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EXPERIMENTAL PAPER

Antioxidant activity, total phenolic and flavonoids contents of three herbs used as condiments and additives in pickles products

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Summary

Heracleum lasiopetalum Boiss, *Kelussia odoratissima* Mozaff., and *Echinophora platyloba* DC. belong the *Apiaceae* family. They are Iranian endemic plants. These three herbs have been used as food additives in traditional preparations such as pickles. Antioxidant activity (AA) of methanol extracts (ME) of the plants was evaluated by three assays, including DPPH, FRAP, and TEAC. From all three assays, comparing all the MEs for their IC_{50} and EC_1 values, *E. platyloba* had the highest AA. Total phenolic content (TPC) of the extracts ranged from 74 to 120 mg TAE/g. The extract of *H. lasiopetalum* exhibited the highest TPC. The flavonoids

content (FC) of the extracts ranged from 7.63 to 14.52 mg RE/g, from which the extract of *E. platyloba* had the highest flavonoids concentration. A positive correlation between the FC and AA in DPPH assay was found. A significant correlation was also found between the TPC and AA in FRAP assay. These results suggested that the level of AA in these plants varied in a great extent. Our results indicated that extract of *E. platyloba* could be an important dietary source of flavonoids compounds with high antioxidant capacity. In addition, *E. platyloba* can be used as an alternative preservative and natural flavor instead of synthetic ones in food industry (especially pickles).

Key words: *Echinophora platyloba, Heracleum lasiopetalum*, *Kelussia odoratissima*, *antioxidant, total phenolic, flavonoids*

INTRODUCTION

Numerous physiological and biochemical processes in the human body may produce oxygen-centered free radicals and other reactive oxygen species as by-products which are known to be carcinogenic [1]. In addition, oxidation, deterioration and microbial reactions occurring in food products may cause the economic loss [2]. Herbs can contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids etc., rich in atioxidant activity (AA) [3-8]. Phenolic compounds are an integral part of the human diet and could be helpful against cancers, atherosclerosis, ischemia, and inflammatory disease, caused by the exposure to oxidative stress [9]. The family *Apiaceae* consists of more than 300 genera and 3000 species of plants. Most species of plants from the *Apiaceae* family are aromatic and can produce monoterpene, sesquiterpenes essences, as well as phenyl components and related resins in their secretory ducts, roots, stem, leaves, flowers, seeds, and fruits [10]. In this study, antioxidant activity (AA) and phytochemical characteristics of the methanol extracts (ME) from three species of plants belonging to the *Apiaceae* family were investigated. The studied plants, including *H. lasiopetalum*, *K. odoratissima*, and *E. platyloba* are wild grown in Iran. These herbs have been utilized as flavoring and food preservative in cucumber and other vegetables (eggplant, cauliflower, Persian shallot, carrots, onion etc.) pickles by Chaharmahali and Bakhtiari tribes [11]. *Kelussia odoratissima* (local name "Kelus or Karafs-e-Bakhtiari") is a wild, erect, glabrous, perennial aromatic herb which grows in a limited area at southwest Iran [12-13]. The leaves of Kelus are edible and used as a wild vegetable. These leaves are also used as a flavoring agent, and are known to be beneficial against indigestion, inflammation, cardiovascular diseases and rheumatism by ethnic communities [11, 14]. *H. lasiopetalum* is known as "Golpar-e-barfi or Karsum", a perennial aromatic herb distributed in Southwest Iran [12]. The fruits of *H. lasiopetalum* have been utilized as traditional medicines for their antiseptic and antimicrobial properties, and also as a flavoring agent and spice for food (especially meat) by indigenous people [11, 15]. *E. platyloba* (local name "Khosharizeh") is a perennial

aromatic herb distributed in Southwest Iran. It is used as seasoning and an anti-mold agent in pickled cauliflower, gherkin, and a kind of native cheese in Chaharmahal va Bakhtiari province [16]. Thus, the objective of this study was to examine total phenolic content (TPC), flavonoid content (FC), and AA of few plants of Apiaceae family and to evaluate them as potential sources of natural antioxidants.

MATERIALS AND METHODS

Plant material

The plants were collected from Chaharmahal va Bakhtiari province, Iran (32°0¢ N; 50°51¢ E; 2071 m above sea level), in May–August, 2011. Identifications were consequently confirmed with the help of the authentic specimens deposited at the Herbarium of I.A.U. of Shahrekord, Chaharmahal va Bakhtiari, Iran (tab. 1).

Table 1.

Iranian herbs used in this study

Scientific name	Family	Habitat	No. voucher	Parts used	Yield extract (%)
Echinophora platyloba DC	*Apiaceae*	Endemic	IAUSHK-157	Stems	5.30
Heracleum lasiopetalum Boiss	*Apiaceae*	Endemic	IAUSHK-150	Leaves	4.30
Kelussia odoratissima Mozaff	*Apiaceae*	Endemic	IAUSHK-149	Leaves and stems	7.30

Chemicals and reagents

$FeCl_3.6H_2O$, acetic acid, $FeSO_4.7H_2O$, $NaC_2H_4O_2.7H_2O$, $FeCl_2.7H_2O$, tannic acid, methanol, and HCl (37%) used in this study were purchased from Merck Co. (Darmstadt, Germany). The 2,4,6-tri-(2-pyridyl)-s-triazin was purchased from Fluka Chemicals (Germany), rutin was obtained from Roth Co. (Karlsruhe, Germany) and the Folin-Ciocalteu reagent, DPPH, and ABTS radical cation were purchased from Sigma–Aldrich Co. (Steineheim, Germany).

Extract preparation

Subsequently, the ground samples were dried at a room temperature (30°C). A 100 g sample was extracted with 1500 mL methanol at 25 °C for 48 hours followed by shaking for two hours. The methanol was subsequently removed under reduced pressure on a rotary evaporator at 40°C. For determination of antioxidant activity by different assays, about one gram extract in 100 ml methanol mixed.

Determination of total phenolic content (TPC)

The TPC in each extract was determined using the Folin–Ciocalteu method following procedure of Singleton and Rossi [17] with some modifications. Briefly, 0.5 ml of the sample was mixed with 2.5 ml of Folin-Ciocalteu's phenol reagent and kept for 5 min at 37 °C. Then 2 ml of saturated Na_2CO_3 (7.5%) was added, and the mixture was brought to 10 ml with addition of deionized-distilled water. The mixture was maintained at a room temperature in the dark for 120 min and then the absorbance of the mixture was measured at 765 nm against a reagent blank using a UV–Vis spectrophotometer (Shimadzu, Japan). Tannic acid equivalent (TAE) was used as a reference standard and the TPC was expressed as mg of TAE equivalents per gram of each extract on dry basis.

Estimation of flavonoids content (FC)

The FC in the extracts was determined spectrophotometrically according to the procedure of Lamaison and Carnat [18], using a method based on the formation of a flavonoid–aluminum complex with a maximum absorptivity at 430 nm. A calibration curve was made using varying concentrations of rutin Diluted samples (2 ml), separately mixed with 2 ml of 2% $AlCl_3 \cdot H_2O$ (w/v) and incubated at a room temperature for 10 min before measuring the absorbance of the reaction mixture at 430 nm. The FC is expressed as mg RE/g dry weight extract (DWE).

Determination of antioxidant activity

The FRAP assay was used to determine the AA, according to the procedure described by Benzie and Strain [19]. The FRAP reagent contained 2.5 ml of a 10 mM TPTZ solution in 40 mM HCl, 2.5 ml of 20 mM $FeCl_3 \cdot 6H_2O$, and 25 m of 300 mM acetate buffer (pH=3.6). A standard curve was prepared using concentrations of 0.1–2 mmol/l $FeSO_4 \cdot 7H_2O$. The reaction mixture was incubated at 37°C for 30 min and the absorbance was measured at 593 nm using the above described spectrophotometer. The antioxidant equivalent concentration ($EC_1=1$) was defined as a concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mmol/l $FeSO_4 \cdot 7H_2O$ and represented the concentration of antioxidant equivalent to theoretical absorbance value of a 1 mmol/l concentration of Fe (II) solution.

Free radical scavenging activity

The free radical scavenging activity was determined using of a stable ABTS radical cation. Total AA values were estimated using TEAC test [20]. Briefly, ABTS was

dissolved in water (7 mM) and the ABTS radical cation ($ABTS^+$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in dark at a room temperature for 16 h before use. The $ABTS^+$ solution was diluted to get an absorbance of 0.70±0.02 at 734 nm. The percentage inhibition was calculated according to the following formula:

(Eq. 1) % inhibition= $\left[\frac{AC(0)-AA(t)}{AC(0)}\right] * 100$, where $A_{C(0)}$ is the absorbance of the control at t = 0 min; and $A_{A(t)}$ is the absorbance of the antioxidant at t=1, 5, 15 and 30 min.

DPPH radical scavenging activity

The DPPH radical scavenging activity of extracts was determined using the method proposed by Brand-Williams et al. [21]. Samples of selected concentrations of the plant extracts were mixed with an equal volume of 0.2 mM methanol solution of DPPH. The disappearance of DPPH was monitored spectrophotometrically at 515 nm beginning immediately after mixing, and after incubation for 1, 5, 15, and 30 min at a room temperature. The absorbance of the DPPH radical without antioxidant against a control was measured daily. The amount of the sample necessary to decrease the absorbance of DPPH by 50% (IC_{50}) was calculated graphically. The percentage inhibition was calculated according to Eq. 1.

Statistical analysis

All assays were done in triplicates and results were expressed as a mean ± standard deviation (SD). The data were statistically analyzed in one-way ANOVA using SPSS (17.0) software. Means of the scavenging activity, EC_1 and IC_{50} of various extracts for different antioxidant assays were compared with least significant different (LSD) test at $p \leq 0.05$ level. The Pearson correlation coefficient (r) was used to show correlation and their significance by using SPSS.

RESULTS AND DISCUSSION

Extraction of yield, total phenolic and flavonoids contents

Yield of the extracts varied from 43 to 73 mg/g of dry plant material (tab. 1).

A significant difference ($p \leq 0.05$) of TPC between the MEs of *H. lasiopetalum* and *E. platyloba* MEs was found (tab. 1). The highest TPC was obtained for the

extract of *H. lasiopetalum* (120±2.12 mg TAE/g DWE) followed by *K. odoratissima* (102±1.89 mg TAE/g DWE) (tab. 2). Ahmadi et al. [14] reported a phenol concentration of 1.03 mg TAE/g dry matter plant for *K. odoratissima* extract. Results of a previous study [22] indicated that the TPC in sub-fractions of *E. platyloba* was varied. Comparison TPC in MEs of seven spices from *Apiaceae* family showed that *Coriandrum sativum* extract (38.83 mg GAE/g extract) had the highest level of phenolics. A high variation in the TPC of ME from 26 Himalayan medicinal plants by Guleria et al. [23] was recorded. Results of a study by Özcan et al. [24] indicated that the TPC of extracts of some Turkish herbal teas was found to be higher when compared with essential oils. They reported that TPC in the essential oil and ME from pickling herb leaves (*Echinophora tenuifolia* L.) were 0.32 and 1.79 moles of GAE/g, respectively. Phenolic compounds possess diverse biological activities including anti-carcinogenic, anti-atherosclerotic and anti-inflammatory which might be related to their AA [25].

Table 2.

Antioxidant activity, total phenolic and flavonoids contents of methanol extracts of three medicinal herbs

Extracts	Total phenolic (TAN/g extract)	Flavonoids (mg rutin/g extract)	FRAP (EC_1) (mg/ml)	DPPH (IC_{50}) (mg/ml)	TEAC-I (IC_{50}) (mg/ml)	TEAC-II (IC_{50}) (mg/ml)	TEAC-III (IC_{50}) (mg/ml)
H. lasiopetalum	120±2.12 a¶	7.63±0.23 b	7.21±0.054 b	6.58±0.93 b	7.11±0.16 b	5.29±0.099	4.75±0.120
E. platyloba	74±4.21 b	14.52±0.45 a	1.15±0.078 a	2.28±0.31 a	1.69±0.12 a	1.84±0.097	1.70±0.096
K. odoratissimaý	102±1.89 ab	12.18±0.19 ab	4.25±0.083 b	4.09±0.34 ab	5.87±0.12 ab	4.76±0.120	4.41±0.098
BHT	–	–	0.99±0.019 a	1.68±0.27 a	1.13±0.026 a	1.37±0.016	1.09±0.071
ANOVA	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p > 0.05$	$p > 0.05$

¶ Mean ±SD ($n=3$); Values in each row having similar letter are not statistically different at $p \leq 0.05$ by LSD test.

The difference in FC for the extracts of *E. platyloba* and *H. lasiopetalum* was found to be statistically significant ($p \leq 0.05$) (tab. 2). The FC of the extracts was in following order: *E. platyloba* ≥ *H. lasiopetalum* ≥ *K. odoratissima*. Very little information, however, is available in the authentic international literature about the phytochemical characteristics of *H. lasiopetalum, K. odoratissima*, and *E. platyloba* extracts. This is in line with results of the only previous study reported by Ahmadi et al. [14] who stated that FC in other ecotype of *K. odoratissima* was 0.595 mg/g DW. Pandey et al. [26] reported that FC for MEs of seven spices of *Apiaceae* ranged from 12.81 in *Carum carvi* to 45.26 mg RE/g extract in *C. sativum*. Flavonoids are naturally occurring compounds in plants and are believed to have positive effects

on human and animal health [27]. Studies on flavonoidic derivatives have shown a wide range of antimicrobial, antiinflammatory, anticancer, and anti-allergic properties [28-30].

Ferric reducing/antioxidant powe (FRAP)

Significant differences in EC_1 values were found among the extract of *E. platyloba* and other extracts. AA of the MEs increased in the order of BHT ≥ *E. platyloba* > *K. odoratissimaý* ≥ *H. lasiopetalum* (tab. 2). The results of a study by Guleria et al. [23] showed that the FRAP values of 26 medicinal plants extracts ranged from 8.66 to 380.9 μmol Fe (II)/g DW. Antioxidant powers divided in four groups by Wong et al. [31]: very low FRAP (<10 μmol Fe(II)/g), low FRAP (10–50 μmol Fe (II)/g), good FRAP (50–100 μmol Fe(II)/g) and high FRAP (100–500 μmol Fe(II)/g).

ABTS radical scavenging activity

ABTS assay is an excellent tool for determining the AA of hydrogen-donating and chain-breaking antioxidants [32]. Statistical analysis indicated that it was a significant difference between the extracts, especially *E. platyloba* and *H. lasiopetalum* in AA by ABTS assay at 2 minutes (tab. 2). Unfortunately, no information is available in the authentic international literature on the AA of essential oil and extract of *H. lasiopetalum, K. odoratissima,* and *E. platyloba* by ABTS assay.

DPPH radical scavenging activity

DPPH* is a stable free radical, widely accepted as a tool for estimating the free radical scavenging activities of antioxidants [33]. In general, results of analysis of variance (tab. 2) and DPPH inhabitation by four concentrations of the MEs at different times (fig. 1, 2 and 3) show the scavenging effect of samples on DPPH radical was in the following order: BHT ≥ *E. platyloba* ≥ *K. odoratissima* ≥ *H. lasiopetalum*. The findings of this study are similar to those of Gholivand et al. [22] which found that the polar sub-fractions of ME of *E. platyloba* provided the highest radical scavenging activity compared to other studied sub-fractions and essential oils. Ahmadi et al. [14] reported that in DPPH and reducing power models the AA of the ME of *K. odoratissima* was generally found to be less effective than that of ascorbic acid, but it was comparable to and/or greater than the activities of α-tocopherol and BHT. Saei-Dehkordi et al. [34] demonstrated that the essential oil of *E. platyloba* exhibited high scavenging and relative antioxidative in DPPH radicals and β-carotene/linoleic acid bleaching assays.

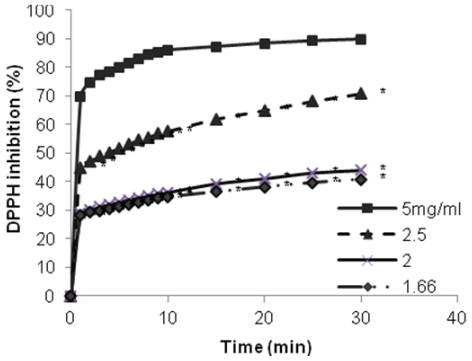

Figure 1.
Inhibition of DPPH by the methanol extract of *E. platyloba*

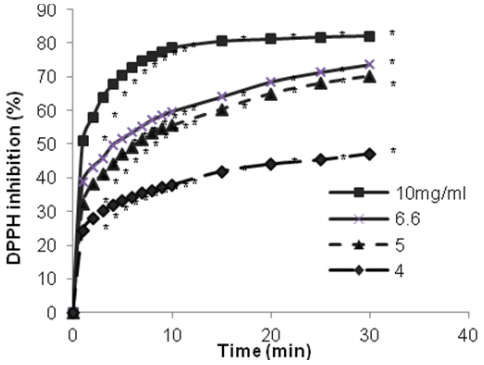

Figure 2.
Inhibition of DPPH by the methanol extract of *K. odoratissima*

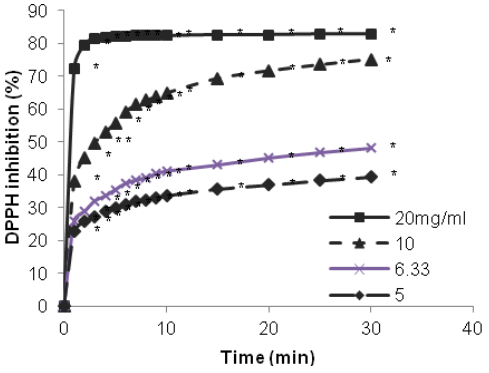

Figure 3.
Inhibition of DPPH by the methanol extract of *H. lasiopetalum*

Correlation between antioxidant capacity and phenolic and flavonoids compositions

The Pearson correlation coefficients between the tests for antioxidants and TPC and FC were calculated and presented in table 3. The TPC correlated significantly with the AA value from the FRAP assay (r=0.994). Other researchers [23, 35-36] have found linear response between TPC and AA in FRAP assays. However, no significant correlation was found between DPPH and TEAC assays and the TPC. The FC correlated significantly with the AA value from the DPPH assay (r=0.997). This can be explained by the fact that the individual flavonoids present in three plants extract had different relative antioxidant potencies. It is well known that phenolic compounds of herbs have the ability to scavenge free radicals, and that the factors such as genetic and environmental conditions (growth season and plant maturity) can cause variations in their values [37]. Miliauskas et al. [38] reported that flavonols content had higher correlation with antiradical activity of twelve extracts as compared with flavonoids.

Table 3.

Correlation matrix showing relationship between antioxidant indices, total phenolic (TP) and flavonoids content (FC)

Characteristics	TPC	FC	FRAP	DPPH	TEAC-I	TEAC-II	TEAC-III
Total phenolic content	1						
Flavenoids content	0.954	1					
FRAP (EC_1)	**–0.994***	–0.981	1				
DPPH (IC_{50})	–0.977	**–0.997***	**0.995***	1			
TEAC-I (IC_{50})	–0.983	–0.882	0.957	0.922	1		
TEAC-II (IC_{50})	–0.968	–0.845	0.933	0.891	**0.997***	1	
TEAC-III (IC_{50})	–0.956	–0.823	0.918	0.871	0.993	**0.999****	1

*: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$

CONCLUSIONS

To the best of our knowledge this is a first report concerning quantitative flavonoid and phenols analysis for Iranian species including *E. platyloba*, *K. odoratissima* and *H. lasiopetalum*. These herbs have been used as food flavoring and preservative agents in pickles of cucumber and vegetables in Iran. The results obtained in this study demonstrated a wide range of AAs by different assays for

the plant extracts. The ME of *E. platyloba* showed the highest AA by three assays (e.g. DPPH, FRAP and ABTS), contained the highest concentration of flavonoids. Stems of *E. platyloba* have been used traditionally as flavoring and preservative in cucumber pickles by indigenous people of Iran. Results of our study also showed the effectiveness of these plant materials in preserving the food, while lacking the harmful side effects of synthetic antioxidants. These herbs can also be served as a valuable source of natural antioxidants for further isolation and purification. However, further toxicological studies should be carried out to ensure their safety *in vitro* and *in vivo*.

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AKTYWNOŚĆ ANTYOKSYDACYJNA, CAŁKOWITA ZAWARTOŚĆ ZWIĄZKÓW FENOLOWYCH I FLAWONOIDÓW W TRZECH ZIOŁACH UŻYWANYCH JAKO PRZYPRAWY I DODATKI DO MARYNAT

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S t r e s z c z e n i e

Heracleum lasiopetalum Boiss., *Kelussia odoratissima* Mozaff. i *Echinophora platyloba* DC. należą do rodziny *Apiaceae*. Są to irańskie rośliny endemiczne. Tych trzech ziół używa się jako dodatków do tradycyjnych produktów, takich jak marynaty. Działanie przeciwutleniające wyciągu metanolowego z tych roślin zostało zbadane trzema metodami: DPPH, FRAP i TEAC. Z trzech badań, podczas których porównano wartości IC_{50} i EC_1 dla wyciągów metanolowych, największe działanie przeciwutleniające wykazano dla *E. platyloba*. Całkowita zawartość związków fenolowych (TPC) w ekstraktach wynosiła od 74 do 120 mg TAE/g. Największą zawartość TPC wykazano dla wyciągu z *H. lasiopetalum*. Zawartość flawonoidów (FC) w wyciągach wynosiła od 7.63 do 14.52 mg RE/g; największe stężenie flawonoidów odnotowano dla wyciągu z *E. platyloba*. W teście DPPH znaleziono dodatnią korelację pomiędzy zawartością flawonoidów a aktywnością antyoksydacyjną. W teście FRAP znaleziono istotną korelację między całkowitą zawartością związków fenolowych a aktywnością antyoksydacyjną. Te wyniki sugerują, że aktywność antyoksydacyjna w tych roślinach bardzo się różni. Nasze wyniki pokazały, że wyciąg z *E. platyloba* może być ważnym źródłem flawonoidów w diecie, działających silnie przeciwutleniająco. Co więcej, *E. platyloba* można stosować jako alternatywny, naturalny dodatek smakowy i konserwujący, zamiast dodatków syntetycznych (szczególnie w marynatach).

Słowa kluczowe: *Echinophora platyloba, Heracleum lasiopetalum*, *Kelussia odoratissima*, *przeciwutleniacze, całkowita zawartość fenoli, flawonoidy*