Vegetable oils and plant extracts have captured a special attention due to their high antioxidant potential and also due to the preference of consumers for natural ingredients in many cosmetic products. The main objective of the present study was represented by the obtaining and characterization of active vegetable oils and extracts by using appropriate biotechnological procedure that allows to maintain a high content and bio-cosmetic potential of ω-3, -6, -9 and of carotenoids. In this context, several vegetable oils, e.g. hemp, milk thistle, safflower and black cumin oils have been obtained by cold pressing of row materials from native Romanian flora and analyzed by FT-IR spectroscopy, gas chromatography coupled with mass spectrometry (GC-MS) and 1H-RMN in order to establish the profile of unsaturated and polysaturated fatty acids. In a further study, two of the previous oils (e.g. safflower and milk thistle oils) have been selected for obtaining and stabilization of carrot extracts with high content in carotenoids. Thus, a rich content of linolenic acid, ω-3 and linoleic acid, ω-6 (e.g. 78% ω-6 in milk thistle oil, 52% ω-6 in black cumin oil etc.) and two carrot extracts with a total content of 147 and 187 mg/100 g extract have been obtained in the present study. The in vitro determination of antioxidant activity achieved on various types of vegetable mixtures (e.g. antioxidant activity values over 80%) encourages their further use in developing vegetable lipid nanostructures with valuable cosmetic potential.

Keywords: milk thistle oil, black cumin oil, GC-MS, antioxidant activity, carrot extracts
Experimental part
Isolation and production of vegetable oils and extracts

The vegetable oils were obtained by cold pressing of the hemp, milk thistle, safflower and black cumin seeds. The herbs were cultivated on organic soils of Hofigal SA. Cold pressing operation was performed by using screw oil press (press extrusion facility/equipment that provides a total separation of oil from plant material). With the screw press oil, the oil obtained from raw vegetable materials is collected in a collector receiver/container and the grist is discharged through the specific pipe. Vegetable oils obtained is passed through a centrifugal filter, are collected in a settling tank, is subjected to a cold rinse operation and then stored for further characterization by GC-MS, FT-IR and H-NMR.

Vegetable extracts rich in carotenoid compounds were obtained from carrot roots by solvent extraction technique followed by concentration of obtained extracts in three kinds of vegetable oils – thistle oil, safflower oil and seabuckthorn oil. To obtain oil extracts rich in carotenoids, plant material was dried under controlled conditions, ground and subsequently extracted with n-hexane at reflux. After removal of the solid residue, the filtrate is subjected to concentration under reduced pressure to obtain carotenoid concentrate. Oily extracts rich in carotenoids (e.g. carrot extract in milk thistle oil, carrot extract in safflower oil and carrot extract in seabuckthorn oil) were subjected to UV-VIS and HPLC analyses to determine the concentration in total carotenoids.

Identification and determination of saponifiable (fatty acids) compounds

FT-IR spectra were recorded using a Thermo Nicolet 6700 series spectrometer fitted with ZnSe crystal/microATR accessory. FT-IR spectroscopy has been used to highlight the characteristic bands from the fatty acids structure (ν_C=O, ν_C=CH(NH), ν.CH=NH, ν.C=O, ν.C–O and ν.C=O). After derivatization to the methyl esters by transesterification of oil triglycerides through alkali-catalyzed methanolysis, the chemical composition was determined by GC-MS using a gas chromatograph Termo – GC with DSQ P 5000 detector. The column used was Macrogol 2000, φ = 0.25 mm, l = 30m, He gas flow = 1 mL/min, injection temperature = 25°C, column temperature = 250°C. To identify the peaks the NIST spectra library has been used.

1H-NMR spectra were recorded on a Bruker Fourier 300 MHz spectrometer, operating at 6.9 T with a resonance frequency of 300.18 MHz for the 1H nucleus. The spectrometer was equipped with a direct detection four nuclei probe head and field gradients on z axis. Samples were analyzed in 5 mm NMR tubes (Norell 507). The chemical shifts are reported in ppm, using the TMS as internal standard. Typical parameters for 1H NMR spectra were: 45° pulse, 5.37 s acquisition times, 6.1 kHz spectral window, 16 scans, 20K data points, delay time 1s. The FID was not processed prior to Fourier transformation. The average acquisition time of the 1H NMR spectra was approximately 2 min.

Determination of non-saponifiable actives (carotenoids) from selected carrots extracts

The concentration of carotenoids from the oily carrot extracts has been determined using UV-VIS absorption spectrometer Cintra 6 at λ = 460 nm. The carotenoids have been calculated from the absorbance by using the calibration curve in the concentration range of 0.5 – 2 µg/mL β-caroten solutions in benzene with a correlation coefficient of R = 0.998, n = 6.

Carrot extracts were treated with benzene and 2-propanol, centrifuged at 3000 rot/min for 30 min, then filtered through a 0.22 µm filter. Different dilutions were tested depending on the concentration of the active compounds. Dionex HPLC (P 540 pump equipped with four elements and capabilities for the gradient); Detector diodes area UV-D 340 U in the 200-600 nm; PR8 Licrosorb thermostatic column, 200 x 4.6 mm x 5 mm; Isocratic system: mobile phase 97% Methanol: 3% water. Detection was performed with a UV detector at a wavelength of 450 nm.

In vitro determination of antioxidant activity

The capacity of the vegetable oils and carrot extracts in several vegetable oils to scavenge the reactive oxygen species has been determined by a chemiluminometer Turner Design TD 20/20, USA. A cyclic hydrazine (luminol) was used as light amplifying substance, which emits light when it is oxidized and it is converted in an excited aminophalate ion in the presence of oxidized species such as superoxide, hydrogen peroxide, hydroxyl radical, free oxygen and lipid peroxide radicals. The free radical generator system was made in buffer solutions of Tris-HCl pH 8.6. The concentration of vegetable oil solutions is 1mg/mL.

The antioxidant activity (percentage of scavenged free radicals) of all vegetable oils and extracts was calculated using the relation:

\[ \% AA = \frac{I_0 - I_s}{I_0} \times 100 \]

where: \( I_0 \) is the maximum chemiluminescence for the standard sample at \( t = 5 \) s; \( I_s \) is the maximum chemiluminescence of the sample at \( t = 5 \) s.

Results and discussions

Considering the complex composition of bio-active mixtures of plants, the primary characterization was performed by IR spectroscopy in order to identify the main fingerprints found in the fatty acids structures (ν_C=O, ν_C=CH(NH), ν.CH=NH, ν.C=O, ν.C–O and ν.C=O) from the selected vegetable oils. The FT-IR spectra of the investigated oils are quite similar (spectra not shown here), the assignment of main characteristic bands of the vegetable oil being summarized in table 1.

As it will be shown later in GC-MS and 1H-RMN analyses, the hemp oil is rich in linolenic acid (55%). The presence of this ω-3 acid is highlighted by the high intensity of the characteristic bands of vinyl groups from the 3000-3100 cm⁻¹ region and 1650 cm⁻¹ (ν_C=C) 913.45 cm⁻¹ (fragmentation pattern out of plane of cis disubstituted vinylic groups) and respectively intense band from 721 cm⁻¹ resulted from fragmentation patern of CH₂ groups and out of plane of cis vinyl groups from unsaturated fatty acids.

According to the literature, the black cumin oil has in its composition variable amounts of vegetal sterols that depend of type of vegetable source [26, 27]. The IR bands of these sterols overlap with bands of fatty acids and can not be highlighted. Additional information on the presence of sitosterols in the black cumin oil was revealed by 1H-RMN (fig. 3).

Identification and quantification of fatty acids by GC-MS and 1H-RMN

The composition of vegetable oils consists mainly of saponifiable compounds (e.g. mono-, di- and triacyl-
glycerols of saturated and unsaturated fatty acids, 98%) and in low amounts of other non saponifiable compounds, such as carotenoids, tocopherols, sterols, phospholipids etc. [28].

The vegetable oils obtained by cold pressing of hemp seed, milk thistle seed, safflower seed and black cumin seeds were firstly saponified and methylated and further analyzed by GC-MS method in order to identify and quantify the total amounts of \( \omega-3 \), \( \omega-6 \) and \( \omega-9 \) fatty acids. The chromatograms of all studied vegetable oils are shown in figure 1 and the fatty acids content is comparatively presented in figure 2.

Analyzing the content of vegetable oils, one can be observed that \textit{safflower oil} is the richest in omega-6 oil having over 77% linoleic acid, while the lowest content of linoleic acid was determined in \textit{milk thistle oil} (fig. 2). Regarding the omega-3 presence, excepting the \textit{safflower} oil, all the analyzed oils have a content ranging between 20-30% oleic acid. The linolenic acid (omega-3) was found in low amounts in vegetable oils, e.g. 10% in the hemp oil and 4% in the milk thistle (fig. 2). The highest content of saturated fatty acids such as the palmitic acid is found in black cumin oil (>17%) and the stearic acid in the milk thistle oil (8%).

In addition to chromatographic analysis, the quantitative assessment of the content of unsaturated fatty acids was also made by \(^1\)H-Nuclear Magnetic Resonance spectroscopy. The common unsaturated fatty acids present in many vegetable oils (e.g. oleic, linoleic and linolenic acids) can be quantified based on protons balance from RMN spectrum. For a good precision each signal from the spectra was integrated in triplicate and the computations was used for the mean integral value [29]. The \(^1\)H-NMR spectra of all organic Romanian vegetable oils are illustrated in Table 1.
Based on the integral values from the 1H-NMR spectra and using the systems of chemometric equation, the composition of oils studied was determined on four classes of fatty acids: tri-unsaturated fatty acids (linolenic acid), di-unsaturated fatty acids (linoleic acid), mono-unsaturated fatty acids (oleic acid) and saturated fatty acids [30, 31]. The results are summarized in table 3. As it can be remarked, the linolenic acid is present in a significant quantity only in the hemp oil. The lack of linolenic acid from the other oils studied can be also observed in the figure 3 were signal A (specific for this acid) is absent. The linoleic and oleic acids were present in large amounts in all studied Romanian vegetable oils. From all oils, milk thistle oil contains the highest quantity of saturated fatty acids.

Besides the characteristic signals of triglycerols in two of the analysed oils, e.g. black cumin oil and milk thistle oil, two singlet signals were found at 1.10-1.15 ppm region that according to literature data [32] are assigned to the methyl groups from 18, 19 position of vegetable sterols structure (table 2).

The results obtained using 1H-NMR method are in good agreement with those reported by GC-MS technique. Moreover, by comparing the GC-MS and 1H-RMN results it is worthwhile to stress that the hemp oil has almost an ideal omega-6: omega-3 ratio of 3 : 1. This ratio provides first information that supports the applicability of the Romanian vegetable oils in preparation of various cosmeceuticals based on natural compounds.

Obtaining and stabilization of carrots extracts in Romanian vegetable oils

Considering the poor stability of carotenes at light and temperature, three vegetable oils (e.g. safflower oil, milk thistle oil and sea buckthorn oil) have been selected for obtaining carrot extracts with high content in carotenoids and desired antioxidant activity for cosmetic purpose.
The concentration of total carotenoids in carrot oil extracts obtained by the repeated extraction with n-hexane was determined by UV-Vis and is shown in figure 4. Introduction of carotenoid concentrate in Safflower and Thistle oils has led to relative moderate concentrations in carotenoids, while by using Seabuckthorn oil a doubling of total concentration in carotenoids was obtained (fig. 4).

Identification of carotenoids in carrot extracts was also performed by high performance liquid chromatography (HPLC). A comparison of the chromatograms obtained on oily extracts of vegetable materials studied revealed that the carrot extract in safflower oil presents a high content of \( \alpha \)-carotene while those in sea buckthorn oil presents significant amounts of lutein and vitamins such as retinol and tocopherol (fig. 5).

Variable concentrations of carotene and other bioactive compounds present in the oily extracts ensure a promising potential for their use in order to develop vegetable lipid nanostructures with cosmetic potential.

**Evaluation of Romanian vegetable oils and carrot extracts to scavenge the reactive oxygen species**

Fruits and vegetables are the most important source of antioxidants. Antioxidants are molecules that interact with free radicals and break the chain reactions before damaging essential molecules [33, 34]. Nowadays it is important to consider vegetable oils and extracts as valuable antioxidant source due to tendencies for natural ingredients demanded by consumers [35].

In vitro evaluation of antioxidant activity of Romanian oils and plant extracts has revealed a significant ability to capture free radicals with antioxidant activity values between 80 and 85% for vegetable oils and higher for oily carrot extracts (ranging between 86 ÷ 92%) (fig. 6). Higher levels of antioxidant activity and oil extracts determined for oily extracts with an increase trend as the carotenol concentration is increased (reaching a maximum of 92.5% for carrot extract in seabuckthorn oil) can be explained by the advantages of omega -3, -5, -9 fatty acids, carotene and vitamins A and E to capture free radicals.

**Conclusions**

In the present study several vegetable oils, e.g. safflower, milk thistle, hemp and black cumin oils have been obtained by cold pressing of row materials from native Romanian flora. A set of suitable analyses methods, FT-IR spectroscopy, gas chromatography coupled with mass spectrometry (GC-MS) and \(^1\)H-NMR have been employed in order to establish the profile of unsaturated and polyunsaturated fatty acids. There was a good agreement between quantitative results obtained by GC-MS and H-NMR techniques.

The rich content of linoleic acid, \( \omega \)-3 and linoleic acid, \( \omega \)-6 detected in the four selected oils (e.g. 78% \( \omega \)-6 in thistle oil, 52% \( \omega \)-6 in black cumin oil etc.) represents a promising approach for their use in cosmetic formulations with nutritive and antioxidant properties. Among the four vegetable oils, hemp oil presents the best advantage for the application in dermato-cosmetic industry, owing to its optimal \( \omega \)-6 : \( \omega \)-3 ratio.
Several vegetable oils have been used as stabilization precursors for obtaining three carrot extracts with high content in carotenoids – carrot extract in safflower oil - 147mg carotenoids, carrot extract in thistle oil - 187mg carotenoids and carrot extract in sea buckthorn oil - 350mg.

The Romanian vegetable oils and carrot extracts have revealed a significant ability to capture free radicals with antioxidant activity values between 80 and 85% for vegetable oils and between 86 ÷ 92% for oily carrot extracts.

The appropriate concentrations and compounds determined in the studied vegetable oils and carrot oily extracts (e.g. omega-3, -6 and -9, carotene and vitamins) guarantee an encouraging premise for their further use in developing of lipid nanostructures with valuable antioxidant extracts (e.g. omega-3, -6 and -9, carotene and vitamins) guarantee an encouraging premise for their further use in developing of lipid nanostructures with valuable antioxidant properties and higher cosmetic potential.

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