts of *D. moldavica* L. The extract of this herb showed a high antioxidant potential mediated by direct free radical scavenging and metal-chelating effects. It showed that polar extracts of Iranian *D. moldavica* to be a more potent for antioxidant activity. The major polyphenol in the extract of this herb was rosmarinic acid, also recognized in plants of Russian and Asian origin [23]. Spinal-Z, a methanolic blend of dried seeds powder of *Peganum harmala* Linn and leaf of *D. kotschyii Boiss* is an Iranian medicine. It has been used for the therapy of several types of cancer for many years. In a research, the cytotoxic and antitumor properties of Spinal-Z and its constituents were investigated. *In vitro* cell proliferation inhibition test using MTT viability assay certified that both Spinal-Z and its ingredients have cytotoxic property against all cell lines tested [24]. In other research on

Dracocephalum genus seventeen secondary metabolites were extracted from *D. tanguticum* that also antioxidant properties of the isolated compounds were specified. This research showed that flavonoids with an OH group displayed important roles for their protective effects against DOX-induced cardiotoxicity and may act as a promising remedial agent for preventing the cardiotoxicity [25]. Also, in other report in 2013 the methanolic extract of *D. moldavica* hairy roots investigated for antioxidant activity and the strongest effects on reducing DPPH radical scavenging. The most active methanolic extract of hairy roots was determined by the highest level of rosmarinic acid and total content of phenolic compounds [26]. Ladanetin-6-O-β-(6"-O-acetyl) glucoside, pedalitin-3'-O-β-glucoside and luteolin-7-O-β-D-glucopyranoside, methyl rabdosiin, 12-methoxy-18-hydroxy-sugiol, 2α, 3α-dihydroxy-11α, 12α-epoxy-urs-28,13β-olide are the major compounds isolated from *Dracocephalum* genus. The compounds limonen-10-al, geranial, neral, β-sitosterol, oleanolic acid, ursolic acid, p-mentha-8-en-1,2- diol, colosolic acid, limonen-10-ol 10-O-β-D-glucopyranoside, limonen-10-ol 10-O-β-D-glucopyranosyl-(1→2)-b-D-glucopyranoside, calycopterin, luteolin-7-O-glucoside, apigenin-7-O-glucoside, luteolin 3′-O-β-d-glucuronide, luteolin, apigenin, cirsimaritin, isokaempferide, penduletin, xanthomicrol, calycopterin and the polyphenol rosmarinic acid are the natural products isolated from *D. Kotschyi* in previous reports [27-29]. All these compounds belong to terpenoids and flavonoids. So these two groups of natural products could be responsible for the biological activities of study *Dracocephalum* species in this work. Further phytochemical investigations and bioactivity estimations could clarify the biological role of phytoconstituents found in this species. In future researches, determination of the chemical compounds of secondary metabolites in *D. Kotschyi* should clarify and explain which of the bioactive compounds provides the highest contribution to antioxidant activity and the *in vivo* antioxidant activity should also be examined.

CONCLUSION

The present work has contributed in the direction of using *D. Kotschyi* not only as a flavoring agent but also to be used as a good phytomedicine to prevent the free radical harm and prevention of some chronic diseases in the body. In conclusion, the potent antioxidant activity of *D. Kotschyi* it

make possible use as a natural antioxidant in food industries and other pharmaceutical purposes.

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