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 vicifolia 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 vicifolia Scop*), linoleic acid contents of oil samples varied from 16.3% (*Onobrychis vicifolia Scop*) and 64.2% (*Trifolium repens*) \( (\rho < 0.05) \). While \( \alpha \)-tocopherol contents of oil samples change between 2.112 (*Pimpinella saxifrage L.*) and 228.279 mg/100 g (*Trifolium pratense*), \( \gamma \)-tocopherol contents ranged from 0.466 (*Phleum pratense*) to 67.128 mg/100 g (*Onobrychis vicifolia Scop*). Also, \( \alpha \)-tocotrienol contents of *Onobrychis vicifolia 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 painting3,4. The most of natural crude oils has limited applications, although majority of seed oils have good structural chemical profiles4,5. 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. 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 vicifolia Scop, Phleum pratense, Pimpinella saxifrage L., Trifolium repens,*
and *Trifolium pratense* were collected from Turkey (Konya province origin) 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 AOAC⁹ method. For fatty acids, fatty acid composition of seed oils were determined by using method defined by Hsiu⁴. 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 ported 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.

### 2.3 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.)⁴⁰.

### 3 Results and Discussion

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

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

| Samples          | Oil content (%) | Palmitic | Stearic | Oleic | Linoleic | Linolenic | Arachidic | Behenic | Girardic | Erucic | Total |
|------------------|-----------------|----------|---------|-------|----------|-----------|-----------|---------|----------|--------|-------|
| *Trifolium repens* | 6.68 ± 0.23^a   | 12.4 ± 0.45^a | 3.8 ± 0.05 | 9.7 ± 0.08 | 9.7 ± 0.08 | 17.4 ± 1.25 | 5.3 ± 0.11 | 0.1 ± 0.01 | 3.2 ± 0.07 | 11.2 ± 0.23 | 64.2 ± 0.18 |
| *Trifolium pratense* | 3.75 ± 0.13^b   | 7.2 ± 0.05 | **     | **     | **     | 12.0 ± 0.09 | 6.4 ± 0.01 | 4.3 ± 0.06 | 27.9 ± 0.17 | 0.9 ± 0.05 | 3.0 ± 0.05 |
| *Pisum sativum* | 7.40 ± 0.07^c   | 15.1 ± 0.17 | 20.5 ± 0.17 | 12.0 ± 0.09 | 12.0 ± 0.09 | 17.1 ± 0.21 | 17.0 ± 0.11 | 0.5 ± 0.05 | 3.5 ± 0.06 | 41.6 ± 0.67 | 90.9 ± 0.50 |

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

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

| Samples          | α-Tocopherol | α-Tocotrienol | γ-Tocopherol | γ-Tocotrienol | Plastochromanol-8 |
|------------------|--------------|---------------|--------------|---------------|------------------|
| *Trifolium repens* | 20.8 ± 0.45 | 2.06 ± 0.02 | 10.0 ± 0.45 | 0.97 ± 0.03 | 2.46 ± 0.02 |
| *Trifolium pratense* | 9.97 ± 0.17 | 0.72 ± 0.05 | 1.0 ± 0.05 | 0.7 ± 0.05 | 0.11 ± 0.06 |
| *Pisum sativum* | 16.3 ± 0.23 | 0.56 ± 0.07 | 16.3 ± 0.23 | 0.56 ± 0.07 | 0.23 ± 0.00 |

*Mean ± standard deviation; **nonidentified; ***Values within each column followed by different letters are significantly different (p < 0.05).*
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(Pimpinella saxifrage L.) (Table 1). Palmitic acid contents of oil samples ranged from 5.5% (Pimpinella saxifrage 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 Pimpinella saxifrage L. were 5.1. Trifolium pratense Scop, and Boreavia orientalis were 30.815 and 23.787 Scop and Boreavia orientalis oils respectively (p < 0.05). Erucic acid was not found in Pimpinella saxifrage L., Trifolium repens, Onobrychis viciifolia Scop, Trifolium pratense and Pimpinella saxifrage L. were 5.1, 39.5, 27.9% and 30.8%, respectively (p < 0.05). Erucic acid was not found in Table 1

Table 1 Total phenol and antioxidant activity of some wild plant seed extracts.

| Samples                  | Total phenolic content (mgGAE/100 g) | Antioxidant activity (%) |
|--------------------------|--------------------------------------|--------------------------|
| Trifolium repens         | 79.73 ± 1.73**                       | 59.23 ± 1.38**           |
| Onobrychis viciifolia Scop | 127.15 ± 1.28**                     | 83.17 ± 1.38**           |
| Phleum pratense          | 144.27 ± 1.49**                      | 98.73 ± 0.65**           |
| Trifolium pratense       | 163.91 ± 0.86**                      | 93.28 ± 3.65**           |
| Pimpinella saxifrage L.  | 67.63 ± 2.68**                       | 56.77 ± 3.87**           |

*mean ± 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. 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. reported that P. saxifraga seed extracts had 280 mg/GAE/100 g total phenol and 190 mgTEAC/100 g (dw) antioxidant activity value. Karamian and Asadbegy reported that total phenolic and 190 mg TEAC/100 g Tannic acid/g of dry extract (19). 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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