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Trachystemon orientalis (L.) G. Don as a valuable source of rosmarinic acid: biological activities and HPLC profiles

Short title: *Trachystemon orientalis*: Source of rosmarinic acid

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ABSTRACT
Objectives: *Trachystemon orientalis* (L.) G. Don, colloquially known in Turkey as Kaldırık, is an edible plant belonging to the Boraginaceae. This plant has been practiced in traditional medicine for many years for its various therapeutic benefits. The effectiveness and chemical composition of the plants can vary depending on their parts, age, and extraction solvent. Therefore, the current study aimed to define the biological activities of various parts and extracts of *T. orientalis*, which were collected in distinct seasons as young and mature, and investigate the main component responsible for these biological effects.
Material and Methods: The plant materials were gathered in the different seasons from the northwest of Turkey. ABTS and DPPH free radical scavenging activities were investigated to assess antiradical and antioxidant potential of the extracts. Anti-inflammatory activity of the extracts was also analyzed. The Folin-Ciocalteu test was conducted to determine the total phenolic content. RP-HPLC-PDA analysis was performed.
Results: Both methanol and aqueous extracts exhibited significant radical scavenging and anti-inflammatory activities in comparison with the control ($p<0.05$). The highest percentage of inhibition on ABTS and DPPH free radicals was obtained in aqueous extracts of mature herbs and roots, respectively. Methanol extracts of mature roots and herbs exhibited the strongest anti-inflammatory capacity. Rosmarinic acid possessed much higher antioxidant and anti-inflammatory effect than the reference compounds used in each assay in our study. The high rosmarinic acid content of the extracts suggests that the compound responsible for the great biological activity potential is rosmarinic acid.
Conclusion: The presence of rosmarinic acid in herbs and roots of *T. orientalis* was shown for the first time in our present study to our knowledge. The phytochemical composition and

effective biological activities of *T. orientalis* explain its traditional use and indicate its significant potential in pharmaceutical industry applications.
Keywords: antioxidant activity, anti-inflammatory activity, rosmarinic acid, RP-HPLC-PDA, *Trachystemon orientalis*

ÖZ
Amaç: Türkiye'de Kaldırık olarak bilinen *Trachystemon orientalis* (L.) G. Don Boraginaceae familyasına ait yenilebilir bir bitkidir. Bu bitki, çeşitli tedavi edici etkileri nedeniyle uzun yıllardır geleneksel tıpta kullanılmaktadır. Bitki ekstrelerinin etkinliği ve kimyasal bileşimi, bitkinin kısımlarına, yaşına ve ekstraksiyon çözücüsüne bağlı olarak değişmektedir. Bu nedenle çalışmamızda, genç ve olgun olarak farklı dönemlerde toplanan *T. orientalis*'in farklı kısım ve farklı ekstrelerinin biyolojik aktiviteleri ve biyolojik aktiviteye neden olacak ana bileşenlerinin belirlenmesi amaçlanmıştır.
Gereç ve Yöntemler: Bitki materyalleri Türkiye'nin kuzey batısından farklı mevsimlerde toplandı. Ekstrelerin antiradikal ve antioksidan kapasitesini değerlendirmek için ABTS ve DPPH serbest radikal temizleme aktiviteleri araştırıldı. Ekstrelerin anti-inflamatuvar aktivitesi de belirlendi. Toplam fenolik içeriği belirlemek için Folin-Ciocalteu yöntemi kullanıldı. RP-HPLC-PDA analizi yapıldı.
Bulgular: Hem metanol hem de sulu ekstreler, kontrol ile karşılaştırıldığında önemli radikal süpürücü ve anti-inflamatuvar aktiviteler sergiledi ($p<0.05$). ABTS ve DPPH serbest radikalleri üzerindeki en yüksek inhibisyon yüzdesi, sırasıyla olgun bitkilerin ve köklerin sulu ekstrelerinde elde edildi. Olgun köklerin ve bitkilerin metanol özleri, en güçlü anti-inflamatuvar kapasiteyi sergiledi. Rosmarinik asit, çalışmamızda her deney için kullanılan referans bileşiklerden çok daha yüksek antioksidan ve anti-inflamatuvar etki gösterdi. Ekstrelerin yüksek rosmarinik asit içeriği, güçlü biyolojik aktivite potansiyelinden sorumlu bileşiğin rosmarinik asit olduğunu düşündürmektedir.
Sonuç: *T. orientalis*'in bitki ve kök kısımlarında rosmarinik asit varlığı bilgilerimize göre ilk kez bu çalışmamızda gösterilmiştir. *T. orientalis*'in fitokimyasal bileşimi ve etkili biyolojik aktiviteleri, geleneksel kullanımını açıklamakta ve farmasötik endüstrisi uygulamalarında önemli bir potansiyeli olduğuna işaret etmektedir.
Anahtar kelimeler: antioksidan aktivite, ani-inflamatuvar akivite, rosmarinik asit, RP-HPLC-PDA, *Trachystemon orientalis.*

Introduction

Trachystemon orientalis (L.) G. Don is a plant belonging to the Boraginaceae family, that is represented by 34 genera and 325 species in Turkey[1]. This plant is distributed in nearly all Black Sea regions in Turkey, East Bulgaria, and West Caucasia. *T. orientalis* is called different names in Turkey, such as Kaldirik, Tomar, Burğı, Hodan, and İspit. It is an edible and medicinal plant. The leafy and budding plant body is consumed as vegetables and roots and petioles are as pickles in several parts of the Black Sea region[2]. It is used as an antipyretic, diaphoretic, diuretic, laxative, anticancer, analgesic, antiflatulent, antirheumatismal plant in medicinal treatments[3-5]. Chronic and cumulative oxidative damage

leads to various pathologic processes such as inflammation, by stimulating the genes incorporated in the inflammatory phases. The occurrence and progression of inflammation-associated conditions like arthritis, atherosclerosis, Alzheimer's disease, autoimmune diseases, ocular diseases, diabetes, and cancer may be triggered by enhancing the number of free radicals. There has been a considerable deal of interest in the role of free radicals in chronic diseases and the protective effect of antioxidants as scavengers of free radicals[6-9]. Inflammatory diseases are commonly treated with steroidal and non-steroidal anti-inflammatory drugs. Besides the beneficial therapeutic effects, they also have crucial side-effects. Much research has been conducted to determine new, safe, pharmacologically active plants and plant-derived compounds with lower side effects[10,11]. Consequently, the target of the current study was to analyse and determine the antioxidant potentials and anti-inflammatory activities of young and mature *T. orientalis* aqueous and methanol extracts from herbs and roots. Our study is the first report showing the antioxidant and anti-inflammatory potentials of herbs and roots of both young and mature *T. orientalis.* Furthermore, to the best of our knowledge, this study identified rosmarinic acid as the major and possibly the most active component of the herbs and roots of this plant for the first time.

Materials and methods

Chemicals

2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), acetonitrile, acetylsalicylic acid (ASA), gallic acid, propyl gallate, rosmarinic acid, trifluoroacetic acid and trolox were bought from Sigma-Aldrich (USA). Sodium carbonate, sodium chloride, potassium persulphate, sodium chloride, dimethyl sulfoxide (DMSO), methanol, ethanol and the other solvents were bought from Merck (Germany) and Sigma-Aldrich (USA).

Plant materials

The plant materials were collected in the different seasons from Düzce, Akçakoca, Tahirli Köyü in Turkey and identified by B. Bıyık. Plant materials were first dried at room temp. Afterwards, the laboratory-scale mill. was used to powder these materials. A voucher specimen was housed in the Herbarium of Ankara University Faculty of Pharmacy (Turkey). Collection site, date and herbarium number of plant samples were noted (*T. orientalis* (L.) G. Don: A3 Düzce, Akçakoca, Tahirli Köyü, 13/02/2016- 25/04/2016, AEF 26813).

Preparation of extracts

Preparation of methanol extract: 5 g of plant materials were powdered for each part. Then, the materials were macerated with 50 ml methanol three times for 8h at 60°C. Afterwards they were filtered and combined. A reduced pressure was applied at 40°C to the mixtures to concentrate them (0.73 g, 14.60% *w/w* young root yield, and 0.42 g, 8.4% *w/w* young herb yield, and 0.66 g, 13.40% *w/w* mature root yield, and 0.31 g, 6.40% *w/w* mature herb yield).
Preparation of aqueous extract: 5 g of the powdered plant material was added to 50 ml of distilled water and boiled for 30 min. The extracts were filtered and afterwards lyophilized (1.15 g, 23.06% *w/w* young herb yield, and 0.73 g, 14.71% *w/w* young root yield, and 1.18 g, 23.70% *w/w* mature herb yield, and 0.92 g, 18.50% *w/w* mature root yield).

Antioxidant activity

The antioxidant activity of the extracts was determined by investigating their scavenging abilities on ABTS and DPPH free radicals.

ABTS free radical scavenging activity

Antioxidant activity was investigated by measuring the scavenging capacity of the samples against ABTS free radicals[12]. Firstly, 7mM aqueous solution of $ABTS^{+}$ reacted with 2.45 mM potassium persulfate and then radical cation solution of $ABTS^{\bullet+}$ was produced. This radical solution was maintained at room temp overnight in the dark. Dilution is applied to this dark radical solution with ethanol until obtaining an absorbance of 0.700±0.05 at 734 nm. Finally,

a working solution was arranged (pH=7.4). The ABTS solution was prepared freshly in each study to prevent degradation. The test sample was mixed with the radical cation solution (100x). Then, the mixtures were held for 6 min at room temp. The reference compound was Trolox in this assay. At the final step of the experiment, the absorbance of the samples was measured at room temp at 734 nm. The experiments were performed at least thrice. The percentage of inhibition was computed for each sample for each concentration. Then the half maximal inhibitory concentration (IC_{50}) values were computed using this values. The final results were presented as IC_{50} ± standard deviation (SD).

DPPH free radical scavenging activity

Both qualitative and quantitative methods were used to determine this activity.

Qualitative DPPH radical scavenging activity

The antioxidant capacity of the samples was assessed using qualitative DPPH radical scavenging assay[13]. 0.1 mM DPPH methanol solution (1 mL) was put to the test tube, then the extracts were inserted in different concentrations and vortexed. The mix was stand at room temp for 30 min. When this DPPH solution is added to a solution containing an antioxidant substance, this dark purple loses color with time.

Quantitative DPPH radical scavenging activity

The DPPH free radical scavenging capacity of the samples was examined to determine their antioxidant activity[14]. DPPH stock solution was prepared firstly. The stock solution of each test compound in methanol was prepared (0.5 mg/ml) and then the serial dilutions in equal amounts of methanol were prepared in wells. DPPH solution was added to each well to initiate the reaction. Then, this mixtures were kept in dark for 30 min. Propyl gallate was served as the reference compound. Absorbance was measured at room temperature at 517 nm. The experiments were performed at least thrice. The percentage of inhibition was computed for each concentration of each sample. Then the IC_{50} values were computed using this values. The final results were presented as IC_{50} ± SD.

Anti-inflammatory activity

The anti-inflammatory activities of the samples were assessed by human red blood cell membrane stabilizing method[15,16]. The protocol was confirmed by the Human Research Ethics Committees of Ankara University, Faculty of Medicine with the number of 14.05.2020/I5-273-20. Fresh human blood was takenfrom the healthy volunteers. The volunteers, without any chronic disease, had not taken any medicine for up to 15 days prior the test (especially steroidals and anti-inflammatory drugs). Then the centrifuge process was applied at 3000 rpm for 10 min. The packed cells were isolated and washed with 0.85% isosaline at least three times (pH=7.2). Then 10% *v/v* cell suspension was arranged. An equal volume of this cell suspension was added to the tubes consist of the test samples, and then incubation was applied at 56°C for 30 min. Afterwards, the tubes were left to cool and the centrifuge process was applied at 2500 rpm for 5 min. Then, the absorbance was determined at 560 nm. ASA was served as the standard compound. The tests were conducted in triplicates. The percentage of inhibition of hemolysis was computed for each concentration of each sample. Then the IC_{50} values were computed and the final outcomes were presented as IC_{50} ± SD.

Phytochemical analysis

Total phenolic content quantification assay

The total phenolic contents of the extracts were examined by the Folin-Ciocalteu method[17]. Folin-Ciocalteu's reagent, 20% (*w/v*) aqueous Na_2CO_3 and samples were mixed and the volume was completed with distilled water. The solution was kept at room temp for 30 min. The absorbance was then determined at room temperature at 765 nm. The same method was applied for gallic acid with the samples and the gallic acid calibration curve was drawn. The results were served as gallic acid equivalent (GAE) (mg GAE/g extract dry weight). All the experiments were performed at least thrice.

Qualitative and quantitative analyzes of rosmarinic acid using RP-HPLC-PDA

Qualitative and quantitative analyzes of rosmarinic acid (536954, Sigma-Aldrich) in the mature leaf and root methanol extracts were assessed using a previously validated method[18]. HPLC system was Agilent 1260 Series. The system is equipped with a quaternary pump, an auto-sampler, a column oven, and a PDA detector. Agilent Chem-station software was practiced for data analysis. The separation was performed on an ACE 5 µ C18 (250×4.60 mm) column with a mobile phase of a mix of trifluoroacetic acid (HPLC grade, ≥99.0%) 0.1% in water (solution A), trifluoroacetic acid 0.1% in methanol (HPLC grade, ≥99.9%) (solution B), and trifluoroacetic acid 0.1% in acetonitrile (HPLC grade, ≥99.9%) (solution C). The gradient profile was (A:B:C), 80:12:8 at 0 min, 75:15:10 at 8 min, 70:18:12 at 16 min, 65:20:15 at 24 min, 50:35:15 at 32 min, 25:60:15 at 40 min and 80:12:8 at 45 min. The period between each run was arranged as 2 min. 10 µL was applied for the injection. The detection UV wavelength was regulated at 330 nm. The column temperature was controlled and arranged to 30°C. All the quantification and validation parameters for rosmarinic acid were given in our previous study[18].

Statistical analysis

All the experiments were assessed at least thrice for all test samples. SPSS 23.0 was used to examine the results. Data were tested for significant differences by one-way ANOVA. Then the post-hoc Tukey test was performed. A p-value below 0.05 was accepted statistically significant.

Results

Antioxidant activity

ABTS free radical scavenging activity

The antioxidant activity of the samples was stated by measuring their radical inhibitory capacity on ABTS. The results were presented in Table 1. All the extracts exhibited significant radical scavenging effect compared to the control ($p<0.05$). This effect was in a concentration-dependent manner. No significant differences were found according to the age of plant. Both mature and young plants were found to be reactive toward ABTS free radical and had considerable reducing ability. Generally, aqueous extracts exhibited higher radical scavenging activity than methanol extracts. The greatest percentage of inhibition was observed in aqueous extracts of mature herbs among all extracts (IC_{50}=21.86±1.63 µg/ml). Rosmarinic acid was found almost 20 fold more effective than trolox, which was used as a standard compound for this assay. IC_{50} value of rosmarinic acid was 0.57±0.02 µg/ml, while trolox was 9.88±0.02 µg/ml.

[Table 1 near here].

DPPH free radical scavenging activity

Qualitative DPPH analysis

The outcomes of the qualitative DPPH analysis demonstrated that all extracts show high antioxidant activities according to inhibition zones (Figure 1).

[Figure 1 near here].

Quantitative DPPH analysis

The DPPH free radical scavenging activity of the samples was assigned as a mark of antioxidant capacity. DPPH inhibition profiles of the extracts was displayed in Table 2. All the extracts exhibited significant DPPH radical scavenging effect compared to control ($p<0.05$). No significant differences between young and mature plants was noted regarding this effect. Both groups were found to be effective in inhibiting DPPH. Generally, aqueous extracts exhibited better DPPH free radical scavenging potential than methanol extracts, correlatively to the outcomes of the other radical assay (ABTS). Aqueous extracts of mature root displayed the highest inhibitory effect on DPPH free radical followed by aqueous extracts of young herbs (IC_{50}=3.50±0.04 and 3.87±0.04 µg/ml, respectively).

[Table 2 near here].

Anti-inflammatory activity

The anti-inflammtory effects of the samples were determined by measuring their protection capacity on human erythrocyte membrane as shown in Table 3. In general, methanol extracts showed stronger protective effects than aqueous extracts and mature plants were more effective than young ones. Methanol extracts of mature roots and herbs exhibited the highest protective effect (IC_{50}=0.30±0.01 mg/ml) that were similar to the reference compound. Rosmarinic acid was found almost 4-fold more effective on this assay with IC_{50} value of 0.07±0.02 mg/ml than that of the reference compound (IC_{50}=0.27±0.05 mg/ml).

[Table 3 near here].

Phytochemical analysis

Total phenolic content quantification

The total phenolic content of the extracts was evaluated by the Folin-Ciocalteu method. Gallic acid calibration curves including gallic acid concentration and absorbance values were shown in Figure 2.

[Figure 2 near here].

All the examined extracts had significant amounts of phenolic compounds. The total amount of phenolic compounds of the samples displayed a narrow range from 472.22±5.50 and 578.33±3.14 mg GAE/g extract (dw) (Table 4). In general aqueous extracts contained higher total phenolic content than methanol ones. The maximum phenolic content was detected in the aqueous extracts of young herbs followed by methanol extracts of young roots.

[Table 4 near here].

HPLC analysis of rosmarinic acid in T. orientalis

Based on the HPLC analysis results, rosmarinic acid was detected as the major compound in both methanol extracts of mature herbs and roots with the contents of 74.56±0.03 mg/g and 77.03±0.01 mg/g, respectively. The HPLC chromatograms of *T. orientalis* herb and root extracts were shown in Figure 3. Also, the overlaid UV spectra of standard rosmarinic acid and rosmarinic acid in the extracts were presented in Figure 3.

[Figure 3 near here].

Discussion

There is a positive correlation between an antioxidant-rich diet and reduction of oxidative damage and inflammation. This antioxidant-rich healthy diet includes a range of plant foods, fruits and vegetables. In addition, not only nutrition, but also natural compounds and trace elements with antioxidant properties can be a solution in regulating key points in oxidative stress and inflammation process[19-23]. Many plants consist of a range of radical scavenging molecules, such as phenolics, flavonoids, anthocyanins, coumarins, alkaloids, and carotenoids. Several studies have indicated natural phenolic compounds and anthocyanins have antioxidant, anti-inflammatory, antidiabetic, and antiproliferative effects[11,24-27]. Rosmarinic acid, one of these phenolic compounds, is an antioxidant found in the structure of many plants from the Boraginaceae family. Some experimental studies have also revealed that rosmarinic acid exhibits inhibitory effects on oxidation and inflammation. Rosmarinic acid represents antioxidant, anti-inflammatory, and hepatoprotective activities and also alleviates the impacts of inflammatory diseases including inflammatory bowel syndrome and rheumatoid arthritis[28,29]. Rosmarinic acid have been reported to inhibit inflammation of colons via binary reducing of NF-κB and STAT3 activation in dextran sulphate sodium-induced mice[30]. Anthocyanin-rich extracts, rosmarinic acid, and in combination, reduced the symptoms of inflammatory bowel disease[24]. *T. orientalis* has been addressed to consist of phenolic compounds, anthocyanins, tannins, essential oils, saponins, and resin and exhibits significant antioxidant activity[31, 32, 33].

The chemical composition of the extracts varies depending on the growing location, environmental conditions, parts and age of the plant, also extraction solvent. Thus, different extracts are expected to elicit various clinical responses. For this reason, *in vitro* antioxidant, anti-inflammatory activities, as well as phytochemical contents of several parts of *T. orientalis* gathered in different seasons were determined in this study. The results of our study informed that all parts of *T. orientalis* exhibited significant antioxidant activity regardless of age. Generally, the aqueous extracts exhibited better free radical scavenging activities than the methanol extracts. All the extracts offered substantial anti-inflammatory activity in comparison with the control. The methanol extracts exhibited better anti-inflammatory activity than the aqueous extracts. Both antioxidant and anti-inflammatory potentials of the extracts were increased dose-dependently. The strong antioxidant and anti-inflammatory effects of the extracts are most often correlated with the high content of total phenols. In our study, the total phenolics of the extracts was found to be high, which supports this information. In addition, rosmarinic acid, a very effective phenolic compound, was found as a main ingredient in our extracts. After obtaining this information, the antioxidant and anti-inflammatory efficacy of not only the extracts but also rosmarinic acid were evaluated. Rosmarinic acid showed higher activity than the reference compounds tested in all methods. The antioxidant activity of rosmarinic acid was ratified and found to be correlated with anti-inflammatory effect in our study. Inhibition of free radical production also protects the cell membranes against oxidative stress and oxidavite damage[34, 35]. This explains the link between rosmarinic acid and *T. orientalis* extracts showing both high free radical scavenging and cell membrane stabilizing effect at the same time. The presence of rosmarinic acid in herbs and roots of *T. orientalis* was shown for the first time in the current study. Moreover, antioxidant and anti-inflammatory effects of *T. orientalis,* which gathered in both young and mature periods were presented for the first time as well. Our data supply evidence that *T. orientalis* herbs and roots can be use as the potential sources of rosmarinic acid.

Conclusion

This is the first report to assess the presence of rosmarinic acid in herbs and roots of *T. orientalis* to the best of our knowledge. Moreover, there is no adequate data comparable to the results obtained in our current study. *T. orientalis* has substantial antioxidant and anti-inflammatory characteristics and rosmarinic acid is probably the main responsible compound contributing to these biological activities. The phytochemical composition and the biological activities of *T. orientalis* explain its traditional use and indicate potential applications in the pharmaceutical and cosmetic industry.

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Conflict of interest

There is no conflict of interest to declare.

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TABLES

Table 1. ABTS free radical scavenging effect of various ages, parts and extracts of *T. orientalis.*

Plant/Reference	Age	Part	Extract	IC_{50} (µg/ml)
T. orientalis	Mature	Herbs	Methanol	30.67±1.51*
			Aqueous	21.86±1.63*
		Root	Methanol	31.22±0.48*
			Aqueous	27.51±0.73*
	Young	Herbs	Methanol	29.84±0.21*
			Aqueous	29.33±0.54*
		Root	Methanol	40.29±0.24*
			Aqueous	25.82±0.61*
Trolox				**9.88±0.02***
Rosmarinic acid				**0.57±0.02***

(*) $p<0.05$; compared with the control, statistically significant. Each value represents mean±SD (independently replicated three times).

Table 2. DPPH free radical scavenging effect of various ages, parts and extracts of *T. orientalis.*

Plant/Reference	Age	Part	Extract	IC_{50} (µg/ml)
T. orientalis	Mature	Herbs	Methanol	4.31±0.04*
			Aqueous	4.33±0.04*
		Root	Methanol	6.12±0.06*
			Aqueous	3.50±0.04*
	Young	Herbs	Methanol	6.24±0.06*

			Aqueous	3.87±0.04*
		Root	Methanol	4.79±0.05*
			Aqueous	4.14±0.04*
Propyl gallate				**1.73±0.02***

(*) $p<0.05$; compared with the control, statistically significant. Each value represents mean±SD (independently replicated three times).

Table 3. Anti-inflammatory activity of various ages, parts and extracts of *T. orientalis.*

Plant/Reference	**Age**	**Part**	**Extract**	**IC_{50} (mg/ml)**

T. orientalis	Mature	Herbs	Methanol	0.30±0.01*
			Aqueous	0.45±0.05*
		Root	Methanol	0.30±0.01*
			Aqueous	0.47±0.03*
	Young	Herbs	Methanol	0.34±0.01*
			Aqueous	0.45±0.02*
		Root	Methanol	0.32±0.01*
			Aqueous	0.51±0.02*
ASA				0.27±0.05*
Rosmarinic acid				0.07±0.02*

(*) $p<0.05$; compared with the control, statistically significant. Each value represents mean±SD (independently replicated three times).

Table 4. Total amounts of phenolic compounds of various ages, parts and extracts of *T. orientalis.*

Plant/Reference	Age	Part	Extract	mg GAE/g extract (dw)
T. orientalis	Mature	Herbs	Methanol	472.22±5.50
			Aqueous	530.56±3.14
		Root	Methanol	462.22±8.64
			Aqueous	559.44±6.29
	Young	Herbs	Methanol	559.44±6.29
			Aqueous	566.11±4.71
		Root	Methanol	570.00±8.64
			Aqueous	578.33±3.14

Each value represents mean±SD (independently replicated three times).

FIGURES

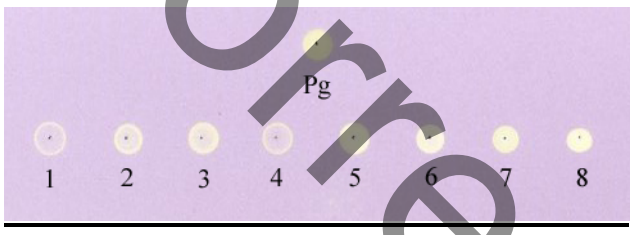


Figure 1. Reactions of extracts with DPPH. Pg: Propyl gallate

1. Young herb methanol, **2.** Young root methanol, **3.** Mature herb methanol, **4.** Mature root methanol,
5. Young herb aqueous, **6.** Young root aqueous, **7.** Mature herb aqueous, **8.** Mature root aqueous

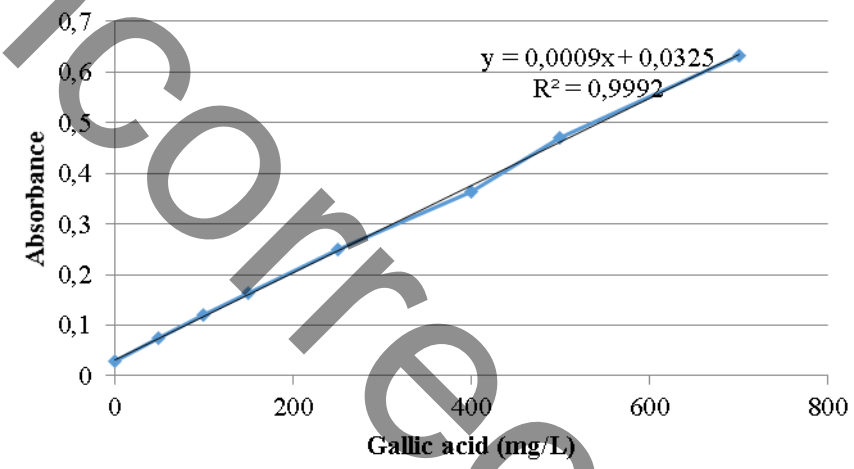
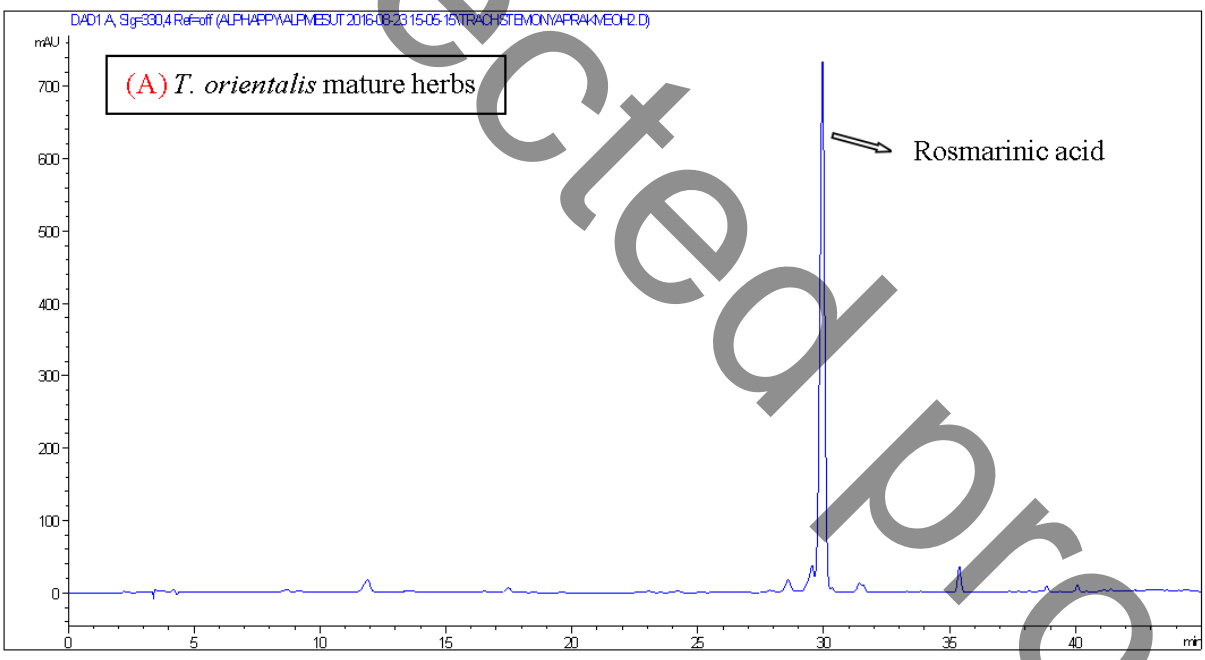


Figure 2. Gallic acid calibration curve

HPLC analysis of rosmarinic acid in *T. orientalis*
(A) *T. orientalis* mature herbs
(B) *T. orientalis* mature roots



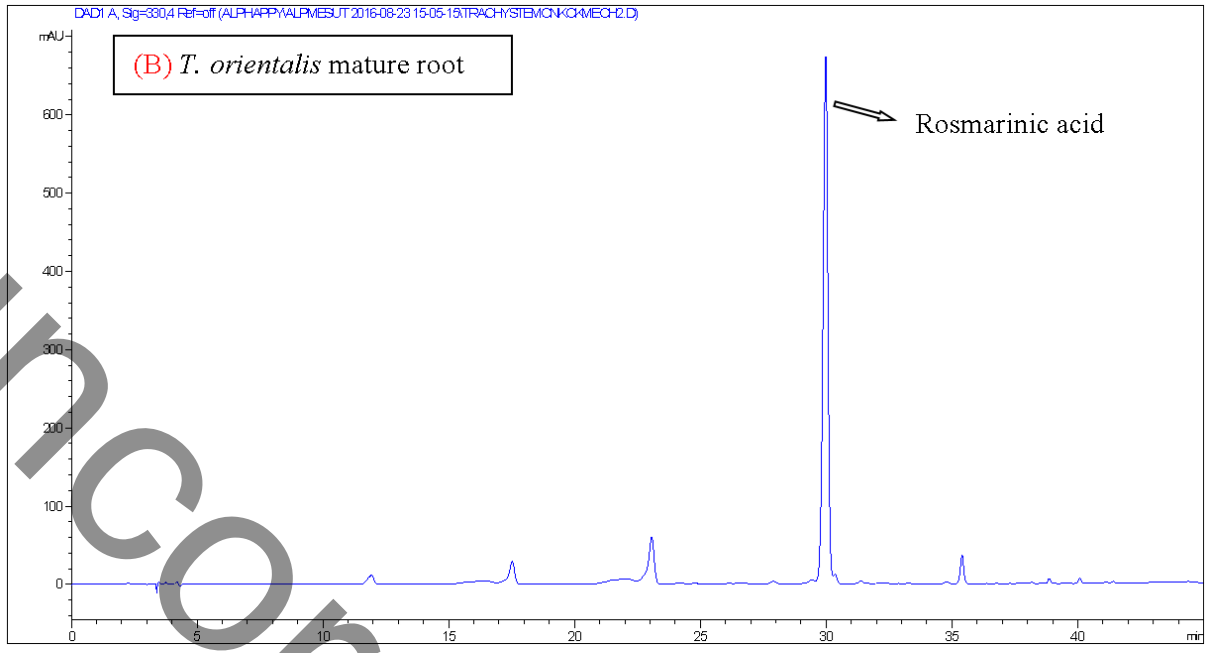


Figure 3. The HPLC chromatograms of herbs and roots of *T. orientalis*

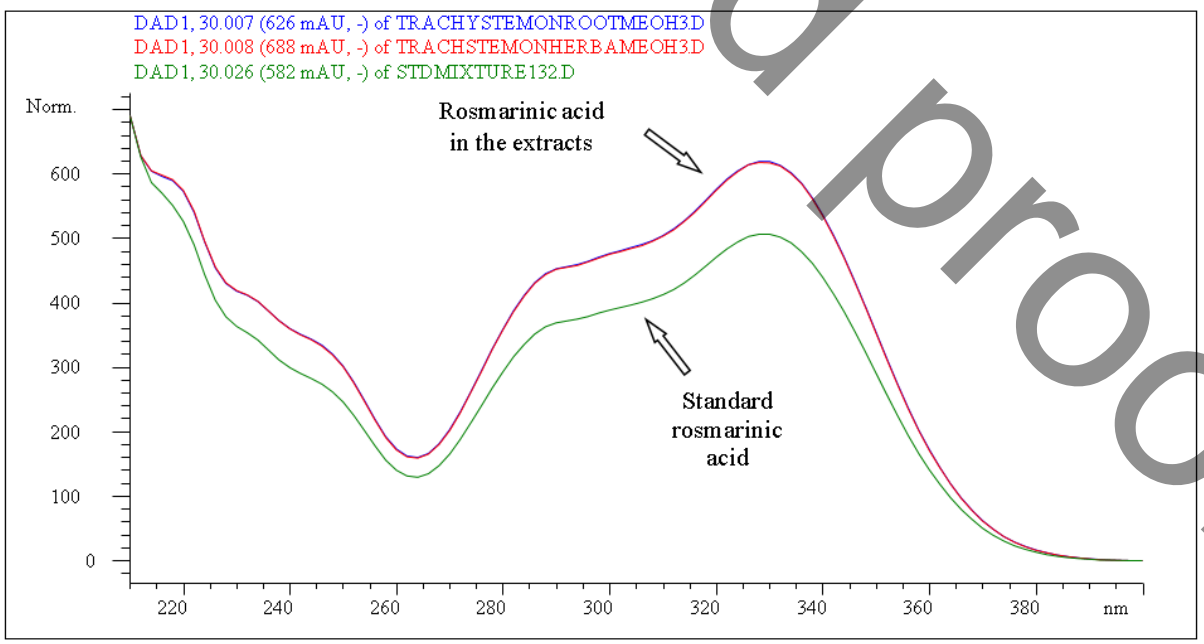


Figure 4. The overlaid UV spectra of standard rosmarinic acid and rosmarinic acid in the extracts.

GRAPHICAL ABSTRACT