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Original article

The Oil Content of Wild Fruits from Different Plant Species Obtained by Conventional Soxhlet Extraction Technique

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SUMMARY

Oils from wild fruits have gained attention due to the presence of the wide variety of natural bioactive lipid components (essential polyunsaturated fatty acids, phytosterols, lipid-soluble vitamins), which have positive effects on human health. The present study describes the extraction of oils from fruits of different plant species, *Sambucus nigra, Prunus spinosa, Sorbus aucuparia* and *Rosa canina*, using the conventional Soxhlet extraction technique with petroleum ether. Before the extraction, mechanical processing of fruit samples (exposure to elevated pressure and temperature) was performed. The yield of oil (%) was expressed as mass of obtained oil per mass of dry plant material (w/w). Moisture content of fruit samples was also determined. Wild fruits showed different moisture contents, ranging from 6.53 to 71.52 g/100 g of fresh weight, in *R. canina* and *S. aucuparia* fruits, respectively. *S. nigra* and *P. spinosa* fruits revealed similar moisture contents, 68.48 g/100 g and 68.89 g/100 g of fresh weight, respectively. Oil yields of the investigated fruit samples showed statistically significant differences ($p < 0.05$) and ranged from 0.38 to 3.22% (w/w) in the *R. canina* and *P. spinosa* fruits, respectively. Dry fruits of *S. nigra* had 2.55% (w/w) of oil, while the oil content in *S. aucuparia* fruits was 0.68% (w/w). The findings obtained from this study might be useful from the aspect of potential utilization of these wild fruits, in particular fruits of *P. spinosa* and *S. nigra*, as raw materials for the extraction of valuable oil for the application in food, cosmetic and pharmaceutical industry.

Key words: **wild fruits, moisture content, Soxhlet extraction, oil yields, practical application of oils**

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INTRODUCTION

There is a growing demand for natural sources of biologically active compounds for food, pharmaceutical or cosmetic industry. Wild fruits are widely consumed in human diet, in fresh or frozen form, as a component of jams, jellies, alcoholic beverages and yogurts. In general, fruits are a rich source of different phytochemicals and a valuable component of functional food, dietary supplements and dermocosmetic products. Due to their constituents (polyphenols, minerals, vitamins, fibers and others) they have significant health benefits in the traditional treatment of many diseases and disorders (1). Fruits from different wild plants, such as elderberry (*Sambucus nigra L., Adoxaceae*), blackthorn (*Prunus spinosa L., Rosaceae*), rowanberry (*Sorbus aucuparia L., Rosaceae*) and dog rose (*Rosa canina L., Rosaceae*), as an interesting and worthwhile source of raw materials, were the subject of our research. The investigated plant species have been used in traditional medicine in our country for many years (2).

Elderberry (black or European elderberry, *S. nigra*) is plant species which grows wild throughout Europe. *S. nigra* fruits are a good source of protein, lipids, phenolic acids, organic acids and vitamins (especially B-group and A-group vitamins, tocopherols, vitamin C*). S. nigra* plant has health-beneficial activity in the treatment of respiratory, cardiovascular, neurodegenerative and inflammatory diseases, diabetes and obesity. Recent studies have shown that *S. nigra* possessed an important antiviral, antiallergic and antibacterial potential and protective activity against harmfull UV radiation (3, 4). *S. nigra* fruit extracts exhibited antioxidant activity determined by different *in vitro* assays as well (5).

P. spinosa (also known as sloe or blackthorn) fruits are used in folk medicine due to their astringent, purgative and diuretic effects. Fruits have a bitter taste and they are a rich source of antioxidants (polyphenols, especially anthocyanins) (6, 7). Fruit preparations have antibacterial and anti-inflammatory properties. Jams, juices, syrups and teas are made from blackthorn fruits and used in human nutrition (8). *In vivo* studies with *P. spinosa* extract incorporated in different semi-solid formulations showed positive effect of the extract on the skin hydration (9).

Rose hips (or false fruits, pseudofruits of dog rose, *R. canina*) and their extracts are largely used in traditional medicine, as laxative and diuretic agents, for the treatment of arthritis, gout, rheumatism, fever, colds and influenza. Rose hips have pro-healthy activities in the lower urinary tracts, kidney and gastrointestinal disorders (7). The main constituents of *R. canina* fruit extracts are phenolic acids, flavonoids, minerals and carotenoids. Also, bioactivity of *R. canina* fruits is attributed to the presence of vitamins, tannins, proanthocyanidins, fatty acids, phospholipids and galactolipids. Due to the high content of vitamin C, hips are used for the treatment of this vitamin deficiency (10). *In vivo* studies with *R. canina* hip extracts and powder suggested their possible use as an anti-aging and skin-lightening agent (11).

Rowanberry (*S. aucuparia*, mountain ash) grows wild in many countries throughout Europe. Fruits from this plant are used in folk medicine for making jams, juices and alcoholic drinks. Further, it represents an important source of phenolics, especially flavonoids and phenolic acids. Because of the antioxidant and anti-inflammatory properties, *S. aucuparia* fruits have positive effects on the treatment of symptoms of diabetes, intestinal obstructions, coronary heart disease and chronic diarrhea (12).

Nowadays, vegetable oils play an important role in human diet as a source of edible lipids, lipid soluble vitamins, squalene, phytosterols and essential fatty acids. Oils provide energy to the body, protect tissues and participate in many functions in the human body (cardiovascular disease prevention, reduction of blood cholesterol levels, prevention of aging-related diseases, improvement of the immune system response, Alzheimer's disease prevention, prevention of various cancers) (13). Plant oils are recognized as healthier in comparison to the animal ones, so their consumption is growing. Alternative oils, like oils from tree nuts (almonds, pistachios, walnuts) and botanicals (borage, evening primrose oils) are becoming more popular (14). Natural vegetable oils (like castor oil, olive oil, jojoba oil and others) are important ingredients in cosmetic products for the treatment of many skin conditions (dry skin, skin infections, atopic dermatitis, rosacea, psoriasis, contact dermatitis, xerosis, eczema, burns), because their lipid composition is similar to the composition of human sebum (15). Also, *in vivo* studies with plant extracts with different vegetable oils (olives, sunflowers or palms), such as St. John's Wort oil extracts, incorporated in cream formulations, revealed beneficial anti-inflammatory effects on the human skin (16).

Therefore, wild fruits might have a great potential to become a valuable source of oils and natural bioactive oil constituents. Oils might be obtained from the plant material by solvent extraction method. Soxhlet extraction with organic solvent, such as petroleum ether, is a conventional technique for oil isolation (17). Investigated fruits from this study might be used as potential renew-

able source of oils that can be further utilized for food, cosmetic applications and pharmaceutical industry. With the aim of examing the possibilities of potential application of oils from different wild fruits, the present study describes extraction of oils, using the Soxhlet technique and comparison of oil yields between the plant species. In order to achieve a higher amount of extracted oil, additional mechanical processing of fruit samples (exposure to elevated pressure and temperature, under controlled conditions, for 2 h) was performed. Moisture content of examined fruits was also determined.

MATERIAL AND METHODS

PLANT SAMPLES AND CHEMICALS

Fruits of four different wild plant species, *S. nigra, P. spinosa, S. aucuparia* and *R. canina,* were investigated. Fruits were collected manually from a wild locality of Vlasina region in Southeastern Serbia. At the time of harvesting (during September to October of 2015), fruits were collected randomly, fully ripened and undamaged. Raw material was authenticated and the voucher specimens (*S. nigra* No. 1121SN, *P. spinosa* No. 2221PS, *S. aucuparia* No. 1407SA and *R. canina* No. 2008RC) were deposited at the Institute for Medicinal Plant Research "Dr Josif Pančić", Department for Pharmaceutical Research and Development in Belgrade (Serbia). Fruit samples were frozen and stored at -20 ^{0}C until further analysis. Before the experiments, fruits were defrosted and crushed in the mill (MKM 6000, Bosch, Germany). Triplicate subsamples were used for each analysis.

Petroleum ether (boiling range 40-60 ^{0}C, density at 15 ^{0}C 0.647-0.654 g/cm^3, purity < 99.5%) was of analytical grade and purchased from Carlo Erba Reagents, Italy.

MOISTURE CONTENT DETERMINATION

The moisture content of wild fruits was determined according to the method AOAC 930.15 (18), by calculating the amount of water evaporated from the fresh fruit material after the drying process. The weight loss after drying the samples was calculated as moisture and results were expressed as % (w/w) of fresh fruit weight. The used equation was:

$$\text{Moisture content (\%)} = \frac{m_f - m_d}{m_f} \times 100$$

where m_f represents the initial mass of the fresh fruits (in grams); and m_d represents the mass of dried plant material (in grams).

OIL EXTRACTION TECHNIQUE

Wild fruits were dried in a drying chamber (FD 23, Binder, Germany) with controlled temperature at 40 ^{0}C, until a constant mass was achieved. Additionally, in order to obtain higher oil content from fruits, dried fruit samples were subjected to high pressure (10 MPa) and temperature of 40 ^{0}C, under controlled conditions, for 2 h. Soxhlet technique using petroleum ether as a solvent was applied to obtain oils from fruit samples. Namely, petroleum ether, as one of the non-polar extraction solvents, expected to have a better selectivity for oil extraction in comparison to the polar ones. Soxhlet extraction is one of the conventional extraction methods for isolating nonpolar components, like oils. A principle of Soxhlet extraction with recycling of solvent is described in the literature (17). Shortly, accurately-weighed amount of dry plant material is packed in a paper thimble, which is placed in extraction chamber. The petroleum ether was added and the obtained Soxhlet system was heated at the boiling point of the extraction solvent. As the solvent was condensed from a laboratory flask, reaching an overflow level, it was aspirated by a siphon. The siphon brought the solvent with extractive components back to the boiling vessel, and the whole reflux process was repeated until the extraction was completed (until the appearance of discoloration). After the extraction, petroleum ether evaporated using a rotary vacuum evaporator (HB 4, Ika-Werke, Germany), under reduced pressure. The residual content was then dried in a drying chamber for 2 h at 30 ^{0}C. The amount of extracted oil was evaluated by weight measuring. Oil samples were stored at 4 ± 2 ^{0}C and protected from light.

DETERMINATION OF EXTRACTION YIELD

The extraction yield was calculated as the weight percentage of extract (oil) obtained from the initial weight of plant material used, using the following equation:

$$\text{Extraction yield (EY) \%} = \frac{m_o}{m_p} \times 100$$

where m_o represents the mass of the fruit oil that was obtained (in grams); and m_p represents the mass of raw plant material (in grams) that was used in the extraction process. The yield of extract (%) was expressed on a dry

matter basis, i.e., mass of obtained oil per mass of dry plant material (w/w).

STATISTICAL ANALYSIS

All analyses for each sample were conducted in three individual experiments. The results were presented as mean value ± standard deviation (SD). Statistical analysis was carried out using the Microsoft Excel 2007 and SPSS Statistics 22.0 program package. The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test.

RESULTS AND DISCUSSION

Moisture content value is one of the important properties which gives information about the composition of fruit material. Nevertheless, the process of oil extraction and consequently oil yield are influenced by the moisture content. Fruits with high amount of moisture are not suitable for oil extraction in fresh form, so they should be dried. As shown in Table 1, the studied wild fruits showed different moisture contents, ranging from 6.53 to 71.52 g/100 g of fresh weight (fw), in *R. canina* and *S. aucuparia* fruits, respectively. *S. nigra* and *P. spinosa* fruits revealed similar moisture contents, 68.48 g/100 g and 68.89 g/100 g of fw, respectively. Our results for blackthorn fruits were similar to the results for the moisture content of *P. spinosa* wild fruits from Portugal (60.86 g/100 g of fw) (7) and Spain (68.06 g/100 g of fw) (19). According to the literature, the moisture content of explored wild *S. aucuparia* fruits was 75.20%, while for *S. aucuparia* different cultivars was within the range from 75.40 to 81.50% (20). To the best of our knowledge, the present work described, for the first time, the moisture content determination of wild *S. nigra* fruits.

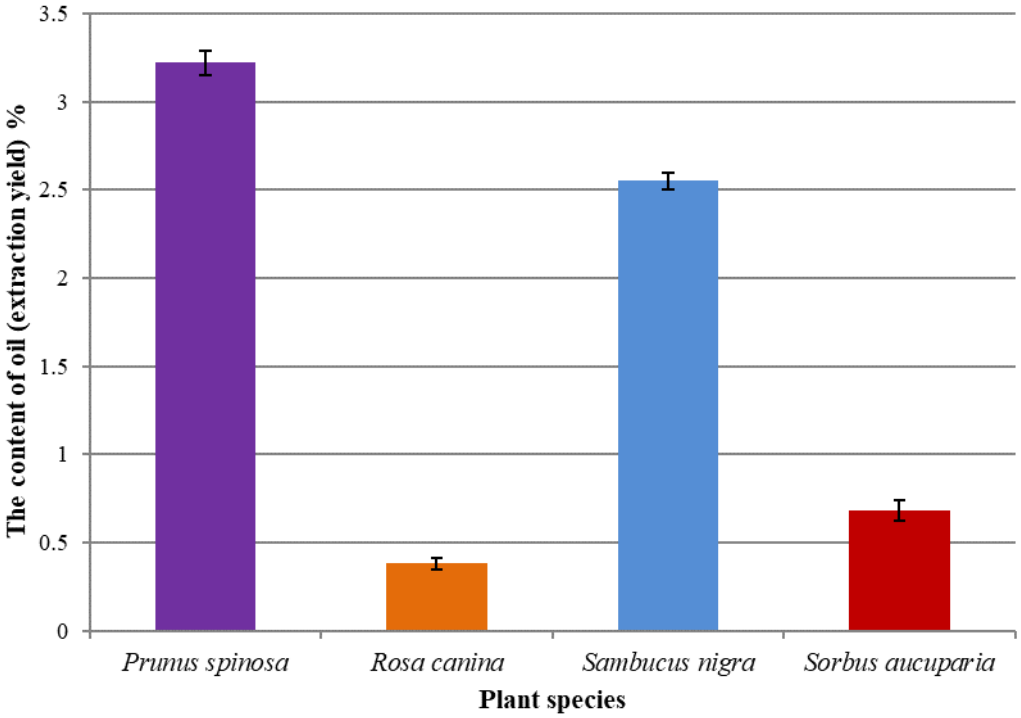
Table 1. **Moisture content of wild fruits of investigated plant species (expressed as g/100 g of fresh weight). Values with different superscripts were significantly different ($p < 0.05$).**

Plant species	Moisture content (%)
Prunus spinosa	68.89 ± 0.97^{A}
Rosa canina	6.53 ± 0.09^{B}
Sambucus nigra	68.48 ± 0.49^{A}
Sorbus aucuparia	71.52 ± 0.95^{C}

The oil contents of fruits obtained by Soxhlet extraction with petroleum ether are presented in Graph 1. The fruit samples showed different content of oils, depending on plant material. The oil contents of *P. spinosa* and *S. nigra* fruits were higher in comparison with fruits from other plant species.

Oil yields of the investigated fruit samples differed statistically significantly ($p < 0.05$) and ranged from 0.38 to 3.22% (w/w) of dry weight (dw) in the *R. canina* and *P. spinosa* fruits, respectively. Dry fruits of *S. nigra* had 2.55% (w/w) of oil, while the oil content in *S. aucuparia* fruits was 0.68% (w/w). There is little data in the literature that could be used to compare the oil content of studied wild fruits, due to the differences in extraction methods and plant materials used.

Study with fresh and frozen *P. spinosa* fruits reported the amount of fat in the fresh fruit was 2.04 g/100 g dw, although the method of analysis was different in comparison to ours (8). In the extraction of *R. canina* hips with n-hexane at 60 ^{0}C for 6 h using Soxhlet extraction, 1.78% of total fat was obtained (21). Barros L. et al. investigated the content of fat from *P. spinosa* and *R. canina* powdered fruit samples from Portugal, using a Soxhlet apparatus and petroleum ether as an extraction solvent. The obtained values were 1.98 g/100 g and 0.65 g/100 g of dry weight, for the *P. spinosa* and *R. canina* powdered fruits, respectively (7), which is in accordance with our results.



Graph 1. **Oil content of fruits of four investigated wild plant species**

As far as *S. nigra* fruits are concerned, in most cases literature provides data about oil content from fruit seeds. In our study, the investigated samples consisted of the whole wild fruits, fruit pulp and seeds. The study of Fazio et al. indicated the amount of *S. nigra* seed oil isolated by Soxhlet extraction with a combination of petroleum ether and hexane. The amount of extracted oil was 1.59 g/10 g of dried seed flour (3). In another study, unripe and ripe fruits of *S. nigra* cultivars were Soxhlet-extracted with dichloromethane - the lipophilic fraction yields ranged from 0.56% to 1.84% of dry weight (22).

There were only a few studies on the oil content of *S. aucuparia* fruits in the literature. The amount of lipids detected in the whole fruit was 6.20% dw, in the seed of *S. aucuparia* was 16.50% dw, while the pulp had the smallest lipid content (23).

CONCLUSION

In the present study, wild fruits of *S. nigra, P. spinosa, S. aucuparia* and *R. canina* plant species, growing on their natural sites in Southeastern Serbia, were analyzed with respect to the moisture contents and oil yields. The study provides useful information about the oil yields of fruits, obtained by Soxhlet extraction with petroleum ether, with mechanical pre-treatment of fruit samples under controlled conditions of elevated pressure and temperature. The findings obtained from this study might be useful in the view of potential utilization of these wild fruits, in particular fruits with the highest oil contents, *P. spinosa* (3.22% w/w) and *S. nigra* (2.55% w/w), as raw materials for the extraction of oils for the application in food, cosmetic and pharmaceutical industry, and consequently for the improvement of human health.

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References

1. Nile SH, Park SW. Edible berries: bioactive components and their effect on human health. Nutrition 2014; 30: 134-44. https://doi.org/10.1016/j.nut.2013.04.007

2. Tucakov J. Healing with plants. RAD, Belgrade, 1997 (in Serbian).

3. Fazio A, Plastina P, Meijerink J, et al. Comparative analyses of seeds of wild fruits of Rubus and Sambucus species from Southern Italy: fatty acid composition of the oil, total phenolic content, antioxidant and anti-inflammatory properties of the methanolic extracts. Food Chem 2013; 140: 817-24. https://doi.org/10.1016/j.foodchem.2012.11.010

4. Sidor A, Gramza-Michałowska A. Advanced research on the antioxidant and health benefit of elderberry (Sambucus nigra) in food – a review. J Funct Foods 2015; 18: 941-58. https://doi.org/10.1016/j.jff.2014.07.012

5. Stanković M, Živković J, Tadić V, Arsić I. Skin protection against solar UV radiation by natural plant products: extracts from elder fruit (Sambucus nigra L.). The Fifth International Conference on Radiation and Applications in Various Fields of Research, RAD Conference Proceedings 2017; 2: 231-6.

6. Fraternale D, Giamperi L, Bucchini A, Ricci D. Antioxidant activity of Prunus spinosa L. fruit juice. Ital J Food Sci 2009; 3: 337-46.

7. Barros L, Carvalho AM, Sa Morais H, Ferreira ICFR. Strawberry-tree, blackthorn and rose fruits: Detailed characterisation in nutrients and phytochemicals with antioxidant properties. Food Chem 2010; 120: 247-54. https://doi.org/10.1016/j.foodchem.2009.10.016

8. Sikora E, Bieniek MI, Borczak B. Composition and antioxidant properties of fresh and frozen stored blackthorn fruits (Prunus spinosa L.). Acta Sci Pol Technol Aliment 2013; 12: 365-72.

9. Arsić I, Tamburić S, Bulatović V et al. Exploring moisturizing potential of naturals: the cases of St. johns wort, chamomile and blackthorn. Euro Cosmetics 2005; 3: 14-21.

10. Lattanzio F, Greco E, Carretta D, et al. In vivo anti-inflammatory effect of Rosa canina L. extract. J Ethnopharmacol 2011; 137: 880-5 https://doi.org/10.1016/j.jep.2011.07.006

11. Marmol I, Sanchez-de-Diego C, Jimenez-Moreno N, et al. Therapeutic Applications of Rose Hips from Different Rosa Species. Int J Mol Sci 2017; 18: 1-37. https://doi.org/10.3390/ijms18061137

12. Mikulic-Petkovsek M, Krska B, Kiprovski B, Veberic R. Bioactive Components and Antioxidant Capacity of Fruits from Nine Sorbus Genotypes. J Food Sci 2017; 82: 647-58. https://doi.org/10.1111/1750-3841.13643

13. Yang R, Zhang L, Li P, et al. A review of chemical composition and nutritional properties of minor vegetable oils in China. Trends Food Sci Technol 2018; In press (doi: 10.1016/j.tifs.2018.01.013) https://doi.org/10.1016/j.tifs.2018.01.013

14. Mahoney N, Molyneux RJ. Rapid Analytical Method for the Determination of Aflatoxins in Plant-Derived Dietary Supplement and Cosmetic Oils. J Agric Food Chem 2010; 58: 4065-70. https://doi.org/10.1021/jf9039028

15. Aburjai T, Natsheh FM. Plants Used in Cosmetics. Phytother Res 2003; 17: 987-1000. https://doi.org/10.1002/ptr.1363

16. Arsić I, Žugić A, Tadić V, et al. Estimation of Dermatological Application of Creams with St. John's Wort Oil Extracts. Molecules 2012; 17: 275-94. https://doi.org/10.3390/molecules17010275

17. Azmir J, Zaidul ISM, Rahman MM, et al. Techniques for extraction of bioactive compounds from plant materials: A review. J Food Eng 2013; 117: 426-36 https://doi.org/10.1016/j.jfoodeng.2013.01.014

18. Official methods of Analysis of AOAC International, 18th edn. Association of Official Analytical Chemists (AOAC), USA, 2005.
19. Morales P, Ferreira ICFR, Carvalho AM, et al. Wild edible fruits as a potential source of phytochemicals with capacity to inhibit lipid peroxidation. Eur J Lipid Sci Technol 2013; 115: 176-85. https://doi.org/10.1002/ejlt.201200162
20. Kylli P, Nohynek L, Puupponen-Pimia R, et al. Rowanberry Phenolics: Compositional Analysis and Bioactivities. J Agric Food Chem 2010; 58: 11985-92. https://doi.org/10.1021/jf102739v
21. Ercisli S. Chemical composition of fruits in some rose (Rosa spp.) species. Food Chem 2007; 104: 1379-84 https://doi.org/10.1016/j.foodchem.2007.01.053
22. Salvador AC, Rocha SM, Silvestre AJD. Lipophilic phytochemicals from elderberries (Sambucus nigra L.): Influence of ripening, cultivar and season. Ind Crops Prod 2015; 71: 15-23. https://doi.org/10.1016/j.indcrop.2015.03.082
23. Raspe O, Findlay C, Jacquemart A. Sorbus aucuparia L. J Ecol 2000; 88: 910-30. https://doi.org/10.1046/j.1365-2745.2000.00502.x

Sadržaj ulja divljih plodova različitih biljnih vrsta dobijen konvencionalnom Soxhlet tehnikom ekstrakcije

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SAŽETAK

Ulja dobijena iz plodova biljaka sve više dobijaju na značaju, upravo zbog prisustva velikog broja prirodnih, bioaktivnih lipidnih komponenata (esencijalnih, polinezasićenih masnih kiselina, fitosterola, lipo-solubilnih vitamina), koji ispoljavaju pozitivne efekte na ljudsko zdravlje. U ovom radu opisan je postupak ekstrakcije ulja iz plodova različitih biljnih vrsta (*Sambucus nigra, Prunus spinosa, Sorbus aucuparia i Rosa canina*), primenom konvencionalne Soxhlet ekstrakcione tehnike sa petrol-etrom. Pre ekstrakcije izvršena je mehanička obrada uzoraka plodova, njihovim izlaganjem povišenom pritisku i temperaturi. Prinos ulja (%) je izražen kao masa dobijenog ulja u odnosu na masu suvog biljnog materijala (w/w). Određen je i sadržaj vlage u uzorcima plodova. Divlji plodovi su se razlikovali po sadržaju vlage, čije su vrednosti bile u granicama od 6,53 g/100 g svežih plodova (*R. canina*) do 71,52 g/100 g svežih plodova (*S. aucuparia*). Biljne vrste *S. nigra* i *P. spinosa* pokazale su sličan sadržaj vlage u plodovima, i to 68,48 g/100 g i 68,89 g/100 g svežih plodova. Prinosi ulja ispitivanih uzoraka plodova razlikovali su se statistički značajno ($p < 0,05$), a vrednosti su bile od 0,38% (kod *R. canina* plodova) do 3,22% (plodovi *P. spinosa*). Kod suvih plodova *S. nigra* dobijeni prinos ulja je iznosio 2,55%, dok je sadržaj ulja u plodovima *S. aucuparia* iznosio 0,68%. Dobijeni rezultati su korisni zbog mogućnosti upotrebe ispitivanih divljih plodova, naročito plodova *P. spinosa* i *S. nigra* (imale su najveći sadržaj ulja), kao polaznih sirovina za ekstrakciju biljnog ulja za primenu u prehrambenoj, kozmetičkoj i farmaceutskoj industriji.

Ključne reči: **divlji plodovi, sadržaj vlage, Soxhlet ekstrakcija, prinos ulja, praktična primena ulja**