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Physicochemical characteristics of seed oil of *Sambucus ebulus*, *Coriandrum sativum* L. and *Silybum marianum* L.

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The physicochemical properties of oil extracted from the seeds of *Sambucus ebulus*, *Coriandrum sativum L.* and *Silybum marianum L.* (0.5:1:1) were examined. The fatty acid composition of the oil mixture was found to contain oleic (51.35%) and linoleic acids (35.01%) predominantly. The characteristic peak in infrared spectrum at about 1740 cm^{-1} was attributed to the C=O stretching in the carboxyl group of the fatty acids, oxidation products exhibit bands in 1730-1670 cm^{-1} region. The O-H stretching of the monomer fatty acid appears at about 3550 cm^{-1}, the signal around 3470 was attributed to the overtone of the CO stretching and the other peaks at 3535, 3621 and 3700 cm^{-1} are related to the stretching vibration of the OH group. The oxidative stability index was determined to be 12.7 h, the total content of tocopherols is 1340 mg/kg and total content of sterols was found at about 0.46%. The plant sterols with highest content that were found in the oil were β-Sitosterol (58.4%) and Δ5-Avenasterol (19.3%). Two fluorescent signals were observed at 570 nm and 680 nm which were assigned to correspond to oxidation products and chlorophyll respectively. Concentration of the toxic elements Cd and Pb were below 5 $ng.g^{-1}$ and 50 $ng.g^{-1}$ respectively.

Key words: ATR FTIR spectroscopy, elements contents, fatty acids composition, fluorescence

INTRODUCTION

Non-traditional vegetable oils are increasingly used in traditional medicine due to their therapeutic properties unique phytochemical composition and antioxidant activity [1-4]. *Coriandrum sativum* L. has pharmacological applications due to its antioxidant [5], antidiabetic [6], anti-lipidemic [7] and antispasmodic [8] properties. The antioxidant properties of coriander are due to the high pigment content, especially carotenoids. The essential oil from coriander qualifies as a natural antioxidant [9].

The fruits of *Sambucus ebulus*, due to their high content of anthocyanins and polyphenols, have high antioxidant activity and have anti-cancer, immunostimulating, antibacterial, antiallergic, antiviral and anti-inflammatory properties [10] and their seeds can be used as raw materials for production of non-traditional oil. *Silybum marianum L.* is a herb, that has been used in European traditional medicine mainly for treatment of various liver diseases. [11]

The chemical properties and fatty acid composition for oils obtained from seeds of *Coriandrum sativum L.* [12], *Sambucus ebulus* [13] and *Silybum marianum L.* [14] were investigated by Nagella, Fazio and Khan.

The authors found no information about the elemental content, optical properties and DSC profile, which are connected with the presence of pigments and oxidative products. The main purpose of this work is to study the physicochemical properties of the oil.

MATERIALS AND METHODS

Samples

The obtained oil was extracted from a previously prepared mixture of the seeds from *Sambucus ebulus* L, *Coriandrum sativum* L. and *Silybum marianum* L in 0.5:1:1 ratio respectively. This ratio dives the optimal fatty acid content in connection with the prospective use of the oil mixture as a dietary supplement.

The oil has been extracted with *n*-hexane in *Soxhlet* apparatus for 8 h. The solvent was partly removed in rotary vacuum evaporator, the residue was transferred in pre-weight glass vessels and the rest of the solvent was removed under stream of nitrogen to a constant weight to determine the oil content [15].

Used methods

Analysis of fatty acids. The fatty acid composition of oils was determined by gas chromatography (GC) after transmethylation of the respective sample with 20 $g.kg^{-1}$ H_2SO_4 in absolute CH_3OH at 50˚C [15]. Fatty acid methyl esters

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(FAME) were purified by thin layer chromatography (TLC) on 20x20 cm plates covered with 0.2 mm silica gel 60 G (Merck, Darmstadt, Germany) layer with mobile phase *n*-hexane: diethyl ether (97:3, v/v). GC was performed on a HP 5890 series II (Hewlett Packard GesmbH, Vienna, Austria) gas chromatograph equipped with a 75 m x 0.18mm x 25 μm capillary column Supelco FP - 2560 and a flame ionization detector. The column temperature was programmed from 140 °C (5 min), at 4 °C/min to 240°C, at 3.0 °C/min to 250°C (9 min), at 40°C/min to 230°C (1 min); injector and detector temperatures were kept at 270 °C and 280 °C. Hydrogen was the carrier gas at a flow rate 0.8 mL/min; split was 1:50. Identification of fatty acids was performed by comparison of retention times with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions [16].

Analysis of tocopherols. Tocopherols were determined directly in the oil by HPLC on a "Merck-Hitachi" (Merck, Darmstadt, Germany) instrument equipped with 250 mm x 4 mm Nucleosil Si 50-5 column (Merck, Darmstadt, Germany) and fluorescent detector "Merck-Hitachi" F 1000. The operating conditions were as follows: mobile phase of *n*-hexane: dioxan 96:4 (by volume), flow rate 1.0 ml/min, excitation 295 nm, and emission 330 nm, 20 μl 1% solution of oil in hexane were injected [17]. Tocopherols were identified by comparing the retention times with those of authentic individual tocopherols. The tocopherol content was calculated on the basis of tocopherol peak areas in the sample versus tocopherol peak area of standard α-tocopherol solution.

Analysis of sterols. Unsaponifiables were determined after saponification of the glyceride oil and extraction with hexane [18]. The total oil sample (sample size about 200 mg, precisely measured) was applied on a 20 cm x 20 cm glass plate with 0.5 mm thick silica gel 60 G layer (Merck, Darmstadt, Germany) and developed with hexane-acetone, 100:8 (by volume) to a front of 19 cm. [19].

Optical measurements. The fluorescence of the samples was studied by exciting them with light emitting diodes (LEDs) emitting at 245, 295, 395, 405 and 435 nm. A 90-degree geometry of light detection in 10x10 mm cuvette was used. Samples were studied without any preliminary solution. For UV illumination the samples is fixed between two quartz plates. Fluorescence and scattering spectra are recorded using fiber-optic spectrometer *Avaspect* with a spectral sensitivity within the 250-1100 nm range.

***Differential scanning calorimetry* (*DSC*).** The thermal characteristics of the oil samples were investigated in the temperature range of –80°C to 40°C by differential scanning calorimeter DSC 204 F1 PHOENIX (NETZSCH, Germany), equipped with intracooler. In order to avoid condensation of water, argon gas was used to purge the furnace chamber at 20 ml.min^{-1}. The DSC apparatus was calibrated with indium standard. The samples with weigh 5 mg were placed into 40 μl aluminium standard crucible and hermetically sealed with aluminium standard lead. An empty aluminium crucible was used as a reference.

Analyse of elements. About 0.3 g oil were weighed in Teflon vessels of microwave digestion system, then 6 ml 67% HNO_3(supra pure) and 2 ml 30% H_2O_2(supra pure). Microwave digestion was performed as follows: 15 min to reach 200°C and 15 min left to stay at this temperature. After cooling the samples were transferred in 50 ml volumetric flask and diluted up to the mark with deionized water. A blank sample was passed through the whole analytical procedure. Inductively coupled plasma-mass spectrometer "X SERIES 2"–Thermo Scientific was used for the determination of elements.

Infrared spectra. Infrared spectra were recorded on a Thermo Fischer Nicolet iS50 FT-IR instrument. The analysed sample (200 μL) was placed between two KBr disks and the transmittance spectrum was recorded.

RESULTS AND DISCUSSION

Several different techniques have been used for characterization of oil extracted from the seeds of *Sambucus ebulus*, *Coriandrum sativum* L. and *Silybum marianum* L. (0.5:1:1). The investigated oil, obtained as mixture of three herb seeds, has acidity value 4.06 mg KOH/g oil, which is higher than oil from *Silybum marianum* L. -1.2 mg KOH/g oil [14], but two times lower than the oil from *Coriandrum sativum* L.- 8.16 mg KOH/g oil [20]. The oxidative stability depends on the composition of the oil. The accelerated stability test showed that this oil has the good oxidative stability 12.7 h, which is higher than the sunflower oil [21]. The oil has total content of tocopherols 1340 mg/kg.

Fatty acid composition of the studied oil is listed in Table 1. Fatty acid composition is one of the main indicators characterizing the nutritional value of the oils and their oxidative stability during storage and heat treatment. The used oil has predominantly oleic acid content, followed by linoleic and palmitic acids. The acid profile of the investigated sample was also studied by FTIR spectroscopy, allowing an easy profile of its major constituents. The characteristic peak at about 1740 cm^{-1} can be attributed to the C=O stretching in the carboxyl group of the fatty acids and

is typically used to assess their concentration. [22] Oxidation products, which can normally be present at small quantities and range from hydroperoxides, aldehydes and ketones, the latter exhibiting bands in 1730-1670 cm^{-1} region. By examining the FTIR spectrum of a diluted (1% CCl_4) oil sample, in a quartz cuvette, a further confirmation of the acid value of the oil sample was obtained. Under these conditions the band, belonging to the C=O groups is not observed due to the absorption of infrared irradiation below 2500 cm^{-1} by quartz. The dilution of the oil allows the observation of the O-H stretching band in the carboxylic group because the fatty acids would exist as monomers in solution rather than dimers. The O-H stretching of the monomer fatty acid appears at about 3550 cm^{-1}. The signal around 3470 is attributed to the overtone of the CO stretching. [23] The other peaks at 3535, 3621 and 3700 cm^{-1} are related to the stretching vibration of the OH group, and specifically the signal at 3535 cm-1 is a characteristic band of the O-H bond in the free acids [23].

Table 1. Fatty acid composition of oil extracted from *Sambucus ebulus, Coriandrum sativum L. and Silybum marianum L.*

Fatty Acid		Content in the investigated oil, %
$C_{16:0}$	palmitic acid	9.31
$C_{18:0}$	stearic acid	4.32
$C_{18:1}$	oleic acid	51.35
$C_{18:2}$	linoleic acid	35.01

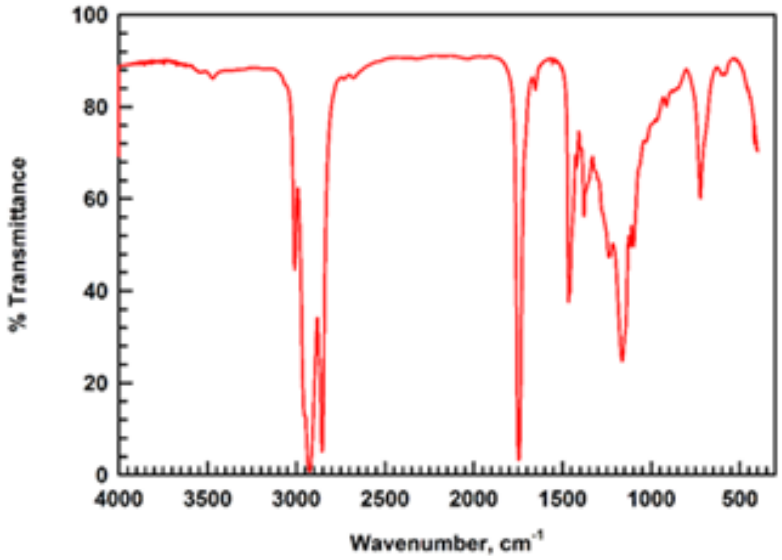

Figure 1. FTIR transmittance spectrum of investigated oil sample.

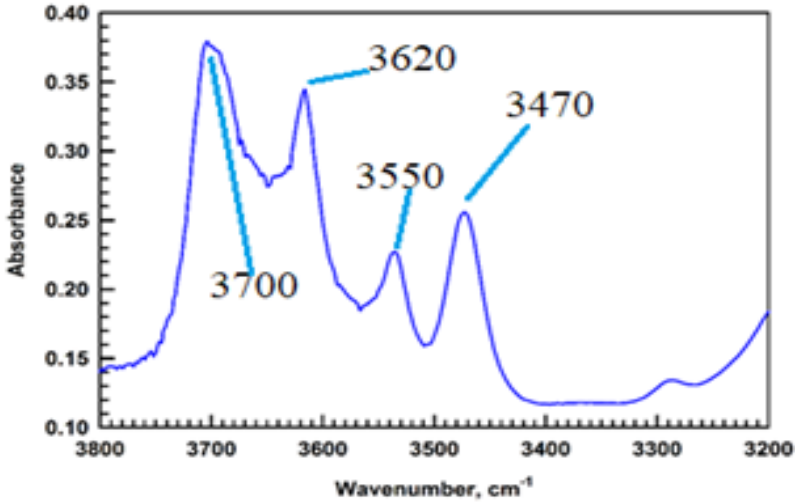

Figure 2. FTIR spectrum of 1% CC_4 solution of the oil in the range 3200-3800 cm^{-1}.

Sterols are present in the called non saponificated part. Their total content in the oil was found to be 0.46 % . The individual sterol composition is presented in Table 2. In the sterol fraction β-

Sitosterol (58.4 %) predominates, what is less than in chia oil from Argentina or Peru [24] (Table 2). Phytosterols (such as Stigmasterol and β-Sitosterol) have various health benefits and their safety profile has been extensively studied and confirmed [25]. Recent studies outline new perspectives for their use in cancer chemotherapy [26].

Table 2. Sterols composition and tocopherol content for oil, extracted from the seeds of *Sambucus ebulus*, *Coriandrum sativum* L. and *Silybum marianum* L.

Type of sterols	Content in the investigated oil, %	Types of tocopherols	Content in the investigated oil, %
Cholesterol	0.1	α-tocopherol	55.9
Campesterol	8.1	α-3 tocopherol	2.1
Stigmasterol	11.2	β - tocopherol	4.8
Δ5- Campesterol	—	γ- tocopherol	21.7
β-Sitosterol	58.4	δ- tocopherol	–
Δ5-Avenasterol	19.3	Total content in oil, mg/kg	1340
Δ7-Stigmasterol	2.5		
Δ-7,24 Stigmasterol	0.4		
Total content in oil, w/w %.	0.46		

α-Tocopherol predominated in the investigated sample – 55.9%, followed by γ-tocopherol. This oil has content of α-tocopherol close to content in *Cucurbita maxima* – 58.1% [27] and β – tocopherol two times greater than oil from *Honeydew melon* – 1.7 [27] (Table 2). The studying of the tocopherol types and contents in various food sources is still an area in which much research is still necessary. Tocopherols possess a high synergic antioxidant capacity and are linked with the prevention of cancer and cardiovascular conditions. [28]

The fluorescence spectra for investigated sample are obtained for excitation wavelengths 295 nm, 395 nm, 405 nm, 415 nm and 435 nm and presented on the Figure 3. They give the connection between the optical and chemical properties of the sample and afford on the opportunity for its quality investigation. Excitation at 295 nm gives a low intensity peak at 380 nm, which corresponds to the total tocopherol content. (Figure 3A) The maxima at 680 nm corresponds to the chlorophyll content. The signals between 500 and 650 nm correspond to the formation of oxidation products. (Figure 3B) Among the primary oxidation products are hydroperoxides which further degrade to secondary products: aldehydes, alcohols, hydrocarbons and ketones [29]. It's important to notice that the known products formed during oxidation of vitamin E group are all non-fluorescent [30]. Changing in the content of tocopherols and phenols is also detected at about 550 - 560 nm are related to the presence of oxidation processes [31] (Figure 3A).

Figure 4 shows the DSC thermogram of investigated oil. Three endothermic peaks are observed on the thermogram, which could be attributed to melting. The lowest temperature peak is a triplet with an on-set temperature -30^{o}C and peak temperature -26.6^{o}C. The relatively low melting temperature of this phase transition could be related to the high amount of polyunsaturated triacylglycerols [32]. It is probably due to the Linalool, an alcohol with melting temperature lower than -20^{o}C, which represents 60-80% of the composition of coriandrum oil [33]. Two other melting transitions could be recognized – at 9.3°C and at 24.4°C. However, their enthalpies of these peaks are less than 30 % of the total melting enthalpy of the oil. This oil can be stored in refrigerator, as it is not expected to partially crystalize.

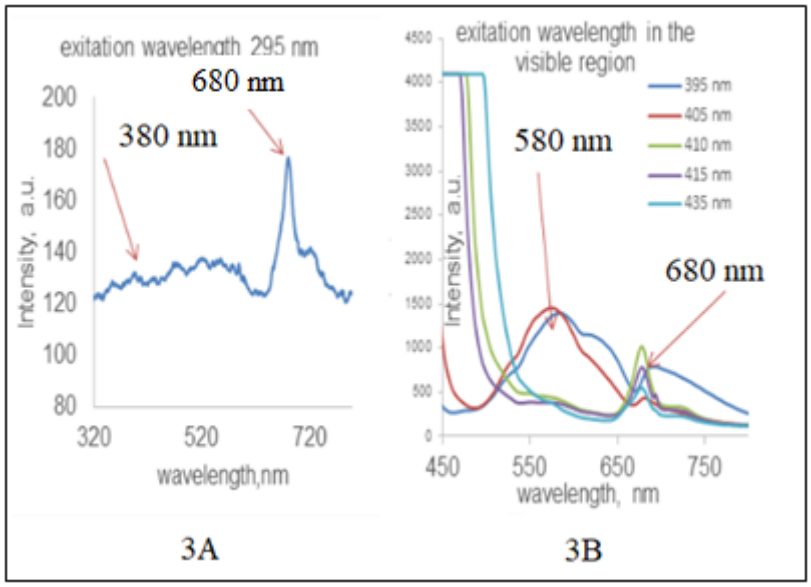

Figure 3. Fluorescence spectra in the UV and visible region for oil from the seeds of *Sambucus ebulus*, *Coriandrum sativum* L. and *Silybum marianum* L.

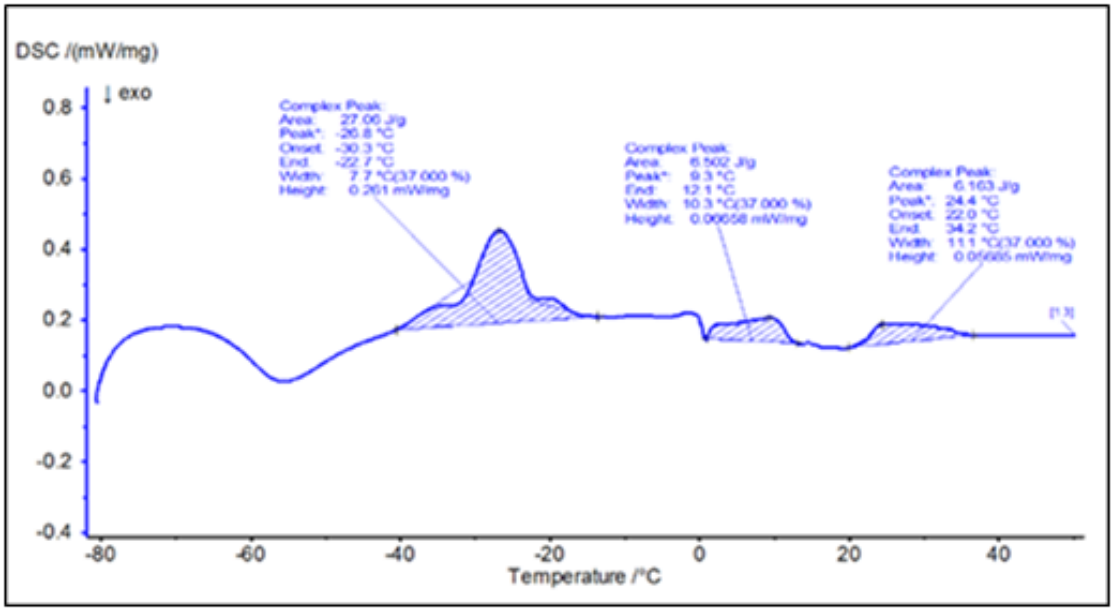

Figure 4. DSC thermogram for oil from the seeds of *Sambucus ebulus* L, *Coriandrum sativum* L. and *Silybum marianum* L.

Content of some toxic and essential elements in seed oil of *Sambucus ebulus*, *Coriandrum sativum* L. and *Silybum marianum* L were determined using ICP-MS. Results shows that Cd, As, Se, Zn, Co and Mo were below 5 ng g^{-1}, the elements Mg (8.07±0.1 μg g^{-1}) and K (5.90±0.1 μg g^{1}) were the major elemental constituents. The chromium and manganese levels were 0.45±0.03 μg g^{-1} and 0.23±0.01 μg g^{-1} respectively. Traces of Pb (40±2 ng g^{-1}) and Ni (30±2 ng g^{-1}) were found as well. The oil is suitable for different applications– cosmetics, medicine etc,

CONCLUSIONS

In this work, we report the physicochemical characterisations of the oil, derived from the seed mixture of *Sambucus ebulus*, *Coriandrum sativum* L. and *Silybum marianum* L. GC and HPLC were used to determine the fatty acid profile and tocopherol contents. The major fatty acids in the sample were oleic (51.35%) and linoleic acid (35.01%). β-Sitosterol and α-tocopherol were with highest concentrations. The infrared analysis confirmed the fatty acid profile and acidic value of the oil sample. The characteristic frequencies of all

expected functional groups were identified in the sample. Concentration of the toxic elements Cd and Pb were below 5 $ng.g^{-1}$ and 50 $ng.g^{-1}$ respectively.

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