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Effects of drought stress on the components of the essential oil of evening primrose (*Oenothera macrocarpa)* and determination of the biological activities of its extracts

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In this study, the essential oil of *Oenothera macrocarpa* belonging to *Onagraceae* family, subjected to drought stress, was studied. First, the plants were grown in three irrigation conditions, including 80% field capacity (control), 60% field capacity (mild stress) and 40% field capacity (intense drought stress). Then, the antioxidant properties of aqueous and ethanolic extracts were evaluated *via* DPPH radical scavenging and beta-carotene bleaching assays. Total phenolic content of the extracts was assessed using the Folin-Ciocalteu reagent.

Results demonstrated that the main components of the plant's essential oil were alkanes and terpene compounds. The antioxidant potency of the ethanolic extract increased by increasing the drought stress.

Keywords: *Oenothera macrocarpa*, Essential oil, Antioxidant, Total phenol

INTRODUCTION

Plants can be considered as the foundation of traditional medicine, the base of phytochemistry and pharmacology; they are the source of unique flavors in the fostod industries and are the exclusive factor of perfumes in the healthcare industries.

By having rapid advancements in science on one hand and economic issues on the other hand, application of medicinal plants has gradually been decreased and industrial drugs have replaced them in the majority of cases. History of treating some diseases with medicinal plants dates back to early humans. By gaining experience and creating science, humans have learned to treat themselves with medicinal plants [1-3].

The extracts of evening primrose are rich in gamma-linoleic and gamma-linolenic acids and are antihypertensive, anti-inflammatory, antispasmodic, antitussive and thrombolytic, having been widely used for treating everything from hang nails to terminal cancer [5].

To the best of our knowledge, all studies on *Oenothera* species have only been performed on their seeds [5-7].

In Italy, Lotti *et al*. (1984), showed that the level of gamma-linoleic acid in the seeds of *Oenothera biennis* grown in the spring seeding, is lower than in those grown in autumn [8]. Levy *et al.* (2002) illustrated that precocious samples of *Oenothera lamarckiana* can be planted successfully in warm zones [9]. Effects of different nitrogen doses demonstrated that the optimum nitrogen doses and row spacing applications for the highest yield and quality were 120 kg ha^{-1} and 40 cm, respectively. However, increasing nitrogen doses negatively affected the gamma-linolenic acid content of evening primrose oil [10].

Mardani *et al*. (2012), reported that the acetone extract of evening primrose flowers (*Oenothera biennis)* has higher level of phenolic and flavonoid compounds and better extraction efficiency in comparison with ethanolic and methanolic extracts. Moreover, the extracts also showed good antibacterial activity against all tested microorganisms [11].

Evening primrose (*Oenothera biennis*) oil has a high GLA content that promotes healthy skin and skin repair. It is usually yellow in color and soothes skin problems and inflammation, making it a good choice for people with eczema, psoriasis, or any type of dermatitis. Evening primrose skin oil helps dry skin conditions and avoids premature aging of the skin [12].

According to our research, two scientific papers had been published on the seeds of *O. macrocarpa*; one is the analysis of the triglycerides of the seeds of *O.macrocarpa* published in 1965 [13] and the other is about the hydrocarbon compounds of its seed reported in 1975 [14].

Here we report the effects of drought stress on the composition of the essential oil of *O. macrocarpa* and investigate the biological activities of its extracts.

MATERIAL AND METHODS

Plant materials

The seeds of *O. macrocarpa* were cultured using the method of Completely Randomized Design (CRD), in 3 replicates (pots) and 3 irrigation conditions including: 80% field capacity (control),

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60% field capacity (mild stress) and 40% field capacity (intense drought stress) in Qom's Agricultural Research Station of Medicinal Plants.

Essential oil extraction

To extract essential oils, distillations were performed using the Clevenger apparatus. The duration of the extraction procedure was 3 hours, after which samples were collected and sodium sulfate was added to them to absorb their probable water content. The samples were kept in a refrigerator.

Gas chromatographic analysis

Analytical gas chromatography of the essential oils was carried out using a Hewlett-Packard 5975B series gas chromatograph with Agilent HP-5 capillary column (30 m×0.25 mm, f.t. 0.25 µm); carrier gas, He; split ratio, 1:10, using a flame ionization detector. The column temperature was adjusted at 50°C which was unchanged for 10 min. It was programmed to rise up to 240°C at the rate of 4°C/min and then stay constant at that temperature for 15 min. GC/MS was performed on an HP 5975B instrument with a Hewlett–Packard 5973 quadruple detector, on a capillary column HP-**5** (30 m×0.25 mm; f.t 0.25 µm). The MS was operated at 70 eV ionization energy. Retention indices were calculated using the retention times of n-alkanes that were injected after the essential oil at the same chromatographic conditions. Quantitative data were obtained from the electronic integration of the FID peak areas. Acquisition mass range was 40-400 m/z.

The components of the oils were identified by comparing their mass spectra and kovats indexes with Wiley library and published books, data bases available and credible websites [15].

Preparation of extracts

Powdered dehydrated flowering branches and leaves of the plant were used for extraction. First, the reflux system was assembled. Then, the cartouche was twice filled with plant samples, put in place and then the extraction took place using two solvents separately: ethanol and water. Finally, ethanol and water extracts that had a blackish green color and contained a lot of solvent were obtained. These solutions were concentrated using a rotary evaporator.

Evaluation of antioxidant activity

DPPH radical assay. Radical-scavenging activities of the plant extracts were determined using a published DPPH radical scavenging activity assaying method with minor modifications [16-17]. Briefly, stock solutions (10 mg/ml each) of the extracts and the synthetic standard antioxidant BHT were prepared in methanol. Dilutions were made to obtain concentrations ranging from 0.8 to 5×10^{-4} mg/ml. Dilute solutions (1 ml each) were mixed with 1 ml of a freshly prepared 1 mg/ml DPPH radical methanol solution and were kept in the dark at room temperature, for 30 min, in order to let the desired reactions to take place. Absorbance values of these solutions were recorded on an UV–Vis spectrometer at 517 nm using a blank containing the same concentration of the extract or BHT without DPPH radicals. Inhibition of DPPH radical in percentage (I %) was calculated as follows:

$$I\% = [(A_{blank} - A_{sample}) / A_{blank}]\times100$$

where A_{blank} is the absorbance value of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance value of the test compound. The sample's concentration is expressed in terms of IC_{50}, which was calculated by drawing the chart of inhibitory percentages against concentrations of the sample. All the tests were carried out in triplicate and IC_{50} values were reported as means ± SD.

β-carotene /linoleic acid bleaching assay. In the β-carotene/linoleic acid test, the antioxidant competes with β-carotene for transferring hydrogen atoms to the proxy radicals (R1R2HCOO.) formed from the oxidation of linoleic acid in the presence of molecular oxygen (O_2) and converts them to hydroperoxides (R1R2HCOOH), leaving the β-carotene molecules intact [18]. Assaying the remaining β-carotene gives an estimation of the antioxidant potential of the sample. A mixture of β-carotene and linoleic acid was prepared by adding 0.5 mg of β-carotene to 1 ml of chloroform (HPLC grade), 50 mg of linoleic acid and 200 mg of Tween 40. The chloroform was then completely evaporated under vacuum and 100 ml of oxygenated distilled water were subsequently added to the residue and mixed gently to form a clear yellowish emulsion. The extract and BHT (positive control) were individually dissolved in methanol (2 g/l) and 350 µl of each of them were added to 2.5 ml of the above-mentioned emulsion in the test tubes and were mixed thoroughly. The test tubes were incubated in a water bath at 50 °C for 2 h together with a negative control (blank) that contained the same volume of methanol instead of the extracts. The absorbance values were measured at 470 nm on an UV–Vis spectrometer. Antioxidant activities (inhibition percentages, I %) of the

samples were calculated using the following equation:

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

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 $A_{\text{initial } \beta\text{-carotene}}$ is the absorbance value of β-carotene at the beginning of the experiments. All tests were carried out in triplicate and inhibition percentages were reported as means ± SD of triplicates.

Total phenol assay. Total phenolic constituents of the extracts of *O. macrocarpa* were determined by literature methods involving the Folin–Ciocalteu phenol reagent and the gallic acid standard [19]. A solution of the extract (0.1 ml) containing 1000 μg of the extract was pipetted into a 50 ml volumetric flask, then 46 ml of distilled water and 1 ml of Folin–Ciocalteu phenol reagent were added to it, and the flask was shaken thoroughly. After 3 min, 3 ml of 2% Na_2CO_3 solution were added and the mixture was allowed to stay for 2 h with intermittent shaking. Absorbance values were measured at 760 nm. The same procedure was performed for all standard gallic acid solutions (0–1000 μg /1 ml) and a standard curve was obtained from the following equation:

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

Total phenols of the extracts, as gallic acid equivalents, were determined using the absorbance values of the extracts measured at 760 nm as the input for the standard curve and the equation. The tests were carried out in triplicate and the gallic acid equivalent values were reported as means ± SD of triplicate.

RESULTS AND DISCUSSION

Chemical composition of the essential oils

The essential oils of the aerial parts of *O. macrocarpa* subjected to three irrigation regimes (80%, 60% and 40% field capacity) were extracted by means of water distillation. Quantitative and qualitative analyses of the plant were performed using the GC-MS method. In the essential oil obtained from the first regime, 23 compounds were recognized, followed by 17 compounds in the second regime and 8 compounds in the third one (Table 1). One interesting point here is that two important compounds in terms of biological activities, thymol and oleic acid, were only observed in the conditions of full irrigation and no drought stress.

ANTIOXIDANT EFFECTS

General results of DPPH experiment on the extracts of O. macrocarpa

In order to investigate the antioxidant activities of the derived extracts, the DPPH method of controlling free radicals and the beta-carotene test were used. The rate of reduction in the absorption of free radicals was measured using a spectrophotometer and the antioxidant activities of aqueous and ethanolic extracts were calculated in different concentrations; also, the IC_{50} level was determined. Among the tested samples that were obtained from the aerial parts of *O. macrocarpa*, the ethanolic extract of the third regime (40% field capacity) and the aqueous extract of the first regime (80% field capacity) had the highest antioxidant activities. Moreover, the beta-carotene test showed that the ethanolic extract of the third regime (40% field capacity) and the aqueous extract of the second regime (60% field capacity) had the highest levels of antioxidant activity.

Determination of total phenolic content

In order to evaluate the phenolic content in the studied extracts, the Folin-Ciocalteu reagent was used with gallic acid as the standard. The aqueous extract of the first regime (80% field capacity) showed the highest level of phenolic compounds, equivalent to 28 μg/mg concentration of gallic acid. The ethanolic extract of the third regime (40% field capacity) showed the highest level of phenolic compounds equivalent to 49 μg/mg concentration of gallic acid (Table 2).

CONCLUSION

Summarizing, from the compounds recognized in the plant's essential oils it can be concluded that most of the compounds are saturated compounds of alkanes and monoterpenes, with a phenolic cycle. By comparing three agricultural capacities, it can be inferred that in 40% field capacity, due to less irrigation, terpene compounds cannot be produced and they were not observed in this stress. Another important point is that the antioxidant potency of ethanolic extract of this plant was increased by increasing the drought stress.

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Table 1. Composition of the essential oils of *Oenothera macrocarpa* in 3 irrigation conditions.

No.	Components	A%	B%	C%	RI
1	n-Octane	1.70	20.62	9.61	800
2	Nonane, 5-methyl	-	0.70	-	959
3	Nonane, 3-methyl	-	1.38	-	971
4	1-Hexyl-3-methylcyclopentane	-	1.15	-	991
5	Decane	3.33	36.90	3.36	1004
6	Undecane	-	-	5.63	1108
7	Dodecane	9.06	9.59	-	1206
8	Citral	-	1.29	-	1279
9	2-Undecanone	1.37	0.53	-	1302
10	Thymol	6.81	-	-	1310
11	Guaiene	0.73	-	-	1353
12	Tetradecane	4.43	2.45	19.91	1406
13	n-Decanoic acid	-	-	14.15	1421
14	Caryophyllene	0.87	-	-	1430
15	Nerol acetate	2.75	-	-	1595
16	Phenol,2,5-bis(1-methyl ethyl)	1.08	-	-	1589
17	Hexadecane	1.52	0.64	-	1605
18	2-Ethylhexyl 2-ethylhexanoate	3.03	1.45	13.84	1612
19	2,2-Dimethyl-7-ethoxy-6-methoxy-4-chromanone	11.63	-	-	1734
20	trans-Nuciferol	1.32	-	-	1743
21	Myristic acid	1.59	1.81	-	1789
22	Octadecane	0.41	-	-	1804
23	Phthalic acid	-	0.48	-	1972
24	Dibutyl phthalate	0.89	-	-	1974
25	n-Hexadecanoic acid	6.84	8.91	-	1996
26	Phytol	0.57	-	-	2038
27	9,12,15-Octa decatrien-1-ol	-	2.83	-	2051
28	Oleic acid	15.68	-	-	2052
29	Tricosane	8.84	6.03	18.45	2090
30	Tetracosane	0.55	-	-	2401
31	Pentacosane	6.12	3.23	-	2501
32	Triacontane	-	-	14.99	2970

Category	A%	B%	C%
Oxygenated monoterpenoids	9.56	1.29	-
Non terpenoid oxygenated hydrocarbons	43.45	16.01	27.99
Non terpenoid hydrocarbons	35.96	82.69	71.95
Sesquiterpenoid hydrocarbons	1.60	-	-
Oxygenated diterpene	0.57	-	-

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

Table 2. Antioxidant activity of the extracts of *Oenothera macrocarpa* in 3 irrigation conditions

Sample	DPPH (IC_{50}µg/ml)	β-Carotene/linoleic acid Inhibition (%)	Folin-Ciocalteu
Aqueous extract FC 40%	40.0 ±.0.9	4.8 ± 0.4	28.0 ± 1.1
Aqueous extract FC 60%	43.0 ± 1.1	15.5 ± 0.6	16.5 ± 0.8
Aqueous extract FC 80%	39.0 ± .0.7	4.6 ± 0.5	28.3 ± 0.9
Ethanolic extract FC 40%	6.1 ± 0.5	49.7 ± 1.1	49.1 ± 1.2
Ethanolic extract FC 60%	14.0 ± 0.3	34.4 ±0.9	41.3 ± 0.7
Ethanolic extract FC 80%	17.0 ± 0.6	44.8 ± 0.8	19.1 ± 0.8
BHT	19.5 ± 0.6	94.2 ± 1.2	-

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ЕФЕКТ НА СТРЕСА ОТ СУША СПРЯМО КОМПОНЕНТИТЕ НА ЕТЕРИЧНО МАСЛО ОТ ВЕЧЕРНА ИГЛИКА (*Oenothera macrocarpa)* И ОПРЕДЕЛЯНЕ НА БИЛОГИЧНАТА АКТИВНОСТ НА ЕКСТРАКТИ ОТ НЕЯ

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(Резюме)

В тази работа е изследвано етеричното масло от *Oenothera macrocarpa*, принадлежаща към семейство *Onagraceae*, подложена на стрес от суша. Най-напред растението е отглеждано при три условия на напояване, включващи 80% полеви капацитет (контрола), 60% (умерен стрес) and 40% (интензивен стрес). След това са оценени анти-оксидантниет свойства на спиртните екстракти от маслата с помощта на DPPH-метода за отстраняване на радикали и β-каротенови тестове. Общото съдържание на феноли в екстрактите е оценено с реагента на Folin-Ciocalteu.

Резултатите показват, че главните компоненти на етеричното масло са алкани и терпени, като анти-оксидантната активност на спиртните екстракти нараства с нарастването на стреса от суша.