PREPARATION AND CHARACTERIZATION OF VEGETABLE OILS AND PLANT EXTRACTS WITH EFFECT IN THE TREATMENT OF VARICOSE VEINS

Natalița IONESCU (BORDEI)[1*], Andreea-Miruna NEAGU[2], Mariana POPESCU[3]

Herbal products are important sources of natural bioactive compounds, commonly used in modern medicine to obtain food supplements and cosmetics. This study aims to investigate the composition of some vegetable oils obtained from Pumpkin, Sunflower and Grape seeds and Rice bran and also plant extracts that were obtained from Wild chestnut, Butcher's Broom and Nettle. They were selected for their well-known role in the treatment of varicose veins. For all the extracts studied, determinations of total flavonoids, total polyphenols and antioxidant activity were performed. The best results were for forest butcher's broom: total flavonoids expressed routinely [10.5 mg / g], total polyphenols expressed in gallic acid: [115.0 mg / g] and antioxidant activity: [1000.80 mmolTrolox / g sample] this being selected for use in the finished product.In the cosmetic formulations with effect in the treatment of varicose veins we selected pumpkin oil rich in palmitic acid: [14%], a compound frequently used in comparative cosmetics with other oils that have a lower content.They were characterized by gas-chromatography coupled with mass spectrometry (GC-MS) and by UV-Vis spectrophotometrical methods.The selected oils and plant extracts will further be used to obtain hydrogels based on nanostructured lipid transporters (NLC).

Keywords: Fatty Acids, Vegetable Fatty Oils, Vegetable Extracts, Bioflavonoids, Gas-Chromatography (GC-MS)

1. Introduction

Fatty oils obtained from plants (pumpkin, sunflower, grapes, rice bran, etc.) using cold pressing extraction techniques or extraction with various solvents are widely used in the food field, but also in other industries (cosmetics, pharmaceuticals, etc.). They have a special role due to the numerous bioactive beneficial compounds that are part of their composition. These bioactive

[1] Department of Inorganic Chemistry, Physical Chemistry and Electrochemistry, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania, e-mail: natychimist1@gmail.com, corresponding author

[2] Department of Organic Chemistry "Costin NENITESCU", Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Bucharest, Romania, e-mail: miru.an@yahoo.com

[3] Faculty of Pharmacy, *Titu Maiorescu* University of Medicine and Pharmacy, Bucharest, Romania, e-mail: mari.popescu@yahoo.com

compounds (both saturated and polyunsaturated fatty acids) are being highlighted using various techniques and some of the most used techniques for the characterization of these oils are UV-Vis spectrometry and gas chromatography coupled with mass spectrometry (GC-MS).

The treatment of varicose veins includes the use of fatty oils as well as various plant extracts such as chestnut, thorns and nettle extracts which are known to be rich in bioflavonoids and polyphenolcarboxylic acids[1].

The production and use of medicinal and aromatic plants in the field of organic farming represents an alternative to the conventional production system, by promoting clean agricultural technologies that exclude the use of synthetic chemicals, but also by promoting healthy agricultural products, which can ensure consumers' health, environmental protection and the conservation of natural resources.

Although medicinal plants have been used for millennia as one of the main sources of treatment as therapeutic agents, their potential in skin therapy remains untapped [2].

Due to the disadvantages of conventional therapy, there is an obvious requirement for the development of new alternatives based on nanostructured topical formulations containing natural active principles and leading to a performant therapeutic action and improved safety profile for the treatment of varicose veins. As part of solving current problems, the development of new advanced pharmaceutical products based on vegetable oils and plant extracts with antioxidant and anticoagulant properties is a priority[3, 4, 5].

Wild chestnut (Aesculus hippocastanum)

Fruits are rich in vitamins C and K, vitamin B - B1, B2, fats, starches, saponosides, triterpenes, bitter principles, tannins, fats, albumin and flavonoid compounds. They have tonic, anti-inflammatory, phlebotonic, vasodilating properties. Antiedematous, anticoagulant and fluidizing for the blood, also have hemostatic, decongestant actions.

Varicose veins, hemorrhoids and venous insufficiency can be treated with wild chestnut preparations. They act on the cell walls of the blood vessels, which they strengthen. At the same time it helps maintain tissue elasticity. Chestnut extracts intensify blood flow, anti-inflammatory and stimulant properties.

Seed cotyledons contain up to 10% saponins, flavonic glycosides, sterols, volatile oil, lipids (6-8%), starch (40-50%). Total saponoside, known as escin, is a complex mixture of glycosides (over 30) that derives structurally from two polyhydroxylated pentacyclic triterpene aglycones: protoescigenol and baringtogenol C.

The vascular action of the stage is biphasic: in the initial phase it acts vasodilator both on the veins and on the arteries, in order to then act by toning

them. However, escin should not be confused with extracts from Hippocastani semen, in which, along with escin, there are also flavonoid compounds that increase the low solubility of escin and, through it, its absorption rate. In addition, other components (proanthocyanidols) act venotonically [6,7].

Butcher's Broom - (Ruscus acculeatus)

The forest thorn contains beneficial ruscogenins and flavonoids, two phytochemicals that support the health of the veins in the legs, being a venous tonic. The forest thorn has been used for decades to support healthy veins, and studies indicate that it helps reduce itching, tingling and cramps caused by the occasional discomfort felt in the lower limbs, especially in middle age. It is also frequently used for attenuation related to hemorrhoids. The major phytocompounds in forest thorns are steroidal saponins. [8] Saponins occur naturally in plants in the form of glycosides and have foaming properties. [9] The specific saponins found in forest thorns are ruscogenin, ruscogenen and neoruscogenin, named for the genus Ruscus. Ruscogenins function as anti-inflammatory agents [10] and are also thought to cause constriction in veins. [11] At present, the mode of action of ruscogenins is not well established, but a proposed mechanism suggests that ruscogenins suppress leukocyte migration by regulating both protein and mRNA. [10]

Newer research has also found that there are polyphenols present in forest thorns, which can also be physiologically active, possibly as antioxidants. [12] [13]

Nettle (Urtica Dioica)

Nettle contains protein and carbohydrates, sterols, volatile oil, acetic acid, formic acid, vitamins A, B2, C and K, mineral salts. In the extracts made from nettle root, the presence of some polysaccharides was highlighted (1.7%), with action on the one hand anti-inflammatory (stronger than indomethacin), and on the other hand immunostimulatory.[14]

2. Experimental

2.1. Materials

In order to obtain fatty vegetable oils rich in unsaturated fatty acids – ω-3, ω-6 and ω-9 – seeds from carefully selected vegetable materials (pumpkin, sunflower, grapes and rice) were used, which were subjected to the cold pressing technique.

The extracts were obtained from the selected plants (*Wild chestnut, Butcher's Broom* and *Nettle*) under temperature-controlled conditions (maximum 40°C); the therapeutic substances were extracted through a 10 day maceration process with ethyl alcohol in various concentrations (45%, 60%, 70%). The

operation is performed in tightly sealed glass vessels. Stirring is important during the entire process. The plants were previously dried and ground. An alternative method to prepare the extracts is multiple percolation, frequently used in the pharmacies, galenic laboratories and industrial installations. The hydroalcoholic extracts have some advantages over aqueous extractive solutions when it comes to the administration mode and the pharmaceutical technology namely that the extraction in the hydro-alcoholic environment is complete.

2.2. Methods

In order to determine the active principles from the studied plant extracts – *Wild chestnut, Butcher's Broom and Nettle* –, the following UV-Vis spectrophotometric methods were used: total polyphenols expressed as gallic acid (Folin–Ciocalteu method) and total flavones expressed as rutin. The antioxidant activity was determined by the CUPRAC method.

Total polyphenol content (TPC) assay: the polyphenols in the extracts are determined colorimetrically using Folin-Ciocalteu phenol reagent. The reagent contains phospho-tungstic acids as oxidants, which on reduction by readily oxidized phenolic hydroxy groups yield a blue colour with a broad maximum absorption at 765 nm. This is due to the formation of so-called tungsten and molybdenum blues. The Folin-Ciocalteu phenol reagent reacts with a wide range of polyphenol compounds and, although the response can vary with the individual components, selection of gallic acid as a calibration standard enables useful total polyphenol data to be obtained.

The flavone derivatives content was determined by the reaction with $AlCl_3$ and total content was expressed in rutin.

Antioxidant capacity CUPRAC assay: the CUPRAC - cupric ion reducing antioxidant capacity - method is a simple and versatile antioxidant capacity assay useful for a wide variety of polyphenols, including phenolic acids, hydroxycinnamic acids, flavonoids, carotenoids, anthocyanins, as well as for thiols, synthetic antioxidants, and vitamins C and E.

The chromogenic oxidizing reagent used for the CUPRAC assay is the bis(neocuproine) copper(II) cation (Cu(II)-Nc) acting as an outer-sphere electrontransfer agent, and the CUPRAC chromophore, formed by reduction of this reagent with antioxidants, is bis(neocuproine) copper(I) cation (Cu(I)-Nc) (Fig. 1). This reagent is useful at pH 7, and the absorbance of the Cu(I)-chelate formed as a result of the redox reaction with reducing polyphenols, vitamins C and E is measured at 450 nm. The orange-yellow color is due to the Cu(I)-Nc chelate formed. CUPRAC reactions are essentially complete within 30 min.

The determination of fatty acids was performed by **gas chromatography coupled with mass spectrometry (GC-MS)** after derivatization to methyl esters by transesterification of the triglycerides present in the studied oils with methanol

in an acidic environment, using a gas chromatograph Termo-GC having a mass spectrometer detector DSQ P 5000. A Macrogol 2000 column was used, with the following characteristics: Φ = 0.25 mm, l = 30 m, helium carrier gas, flow rate = 1 mL/min, injection temperature = 25°C, column temperature = 250°C; in order to identify the peaks corresponding to the analyzed compounds, the NIST spectrum library was available.

3. Results and discussions

The results for the content in bioflavonoids, polyphenolcarboxylic acids and antioxidant activity in the hydroalcoholic extracts from chestnut, thorns and nettle are presented in Table 1.

Fig. 1 shows the GC–MS chromatograms as well as saturated and polyunsaturated fatty acids (ω-3, ω-6 and ω-9) for the studied fatty oils (pumpkin, sunflower, grapes and rice).

Table 1

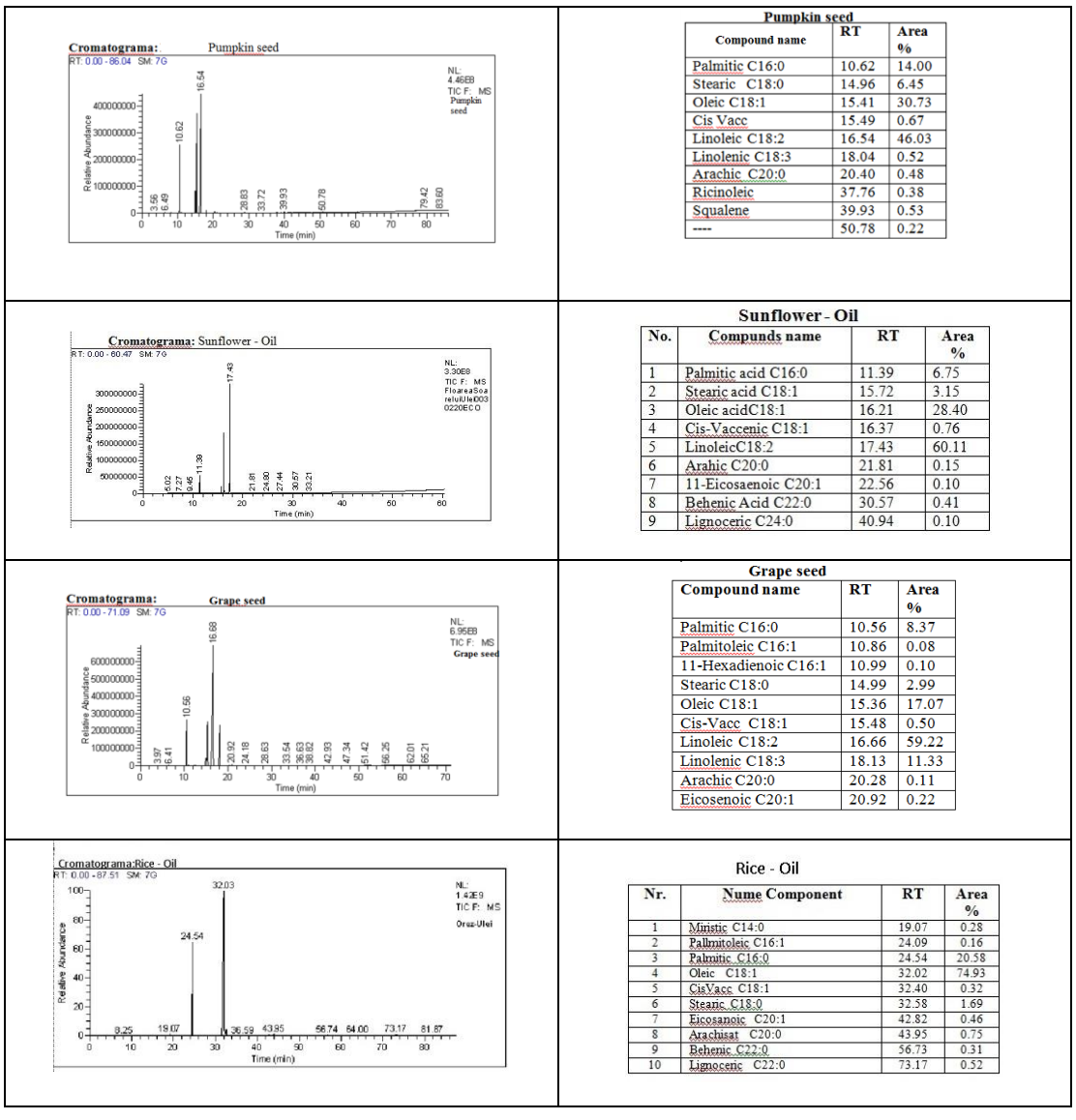
Physico-chemical determinations for the plant extracts

Analyzes	Vegetal material – extracts conc. 5:1		
	Wild chestnut	**Butcher's Broom**	**Nettle**
Total flavones expressed as rutin [mg/g]	8,0	10,5	5,3
Total polyphenols expressed as galic acid [mg/g]	38,5	115,0	35,5
Antioxidant activity [mmol Trolox /g sample]	360,45	1000,80	475,50

From Table 1 we can observe the richer content of bioflavonoids, polyphenolicarboxylic acids and antioxidant activity for the hydroalcoholic extract of Butcher's Broom (10.5 mg/g, 115.0 mg/g respectively 1000.8 mg Trolox equiv/g sample), hydroalcoholic extracts of Wild chestnut and nettle have lower concentrations of bioflavonoids, polyphenolcarboxylic acids and antioxidant activity.

As shown in Fig. 1, Omega 3 and 6 fatty acids are of interest for the cosmetic formulations with therapeutic effect in the treatment of varicose veins. Omega 3 fatty acid is found in a considerable percentage only in grape seed oil (11.33%), the other oils being richer in polyunsaturated fatty acids Omega 6 and Omega 9. The highest percentage of Omega 6 fatty acids is found in sunflower and grape seeds oils (60% and 59% respectively), followed by pumpkin oil (46%); in rice oil it was below the detection limit. Rice oil has a high content of Omega 9 fatty acid (75%), for the other fatty oils the Omega 9 fatty acid content is between 20-30%. In the cosmetic formulations with effect in the treatment of

varicose veins we selected pumpkin oil rich in palmitic acid: [14%], a compound frequently used in comparative cosmetics with other oils that have a lower content



Pumpkin seed

Compound name	RT	Area %
Palmitic C16:0	10.62	14.00
Stearic C18:0	14.96	6.45
Oleic C18:1	15.41	30.73
Cis Vacc	15.49	0.67
Linoleic C18:2	16.54	46.03
Linolenic C18:3	18.04	0.52
Arachic C20:0	20.40	0.48
Ricinoleic	37.76	0.38
Squalene	39.93	0.53
----	50.78	0.22

Sunflower - Oil

No.	Compunds name	RT	Area %
1	Palmitic acid C16:0	11.39	6.75
2	Stearic acid C18:1	15.72	3.15
3	Oleic acidC18:1	16.21	28.40
4	Cis-Vaccenic C18:1	16.37	0.76
5	LinoleicC18:2	17.43	60.11
6	Arahic C20:0	21.81	0.15
7	11-Eicosaenoic C20:1	22.56	0.10
8	Behenic Acid C22:0	30.57	0.41
9	Lignoceric C24:0	40.94	0.10

Grape seed

Compound name	RT	Area %
Palmitic C16:0	10.56	8.37
Palmitoleic C16:1	10.86	0.08
11-Hexadienoic C16:1	10.99	0.10
Stearic C18:0	14.99	2.99
Oleic C18:1	15.36	17.07
Cis-Vacc C18:1	15.48	0.50
Linoleic C18:2	16.66	59.22
Linolenic C18:3	18.13	11.33
Arachic C20:0	20.28	0.11
Eicosenoic C20:1	20.92	0.22

Rice - Oil

Nr.	Nume Component	RT	Area %
1	Miristic C14:0	19.07	0.28
2	Palmitoleic C16:1	24.09	0.16
3	Palmitic C16:0	24.54	20.58
4	Oleic C18:1	32.02	74.93
5	CisVacc C18:1	32.40	0.32
6	Stearic C18:0	32.58	1.69
7	Eicosanoic C20:1	42.82	0.46
8	Arachisat C20:0	43.95	0.75
9	Behenic C22:0	56.73	0.31
10	Lignoceric C22:0	73.17	0.52

Fig. 1. GC-MS chromatograms and compounds for the vegetable oils

4. Conclusions

The selected oils and the plant extracts will be used to obtain hydrogels based on nanostructured lipid transporters (NLC) for encapsulation and subsequent controlled distribution of bioflavonoids present in the studied plant

extracts, in order to improve the bioavailability of active ingredients and the therapeutic efficacy of the formulation.

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