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Evaluation of Physicochemical Properties of Nettle Leaf Oil

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ABSTRACT: In this project, different nettle plants from north regions of Iran including Tonekabon, Amol and Gorgan were collected. The oil was extracted using soxhlet extraction apparatus and was subjected to series of physical and chemical tests and determinations consisting of refractive index, melting point, smoke point, nonsaponifiable matter, Iodine value, saponification value, peroxide value, totox value, acid values, kreis test and fatty acids composition. Among the samples collected the one obtained from Gorgan had the highest oil content. Fatty acid analysis of the extracted oils indicated a high degree of unsaturation where linolenic acid accounted for 28.7 % of the total fatty acids with an unusual concentration of C17:0. The oil also showed a high concentration of nonsaponifiable matter where the sterol constituted the major part of this fraction.

Keywords: *Fatty Acids, Nettle Leaf Oil, Physicochemical Properties.*

Introduction

Nettle plant belongs to the family of Ceaeurtica, with the common name of stinging nettle perennial with 150-30 cm height. This plant has tetragonal green to purple stem which covers with cone-shaped hooks and piles (Bnouham *et al*., 2011; Constantine *et al*., 2006; Hirono *et al*., 2012). The root of this plant lies up and creepy. Since the leaves and stems are covered with cone-shaped piles therefore by touching these villis, they stick to the skin and their sting can produce itching and burning effect. Figure 1 present a conventional nettle plant structure. Different varieties of nettle consist of urtica dioica, urtica urens, urtica cannabinal L, urtica membranaceapoire, urtica Kiovensis Rogoff, urtica Pilulifera L. Among these varieties, urtica dioica has been highly regarded as a medicinal plant. Nettle Plant has dark and little seeds quite similar to linseed (Aksu & Kaya, 2004; Hirono *et al*., 2012).

Fig. 1. Nettle plant

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This plant is found in most mild and moderate regions of the world. In humid areas of Iran, especially in northern, western and central areas such as Shahrood, Shemiranat, Bastam, Esfahan, Kandovan, Karaj, Roudbar, Astara,… nettle might be grown. The most important chemical compounds of nettle consist of triglycerides, lecithin, proteins, tannin, formic acid, potassium nitrate, calcium nitrate, iron, vitamins and glycoside components (Aksu & Kaya, 2004; Farzami *et al.*, 2003).

Nettle herb is used for the treatment of infectious diseases, cure of anemia, improvement of prostatitis, reducing of liver toxicity, especially reducing of symptoms of arthritis (Lanteri *et al.*, 2002; Obertreis *et al.*, 1996). The natural compounds such as phenolic components are present in nettle oil that might be used as a secondary protection material. Nettle herb has antioxidant activities as well as antimicrobial effects (Miltman, 1990). Fatty acid analysis of nettle seeds has indicated a high degree of unsaturated and saturated fatty acids (Bnouham *et al.*, 2011; Constantine *et al.*, 2006). The quality of nettle oil depend on the quantity and kind of chemical compounds present in the oil. The concentration of polyphenols and tocopherols as well as fatty acid composition depends on the oil variety, method of extraction, climatic conditions and plant cultivation.

Therefore the aim of this study is to investigate the physicochemical characteristics of nettle leaf oil in three different regions of Iran's consisting of Tonekabon, Amol and Gorgan.

Materials and Methods

- Sample Preparation

Nettle plant samples randomly were taken from three different regions in north part of Iran and coded according to Table 1.

The leaves after cleaning and washing were dried in an oven at 80 °C for 3 h and were milled separately. Each sample is placed in a soxhlet extractor and the oil was extracted using n-hexane. All the chemicals used for these experiments were purchased from Merck chemical company of Germany.

Table 1. Harvesting regions and coding the samples

Samples	Harvesting area
A	Tonekabon
B	Amol
C	Gorgan

- Physical and Chemical tests

The amount of oil was determined by the application of Soxhlet method using hexane as the solvent for eight hours in triplicate order. Fatty acid composition of the oil was determined by methylation of the fatty acids followed by the application of methyl esters on to a HP-5890, Hewlett-Packard GC equipped with a 60m cpsill88 capillary column and flame ionization detector according to AOAC method number 963.22 where Helium was used as the carrier gas.

Refractive index, melting point, smoke point, iodine value, saponification value, P-anisidine value, acid value, peroxide value, totox value and nonsaponifiable matter determinations and fractionation and identification of fractions of nonsaponifiable matter were carried out according to Ghavami *et al.* (2008).

Results and Discussion

Figure 2 shows the oil contents of nettle leaves collected from different regions of Tonekabon, Amol and Gorgan. Among the samples examined, sample C had the highest oil concentration based on the dry weight. The moisture content of fresh nettle leaves is about 90%.

Figure 3 and table 2 show the fatty acid profile and composition of nettle oil obtained from Gorgan sample. The result indicated that margaric acid and alpha-Linolenic were the predominant fatty acids respectively.

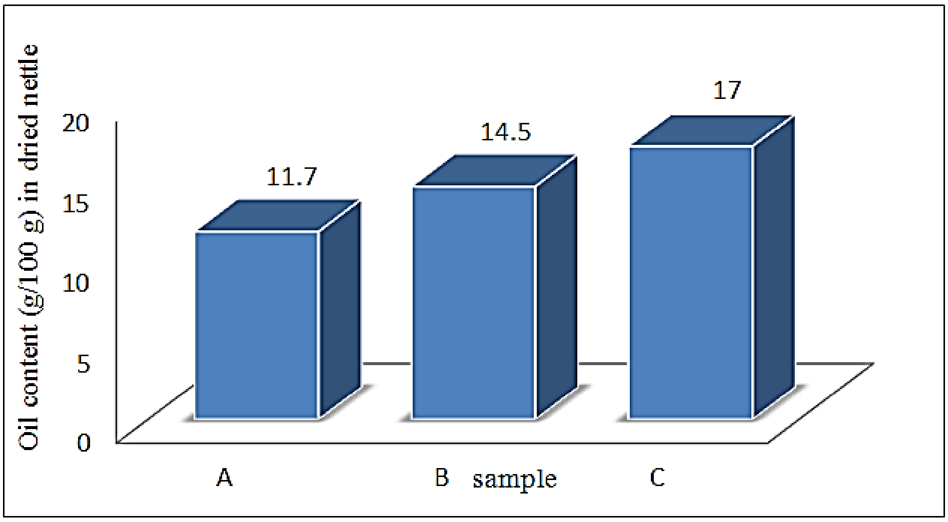

Fig. 2. Oil concentration in the samples of dried nettle leaves

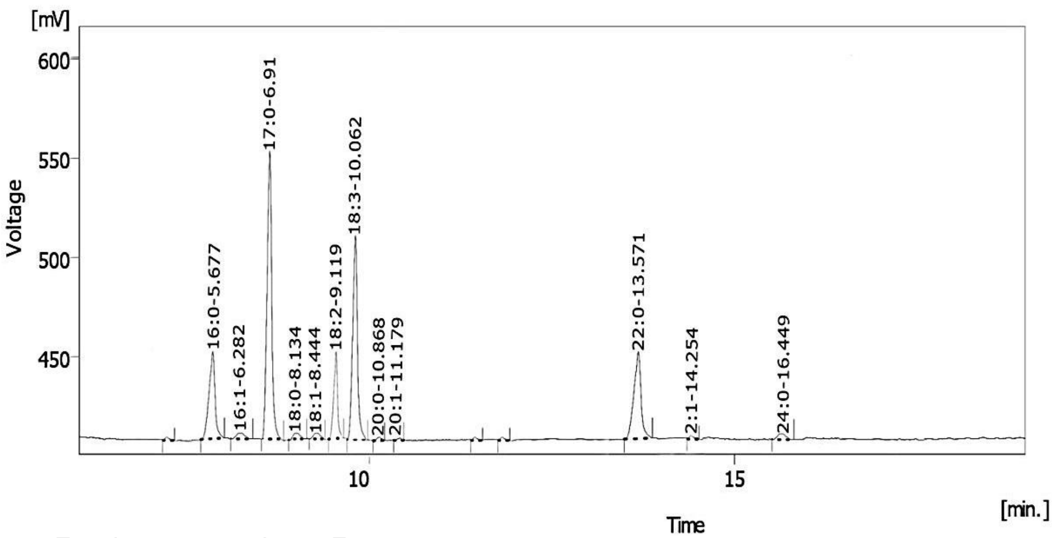

Fig.3. Fatty acid composition of nettle leaf oil

Margaric acid, the odd carbon and saturated fatty acid was the predominant fatty acid present. A result that was quite unexpected. A high concentration of alpha linolenic acid is present that make the oil quite susceptible to oxidation chain reactions and unfit for human consumption. However the oil might be employed for some other industrial applications. Linoleic acid and alpha - linolenic acid are essential fatty acids that beside controlling the blood cholesterol level, reduce the risk of heart diseases (Mavi *et al.*, 2004). It is therefore expected that nettle oil has also valuable nutritional properties. Fatty acid composition of nettle oil depends on several factors including species, growing region, climate and maturity of the plant (Mendez *et al.*, 1996).

High Calculated Oxidizability Value (Cox value) shows that the oxidative stability of nettle oil is low which can be attributed to the high levels of PUFA particularly alpha linolenic acid. The calculated Cox value nettle oil and its comparison with other edible oils are presented in Tables 3 and 4. The Cox value of oils is calculated by the percentage of eighteen-carbon unsaturated fatty acids that includes Oleic, linoleic and linolenic acids.

$$\text{Cox value} = \frac{[1(C18:1\%) + 10.3(C18:2\%) + 21.6(C18:3)]}{100}$$

Table 2. Fatty acid composition of nettle leaf oil

Fatty acid	**(%)**
C16:0	11.7
C16:1	0.4
C17:0	35.0
C18:0	0.3
C18:1	0.4
C18:2	11.2
C18:3	28.7
C20:0	0.3
C20:1	0.4
C22:0	11.1
C22:1	0.2
C24:0	0.3

One of the criteria for assessing nutritional properties of nettle oil, is the ratio of Omega-6 to Omega-3 fatty acids (ratio of linoleic acid to linolenic acid (C18: 2/C18: 3). This value in nettle oil is significant. MUFA / PUFA ratio is the symbol of oil resistance to oxidation reactions and on the other hand, the lower the resistance the higher is the nutritional value of oil due to presence of essential fatty acids. Nettle oil has valuable nutritional properties but low oxidative stability. In addition, the high ratio of PUFA / SFA in this oil confirms its high nutritional value.

Table 4 compares the Cox values of nettle oil with some edible oils such as olive, peanut, rice bran, canola, sesame, cottonseed, corn, sunflower seed and soybean oils. It should be considered that this oil shows low oxidative stability. The work carried out by Mendez *et al.* (1996) for the assessment of relative stability of fish oil using Rancimat method indicated that high ratio of polyunsaturated fatty acids to saturated fatty acids (PUFA / SFA) is a criterion for unsaturated content of oils and fats and represents a high oil affinity to oxidation reactions (Mendez *et al.*, 1996).

Table 3. Calculated oxidizability value of nettle leaf oil

Saturated fatty acids (SFA)	**58.7**
Monounsaturated Fatty Acids (MUFA)	1.4
Polyunsaturated Fatty Acids (PUFA)	39.9
C18:2/C18:3	0.39
PUFA/SFA	0.679
MUFA/PUFA	0.035
Calculated oxidizability value (Cox value)	7.35

Figure 5 represents the refractive indices of the oil samples. Refractive index and melting point are directly related to each other. The higher the melting point the higher is the refractive index. The results of figure 5 confirm this matter. Figure 6 presents the smoke point of the oil samples. It is worth to mention that nettle oil is relatively sensitive to thermal process and quickly is oxidized and converted into secondary oxidation products namely aldehydes, ketones, acids and alcohols.

Figure 4 shows the melting points of three samples of nettle oil. As the results show sample A has the highest melting point followed by samples C and B.

Table 4. Comparison of calculated oxidizability value nettle leaf oil and other edible oils

Vegetable oil	**Oleic acid**	**Linoleic acid**	**Linolenic acid**	**Calculated oxidizability value**
Nettle leaf oil	0.4	11.2	28.7	7.35
Olive oil	55-83	3.5-21	0-1.5	2.15
Peanut oil	41.3-67.4	13.9-35.4	-	3.08
Rice bran oil	35-50	45	0.5-1.8	4.48
Canola oil	61.6	21.7	9.6	4.92
Sesame oil	35.5-50	35.5-50	1	5.04
Cottonseed oil	13-44	33-58	0.1-2.1	5.21
Corn oil	19.5-30.4	43-65.3	1.2-2.1	6.69
Sunflower oil	18.6	68.2	0.5	7.31
Soybean oil	23.4	53.2	7.8	7.55

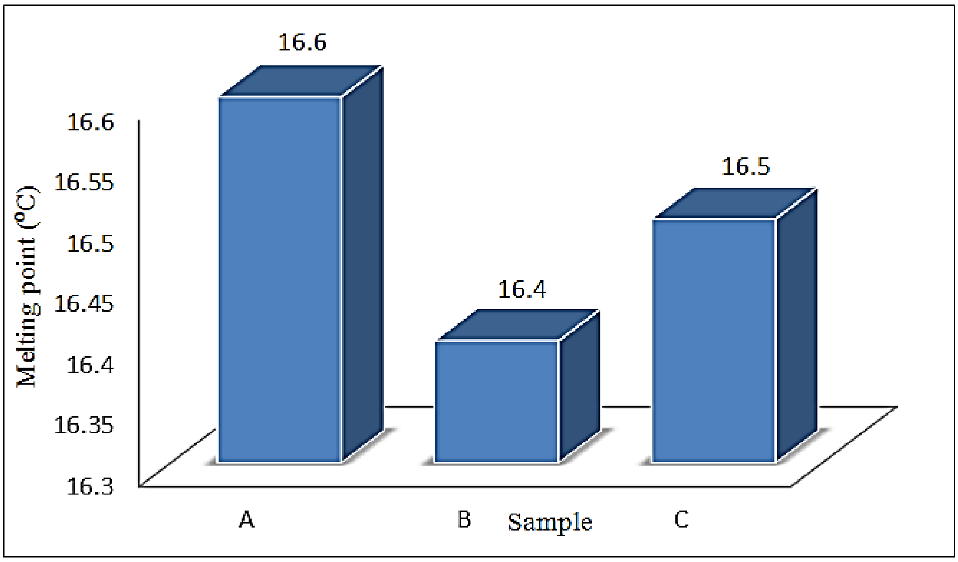

Fig. 4. Melting point of nettle leaf oil samples

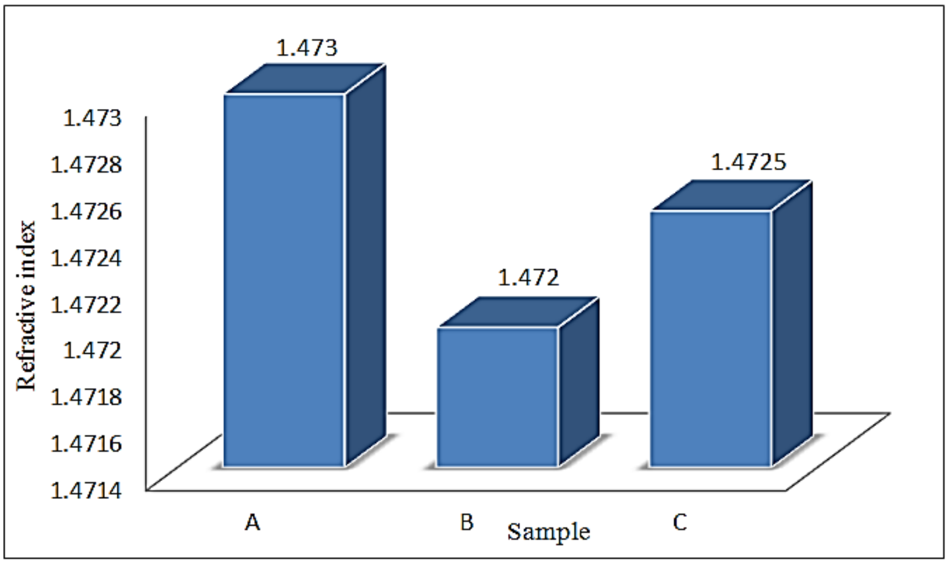

Fig. 5. Refractive indices of nettle leaf oil samples

The Iodine and saponification values of the oil samples are presented in figures 7 and 8. Iodine value of nettle leaf oil is in the range of 175-180 that is higher value than other vegetable oils such as sunflower; 113-143, corn; 109-133, cottonseed; 99-133, soybean; 117-141, oils (Lanteri *et al.*, 2002). The higher the unsaturation of oil the lower its resistance against oxidation. Among the samples examined sample C had the highest amount of unsaturation.

Saponification value that indicates the average molecular weight of the fatty acids decreases with increasing the length of fatty acids chain (Miltman, 1990). Nettle oil contains long chain fatty acids therefore its saponification value is lower than other common oils like olive, sesame, soybean, peanut and corn oils.

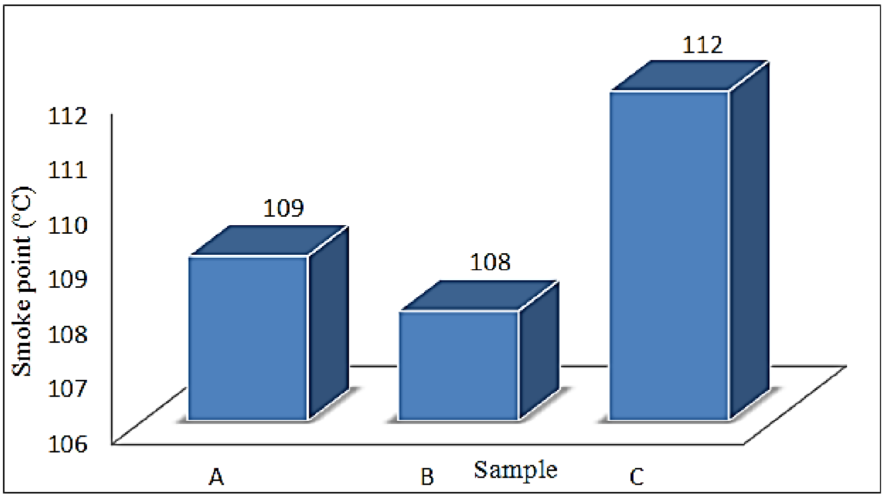

Fig. 6. Smoke point of nettle leaf oil samples

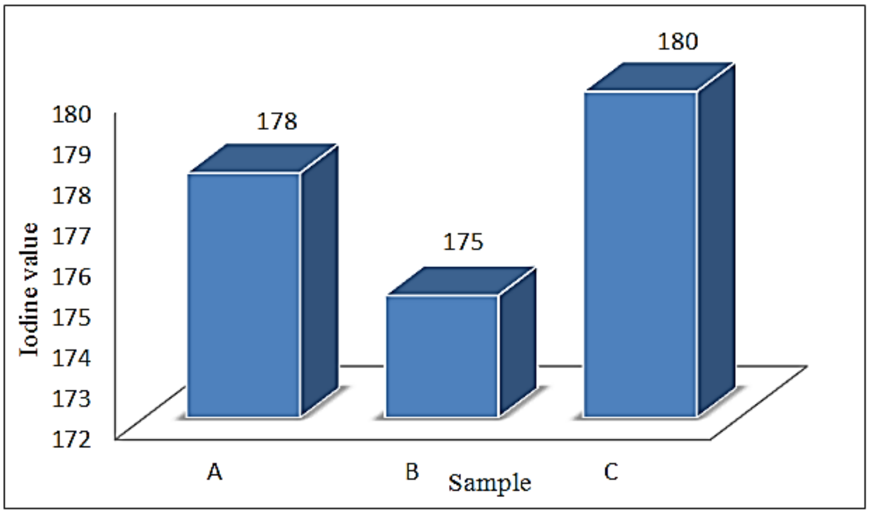

Fig. 7. Iodine value of nettle leaf oil samples

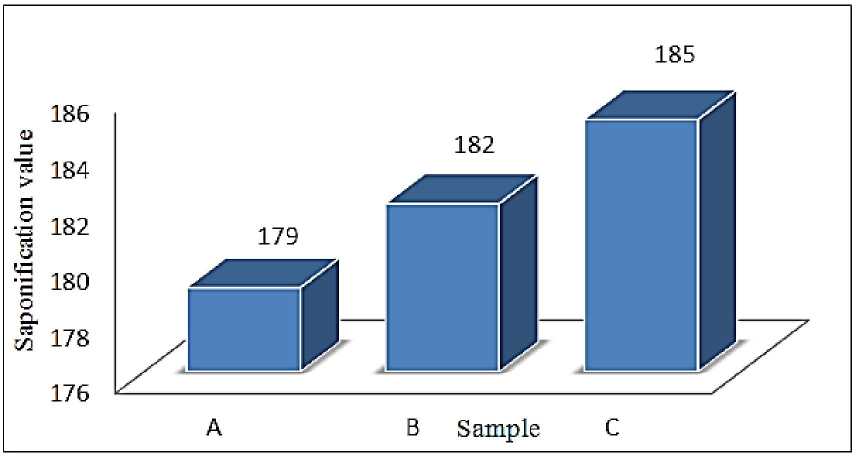

Fig. 8. Saponifiable matters of nettle leaf oil samples

Figure 9 shows the amount of nonsaponifiable matters as g per 100 g of the oil. Vegetable oils are mainly composed of triacylglycerols together with small quantities of free fatty acids, monoacyl glycerols, diacylglycerol and nonsaponification matters. The glyceride part of the lipid composition reacts with KOH or NaOH and turns into water soluble soap. This part is called saponifiable part of the oils. Those compounds that do not react with NaOH or KOH are called nonsaponifiable parts of the oils. Although, this part is not considerable in term of quantity but have interesting functions and play important roles in preventing the oils and fats from spoilage. The nonsaponifiable matters consist of different classes of chemical compounds and each class is made of different components like tocopherols, sterols and hydrocarbons. In general, edible oils depending on the severity of refining operation contain varying amounts of unsaponifiable matters. Therefore, these compounds might be used as an indicator for originality and control of purification process (Obertreis, 1996). The amount of nonsaponifiable matters in vegetable oils is usually less than 2% (Gülçin *et al.*, 2004; Gulcan *et al.*, 2006). Nettle oil contains 6.2% nonsaponifiable matters.

Figure 10 shows the identification of different components of nonsaponifiable matters on Thin Layer Chromatography. As it is observed the sterol fraction constitutes the major fraction of the nonsaponifiable matter.

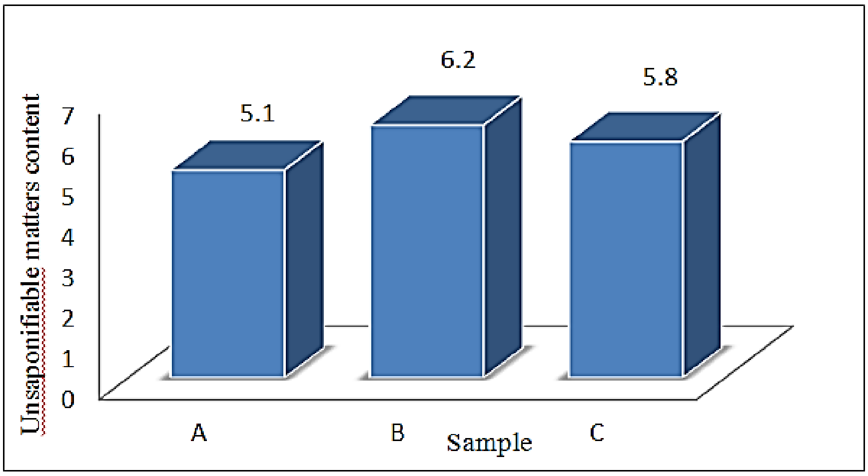

Fig. 9. Nonsaponifiable matters of nettle leaf oil samples

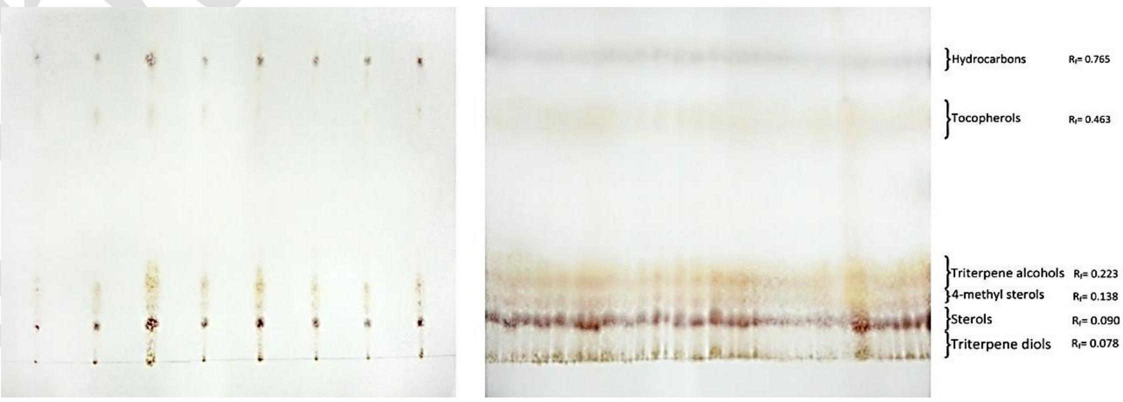

Fig. 10. TLC fractionation of the nonsaponifiable matter of nettle leaf oil

The results of Kreis test were negative for all the three samples examined. Figure 11 shows the acidity or percentage of free fatty acids of nettle oil samples. This value shows the degree of hydrolysis of the oil in the presence of lipase or by heating in the vicinity of humidity.

Figure 12 indicates the peroxide value of the extracted oil. Peroxide value measures the primary oxidation product. In the presence of pro-oxidant such as light, heat, oxygen and metals like iron and copper, free radicals are created. These radicals, with their high affinity to bind with oxygen, turn into peroxide radicals and eventually hydroperoxides. Since the hydroperoxide is an unstable composition at elevated temperature and quickly turns into the secondary oxidation products such as aldehydes, ketones, acids and alcohols, therefore this method might not be a reliable method for evaluating the condition of the oil, however the figures determined indicate that the oil samples have acceptable peroxide value.

Figure 13 shows Totox value that is based on hydroperoxide, the primary oxidation product and also the anisidine value the secondary oxidation product.

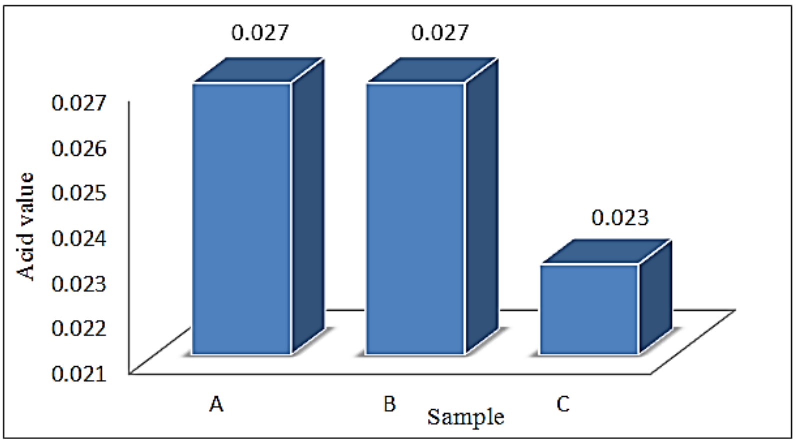

Fig. 11. Acid value of nettle leaf oil samples

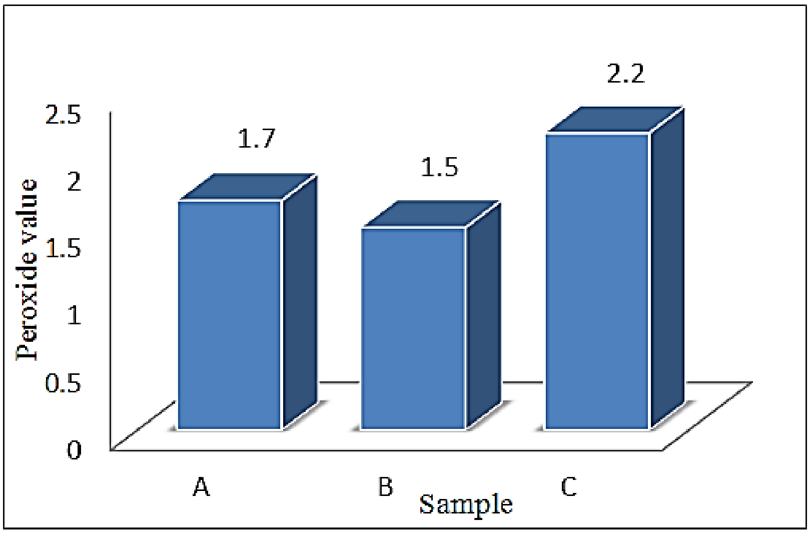

Fig. 12. Peroxide value of nettle leaf oil samples

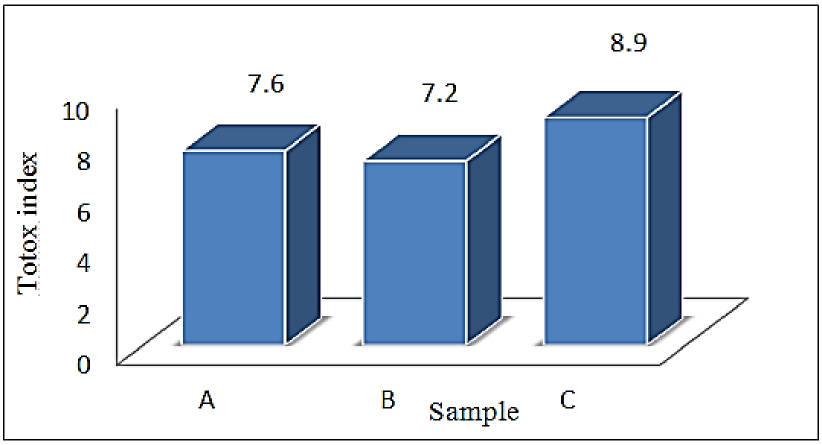

Fig. 13. Totox index of nettle leaf oil samples

Conclusion

Nettle (Dioica) is a medicinal plant that grows in many parts of Asian, European and American countries. The stem, root and leaf of nettle are used to cure many diseases. The young and fresh stems and leaves of nettle are rich in ascorbic acid which is used to treat Scurvy (disease due to lack of vitamin C). The most important and effective compounds present in nettle are acetylcholine, caffeic acid, formic acid and flavonoids. The root of nettle plant contains tannins, coumarins, triterpenes and sterols namely β-sitosterol. β-sitosterol is the major sterol present in nettle leading the relief of symptoms associated with hypertrophy of prostate tissue. Fatty acid composition of nettle leaf oil shows the presence of valuable essential fatty acids. The oil is rich in antioxidants such as tocopherols. The antimicrobial and antioxidant effects of nettle oil might recommend this oil to be employed in food, pharmaceutical and cosmetic industries.

References

Aksu, M. I. & Kaya, M. (2004). Effect of usage Urticadioica L. on microbiological quality of Turkish sucuk (Turkish style dry-fermented sausages). Food Control, 90, 2, 591-598.

Bnouham, M., Merhfour, F.Z., Ziyyat, A., Mekhfi, H., Aziz, M. & Legssyre, A. (2011). Antihyperglycemic activity of the aqueous extract of Urticadioica. Fitotrapia, 74, 677–681.

Constantine, F. D., Karmen, G. & Baroody, G. M. (2006). Effect of Urticadioica extract upon blood lipid profile in the rats.Fitoterapia, 77, 183–188.

Farzami, B., Ahmadvand, D., Vadasbi, S., Majin F. & Khaghani, K. H. (2003). Induction of insulin secretion by a component of urticadioica leave extract in perifused islets of langerhans and its in vivo effects in normal and streptozotocin diabetic rats. Journal of Ethnopharmacology, 89, 47–53.

Firestone, D. (1997). Official Methods of Analysis of the Association of Official Analytical Chemists, 17 th edn., Arlington, USA.

Ghavami, M., Gharachorloo, M. & Ghiassi Tarzi, B. (2008). Laboratory Techniques Oils and Fats, Islamic Azad University, Tehran Science and Research Branch Publisher.

Gulcan, A., Esra, K., Abdullah, E., Erdem Y. & Kucukkurt, I. (2006). Antihypercholesterolaemic and antioxidant activity assessment of some plants used as remedy in Turkish folk medicine. Journal of Ethnopharmacology, 107, 418–423.

Gülçin, İ., Küfrevioğlu, Ö.İ., Oktay, M. & Büyükokuroğlu, M. E. (2004). Antioxidant, antimicrobial, antiulcer and analgesic activities of netle (Urticadioica L.). Journal of Ethnopharmacology, 90, 205-215.

Hirono, T., Homma M. & Oka, K. (2012). Effects of stringing Nettle root extract and their steroidal components on the Na, K ATPase of the benign prostatic hyperplasia. Plantaedica, 60, 30– 33.

Lanteri, S., Armanino, C., Perri, E. & Palopoli, A. (2002). Study of oils from calabrian olive cultivars by chemometric method. Food Chemistry, 76, 501–507.

Mavi, A., Terzi, Z., Ozgen, U., Yildirim, A. & Coskun, M. (2004). Antioxidant Properties of Some Medicinal Plants: Prangosferulacea (Apiaceae), Sedum sempervivoides (Crassulaceae), Malvaneglecta (Malvaceae), Cruciatataurica (Rubiaceae), Rosa pimpinellifolia (Rosaceae), Galiumverum subsp. verum (Rubiaceae), Urticadioica (Urticaceae). Biological & Pharmaceutical Bulletin, 27, 5, 702–705.

Mendez, E., Sanhueza, J., Speisky, H. & Valenzuela, A. (1996). Validation of the Rancimat test for the assessment of the relative stability of fish oils. Journal of the American Oil Chemists' Society, 73, 1033–1037.

Miltman, P. (1990). Randomized, double-blind study of Urticadioica in the treatment of allergic rhinitis.PlantaMedica, 56, 44–47.

Obertreis, B., Giller , K., Teucher, T., Behnek B. & Schmitz, H. (1996). Anti-inflamatory effect of urticadioica folia extract in comparision to caffeic malic acid. Arzneimittelforschung, 46, 1, 52–56.