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NETTLE (*URTICA DIOICA* L.) SEED OIL: EXTRACTION, CHEMICAL CHARACTERISATION AND ANTIOXIDANT ACTIVITY

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Abstract

Nettle seed oil is good source of linoleic and oleic acid, vitamin E, phytosteroles and antioxidants. There are data on its and antioxidant activity and potential activity in ameliorating colonic inflammation. The aim of this research is to determine the differences in yield and antioxidant activity of the oils obtained by different solvents.

The oil from dry nettle seeds, purchased from "Jeligor", Svrljig in Serbia, was extracted by using reflux technique with n-hexane and trichlorethylene, at the boiling point of the solution. The oils were characterized by chemical numbers (saponification, acid, iodine and ester) by using the standard volumetric analysis methods. The antioxidant activity was determined by the method of determination of scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH SC). The statistical analysis by t-test, at 5% level was done.

There is no statistically significant difference ($p > 0.05$) in the yield of the oil obtained by n-hexane (21.11 ± 1.98 g per 100 g of dry seed) and the oil obtained by trichlorethylene (21.16 ± 2.35 g per 100 g of dry seed). Also, there is no statistically significant difference in the value of the saponification number between oil obtained by n-hexane and trichlorethylene (236.97 ± 14.36 and 238.73 ± 18.64 mg KOH per g of oil, respectively) and the iodine number (111.66 ± 14.25 and 117.54 ± 12.22 g iodine per 100 g oil, respectively), but there is a statistically significant difference in the value of the acid number (14.65 ± 7.89 and 45.95 ± 8.26% of the oleic acid, respectively) and the ester number (207.81 ± 4.45 and 147.32 ± 17.65 mg KOH per g of oil, respectively). The antioxidant activity of determined as scavenging capacity of DPPH radicals showed that the oil obtained by trichlorethylene had a slightly better antioxidant activity (IC_{50} value was 30.95 mg/mL) than oil obtained by n-hexane (IC_{50} value was 33.87 mg/mL).

As the trichlorethylene is the cheaper solvent than hexane, results show it can be successfully to obtain the oil from nettle seeds in order to further analyze its antioxidant and other properties.

Key words: *Nettle, Seeds, Oils, Solvents, Chemical numbers, Antioxidant activity*.

1. Introduction

Plant seeds represent important sources of oils with a high nutritional, nutraceutical and industrial importance. These oils are mainly composed of tri- acylglycerols, di- acylglycerols, free fatty acids, phospholipids, glycolipids and other components. Triacylglycerols make the primary component in oils containing long-chain unsaturated fatty acids, with a great importance in the human diet, both in terms of nutritional value and in terms of organoleptic properties (smell and taste). Among the other components such as unsaponifiable components include phenolic compounds, squalene, phytosterols, tocopherols and carotenoids. These components are known as antioxidants that have the ability to extend shelf life of the oils, reduce the risk of cardiovascular, carcinogenic and other diseases [1 - 4].

Stinging nettle (*Urtica dioica* L.) is a perennial cosmopolitan plant which belongs to the *Urticaceae* family [5], and it is widespread throughout the Europe and USA [6]. Many studies have shown that nettle is a good source of proteins, carbohydrates, fats and dietary fibber [7], vitamins, pro-vitamins and minerals [8] and phytochemicals among which are phenolics. Nettle have been used in traditional medicine since ancient times due to its components have a wide spectrum activities, such as antioxidant, antimicrobial, antiulcer, analgesic etc. [9].

Nettle seed oil also has health-beneficial aspect due to its composition. It is rich in unsaturated fatty acids (oleic (C18:1, 19.88%) and linoleic (C18:2, 66.37%) [10], natural antioxidants, such as phenolics and tocopherols, which

play a role in protecting against free radicals reaction and oxidative stress. It was found that the oleic acid as a monounsaturated fatty acid have important physiological role [11]. Oxidation of polyunsaturated fatty acids has always been the biggest problem for sustainability of oils. Unsaturated fatty acids with two or three unsaturated bonds in a molecule are subject to oxygen addition, whereby hydroperoxides being formed as the primary oxidation products [12]. The primary oil oxidation products are highly unstable and are easily decomposed to secondary oxidation products such as aldehydes, ketones, alcohols and acids, which lead to lowers the sensory and nutritional quality, as well as the great economic loses to the food industry [13]. On the other hand, most secondary oxidation products can interact each other and cause chain oxidation processes in vivo, which contribute to the development of many diseases. Phytochemicals, such as tocopherols, carotenoids, fatty acids, phenolic compounds present in the oils [14, 15] provide protection of the oil against the oxidation reaction and at this way reduce the ability to create primary and secondary products.

In the present study the oil from nettle seeds was extracted by using reflux and n-hexane and trichloroethylene as solvents and chemical numbers and antioxidant activity were determined. The aim of study was to determine the differences between the obtained oils in chemical composition and antioxidant activity and to select the solvent for further oil extraction and analyse its properties.

2. Materials and Methods

2.1 Plant material

The nettle seed ("Jeligor" from Svrljig in Serbia) was purchased in the local store. For analysis, the seed was milled to the overall particle size of 0.4 mm.

2.2 The extraction and content of oil

The milled nettle seeds (30 g) were put into an Erlenmeyer flask 300 mL of n-hexane (VWR, Czech Republic) or trichloroethylene (MosLab, Serbia) was added and extracted for 30 minutes, under reflux and by mixing (200 min^{-1}) at solvent boiling temperature. The extract was separated under the weak vacuum, eluted by water (3 × 30 mL) and the eluted extract volume was recorded. An aliquot (3 mL) was taken and put into the disk plate analyser (Scaltec SMO 01, Scaltec instruments, Germany) to dry at 110 ^{0}C to a constant weight. The content of the oil residue was read out on the display, yield calculated and expressed in g of oil per 100 g of dry seed.

2.3 Chemical numbers

The acids (AN), saponification (SN), and peroxide (PN) number of oils were determined by using the standard procedures by Official AOCS Methods [22], AOCS Official Method Cd 3-25 [23], and AOCS Official Method Cd 8b-90 [24], respectively. The ester number (EN) was calculated as difference in saponification and acids number (SN - AN).

2.4 HPLC analysis

For the HPLC analysis, Holčapek *et al.*, [16], modified method as it was already described [17]. The mono-, di- and tri-acilglycerols were identified comparing retention times of the lipid components to retention times of standards. The percentage of the mono-, di-, and tri-acylglycerols in the oil was computed based on HPLC peak area and and expressed g of the acylglycerols per 100 g of oil.

2.5 DPPH radical scavenging capacity (DPPH SC)

The DPPH SC of the oil extracts was determined by the method described by Mensor *et al.*, [18]. The method has been modified so that ethyl acetate was used to dissolve the oil and DPPH instead of the methanol, which is usually used when the antioxidant activity of extracts of the phenolics is investigated. The oil was dissolved in ethyl acetate in a ratio of 1 : 4 v/v, the absorbance at 517 nm was measured and converted into the percentage of radical SC (%):

$$\text{DPPH SC (\%)} = 100 - \left[\left(A_{sample} - A_{blank} \right) \frac{100}{A_{control}} \right] \quad (1)$$

Where: A_{sample} is the absorbance at 517 nm of the ethyl acetate solution of the oil (2.5 mL) treated by the DPPH radical solution dissolved in ethyl acetate, A_{blank} is the absorbance at 517 nm of the ethyl acetate solution of the oil (1 mL of ethyl acetate added to 2.5 mL of oil), and $A_{control}$ is the absorbance at 517 nm of ethyl acetate solution of the DPPH radical (1 mL of a 0.3 mM added to 2.5 mL of ethyl acetate). The IC_{50} value was determined by using Microsoft Excel ed50plus v1.0 software (INER/México) and expressed as mg of the oil per mL in ethyl acetate, and tocopherol was used as a standard.

2.6 Statistical analysis

The results were presented as the mean of three determinations ± standard deviation. In order to compare the differences in mean values a statistical analysis by t-test, at 5% level was done.

3. Results and Discussion

The results of the oil yield extracted by using two solvents, n-hexane and trichloroethylene and their chemical characterization by chemical number are presented in Table 1.

Table 1. The yield and chemical numbers of oil obtained by extraction by using n-hexane and trichloroethylene*

Yield of the oil and chemical number / Solution for oil extraction	n-Hexane	Trichloroethylene
Oil content (g per 100 g of dry seed)	21.11±1.98[a]	21.16 ± 2.35[a]
Acids number (% of oleic acid) (mg KOH per g of oil)	14.65 ± 3.89[a] 29.16 ± 5.21[a]	45.95 ± 8.26[b] 91.41 ± 11.32[b]
Saponification number (mg KOH per g of oil)	236.97 ± 14.36[a]	238.73 ± 18.64[a]
Iodine number (g iodine per 100 g oil)	111.66 ± 14.25[a]	117.54 ± 12.22[a]
Ester number (mg KOH per g of oil)	207.81 ± 4.45[a]	147.32 ± 17.65[b]

Legend: *Within rows, values assigned to different letters are significantly different at ($p < 0.05$).

Results showed there was no statistically significant difference ($p > 0.05$) of the yield of the oil obtained by n-hexane (21.11 g/100 g of dry seeds) and the oil obtained by trichloroethylene (21.16 g/100 g of dry seeds). In the literature there are data on yield of the oils obtained from nettle seeds after n-hexane extraction in Soxhlet apparatus [10]. The result was higher (30.68%) than these presented in our study, and this difference may be attributed to different technique of the oil extraction or geographic origin of seeds. Also, there is no statistically significant difference in the value of the saponification and the iodine number between investigated oils, but there is a statistically significant difference in the value of the acid and the ester number. This difference and higher acid number could indicate the oil obtained by trichloroethylene has a higher content of free and a lower content of ester-bound fatty acids in the acylglycerols, compared to the oil obtained by n-hexane. The chemical characterisation of oil extracted by petroleum ether from mature nettle seeds showed that the acid number, saponification and iodine number was 6.1 mg KOH per g of oil, 186.8 mg KOH per g of oil, 151.2 g iodine per 100 g of oil, respectively [19]. This research suggests a lower content of free fatty acids, a higher content of fatty acids with higher molecular weight in composition of acylglycerols and a higher degree of unsaturation of oil, compared to oils obtained in our research. These differences in addition to the origin of the seeds may also be due to the different solvent used.

The HPLC chromatograms of the oils extracted by using n-hexane and trichloroethylene are shown In Figure 1.

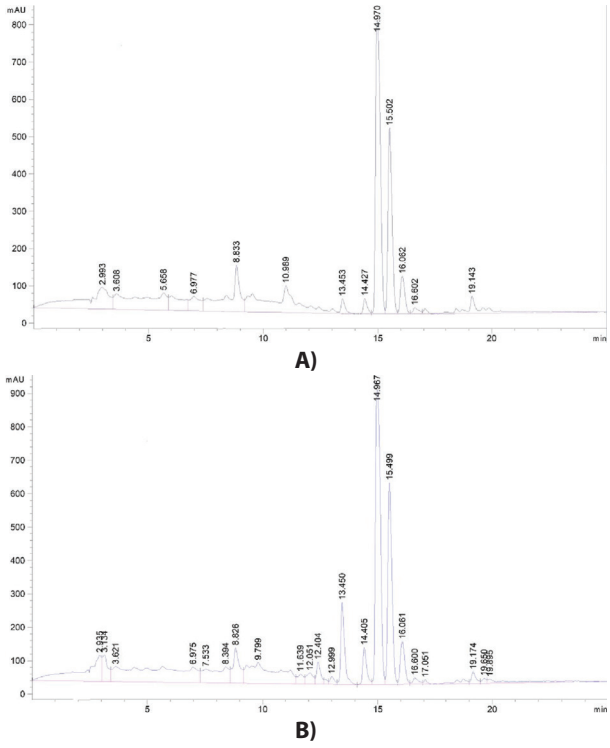

Figure 1. HPLC chromatogram of the oil extracted by using n-hexane (A) and trichloroethylene (B)

The statistically significant difference between oils extracted by n-hexane and trichloroethylene also existed in the content of monoacylgycerols and the higher content of monoacylglycerols was found in the oil extracted by trichloroethylene. In the literature there are data on the content of acylglycerols in soybean oil obtained by trichloroethylene by using reflux extraction [17]. This research demonstrate the lower values of content of the mono-, di- and tri-acylglycerols in comparison with values of these components in nettle oil extracted by trichloroethylene in our research. Also, the research of authors D'Alonzo *et al.*, [20], show the lower content of mono-, di- and tri-acylglycerols in the oil from rapeseed, which was analysed by high-temperature glass capillary column gas chromatography after derivatization of fatty acids, mono- and diacylglycerol with (N,O)-bis (trimethylsilyl) trifluoroacetamide. The reason for lower acylglycerols content could be due to the origin of the seed from different plant species and different method of quantification of acylglycerols in the oil.

The results also indicate that the choice of solvent for the extraction of the oil from the nettle seeds is important and has an effect on the chemical characteristics of the obtained oil such as acid number, ester number and monoglycerols content.

The results of the content of acylglycerols in the oil from nettle seeds extracted by using the n-hexane and trichloroethylene are presented in Table 2. The results showed, among acylglycerols, the content of triacylglycerols was the highest (over 95%), and the statistically significant difference ($p > 0.05$) between the oil obtained by n-hexane and oil obtained by trichloroethylene existed by the content of monoacylglycerols.

Table 2. The content of acylglycerols in the oil from nettle seeds extracted by using the n-hexane and trichloroethylene*

Acylglycerols/ The solvent for the oil extraction	n-Hexane	Trichloroethylene
Monoacylglycerols (g per 100 g of oil)	0.03 ± 0.02^a	1.38 ± 0.09^b
Diacylglycerols (g per 100 g of oil)	1.33 ± 0.38^a	2.92 ± 0.51^a
Triacylglycerols (g per 100 g of oil)	98.64 ± 6.32^a	95.70 ± 7.22^a

Legend: *Within rows, the values assigned to different letters are significantly different at ($p < 0.05$).

The results of DPPH scavenging capacity of the obtained oils depending on the oil concentrations are shown in Figure 2.

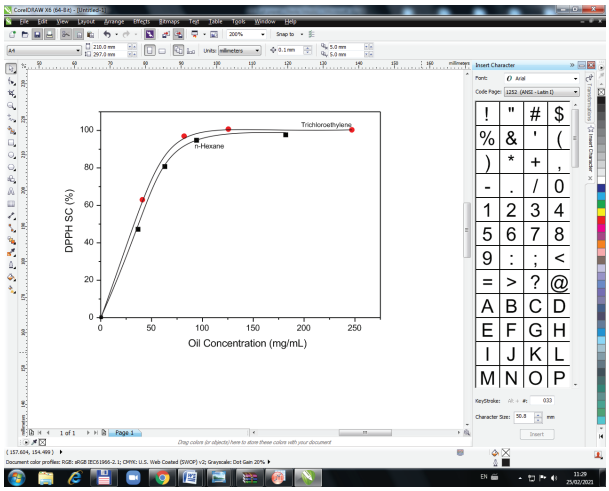

Figure 2. DPPH scavenging capacity of the oil obtained by n-hexane and trichloroethylene depending on the oil concentrations 2

Based on the obtained curves the IC_{50} values are obtained as concentration required to neutralize the 50% of presented DPPH radicals. The results showed that the oil obtained by trichloroethylene had a slightly better antioxidant activity (IC_{50} value was 30.95 mg/mL and 19.98 µg of tocopherol equivalent per mL) than the oil obtained by n-hexane (IC_{50} value was 33.87 mg/mL and 24.11 µg of tocopherol equivalent per mL). The acid number of oil extracted by trichloroethylene was higher than of the oil extracted by n-hexane, and the content of free fatty acids could be responsible for this. In literature there are data that support this thesis as fatty acid methyl esters from vegetable oils show antioxidant potential by the scavenging effect on DPPH radicals [21]. According to research of Uluata and Özdemir, [10], the DPPH-scavenging capacity of nettle seed oils was 46.01 mg Trolox/100 g of oil. However, since the results are presented in mg Trolox per 100 g of oil, it is hard to make proper comparison of these results to results obtained in our study where they are expressed as IC_{50} value in mg per mL.

Further analyses are planned to test the antioxidant activity of the nettle oil by other methods (RRAP, reducing power, etc.) and to analyse the fatty acids composition. Based on results obtained in this research, the trichloroethylene as a cheaper solvent than n-hexane can be used for extraction the oil from nettle seed.

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4. Conclusions

- The results of scavenging capacity of DPPH radicals showed that the oil obtained by trichloroethylene had a slightly better antioxidant activity (IC_{50} value was 30.95 mg/mL) than the oil obtained by n-hexane (IC_{50} value was 33.87 mg/mL), and the content of free fatty acids could be responsible for this.

- These findings indicate that from nettle seeds the oil can be obtained, which, because of its antioxidant potential, can be used for food, cosmetics and pharmaceutical applications.

- The results also show the trichloroethylene can be used to extract the oil from nettle seed for purpose further analyse of its antioxidant properties and composition.

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