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The effects of drought stress on the components of the essential oil of *Hyssopus officinalis* L. and determining the antioxidative properties of its water extracts

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ABSTRACT

The goals of this study are to investigate the effects of drought stress on the essential oil and water extract of *Hyssopus officinalis* L. which is a very important medicinal plant. Three irrigation conditions were studied including: **1**)80% field capacity, non-stress, **2**) 60% field capacity, mild drought stress and **3**) 40% field capacity, intense drought stress. Plants were kept in pots under controlled conditions.

Essential oils were extracted from the aerial parts of the plant by the hydrodistillation method and the analyses were performed using GC-FID and GC-MS. 31 compounds in 80% field capacity, 27 in 60% field capacity and 42 in 40% field capacity were identified. In all 3 irrigation levels, trans-pinocamphone (33.08-15.31), cis-pinocamphone (45.32-25.67) and β-pinene (11.44-6.09) were the most found compounds.

The antioxidant activities of the water extracts of this plant were studied using DPPH and *β*–Carotene/linoleic acid assays and total phenolic compounds were evaluated.

Results showed that the water extract of this plant in normal irrigation conditions has the most antioxidant activity (inhibition percentage = 36.38, IC_{50} = 0.083 µg/ml).It is worth mentioning that this plantshowed considerable antioxidant activity even in intense drought stress.

KEY WORDS: *Hyssopus officinalis*; essential oil; extract; antioxidant; drought stress

INTRODUCTION

Hyssopus officinalis L. belongs to the family Lamiaceae and is a roughage and perennial plant. The source of this plant is reported to be Asia and it grows in areas from the Caspian Sea to the Black sea and also in some parts of the Mediterranean region[1].Since the hyssop plant essential oil contains a variety of chemical compounds, it is one of the most important medicinal plants that is used in food, health and cosmetic industries all around the world. In traditional medicine, this plant is used to treat bronchitis and respiratory infections especially when production of mucus is high. It is also used as a sedative to treat asthma in kids and adults [2].

In veterinary, this plant is used to treat the gastrointestinal tract disorders of animals [3].

Antibacterial and antifungal activities of the essential oil of Hyssop have been shown by a number of researchers [4-7].

The tea that is made from its stem and leaves is effective in treating neurological disorders and toothache [8].

The extract obtained from its leaves has antimicrobial and antispasmodic properties and strong antiviral activity against the HIV virus [9].

In all the reports from Turkey (2010), Serbia (2000), Poland (2010) and Lublin (2013), cis-pinocamphone had been the main component within the range of 27.5-57.27% [10-13].

In a report from Spain (2011) about the effects of farming methods on the volatile components of *H.officinalis*L., the results revealed that all the samples, both irrigated and unirrigated, contained the two major terpenes: pinocamphone and iso-pinocamphone (35-40%) [14].

However, in a study conducted in 2011 in the city of Tabriz, western Iran, the most prominent compound of this plant was reported to be Myrtenyl acetate (74.08%) [15].

Also in a report (2012) about this plant from the city of Shiraz, southern Iran, thymol (18.95%), n-Decane (11.76%), Bisabolol (10.62%) and carvacrol (7.73%) were mentioned as the main compounds [16].

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About the antioxidant properties of the water extract of this plant by means of the maceration method, there are two reports in the literature that show the water extract possesses medium antioxidant activity [17-18].

Water is one of the most important environmental factors that has major effects on the growth of medicinal plants. Water deficit can impact the quantity of essential oils and their components [1].

In addition, antioxidant properties of the extracts of plants are influenced by the amount of available water at the stages of growth. Since Iran as a whole is classified as a dry region and faces lack of rain, thus we conducted this study to evaluate the effects of water deficit on the quantity of the obtainable essential oil of Hyssop and its components, and also on its antioxidant properties.

MATERIALAND METHODS

Plant material

The seeds of *H.officinalis* L. were cultured by Completely Randomized Design (CRD),in 3 replicates (pots) and 3 irrigation conditions including **1**) 80% field capacity (control), **2**) 60% field capacity (mild stress) and **3**) 40% field capacity (intense drought stress)in April 2014 in Qom Agricultural Research Station of Medicinal Plants.

Essential oilextraction

The hydrodistillation method with a Clevenger-type apparatus was used on air-dried plant materials and the procedure took 3 hours. Separated materials were kept in a firmly sealed vial in 4°C until they were analyzed [19].

Production of the extract

The act of extraction from the plant using the water solvent was performed by the reflux method. The obtained extracts were placed in a rotary evaporator that was set on 90°C in order to vaporize its solvent [20].

Gas Chromatography analysis

Analytical gas chromatography of the essential oil was performed using a Hewlett-Packard 5975B series gas chromatograph with Agilent HP-5 capillary column (30 m×0.25 mm, f.t 0.25 µm); the carrier gas was Heand split ratioof 1:10, and a flame ionization detector was used. Temperature of the column was adjusted at 50°C which was unchanging for 10 min and was programmed in order to rise up to 240°C at the rate of 4°C/min and stay unvarying at that temperature for 15 min. GC/MS analysis was carried out on a HP 5975B having a Hewlett–Packard 5973 quadrupole detector, on a HP-**5** (30 m×0.25 mm; f.t 0.25 µm) capillary column;

The MS operated at 70 eV ionization energy. Retention indexes were all calculated using the retention time of n-alkenes which were injected following the volatile oil at identical chromatographic conditions. Quantitative data were acquired from electronic integration of the FID peak areas. Oils components were categorized by comparing their mass spectra and kovats indexes with published books and the Wiley library, available data bases and credible websites[21].

Evaluation of antioxidant activity

DPPH radical assay

The ability of different water extracts to give electrons is tested by the amount that they bleach the violet solution of DPPH (2,2-Diphenyl-1-picrylhydrazyl) in methanol. Concisely, stock solutions (10 mg/ml each) of the extract and the synthetic standard antioxidant BHT were formulated in methanol. Several dilutions were made so to acquire certain concentrations that ranged from 0.8 to 5×10^{-4} mg/ml. The obtained diluted solutions (1 ml each) were mixed with 1 ml of a newly prepared 1mg/ml DPPH radical methanol solution; then after 30 min of keeping them at room temperature, the absorbance rate of the samples was read at the wavelength of 517 nm. The inhibition percentage of DPPH free radicals was obtained through the equation below:

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

In this formula A_{blank} is the absorbance value of the control reaction (containing all the reagents but the test compound) and A_{sample} is the absorbance value of the test compounds. After that IC_{50} was calculated using a graph. In this section BHT synthetic antioxidant was used as positive control and all the experiments were done in triplicate [22].

β-carotene /linoleic acid bleaching assay

In this assay, determination of antioxidant capacity is done by measuring the inhibition of organic volatile compounds and the conjugated dienehydro peroxides arising from linoleic acid oxidation [23].A mixture of *β*-carotene and linoleic acid was prepared by mixing0.5 mg of *β*-carotene with 1 ml of chloroform, 50 mg of linoleic acid and 200 mg of Tween40. Then chloroform was completely evaporated under vacuum conditions and after that, oxygenated distilled water (100 ml) was added to what was remaining; then, the mixture slowly turned into a light yellow emulsion. Incubation of the test tubes took place in a water bath using a negative control (blank) that contained the exact same volume of methanol as a substitute of the extracts at 50 °C which took2 h. An ultraviolet and visible (UV–Vis) spectrometer measured the absorbance values at 470 nm.

All determinations were performed in triplicate. Total antioxidant activity was calculated using the equation below:

$$I\% = (A_{\beta\text{-carotene after 2 h assay}} / A_{\text{initial } \beta\text{-carotene}}) \times 100$$

Where $A_{\beta\text{-carotene after 2 h assay}}$ is the absorbance value of β-carotene after 2 h assay remaining in the samples and $I_{\text{initial } \beta\text{-carotene}}$ is the absorbance value of β-carotene at the beginning of the experiments.

Assay for total phenolic

Total phenolic compounds of the water extracts of Hyssop were determined using the Folin-Ciocalteu method [24, 25].

A solution (0.1 ml) that contained1000 µg of the extract was pipetted into a 50 ml volumetric flask, then 46 ml of distilled water and 1 ml of Folin–Ciocalteu's phenol reagent were added to it, then the flask was shaken thoroughly. 3 min later, 3 ml of 2% Na_2CO_3 solution were added and the obtained solution was unchanging for 2 h with intermittent shaking. Total polyphenol content was determined by a spectrophotometer at 760 nm.

For all the standard Gallic acid solutions (0–1000 µg /1 ml) the same procedure was repeated and so a standard curve was obtained from the equation below:

$$\text{Absorbance} = 0.0012 \times \text{Gallic acid } (\mu g) + 0.0033$$

Total phenolic compounds existing in the extracts were determined based on the Gallic acid equivalents using the Gallic acid equation.

RESULTS AND DISCUSSION

Essential oils composition

Medicinal plants react differently to drought stress in terms of performance and production of effective materials. Regarding the importance of medicinal plants especially in the pharmaceutical industry and their rarity in nature, studying the various agricultural aspects of these plants is of great importance. Hyssopis a medicinal plant that due to having antibacterial, anti-cancer and antiviral properties is quite important [26].

In order to study the effects of drought stress, the pot experiment was performed in the form of randomized complete block design (RCBD) in three irrigation levels. The irrigation conditions were:**1**) 80% field capacity (non-stress), **2**) 60% field capacity (mild drought stress) and **3**) 40% field capacity (intense drought stress).

According to table 1, 31 compounds were identified in the essential oil of the plant in the conditions without drought stress, representing 100% of the oil constituents.

The main components of the essential oilswere trans-pinocamphone, cis-pinocamphone and β-pinene, which shows that the essential oils obtained from the central regions of Iran (Qom) are not similar to the ones of southern and western regions of the country, but rather similar tothe components of the essential oil reported in Serbia [11,15,16].

In the irrigation condition of 60% field capacity, 27 compounds were extracted from the essential oil of Hyssop which are equal to 96.97% of total compounds and also in the irrigation conditions of 40% field capacity, 42 compounds were identified that are equal to 99.27% of total essential oil of the sample.

18of 31 compounds of the composition of the essential oil obtained in normal irrigation conditions, were found with different values in conditions of 60% and 40% field capacities.

Table 1.Composition of the essential oil of *Hyssopus officinalis* L. in 3 irrigation conditions

Compound	A%	B%	C%	RI	Compound	A%	B%	C%	RI
Heptane, 3-methyl	0.28	0.16	-	771	Myrtenol	1.66	3.76	1.68	1208
Octane	1.55	1.16	2.11	803	Bicycloelemene	0.17	-	-	1345
α-Thujene	0.41	-	0.17	932	1,5,5-Trimethyl-6-methylene-cyclohexene	-	-	0.32	1346
α-Phellandrene	-	0.19	-	932	β-Bourbonene	0.66	0.53	0.98	1394
α-Pinene	0.63	0.26	0.45	940	β-Elemene	-	-	0.22	1401
Camphene	0.14	-	0.09	956	Tetradecane	-	-	0.08	1405
Sabinen	2.04	1.13	-	980	α-Gurjunene	-	-	0.45	1419
β-Pinene	10.24	6.09	11.44	985	β-Caryophyllene	0.56	1.45	2.16	1429
β-Myrcene	1.27	0.89	1.06	997	β-Cubebene	1.32	-	0.22	1439
Decane	0.29	0.44	0.57	1004	Humulene	-	0.18	0.37	1463
Terpinolene	-	0.23	-	1023	Alloaromadendren	0.25	0.82	0.81	1470
α-Terpinene	0.30	-	-	1024	D-Germacrene	-	2.78	2.52	1491
Benzene, 4-ethyl-1,2-dimethyl-	-	0.15	-	1032	Bicyclogermacrene	2.09	3.52	3.32	1506
β-Phellandrene	1.82	1.09	1.50	1035	γ-Cadinene	-	-	0.1	1525
D-Limonene	-	-	0.15	1043	Elemol	3.87	8.88	8.82	1561
β-trans-Ocimene	0.45	0.17	0.39	1054	δ-Cadinene	0.36	-	0.29	1533
γ-Terpinen	0.60	0.67	0.09	1066	Spathulenol	0.40	-	0.4	1590
trans-Sabinene hydrate	0.47	2.44	0.13	1077	γ-Gurjunene	-	-	0.18	1598
Isoterpinolene	-	0.16	-	1096	Selina-3,7(11)-diene	-	-	0.14	1617
Linalool	0.46	0.59	0.78	1109	γ-Eudesmol	0.35	-	0.38	1646
β-Thujone	-	-	0.13	1127	β-Eudesmol	-	-	0.25	1665
1(7),3,8-o-Menthatriene	3.16	-	-	1167	α-Eudesmol	0.49	-	0.36	1667
1(7),5,8-o-Menthatriene	-	-	2.70	1169	Mintsulfide	-	-	0.14	1750
trans-Pinocamphone	18-00	33.08	15.31	1171	Neophytadiene	-	-	0.59	1845
cis-Pinocamphone	45.32	25.67	36.79	1186	Hexadecanoic acid	-	-	0.17	1980
α-Terpinol	0.40	0.48	0.44	1202	Total	100	96.97	99.27	-

A: Composition of essential oil in non-stress condition; B: Composition of essential oil in mild drought stress condition;
C: Composition of essential oil in intense drought stress condition;
RI: Relative retention indices to C8–C24 n-alkanes HP-5 MS column

The comparison of the main compounds of the essential oils of this plant under different test conditions showed that implementation of the 2 conditions of intense drought stress and no stress had the same effect on the main compounds, whereas the conditions of mild stress caused a very significant difference in the percentage of these compounds.

For instance, in the conditions of non-stress, the main compounds were cis-pinocamphone (45.32%)andβ-pinene (10.24%) but these compounds in the conditions of mild stress were respectively diminished to 19.65 and 4.15%.Trans-pinocamphone was another prominent compound that its values were significantly increased in the conditions of mild drought stress(Table 1).Out of 34 dissimilar compounds identified in these three different water-deficit conditions, 19 were only produced under the effects of intense drought stress, from which we can point out to germacrene D. This compound is a sesquiterpene identified in volatile oils and possesses antibacterial properties and is an insect repeller [27].

Determination of antioxidant activity

In order to study the antioxidant potency of the water extract of Hyssop, DPPH free radical elimination method and β-carotene bleaching test were used. Also, total phenolic compounds of these extracts were estimated.

Determination of free radical-scavenging activity

Results obtained from table 2 show that IC_{50} values of the water extracts of the 3 conditions of non- stress, mild stress and intense stress, are highly lower than BHT as positive control; and this reveals that the antioxidant potency of the water extracts of this plant is very high regardless of irrigation conditions. These results are different from those obtained from Turkey and Romania, as antioxidant properties of the water extracts of this plant reported from those countries are not that favorable[17,18].

Also, by increasing the drought stress, the antioxidant potency in the water extract of this plant diminishes. It is worth mentioning that even the highest observed IC_{50} values of the water extracts, show considerable antioxidant activity in Hyssop that can introduce this plant as a natural source for antioxidants to food and pharmaceutical industries.

Determination of β-carotene /linoleic acid bleaching

Unsaturated fatty acids such as linoleic acid are highly sensitive toward the oxidation process; therefore, inhibition of oxidation of this substance is being used as a valuable method in determination of antioxidant activity. In this test, as it is shown in table 2, the sample of the water extract of this plant from the conditions without drought stress, had the most inhibition percentage (36.38%) and the sample from intense stress had the least inhibition percentage (31.04%). So extracts of this plant show less antioxidant potency by increasing the drought stress; and this trend was also observed in the results obtained from the DPPH free radical-scavenging method.

Table 2. Antioxidant activity of extracts of *Hyssopus officinalis* L. in 3 irrigation conditions

Sample	DPPH IC50 (µg/ml)	β-Carotene/linoleic acid Inhibition (%)
Water extract in non-stress condition	0.08± 0.02	36.38± 0.74
Water extract in mild drought condition	0.12± 0.05	32.38± 0.70
Water extract in intense drought condition	0.15± 0.04	31.04± 0.52
BHT	19.72 ± 0.82	88.34 ± 0.93
Negative control	NA	5.50 ± 0.46

Determination of total phenolic content

Results of the studies performed before, show that high levels of phenolic compounds can be the reason for high antioxidant activity of the extracts. Based on available evidence, there is a positive relationship between the amount of phenolic compounds and antioxidant potency of plants [28].

In this experiment, the obtained results from studying the phenolic compounds of the water extracts of 3 conditions of non-stress, mild stress and intense stress, respectively were 87.5, 73.3and 65.8 µg Gallic acid equivalent. Comparison of these results with the ones obtained from Turkey and Romania shows that the amount of phenolic compounds of the water extract of this plant in the samples of this study is much more than what it had been in those countries [17, 18].

CONCLUSION

The results of this study reveal that with respect to the effects of water deficit on essential oil compounds of medicinal plants, it is possible to create certain conditions in which the effective materials are at their highest and at the same time, water is used thrifty; this matter is crucial for regions that struggle with lack of water.

Since water extracts are very important in traditional medicine and the way we use medicinal plants (mostly brewed and boiled), in this study, the antioxidant potency of the water extracts was evaluated in different stresses by two methods of DPPH and β–Carotene bleaching test and, the results showed that decrease in antioxidant potency is proportionate to increase in drought stress. This trend was also observed in the total phenol experiment. As a whole, the most significant characteristic observed in this plant is that its antioxidant potency is very high despite being grown in drought-stress conditions.

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