Biologically active components and health benefits of nettle seed oil

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SUMMARY: The biologically active components of nettle seed oil and important lipid indices, which are criteria for the health benefits of the oil, have been examined. Linoleic acid predominates in triacylglycerols (77.7%), followed by oleic (16.2%). Sterols in the lipids are present at 1.1% and β-sitosterol is the main component (90.1%). The oil contains 711 mg/kg tocopherols and γ-tocopherol predominates (36.1%), followed by α-tocopherol (28.9%) and δ-tocopherol (26.9%). Atherogenicity and thrombogenicity index values are significantly low, which determine the best anti-atherogenic and anti-thrombogenic properties of the oil. The cholesterolemic index and the ratio of polyunsaturated and saturated fatty acids are considerably higher than 1.0 and reveal good hypocholesterolemic potential and nutritional value. The content of biologically active components of nettle seed oil indicates that it is a rich source of essential fatty acids, sterols and tocopherols and this oil can be used in food, cosmetics, and pharmaceutical products.

KEYWORDS: *Atherogenicity; Biologically active components; Cholesterolemic index; Health benefits; Nettle seed oil; Thrombogenicity*

RESUMEN: ***Componentes biológicamente activos y beneficios para la salud del aceite de semilla de ortiga.*** Se han determinado los componentes biológicamente activos del aceite de semilla de ortiga y los índices lipídicos más importantes, como criterios sobre los beneficios para la salud del aceite. El ácido linoleico predomina en los triacilgliceroles (77,7%), seguido por oleico (16,2%). Los esteroles son el 1,1% siendo el β-sitosterol el componente mayoritario (90,1%). Los tocoferoles son 711 mg/kg y predomina el γ-tocoferol (36,1%), seguido por α-tocoferol (28,9%) y δ-tocoferol (26,9%). Los valores de los índices de aterogenicidad y trombogenicidad son significativamente bajos, lo que determina las buenas propiedades antiaterogénicas y antitrombogénicas del aceite. El índice hipo/hipercolesterolémico y la proporción de ácidos grasos poliinsaturados y saturados presentan valores altos (superiores a 1,0), lo cual indica un buen potencial hipocolesterolémico y valor nutricional del aceite. El contenido de componentes biológicamente activos del aceite de semilla de ortiga muestra que es un producto rico de ácidos grasos esenciales, esteroles y tocoferoles, y por eso se puede utilizar en alimentos, cosméticos y productos farmacéuticos.

PALABRAS CLAVE: *Beneficios para la salud; Componentes biológicamente activos; Índice de aterogenicidad; Índice de trombogenicidad; Índice hipo/hipercolesterolémico*

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1. INTRODUCTION

Nowadays, many studies are focused on foods with a presence of biologically active components which not only meet nutritional needs, but also aid in the prevention of some diseases and disorders when the food is consumed regularly. These foods are stated as functional foods or nutraceuticals and according to DeFelice (1992) and Lobo *et al.*, (2010) they are foods or part of the foods that possess health and medical benefits to human mental and physical well-being. Many medicinal plants have been investigated in order to assess their health benefits, including the determination of major biologically active components such as essential fatty acids, sterols, tocopherols, phospholipids, etc. On the other hand, numerous plant species are not sufficiently investigated and one such plant is the stinging nettle (*Urtica dioica* L.).

The stinging nettle belongs to the Urticaceae family and represents a perennial plant. It is widespread throughout Europe, America and Asia in different areas from temperate to tropical and it is easily adapted to many climatic conditions (Di Virgilio *et al.*, 2015). The nettle is a food with high nutritional value and it is widely used for cooking (Guil-Guerrero *et al.*, 2003). The plant is also rich in essential amino acids, minerals, vitamins, unavailable carbohydrates and carotenoids (Guil-Guerrero *et al.*, 2003). Nettle oil is abundant in linoleic and linolenic acids (Guil-Guerrero *et al.*, 2003; Kamyab *et al.*, 2015; Rafajlovska *et al.*, 2001; Uluata and Özdemir, 2012), which not only increase the nutritional value of foods, but also exert beneficial effects on coronary heart disease in people (Guil-Guerrero *et al.*, 2003; Simopoulos, 2000).

Though the medicinal benefits of nettles have been known for centuries, the investigations on this plant were mainly focused on their leaves. Few researchers (Guil-Guerrero *et al.*, 2003; Uluata and Özdemir, 2012) examined the lipid composition of the nettle seeds and there were no thorough analyses on the biologically active components of their lipids. Therefore, the aim of the present study was to determine the main biologically active components (fatty acids, sterols and tocopherols) of nettle seed oil and to define atherogenicity, thrombogenicity, cholesterolemic indexes and the ratio of polyunsaturated and saturated fatty acids, which are important for the therapeutic effect of the oil.

2. MATERIALS AND METHODS

2.1. Materials

The plant used was identified as *Urtica dioica* L. grown in Southern Bulgaria. The weight of 1000 seeds was 0.15 g. The moisture of the seeds was 8.4%. The oil was obtained by cold pressing of the nettle seeds in a small factory in Bulgaria. The oil yield was 22.7% (on dry basis).

2.2. Fatty acid composition

The fatty acid composition of triacylglycerols was determined by gas chromatography (GC) (ISO 12966-1:2014). Fatty acid methyl esters (FAMEs) were prepared by pre-esterification of the triacylglycerols with sulfuric acid in methanol (ISO 12966-2:2017). The determination of FAMEs was performed on HP 5890 gas chromatograph equipped with a 75 m x 0.18 mm x 25 μm (film thickness) capillary Supelco column and a flame ionization detector. The column temperature was programmed from 140 °C (held 5 min), at 4 °C/min to 240 °C (held 3 min); the injector and detector temperatures were set at 250 °C. Identification was performed by comparison of the retention times with those of a standard mixture of FAME (Supelco, USA 37 comp. FAME mix) subjected to GC under identical experimental conditions. The limit of detection in GC was 0.05%. For the quantification of fatty acids ISO 12966-1:2014 was used.

2.3. Tocopherols

Tocopherols were determined directly in the oil by high performance liquid chromatography on a Merck-Hitachi (Merck, Darmstadt, Germany) instrument equipped with 250 mm x 4 mm Nucleosil Si 50-5 column and fluorescent detector Merck-Hitachi F 1000. The operating conditions were mobile phase of hexane: dioxane, 96:4 (v/v) and flow rate 1 mL/min, excitation 295 nm, emission 330 nm. 20 μL 2% solution of crude oil in hexane were injected. Tocopherols were identified by comparing the retention times with those of authentic individual ones. The tocopherol content was calculated on the base of tocopherol peak areas in the sample vs. tocopherol peak area of standard tocopherol solution (ISO 9936:2016). The limit of detection in HPLC was 0.05%.

2.4. Sterols

Unsaponifiables were determined after saponification of the glyceride oil and extraction with hexane (ISO 18609:2000). Quantification of sterols was carried out spectrophotometrically (at 597 nm), after the isolation of sterols from other unsaponifiable matter by TLC (Ivanov *et al.*, 1972). For the determination of total sterols an analytical curve was constructed by using a standard solution of β-sitosterol – the concentration ranged from 0 to 3000 μg/mL. The linear regression coefficient (R^2) was 0.9985, the limit of detection (LOD) was calculated to be 95 μg/mL and the limit of quantification (LOQ) was 315 μg/mL.

Sterol composition was determined on a HP 5890 gas chromatograph equipped with 25 m x 0.25 mm DB – 5 capillary column and flame ionization detector. Temperature gradient from 90 °C (held 3 min) up to 290 °C at a rate of change of 15 °C/min and then up to 310 °C at a rate of 4 °C/min (held 10 min); detector temperature – 320 °C; injector temperature – 300 °C hydrogen as carrier gas. Identification was confirmed by the comparison of retention times with those of a standard mixture of sterols containing cholesterol (stabilized, purity 95%, New Jersey, USA), stigmasterol (Sigma-Aldrich, purity 95%, St. Louis, MO, USA) and β-sitosterol (with ca 10% campesterol, ca 75% β-sitosterol, New Jersey, USA) (ISO 12228-1:2014). The limit of detection in GC was 0.05%.

2.5. Lipid indices

Index of atherogenicity. The index of atherogenicity (IA) was calculated on the basis of the fatty acid composition of the oil according to the following formula (Ulbricht and Southgate, 1991):

$$IA = \frac{C_{12:0} + 4 X C_{14:0} + C_{16:0}}{\sum MUFA + \sum PUFA} \qquad (1)$$

Where ΣMUFA is the amount of monounsaturated fatty acids; ΣPUFA – polyunsaturated fatty acids; $C_{12:0}$ – lauric acid; $C_{14:0}$ – myristic acid; $C_{16:0}$ – palmitic acid.

Index of thrombogenicity. The index of thrombogenicity (IT) is defined as the ratio between prothrombogenic (SFA) and antithrombogenic (MUFA, n-3 and n-6 PUFA) fatty acids (Ulbricht and Southgate, 1991). It was calculated according to the formula:

$$IT = \frac{C_{14:0} + C_{16:0} + C_{18:0}}{0.5 \times \sum MUFA + 0.5 \times \sum n-6PUFA + 3} \qquad (2)$$
$$\times \sum n-3PUFA + \frac{\sum n-3PUFA}{\sum n-6PUFA}$$

Where ΣMUFA is the amount of monounsaturated fatty acids; Σ n-6 PUFA – polyunsaturated fatty acids (n-6); Σ n-3 PUFA – polyunsaturated fatty acids (n-3); $C_{14:0}$ – myristic acid; $C_{16:0}$ – palmitic acid. $C_{18:0}$ – stearic acid.

Cholesterolemic index. Cholesterolemic index (h/H) was calculated by the following formula (Santos-Silva *et al.*, 2002):

$$(h/H) = \frac{\begin{array}{c}C_{18:1n-9} + C_{18:2n-6} + C_{18:3n-3} + C_{18:3n-6} + C_{20:2n-6} \\ + C_{20:3n-3} + C_{20:4n-6} + C_{20:5n-3} + C_{22:6n-3}\end{array}}{C_{12:0} + C_{14:0} + C_{16:0}} \qquad (3)$$

$C_{18:1n-9}$ – oleic acid; $C_{18:2n-6}$ – linoleic acid; $C_{18:3n-3}$ – α-linolenic acid; $C_{18:3n-6}$ – γ-linolenic acid; $C_{20:2n-6}$ – eicosadienoic acid; $C_{20:3n-3}$ – eicosatrienoic acid; $C_{20:4n-6}$ – arachidonic acid; $C_{20:5n-3}$ – eicosapentaenoic acid; $C_{22:6n-3}$ – docosahexaenoic acid; $C_{12:0}$ – lauric acid; $C_{14:0}$ – myristic acid; $C_{16:0}$ – palmitic acid.

2.6. Statistical analysis

All measurements were performed in triplicate (n = 3) and the results were presented as mean value with the corresponding standard deviation (SD) (Microsoft Excel software).

3. RESULTS AND DISCUSSION

3.1. Biologically active components

Phytosterols, tocopherols, phospholipids and essential fatty acids are considered bioactive compounds. The latter ones are such compounds that possess pharmacological or toxicological effects on humans and animals (Bubalo *et al.*, 2018).

The content of biologically active components (sterols and tocopherols) of nettle seed oil is shown in Table 1.

TABLE 1.   Biologically active components of nettle seed oil[*]

Biologically active components	Content
Unsaponifiable matters, %	3.2 ± 0.2
Total sterols, %	1.1 ± 0.2
Total tocopherols, mg/kg	711 ± 12

*Values are means ± SD (n = 3)

The unsaponifiable matter was detected at 3.2% from all lipids and total sterols were found to be 1.1% in the oil. This was in agreement with the sterol content of some commonly-used vegetable oils (from 0.1 to 1.3%) (Codex Stan 210, 1999). Sterols are regarded as preventive compounds that have the capacity to reduce plasma cholesterol and LDL cholesterol (Popova *et al.*, 2018). The total tocopherol content of nettle seed oil was rather high (711 mg/kg). This result differed from previous studies where total tocopherols were lower (411.05 mg/kg in the oil) (Uluata and Özdemir, 2012). It is well-known that tocopherols are natural antioxidants and the most common in plants is α-tocopherol, mainly in the green parts, but tocotrienols can also be found in the seeds. These compounds have the ability to scavenge the lipid peroxy radicals and threfore prevent the oils from lipid peroxidation (Munné-Bosch and Alegre, 2002).

Tocopherol and sterol composition of the examined nettle seed oil is presented in Table 2.

TABLE 2. Tocopherol and sterol composition of nettle seed oil*

Tocopherols (% of total tocopherols)		Sterols (% of total sterols)	
α-Tocopherol	28.9 ± 0.2	Cholesterol	0.7 ± 0.1
β-Tocopherol	6.2 ± 0.1	Campesterol	0.7 ± 0.2
γ-Tocopherol	36.1 ± 0.3	Stigmasterol	8.5 ± 0.2
γ-Tocotrienol	2.1 ± 0.1	β - Sitosterol	90.1 ± 0.4
δ-Tocopherol	26.9 ± 0.2		

*Values are means ± SD (n = 3)

γ-Tocopherol (36.1%) predominated in the oil, followed by α-tocopherol (28.9%) and δ- tocopherol (26.9%). The content of β-tocopherol (6.2%) in the nettle seed oil was rather high and a small amount of γ-tocotrienol (2.1%) was also detected. The present results differed from those reported by Uluata and Özdemir (2012), who established that the content of γ-tocopherol was considerably higher (379.29 mg/kg or 90.6% of the tocopherol fraction), while α-tocopherol and δ-tocopherol were present at 34.96 mg/kg (8.5%) and 3.80 mg/kg (0.9%), respectively. On the other hand, these authors did not identified β-tocopherol or γ-tocotrienol.

The sterol composition of nettle seed oil was determined for the first time and the major sterol in the oil was β – sitosterol (90.1%). A small quantity of stigmasterol was also detected (8.5%) and negligible amounts of cholesterol (0.7%) and campesterol (0.7%) were identified. The high content of β – sitosterol make nettle seed oil suitable for regular consumption in order to decrease serum cholesterol.

3.2. Fatty acids

The fatty acid composition of the examined nettle seed oil is presented in Table 3.

Eleven fatty acids were identified in the nettle seed oil. Linoleic acid (77.7%) predominated in the triacylglycerol fraction, followed by oleic acid (16.2%). The amount of palmitic acid was considerably lower (3.7%). In the lipid fraction of the examined glyceride oil n-3 fatty acids such as linolenic (0.4%), eicosatrienoic (0.2%) and eicosapentaenoic (0.1%) acid were also identified. The other fatty acids were present in small quantities from 0.1% to 0.8%.

The current results on the fatty acid composition of nettle seed oil did not correspond to the findings of Guil-Guerrero *et al.*, (2003), who reported that the quantity of linoleic acid was much lower (22.7%), while the content of linolenic acid was higher (6.6%) than in the present study. On the other hand, Uluata and Özdemir (2012) reported that the contents of linoleic, oleic and palmitic acid were 66.37, 19.88 and 7.57%,

respectively. Interestingly enough, the fatty acid composition of nettle seed oil and nettle leaf oil differed to a great extent. According to Kamyab *et al.*, (2015) the major fatty acids in the oil from the leaves were margaric (35.0%) and linolenic acid (28.7%). Rafajlovska *et al.*, (2001) examined the fatty acid composition of nettle leaf oil obtained by supercritical carbon dioxide extraction and established that the content of palmitic, stearic, oleic, linoleic and linolenic acids were as follow: 6.8, 1.1, 3.6, 20.2 and 12.4%, respectively.

TABLE 3. Fatty acid composition of nettle seed oil*

Fatty acids, %		Content
$C_{14:0}$	Myristic	0.1 ± 0.0
$C_{14:1}$	Myristoleic	0.1 ± 0.0
$C_{16:0}$	Palmitic	3.7 ± 0.1
$C_{17:0}$	Margaric	0.6 ± 0.2
$C_{17:1}$	Heptadecaenoic	0.1 ± 0.0
$C_{18:0}$	Stearic	0.8 ± 0.1
$C_{18:1}$	Oleic	16.2 ± 0.3
$C_{18:2}$	Linoleic (n-6)	77.7 ± 0.5
$C_{18:3}$	Linolenic (n-3)	0.4 ± 0.1
$C_{20:3}$	Eicosatrienoic (n-3)	0.2 ± 0.0
$C_{20:5}$	Eicosapentaenoic (n-3)	0.1 ± 0.0
SFA[a]		5.2 ± 0.4
MUFA		16.4 ± 0.3
PUFA		78.4 ± 0.6

[a]SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids
*Values are means ± SD (n = 3)

The contents of saturated (SFA), mono- (MUFA) and polyunsaturated fatty acids (PUFA) in nettle seed oil is also shown in Table 3. Unsaturated fatty acids were the major fatty acids in nettle seed oil and comprised 94.8% of all fatty acids in the triacylglycerols. The amount of polyunsaturated fatty acids (PUFA) was significantly high (78.4%), where linoleic acid constituted 99.1% of the entire amount of PUFA. The presence of the latter fatty acid in the oil reveals its ability to reduce the incidence of heart disease and cancer (Uluata and Özdemir, 2012). The obtained results slightly differed from those reported by Uluata and Özdemir (2012), who established that the content of PUFA was 67.19%, MUFA – 19.94% and SFA – 12.87%.

3.3. Lipid indices

The biological completeness of oil depends largely on the content of certain groups of fatty acids which have a positive effect on human health. It is evaluated on the basis of basic lipid indices, a ratio between polyunsaturated and saturated fatty

TABLE 4. Ratio of polyunsaturated (PUFA) and saturated (SFA) fatty acids, atherogenic, thrombogenic and cholesterolemic indices of nettle seed oil*

Indices	Values
PUFA/SFA	15.1 ± 1.5
Index of atherogenicity	0.043 ± 0.002
Index of thrombogenicity	0.093 ± 0.005
Cholesterolemic index	24.993 ± 0.811

*Values are means ± SD (n = 3)

acids, the atherogenic, thrombogenic and cholesterolemic indexes (Table 4).

The ratio of polyunsaturated and saturated fatty acids plays an important role in determining the different properties of cell membranes that help maintain normal metabolism in cells. The PUFA/SFA ratio, which leads to reducing the risk of cardiovascular disease, is 1.0 – 1.5 (Kang *et al.*, 2005). This ratio was significantly higher in nettle seed oil than the recommended ratio, due to the high amount of polyunsaturated fatty acids in the oil. The PUFA/SFA ratio of the examined nettle seed oil was much higher than the same ratio of soy bean oil (4.39), maize oil (4.10), perilla oil (9.11), sesame oil (2.94) and palm oil (0.18) (Kang *et al.*, 2005).

The index of atherogenicity reveals the relationship between the sum of the major saturated fatty acids which are considered pro-atherogenic (fatty acids that cause an increase in cholesterol in blood because they are easily deposited onto the artery walls) and the major unsaturated fatty acids which have an anti-atherogenic effect (Cottin *et al.*, 2011). Anti-atherogenic lipids inhibit plaque accumulation and reduce the levels of esterified fatty acids and cholesterol, thus preventing the occurrence of micro- and macro-coronary diseases (Hooper *et al.*, 2006). The index of thrombogenicity determines the tendency for thrombogenesis in blood vessels. The indices of atherogenicity and thrombogenicity of nettle seed oil were extremely low (0.043 and 0.093, respectively), which showed good anti-atherogenic and anti-thrombogenic properties of the lipids (Ulbricht and Southgate, 1991).

The cholesterolemic index is related to the ratio of hypocholesterolemic and hypercholesterolemic fatty acids, which includes mainly saturated fatty acids – lauric, myristic and palmitic (Bonanorme and Grundy, 1988). The values of the cholesterolemic index above 1.0 are believed to be indicative of a better hypocholesterolemic potential of the lipids which makes them more suitable for consumption and their regular intake reduces the risk of cardiovascular disease (Barter *et al.*, 2007). The values for this index in the nettle seed oil was considerably higher than 1.0 (24.993) which revealed its good hypocholesterolemic potential.

The indices of atherogenicity and trombogenicity of nettle seed oil are significantly lower than those of olive oil (0.1250 and 0.3230, respectively), argan oil (0.1577 and 0.4498) and sesame oil (0.1235 and 0.3623), while the cholesterolemic index was about three times higher than of the mentioned oils (7.9151, 6.3417 and 8.0962, respectively) (Misajel and Carolina, 2017).

4. CONCLUSIONS

Biologically active components (fatty acids, sterols and tocopherols) of nettle seed oil were examined and important indices (atherogenicity, thrompogenicity, cholesterolemic index and ratio of PUFA/SFA) related to evaluation of the health benefits of the oil were determined for the first time. The oil is a rich source of biologically active components (sterols and tocopherols) and essential fatty acids (mainly linoleic acid). The low values of the indices of atherogenicity and thrombogenicity as well as the high value of the cholesterolemic index depict good anti-atherogenic, anti-thrombogenic properties and hypocholesterolemic potential of nettle seed oil. The ratio of PUFA/SFA was also high which confirmed the nutritional value of the oil. Based on the results, nettle seed oil possesses health benefits and can be a potential source for the isolation of bioactive compounds with possibilities for application in food, cosmetics, and pharmaceutical products.

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