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Original article

Elemental Analysis And Antioxidant Activities Of *Tripleurospermum parviflorum* (Willd.) Pobed. And *T. tenuifolium* (Kit.) Freyn Ex Freyn

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Summary. In this study, we determined the trace element contents of *Tripleurospermum parviflorum* (Willd.) Pobed. and *T. tenuifolium* (Kit.) Freyn ex Freyn from Izmir, Turkey. The complete phenolic and flavonoid contents, as well as the antioxidant properties of methanol extracts of *Tripleurospermum parviflorum* and *T. tenuifolium* aerial parts, were also examined. Nine minerals (Na, Ca, Mg, K, Si, Al, P, S and Ti), six trace elements (Fe, Zn, Ni, Br, Ag and Ta) were determined. The heavy metal content in percentages were determined as 9.78-11.08% and 21.63-19.94% for powdered drug and water extracts of *Tripleurospermum parviflorum* and *T. tenuifolium*, respectively. *T. tenuifolium* water extract was found to posses highest concentration of K. In addition, Na, Cl and Ca were also obtained in significant amounts in water extracts of two species. The presence of K and Ca in examined two *Tripleurospermum* species might contribute to explain the traditional use of these drugs against cardiac disorders. The methanolic extract of *T. parviflorum* demonstrated the higher antioxidant activity than *T. tenuifolium.* The antioxidant activity assay findings were found to have significant correlation with the total phenolics and flavonoid content of the extracts.

Key words: Antioxidant activity, Trace elements, *Tripleurospermum parviflorum, Tripleurospermum tenuifolium,* X-ray fluorescence

Introduction

The genus *Tripleurospermum* Sch. Bip. is a member of the Asteraceae (Compositae) family, which includes 40 species in Europe, subtropical Asia, and Northern Africa. In Turkey's flora, there are 31 species, 14 of which are endemic to the genus (1). In Anatolia, *Tripleurospermum* species are popularly known as "Akpapatya" which are used for food. In traditional medicine, different *Tripleurospermum* species are used as tea against cough, stomachache, asthma, throat diseases, vaginitis, cardiac disorders and hair problems (2-4). The flowers of *Tripleurospermum parviflorum* are used to treat anxiety and depression in Shahrekord folk medicine in Iran (5). In our country, some *Tripleurospermum* species are also used as wound healing, against urinary system infections, external infections, acne, diabetes disease, and for headache in Turkish folk medicine (6).

The phenolic compounds apigenin, quercetin, luteolin, chrysoeriol, and their glycosides are determined in some species from *Tripleurospermum* genus (7). Terpenes, hydrocarbons, steroids, oxygen compounds, alcohols, acids and aromatic compounds have been isolated from the *Tripleurospermum* species in previous studies (8,9) and they were found to possess antioxidant (10-12), anti-inflammatory (13), antiulser (14) and antifungal activities (15). In a previous study, Souri *et al.* searched for the antioxidant activity of the flowers of *Tripleurospermum dicsiforme* from Iran,

by the linoleic acid peroxidation method (10). The antioxidant activity of *Tripleurospermum conoclinium* essential oil from Turkey was investigated (11). *T. insolarum* from Turkey was also studied for its chemical composition and antioxidants activity (12). However, there is no report on the antioxidant activities of *Tripleurospermum parviflorum* and *T. tenuifolium*.

The human needs some minerals for nutrition and for benefit of health. Plants contain substances that display medicinal or theurapetic effects (16). The use of plants for medical purposes may cause side effects from some metals and environmental pollution. Trace elements can be used as preventive and theurapeutic in diseases. Metallic elements which are present in plants, can also be toxic for human body (17). This is why it is necessary to prevent contamination of medicinal plants with metals (18). The decoction and infusion of some *Tripleurospermum* species are known to be used for the treatment of some diseaes in traditional medicine (2-6). Hence this study aimed to investigate the major minerals and trace elments of *Tripleurospermum parviflorum* and *T. tenuifolium* by using X-ray fluorescence spectrometry. In addition, the antioxidant activities with total phenolic and flavonoid contents of mentioned plants are investigated.

Materials and Methods

Plant materials

In May 2018, *Tripleurospermum parviflorum* (Willd.) Pobed. and *Tripleurospermum tenuifolium (Chrysantemum tenuifolium)* (Kit.) Freyn ex Freyn were collected during flowering periot in Izmir-Bozdağ. The voucher samples (herbarium numbers 1366 and 1368 for *T. parviflorum* and *T. tenuifolium*, respectively) are deposited at the Faculty of Pharmacy, Ege University in Izmir, Turkey.

Extraction

Methanol extracts were prepared from 40 g aerial plant parts which were air-dried and powdered before extracting with 400 ml methanol in a Soxhlet apparatus for 12 hours. In rotary evaporator (Buchi-R200) (40 °C), the solvents were evaporated to dryness. The yields of methanol extracts of *Tripleurospermum parviflorum* and *T. tenuifolium* were 18.66% and 21.04%, respectively.

Antioxidant activity assays

DPPH radical scavenging assay. The method defined by Fukumoto and Mazza (19) was used to calculate the DPPH (2,2'-diphenyl-1-picrylhydrazyl) scavenging action of methanol extracts. 4 ml of 0.004 percent DPPH in methanol is added to 1 ml of 1 mg/ml methanol extracts. After 30 minutes, the absorbance was measured at 517 nm. The percentage of free radical inhibition was determined as follows:

$$I\% = [(Ab\text{-}As)/Ab] \times 100$$

(Ab: the absorbance of the control, and As: the absorbance of the test sample) The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration. Trolox was used as standard.

ABTS radical cation decolorazition activity. [2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diamonium salt] solution was mixed with potassium persulfate ($K_2S_2O_8$) and the mixture was allowed to react for 15-16 h in the dark at room temperature (20). The radical solution was diluted with acetic acid (20 mM, pH 4.5) to obtain a solution with an absorbance of 0.70±0.02 at a wavelength of 734 nm, ABTS.+ solution (2 ml) was added to 200 µL of diluted sample (1 mg/ml). Absorbance was scanned for 15 minutes in 734 nm. Trolox was utilized for standard solution. The percentage decrease of absorbance against a blank sample (distilled water) was calculated using the following equation:

Inhibiton % = 100 x [(Abs1– Abs2) / Abs1]

(Abs1: the initial absorbance, Abs2: the absorbance at 15 min).

Cupric ion reducing antioxidant capacity (CUPRAC assay). As indicated by Apak *et al.* with certain alterations Cu (II) reducing force was analysed (21). After setting up the mixture of neocouprin and Cu (II) solutions (pH 7), with sample solutions in different fixations the absorbance were observed at 450 nm after holding up 30 minutes at room temperature. Trolox solution at various concentrations was used as standard.

Determination of total phenolic and flavonoid contents

The amount of total phenolics content (TPC) in extracts was determined according to Folin-Ciocalteu method (22). 0.2 µl of sample solution (1mg/ml) were observed at into test tube containing 1 ml of Folin-Ciocalteu's reagent and 2 ml of Na_2CO_3 (7.5%). The final volume was brought up to 7 ml with deionized water. After 2 h incubation at room temperature, the absorbance was measured at 765 nm with spectrophotometer (Shimadzu, UV-1800). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram of extract (mg GAE/g extract).

Total flavonoid content (TFC) of the extract was determined according to repored method in literature (23). 0.5 ml of sample solution (1 mg/ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml 5% of $NaNO_2$ solution. After 6 min incubation, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, followed by adding 2 ml of 4% NaOH solution to the mixture. The mixture was made up to 5 ml with methanol and mixed well. The absorbance was measured at 510 nm after incubation for 15 min. The total flavonoid content was expressed in milligrams of rutin equivalents (RE) per gram of extract.

Statistical analysis

All examination of each example were completed three times and obtained results were appeared as means±SD. One-way-analysis of variance (ANOVA) ($p<0.05$) was applied and Person's correlation was utilized to decide the correlation coefficient of total phenolic substance and antioxidant activity. (Microsoft excel 2013).

Determination of mineral content

Plant materials that had been air-dried were cut into small pieces and finally ground. The water extract of plants materials were prepared by 2% infusion and then filtered. Using a rotary evaporator (Buchi-R200) the filtrate was evaporated and stored at 20°C before analysis. XRF technology was used to examine the powdered plant material and water extract for major minerals and trace elements. The elemental composition was calculated using a SPECTRO IQ (Ametek, Germany) with a silicon drift detector SDD (resolution of 145 eV at 10000 pulses). The HOPG goal is a Bragg crystal and a strongly ordered pyroltic garphite. Under helium atmosphere, the samples (0.1 g) were measured for 300 seconds at voltages of 25 kV and 50 kV and currents of 0.5-1.0 mA.

Results

Table 1 shows the antioxidant activities of *Tripleurospermum parviflorum* and *T. tenuifolium* extracts. The total phenolic content of *T. parviflorum* extract was higher than *T. tenuifolium* extract ($p < 0.05$). For the antioxidant activities "DPPH, ABTS and CUPRAC assays were determined for" methanol extracts of *T. parviflorum,* with Trolox equivalent values of 3.248 mg/ml (DPPH), 1.948 mg/ml (ABTS) and 2.756 mg/ml (CUPRAC) was found to have higher antioxidant activity than *T. tenuifolium* with 1.894 (DPPH), 1.042 (ABTS) and 1.963 (CUPRAC) TEAC mg/ml. The TPC of the extracts of *T. parviflorum* and *T. tenuifolium* were determined as 645.41, and 543.08 mg gallic acid equivalents of the dry matter (mgGAE/g). TFC of MeOH extracts of *T. parviflorum* and *T. tenuifolium* were calculated as 5.49, and 4.86 mgRE/g, respectively.

The concentration of main minerals and trace elements were specified in the water extracts and powdered materials of *T. tenuifolium* and *T. parviflorum* by using XRF (X-ray fluorescence) spectroscopy. The findings are presented in Table 2. In the present analysis, nine minerals (Na, Ca, Mg, K, Si, Al, P, S and Ti), six trace elements (Fe, Zn, Ni, Br, Ag and Ta) and other elements were detected in *T. parviflorum* and *T. tenuifolium*. K was found as major element in two species (*T. tenuifolium* 3.05% and *T. parviflorum* 3.268%).

Discussion

A positive correlation was found between the total flavonoid and phenolic contents of the active extract and its antioxidant activity. Similarly considerable variations in antioxidant capacity values of

Table 1. Results of antioxidant activities, total phenolic and flavonoid contents of *Tripleurospermum parviflorum* and *T. tenuifolium*

	Yields (%)	DPPH (mgTEAC/ml)	ABTS (mgTEAC/ml)	CUPRAC (mgTEAC/ml)	TPC (mgGAE/g)[a]	TFC (mgRE/g)[b]
T. parviflorum	18.66	3.248±0.95 [c]	1.948±0.88	2.756±1.29	645.41 ± 1.35	5.49 ± 1.12
T. tenuifolium	21.84	1.894±2.01	1.042±1.86	1.963±0.54	543.08±0.94	4.86±2.02
Trolox	-	0.041±0.002	-	0.045±0.012	-	-
Acetic acid	-	-	0.920±0.011	-	-	-

[a] Total phenolic content expressed as gallic acis equivalent (mg GAE/g extract)
[b] Total flavonoid content expressed as rutin equivalent (mg RE/g extract)
[c] Results are mean ±SD of three replicate analysis

Table 2. Metallic content of water extract and powdered drug of *Tripleurospermum parviflorum* and *T. tenuifolium*

Element	TT pd	TT we	TP pd	TP we
Na	0.584	1.636	0.594	0.869
Mg	0.4193	0.738	0.4251	0.4754
Al	0.5129	0.3452	1.071	0.2355
Si	0.909	<0.00051	2.493	<0.00011
P	0.2734	0.4321	0.2657	0.3506
S	0.7089	0.9222	0.5587	0.8309
Cl	0.8626	4.721	0.8548	1.564
K	3.05	11.19	3.268	4.686
Ca	1.356	1.419	1.136	0.8281
Ti	0.1419	<0.00051	0.0358	<0.0088
Fe	0.839	0.062	0.2593	0.0158
Ni	0.0196	0.00371	0.00218	0.00214
Zn	0.00288	0.00635	0.00421	<0.0084
Ga	0.00489	0.0106	0.00622	0.0047
Br	0.00048	0.00216	0.00075	0.00156
Ag	0.00094	0.0018	0.00051	0.00182
I	<0.00071	<0.00071	0.00023	<0.00071
Ta	0.0972	0.1358	0.1048	0.1066
Pb	<0.0001	0.00129	<0.0001	<0.0048

*TT: *Tripleurospermum tenuifolium*, TP: *T. parviflorum*, pd: powdered drug, we: water extract

Tripleurospermum species were reported in previous studies (10-12). Souri *et al.* tested the chloroform extract of *T. disciforme* by linoleic acid peroxidation for antioxidant activity utilizing 1,3-diethyl-2-thiobarbituric acid. This extract was found active (IC_{50}=10.75 µg/ml), comparable to α-tocopherol (IC_{50}=14.75 µg/ml) as positive control (10). In a previous analysis, the essential oil of flowers of *T. conoclinium* and leaves of *T. conoclinium* showed moderate antioxidant activity with IC_{50} values of 572.80 and 385.80 µg/ml against ABTS radical (11). DPPH and FRAP methods were used to determine the antioxidant function of *T. insularum* essential oil. Previous research has found that the antioxidant activities of Asteraceae plant extracts are related to the presence of essential oils, especially sesquiterpenes and caffeoyl derivates (12). Many

flavonoids such as apigenin, quercetin, luteolin, chrysoeriol, and their glycosides were isolated from different *Tripleurospermum* species (7).

Therefore, their antioxidant and radical scavenging activities, reducing powers could be caused by these flavonoids. Epidemiologic studies have shown an inverse correlation between foods with high antioxidant content and the rate of death from diseases of degenerative origin such as cancer and cardiovascular diseases (24). Therefore *Tripleurospermum parviflorum*, *T. tenuifolium* and their phenolic compounds are important as natural sources of antioxidants and can be used in nutrition and for many pharmacological applications.

Potassium is required for photosyntesis, enzyme energizing protein synthesis and water use efficiency in plants. Potassium is an essential macroelement in human nutrition and is important in mainting fluid and electrolyte balance in the body (25). Supplements of potassium to reduce tension are used in conjuction with diuretics and thiazides. Potassium is also important in many responses such as signal transduction, hormon release, insulin secrection, vascular tone, immune response and regulation of membrane potential (26, 27).

The concentration of Ca was specified in *T. tenuifolium* as 1.356% and 1.419% for pd and we respectively. Ca was determined in *T. parviflorum* as 1.136% and 0.826% for pd and we, respectively. Ca is the most abundant element in the skeleton and is needed for a variety of vital functions, including nerve and muscle function, hormonal activity, and blood clotting (28).

Silicon was only detected in *T. parviflorum* and *T. tenuifolium* powdered drugs with 2.493% and 0.909%, respectively. Silicon is needed for synthesis for elastin, collagen and also the aorta contains the high quantity of them (29). This might contribute to explain the use of *Tripleurospermum* genus against hearth diseases in folkloric medicine.

Chloride (4.721%) was found as major element in *T. parviflorum* we. Chloride is necessary for turning food into energy and it helps the acid-base balance in human body. The highest amount of iron (0.839%) and nichel (0.0196%) was found in *T. tenuifolium* pd while the amount of zinc (0.00635%) was in higher concentration in *T. tenuifolium* we. Iron is necessary trace element for all living organisms and essential for microorganisms and plants. It procures carbonhydrates oxidation and it helps to control of body weight. Zinc is responsible for immune response, vitamin A transport, fetus and sperm development.

In this study, K and Ca were found as major elements in *T. tenuifolium* and *T. parviflorum.* The highest K concentration (11.19%) is determined in *T. tenuifolium* we. The presence of K and Ca in two *Tripleurospermum* species explain the traditional use especially against cardiac disorders of these drugs.

In conclusion, in the present study, antioxidant activities and trace metal compositions of *Tripleurospermum tenuifolium* and *T. parviflorum* were investigated for the first time. This work showed that the species is a good natural source of K and Ca elements and has significant antioxidant activity. Some further analysis should be conducted for the structural elucidation and isolation of effective compounds.

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