Determination of Bioactive Lipid and Antioxidant Activity of *Onobrychis*, *Pimpinella*, *Trifolium*, and *Phleum* spp. Seed and Oils

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Abstract: In this study, bioactive lipid components such as fatty acid composition, tocopherol and total phenolics content and antioxidant activity of few wild plant seed extracts were determined. The oil contents of seed samples changed between 3.75 g/100 g (*Onobrychis viciifolia* Scop) and 17.94 g/100 g (*Pimpinella saxifrage* L.). While oleic acid contents of seed oils change between 10.4% (*Trifolium repens*) and 29.5% (*Onobrychis viciifolia* Scop), linoleic acid contents of oil samples varied from 16.3% (*Onobrychis viciifolia* Scop) and 64.2% (*Trifolium repens*) ($p < 0.05$). While α -tocopherol contents of oil samples change between 2.112 (*Pimpinella saxifrage* L.) and 228.279 mg/100 g (*Trifolium pratense*), γ -tocopherol contents ranged from 0.466 (*Phleum pratense*) to 67.128 mg/100 g (*Onobrychis viciifolia* Scop). Also, α -tocotrienol contents of *Onobrychis viciifolia* Scop and *Phleum pratense* were 30.815 and 23.787 mg/100 g, respectively. Results showed some differences in total phenol contents and antioxidant activity values of extracts depending on plant species. The present study indicates that this seed oils are rich in fatty acid and tocopherol.

Key words: antioxidant activity, fatty acids, medicinal plant, seed oil, tocopherols, total phenol

1 Introduction

Seed oils have shown various health benefits due to high concentration of bioactive lipid components¹. In addition, these oils are important macromolecules for industrial applications such as cosmetic and painting^{2,3}. The most of natural crude oils has limited applications, although majority of seed oils have good structural chemical profiles^{4,5}. In addition to the fatty acid composition, the tocopherol is an important characteristic feature to describe the identity of several plant seed oils. The function of tocopherols is believed to be the protection of polyunsaturated fatty acids against peroxidation⁴. Fatty acids, tocopherols, tocotrienols and sterols are important bioactive compounds of oil seeds⁷. Tocopherol (vitamin E) is the major lipophilic antioxidant⁶. In addition to the fatty acid composition, the tocopherol is an important characteristic feature to describe the identity of several plant seed oils. The main biochemical function of tocopherols is believed to be the protection

of polyunsaturated fatty acids against peroxidation⁴. The important antioxidants that exist in plants are tocopherols and tocotrienols as fat-soluble antioxidants (vitamin E)^{6,7}. There is an important role of vitamin E at the preventing lipids and lipid containing foodstuffs from oxidation during storage in food production⁸. Limited studies were conducted on phytochemical properties of these plant seeds. The purpose of current study was to determine bioactive lipid components such as fatty acid and tocopherol contents and *in vitro* total phenolics content and antioxidant activity of few wild plant seed extracts.

2 Material and Methods

2.1 Material

Plant seeds (*Onobrychis viciifolia* Scop, *Phleum pratense*, *Pimpinella saxifrage* L., *Trifolium repens*,

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and *Trifolium pratense*) were collected from Turkey (Konya province origine) in August, 2019. Seed were transported to the laboratory. They were cleaned in an air screen cleaner to remove all foreign matter such as dust, dirt, stones and chaff, and immature. Seeds were kept at +4°C till analysis duration. Quality of the solvent used in text is analytical and HPLC grade.

2.2 Methods

After sample powder (about 2 g) was extracted in a Soxhlet apparatus for 6 h., the solvent was removed at 40°C. The oil obtained was kept at +4°C by using. Oil contents of samples were determined according to AOAC⁽⁹⁾ method. For fatty acids, fatty acid composition of seed oils were determined by using method defined by Hışıl⁽¹⁰⁾. Oil samples were converted to fatty acid methyl esters. Fatty acid methyl esters (1 microliter) were analyzed by 6890 Agilent branded gas chromatography with flame ion detector (FID) with a capillary column, CP-Sil 88 (Varian Inc., Darmstadt, Germany) (100 m long, 0.25 mm ID, film thickness 0.2 mm). The temperature program was as follows: from 155°C; heated to 220°C (1.5°C/min), 10 min isotherm; injector 250°C, detector 250°C; carrier gas 36 cm/s hydrogen; split ratio 1:50; detector gas 30 mL/min hydrogen; 300 mL/min air and 30 mL/min nitrogen and manual injection volume less than 1 mL. The standard mixture of fatty acids (Sigma Chemical Co.) was used for identification of peaks. For determination of tocopherols, a solution of 250 mg of oil in 25 mL of *n*-heptane was directly used for the HPLC. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with a L-6000 pump, a Merck-Hitachi F-1000 fluorescence spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm), and a D-2500 integration system. The mobile phase used was *n*-heptane/*tert*-butyl methyl ether (99 + 1, v/v⁽¹¹⁾).

Total phenolic contents of seed extracts were identified by using Folin-Ciocalteu (FC) reagent as described by Yoo *et al.*⁽¹²⁾ with some modifications. The results are given as mg gallic acid equivalent (GAE)/100 g of fresh weight. The free radical scavenging activity of extracts was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) according to Lee *et al.*⁽¹³⁾.

2.3 Statistical analyses

A complete randomized split plot block design was used analysis of variance (ANOVA) was performed by using JMP version 9.0 (SAS Inst. Inc., Cary, N.C.U.S.A)⁽¹⁴⁾.

3 Results and Discussion

The oil contents of seed samples changed between 3.75 g/100 g (*Onobrychis viciifolia* Scop) and 17.94 g/100 g

Table 1 Oil content and fatty acid composition of plant seeds (%).

Samples	Oil	Palmitic	Palmitoleic	Stearic	Oleic	Vaccenic	Linoleic	Linolenic	Arachidic	Gadoleic	Behenic	Erucic	Total
<i>Trifolium repens</i>	6.08 ± 0.23 ^{ac}	12.6 ± 0.45 ^a	0.1 ± 0.01 ^b	3.8 ± 0.03 ^a	10.4 ± 0.13 ^c	0.7 ± 0.01 ^a	64.2 ± 0.28 ^a	5.1 ± 0.11 ^d	0.7 ± 0.01 ^b	0.4 ± 0.01 ^b	0.7 ± 0.01 ^b	0.4 ± 0.01 ^a	99.3
<i>Onobrychis viciifolia</i> Scop.	3.75 ± 0.11 ^d	7.7 ± 0.03 ^c	— **	2.7 ± 0.7 ^c	29.5 ± 0.17 ^a	1.0 ± 0.03 ^b	16.3 ± 0.31 ^c	39.5 ± 0.23 ^a	0.5 ± 0.01 ^c	0.6 ± 0.03 ^a	0.9 ± 0.03 ^a	0.3 ± 0.01 ^b	99.0
<i>Trifolium pratense</i>	9.77 ± 0.45 ^{b***}	9.2 ± 0.09 ^b	0.8 ± 0.01 ^a	2.6 ± 0.03 ^c	12.0 ± 0.09 ^c	0.6 ± 0.01 ^a	43.4 ± 0.56 ^b	27.9 ± 0.17 ^c	0.9 ± 0.03 ^a	0.3 ± 0.01 ^b	0.8 ± 0.05 ^a	0.1 ± 0.01 ^c	98.8
<i>Pimpinella saxifraga</i> L.	17.94 ± 1.23 ^a	5.5 ± 0.11 ^d	0.1 ± 0.0 ^b	3.2 ± 0.08 ^b	17.1 ± 0.21 ^b	0.5 ± 0.03 ^c	41.8 ± 0.61 ^b	30.8 ± 0.56 ^b	0.5 ± 0.01 ^c	0.1 ± 0.03 ^c	0.1 ± 0.03 ^c	—	99.8

*mean ± standard deviation; **nonidentified; ***Values within each column followed by different letters are significantly different ($p < 0.05$).

Table 2 Tocopherol contents of wild plant seed oils (mg/100 g).

Samples	α-Tocopherol	α-Tocotrienol	β-Tocopherol	γ-Tocopherol	β-Tocotrienol	Plastocholesterol-8	γ-Tocotrienol	δ-Tocopherol	δ-Tocotrienol	Total
<i>Trifolium repens</i>	97.89 ± 0.87 ^{ab}	—	0.91 ± 0.09 ^b	2.46 ± 0.03 ^d	—	0.45 ± 0.07 ^b	—	—	—	101.70
<i>Onobrychis viciifolia</i> Scop.	— **	30.82 ± 0.17 ^a	—	67.13 ± 0.67 ^a	—	1.78 ± 0.11 ^a	—	8.92 ± 0.46 ^c	1.85 ± 0.09 ^b	110.50
<i>Phleum pratense</i>	14.68 ± 0.49 ^{c***}	23.79 ± 0.28 ^b	7.49 ± 0.21 ^a	0.47 ± 0.03 ^c	13.94 ± 0.87 ^a	—	—	1.24 ± 0.11 ^b	2.81 ± 0.11 ^a	64.42
<i>Trifolium pratense</i>	228.28 ± 0.91 ^a	—	0.82 ± 0.09 ^b	5.84 ± 0.57 ^c	0.25 ± 0.03 ^b	0.21 ± 0.01 ^b	—	—	—	235.38
<i>Pimpinella saxifraga</i> L.	2.11 ± 0.23 ^d	—	—	40.78 ± 0.83 ^b	—	—	0.07 ± 0.01	0.35 ± 0.03 ^c	—	43.30

*mean ± standard deviation; **nonidentified; ***Values within each column followed by different letters are significantly different ($p < 0.05$).

(*Pimpinella saxifraga* L.) (Table 1). Palmitic acid contents of oil samples ranged from 5.5% (*Pimpinella saxifraga* L.) to 12.6% (*Trifolium repens*) (Table 1). In addition, while oleic acid contents of seed oils change between 10.4% (*Trifolium repens*) and 29.5% (*Onobrychis viciifolia* Scop), linoleic acid contents of oil samples varied from 16.3% (*Onobrychis viciifolia* Scop) and 64.2% (*Trifolium repens*) ($p < 0.05$). Linoleic acid contents of *Trifolium pratense* and *Onobrychis viciifolia* Scop were found partly similar. Linolenic acid contents of *Trifolium repens*, *Onobrychis viciifolia* Scop, *Trifolium pratense* and *Pimpinella saxifraga* L. were 5.1%, 39.5%, 27.9% and 30.8%, respectively ($p < 0.05$). Erucic acid was not found in *Pimpinella saxifraga* L., *Trifolium repens*, *Onobrychis viciifolia* Scop. Generally, total fatty acid contents of oil samples changed between 98.8% (*Trifolium pratense*) and 99.3% (*Trifolium repens*) (Table 1). It was observed statistically significant differences among the amounts of fatty acids of seed oils ($p < 0.05$). In previous study, wild plant seeds contained between 6.97% (*Cardaria draba*) and 36.87% (*Boreava orientalis*) oils³. Tonguç and Erbaş³ reported that some wild plant seed oils contained 0.65 to 24.43% palmitic, 2.07 to 9.24% stearic, 9.02 to 51.15% oleic, 7.04 to 66.22% linoleic, 13.56 to 40.52% linolenic and 2.12 to 56.63% erucic acids. Ksouda *et al.*¹⁵ reported that *Pimpinella saxifraga* seed oil contained 7.85% palmitic, 1.41% stearic, 13.91% oleic, and 14.4% linoleic acids. Results showed some differences with results of previous studies^{3, 5}. These differences can be likely due to plant species, maturation, climatic factors and some analytical conditions³⁻⁵.

The tocopherol content of the seed oils investigated in present study are shown in Table 2. While α -tocopherol contents of oil samples change between 2.112 (*Pimpinella saxifraga* L.) and 228.279 mg/100 g (*Trifolium pratense*), γ -tocopherol contents ranged from 0.466 (*Phleum pratense*) to 67.128 mg/100g (*Onobrychis viciifolia* Scop). Also, α -tocotrienol contents of *Onobrychis viciifolia* Scop and *Phleum pratense* were 30.815 and 23.787 mg/100 g, respectively. Total tocopherol contents were

found between 43.3 mg/100 g (*Pimpinella saxifraga* L.) and 235.38 mg/100 g (*Trifolium pratense*). Tested plants showed differences in their tocopherol and tocotrienols contents, and the differences were significant at the level of $p < 0.05$. In previous study, it was determined α -tocopherol content in 62 edible tropical plants and α -tocopherol contents of these plant oils changed between 1.23 mg/100 g (*Pochyrrhizus erosus*) and 79.65 mg/100 g (*Saluopus androgynus*)²⁰. It was observed statistically significant differences among the amounts of tocopherols of seed oils ($p < 0.05$). As a result, these differences of bioactive compounds of seed oils may be probably due to growth conditions, varieties, genetic factors, harvesting time, soil properties, geographical variations and analytical conditions^{4, 5}. Minzangi *et al.*⁷ determined 39.5% (*Carapa grandiflora*) to 79.3% (*Maesopsis eminii*) oleic and 17.3% (*Maesopsis emini*) to 53.4% (*Carapa grandiflora*) stearic in several wild plant seed oils. The present study indicates that this seed oils are rich in fatty acid (oleic, linoleic and linolenic acids) and tocopherol (α -tocopherol). The findings are important for the nutrition science due to bioactive compounds which have considerable effects on health¹.

The total phenol contents and antioxidant activity values of the wild plant seed extracts investigated in current study are illustrated in Table 3. Results showed some differences in total phenol contents and antioxidant activity values of extracts depending on plant species. While total phenolic contents of extracts change between 67.63 mgGAE/100 g (dw) (*Pimpinella saxifraga* L.) and 163.91 mgGAE/100 g (dw) (*Trifolium pratense*), antioxidant activity values of seed extracts were determined between 56.77% (*Pimpinella saxifraga* L.) and 98.73% (*Phleum pratense* L.). In general, the highest total phenol and antioxidant activity values were found in seed extracts of *Trifolium pratense*, *Phleum pratense* and *Onobrychis viciifolia* Scop plants. It was observed statistically significant differences among the amounts of total phenol contents and antioxidant activity values of seed extracts ($p < 0.05$). In previous study, Sarker *et al.*¹⁶ reported that *Phleum*

Table 3 Total phenol and antioxidant activity of some wild plant seed extracts.

Samples	Total phenolic content (mgGAE/100 g)	Antioxidant activity (%)
<i>Trifolium repens</i>	79.73 $\pm$ 1.73 ^{*d}	59.23 $\pm$ 1.38 ^d
<i>Onobrychis viciifolia</i> Scop.	127.15 $\pm$ 1.28 ^{*c}	83.17 $\pm$ 1.38 ^c
<i>Phleum pratense</i>	144.27 $\pm$ 1.49 ^{b**}	98.73 $\pm$ 0.65 ^a
<i>Trifolium pratense</i>	163.91 $\pm$ 0.86 ^c	93.28 $\pm$ 3.68 ^b
<i>Pimpinella saxifraga</i> L.	67.63 $\pm$ 2.68 ^a	56.77 $\pm$ 3.87 ^c

*mean $\pm$ standard deviation; ** Values within each column followed by different letters are significantly different ($p < 0.05$).

pratense seed extract had 145 µg/mL antioxidant activity value. In other study, Esmaeili *et al.*¹⁷⁾ determined 46.88 mg GAE/g(dw) total phenol and 205.47 µg/mL antioxidant activity value in methanol extracts of callus tissue of red clover (*Trifolium pratense*) (*in vitro*). Ksouda *et al.*¹⁵⁾ reported that *P. saxifraga* seed extracts had 280 mgGAE/100 g total phenol and 190 mgTEAC/100 g(dw) antioxidant activity value. Karamian and Asadbegy¹⁸⁾ reported that total phenolic contents of *Onobrychis* Species changed between 6.25 and 10.38 mg/g(dw). Total phenolic acid contents of different extracts (methanol, acetone, distillation water and ethyl ethanoate) of *O. viciifolia* were determined between 11.35 ± 0.82 and 38.26 ± 0.15 µg Tannic acid/g of dry extract¹⁹⁾. When results were compared with literature, results showed partially variations. These variations can be probably due to plant species, growing factors, seed maturation and climatic factors.

4 Conclusion

There were observed statistically significant differences among the amounts of fatty acids of seed oils. Tested plants showed differences in their tocopherol and tocotrienols contents, and the differences were significant at the level. The present study indicates that this seed oils are rich in fatty acid (oleic, linoleic and linolenic acids) and tocopherol (α -tocopherol). Results showed some differences in total phenol contents and antioxidant activity values of extracts depending on plant species.

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