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EVALUATION OF ANTIOXIDANT CAPACITY FOR SOME WILD PLANT EXTRACTS USED IN COSMETICS

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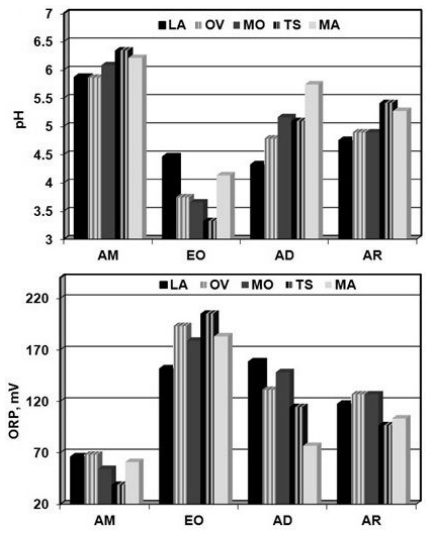
Herbal cosmetics are quickly absorbed by the superficial layers of the skin and are usually hypo-allergenic. Organic cosmetic ingredients obtained from plant species contain a higher quantity of natural antioxidants and could successfully replace the synthetic ingredients or synthetic additives used to stabilize cosmetics, most often, harmful for the skin. The study aims to present original results concerning some characteristic parameters of wild lavender (*Lavandula angustifolia)*, lemon balm (*Melissa officinalis)*, water mint (*Mentha aquatica*), oregano (*Origanum vulgare*) and thyme (*Thymus serpillum*) extracts used in cosmetics. The wild plants, collected from Constantza and Buzau unpolluted areas, have been processed to obtain aqueous distillate, alcoholic macerate, etheric oils by hydrodistillation and aqueous mixture remained after hydrodistillation. Further, the studied plant extracts were analysed determining the following parameters: pH, ORP, total phenols content using an adapted Folin Ciocalteu method related to antioxidant capacity and DPPH Radical Scavenging test. From all analysed extracts, essential oils have the highest ORP and polyphenol compounds content. Thyme and mint contain the highest poliphenol content from wild plant considered in this study. The obtained results show that the bioactive compounds from the studied plant extracts ensure optimum pH values and explain the long-term stability of organic cosmetics, with 0% chemicals added.



INTRODUCTION

Plants are a source of compounds that may be used as pharmacologically active products.[1] It was a time when nature was the only source to treat many diseases or to maintain skin health. Beautifying products containing plant material with therapeutic properties are known and used since the age of queen Hetephexes, the mother of pharaoh Keops. Nowadays, while technology has grown enormously, plant extracts are replaced with synthetic compounds in many products, especially in cosmetics, due to low costs and high demand. In Romania, women spent almost 700 milions $/year on cosmetics, while worldwide, the numbers are impressive: more than 55 billion $ are spend on different types of cosmetics. Cosmetics industry is one of the richest industry with fast development. But do people really know what they buy?

In ancient times and in the Roman Empire, especially in Rome, there was a particular concern for beautifying using plant extracts. It is known that the Romans mainly used to live in relation to the philosophy "Mens Sana in corpore sano",

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meaning "a healthy mind in a healthy body". Enhancing people's beauty with the help of nature it was a priority. They were interested in many plant species like chamomile, mint, fir, lavender and many others, to study their potential in healing different skin diseases. Nature represented their first choice when looking for a cure.

Lavender (*Lavandula angustifolia*) is a plant with a number of beneficial properties for the human body. Besides its application in herbal treatment, lavender is widely used in the cosmetic, perfume, food, and aroma therapeutic industries. It contains essential oil (linalool, linalyl acetate, ocimene etc.) anthocyanins, phytosterols, sugars, minerals, coumaric acid, glycolic acid, valeric acid, ursolic acid, herniarin, coumarin and tannins.[2,3]

Origanum vulgare L. a member of the Lamiaceae family, used in medicine, contains mainly thymol, carvacrol, polyphenols (flavonoides, flavones), monoterpenes, monoterpenoids. Recently, advanced studies regarding the phenolic compounds determination from ethanol extract of whole plants of origanum and their therapeutic effects have been done.[4, 5]

Melissa officinalis L. commonly known as lemon balm (family Lamiaceae) is one of the oldest and still most popular medicinal plant. Pharmacological reports revealed that the most commonly known therapeutic properties of M. officinalis extract are sedative, carminative, antispasmodic, antibacterial, antiviral, anti-inflammatory, antioxidant and neuro-protective.[6] Lemon balm has a complex chemical composition. It contains up to 6% hydroxycinnamic acids and up to 0.37% of an essential oil composed of monoterpenes, sesquiterpenes, and terpenoids (citral, citronellal, geraniol, nerol, linalool, farnesyl acetate, humulene, β-caryophyllene, eremophilene etc). There are also other constituents, such as flavonoids, e.g. glycosides of luteolin, quercetin, apigenin and kaempferol, as well as tannins and acidic triterpenes (ursolic and oleanolic acids). Some pharmacological properties have been attributed to the principal constituents.[7,8]

Water mint (*Mentha aquatica*), as other plants of the genus Mentha are widely used in cooking, cosmetics and popular medicine (to cure abdominal and chest pains). The major chemical compounds so far identified in *M. aquatica* essential oil are monoterpenes and monoterpenoids (including α-pinene, limonene, trans-β-ocimene, α-terpinene, linalool, linalyl acetate, 1,8-cineole, pulegone, menthofuran, menthone, menthol, isopinocamphone, piperitenone oxide and dihydrocarveyl acetate) and common sesquiterpene compounds (β-caryophyllene, germacrene D, elemol, viridiflorol, caryophyllene oxide).[9,10]

Thymus serpillum (thyme), another member of Lamiaceae family contains volatile oil of variable composition (thymol, carvacrol, 1,8-cineole, borneol, geraniol, linalool, bornyl and linalyl acetate, thymol methyl eter and α-pinene), flavonoids (apigenin, luteolin, thymonin, naringenin et al.), tannins, labiatic and caffeic acids etc.[11] Various thymus species have been reported to possess a broad spectrum of bioactivities, including antioxidant, anti-inflammatory and antimicrobial activities.[12]

Natural antioxidants protect the human body from free radicals, prevent oxidative stress and associated diseases. For these reasons they play a very important role in health care. Plants are a source of compounds with antioxidant activity such as phenolic acids, flavonoids (including anthocyanins and tannins), vitamins and carotenoids that may be used as pharmacologically active products.[13]

Widespread empirical use of wild plants demands accurate and reliable information on their phytochemicals and antioxidant activity, as well as on the potential benefits and prospective products, such as nutraceuticals and phytomedicines. Numerous tests have been developed for measuring the antioxidant capacity of food and biological samples. However, there is no universal method that can measure the antioxidant capacity of all samples accurately and quantitatively.[14]

None of the studies conducted with vitamins or other compounds (such as polyphenols) can give precise information about any single product, even after supplementation, because foods provide the intake of many of them all together. The final activity belongs to the combination of a variety of antioxidants. As a consequence, sophisticated statistical analysis had to be applied to the data to isolate the effect of a given compound. Despite this effort, it is very hard to define the activity of a single product.[1] In the same time, natural extracts used to formulate cosmetic products have benefic effects on the skin like acnee healing, anti inflamatory effect, cell regeneration process and retarding aging etc.[15]

The aim of this paper is to present original results concerning some characteristic parameters of wild lavender, oregano, lemon balm, thyme and water mint different plant extracts used in cosmetics: pH, ORP, total phenols content using an adapted Folin Ciocalteu method related to antioxidant capacity and DPPH Radical Scavenging test. The four considered different plant extracts were aqueous distillate, alcoholic macerate, etheric

oils by hydrodistillation and aqueous mixture remained after hydrodistillation.

RESULTS AND DISCUSSION

Most of the scientific paperwork studies alcoholic or aqueous plant extracts and essential oils derived from wild or crop plants. In this investigation, beside the usual plant extracts, two other products were taken into study: the aqueous distillate and the aqueous mixture remained after obtaining the essential oil by hydrodistillation. We believe that in order to evaluate the skin benefits produced by various extracts, it is important to make a characterization of all products obtained from plants that were left in contact with different solvents, in different conditions.

pH and oxidation reduction potential

Starting from the skin benefits observed during many years of experience by using original cosmetic products containing sea buckthorn extracts, in a previous work we report interesting shapes of pH-ORP evolution for aqueous distillates, concluding that low pH values explain the hydrating action and ORP data are related to bioactive compounds.[16]

Figures 1 and 2 present the evolution of two physico chemical parameters determined in all wild plants extracts taken into study. We noticed that essential oils recorded the lowest values of pH and the highest values of ORP, while in alcoholic extracts the situation is the opposite: higher values of pH (5.8-6.3) and lower oxidation reduction (ORP -70 mV). The two aqueous products potential (the distillate and the residue after hydrodistillation) have similar values of this two parameters but aqueous distillates derived from lavender, oregano, lemon balm and thyme, which are used for skincare, have superior properties compared to residues derived from the same plants.

Antioxidant activity

To effectively characterize the 4 extracts obtained from 5 wild plant species used in organic aesthetic, the results obtained after determining the total phenol content and reinterpreted DPPH test are reported. We consider that the expression of antioxidant activity resulting from DPPH test in mg gallic acid equivalents or ascorbic acid is more useful.

Table 1 presents the values obtained for total phenolic content and antioxidant activity in wild plants extracts taken into study.

It can be observed that essential oils have the highest total phenols compounds content, significantly higher than in alcoholic macerates, aqueous distillates and aqueous residues. These results are in good correlation with the pH and ORP values. Plants with highest polyphenol content in essential oils are thyme and water mint.

Antioxidant capacities expressed as mg GAE/mL and mg AAE/mL increase in order AD<EO<AM<AR.

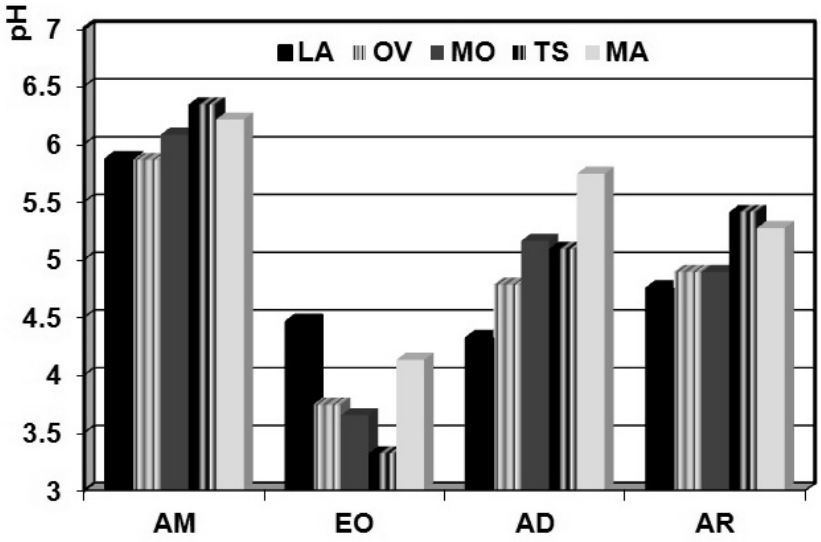

Fig. 1 – pH values for studied wild plant extracts used in cosmetics (AM – alcoholic macerate, EO – essential oil, AD – aqueous distillate, AR – aqueous residue); LA – lavender, OV – oregano, MO – lemon balm, TS – thyme, MA – water mint.

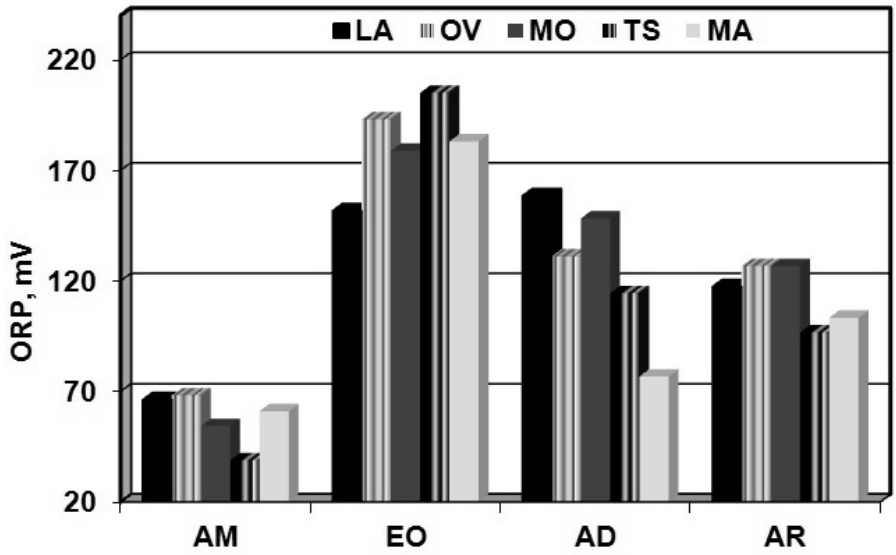

Fig. 2 – Oxidation- reduction potential (ORP) values for studied wild plant extracts used in cosmetics (AM- alcoholic macerate, EO – essential oil, AD - aqueous distillate, AR – aqueous residue); LA – lavender, OV – oregano, MO – lemon balm, TS – thyme, MA – water mint.

Table 1

Total phenols and antioxidant capacities for studied wild plant extracts used in cosmetics

Specification		TP, mg GAE/mL	DPPH	
			mg GAE/mL	mg AAE/mL
AM	LA	0.203	35.498	9.49
	OV	0.228	28.79	9.71
	MO	0.2055	19.09	9.90
	TS	0.2528	18.69	9.48
	MA	0.039	13.68	33.91
EO	LA	16.869	1.044	2.691
	OV	20.322	4.778	2.507
	MO	10.812	1.031	3.444
	TS	34.542	6.550	2.429
	MA	34.877	6.977	19.166
AD	LA	0.0067	0.054	0.037
	OV	0.0041	0.053	0.037
	MO	0.0203	0.0855	0.236
	TS	0.0165	0.086	0.233
	MA	0.018	0.0838	0.226
AR	LA	0.2195	87.58	239.15
	OV	0.2137	76.65	28.79
	MO	0.222	91.9	237.37
	TS	0.203	86.75	237.38
	MA	0.236	89.83	246.5

EXPERIMENTAL

Wild plant species are known to have a higher quantity of bioactive compounds than crop plants due to geographical areas less polluted and more oxygenated. The studied wild plants were collected from Constantza and Buzau unpolluted areas: *Lavandula angustifolia, Melissa officinalis*, *Mentha aquatica, Origanum vulgare* and *Thymus serpillum***.** The plants were picked at full bloom and naturally dried on nets situated in an airy space.

Samples preparation

The natural extracts taken into study are: (i) **alcoholic macerate (AM)** obtained by the contact for 10 days of plant with homemade alcohol in ratio of 2:1 – 1:1, followed by separation of the supernatant; (ii) **essential oil (EO)** that represents volatile components separated from plants by hydrodistillation in Neoclevenger device; for spectrometric measurements there were used alcoholic solutions obtained from weighted quantities of oil diluted with ethanol in 10 mL calibrated flask; (iii) **aqueous distillate (AD)** obtained by

distillation of the mixture between plant and pure water in ratio 1:3 – 1:10; and (iv) **aqueous residue (AR)** that represents the remained mixture after the eteric oils obtaining by hydrodistillation process.

Determinations

For plant extracts characterization the next determinations have been done: pH and oxidation reduction potential (ORP) using a pH – meter pH 300 with a combined electrode, total phenols content (TPC) with adapted Folin Ciocalteu method and antioxidant capacity by reinterpreted DPPH Radical Scavenging test.

The spectrometric measurements have been performed using a Camspec M 330 scanning spectrophotometer.

The used method for TPC determination is based on the reduction of a phosphowolframate – phosphomolibdate complex to blue products by soluble phenolic compounds, in sodium carbonate media and the measurement of the absorption of the formed complex at the wavelength of 681 nm. The absorbance relative to a gallic acid standard curve was measured and results are expressed as gallic acid equivalents (mg GAE/ mL for AM, AD and AR, mg GAE/g oil for EO).

To plot the calibration curve in the range of 0.68 – 4.76 mg GAE/L, 1 mL Folin Ciocalteu reagent 1:2 was added in 50mL calibrated flasks to different volumes of standard gallic acid solution, then 1 mL sodium carbonate solution 20%, then the mixture was mixed and let standing 10 min at room temperature and fill up to the mark with distilled water. At the end the mixture was homogenized and let under room temperature 30 minutes for the color stabilization and after that the absorbance was read at 681 nm. The correlation coefficient was 0.99769.

To measure the total phenols content, various volumes of samples (20 mL AD, 2 mL AM and AR and 1mL EO solution) were added in 50 mL calibrated flasks, then 1 mL Folin Ciocalteu reagent 1:2, 1 mL sodium carbonate solution 20% and the process was the same like those used for calibration.

The antioxidant capacity was evaluated using DPPH Radical Scavenging test. Two standard compounds were considered to plot calibration curves: gallic acid (GA) and ascorbic acid (AA) and the results are expressed as equivalents (mg GAE or AAE). In 25 mL calibrated flasks different volume of gallic acid or ascorbic acid solutions were added, then 5 mL DPPH 1.268 mM in methanol, filled up to the mark with methanol and let in the dark, to the room temperature for 45 minutes before the absorbance registration at 530 nm versus methanol. Previously, the DPPH solution spectrum was recorded and the maximum absorbance was registered at 530 nm.

The solutions absorbance' decrease due to the antioxidant capacity of standard compounds determined the downward allure of calibration curves.

The calibration curve with gallic acid as standard was linear in 0 – 2.72 mg GAE/L range and the correlation coefficient was 0.99818. The calibration curve with ascorbic acid as standard was linear in 0 – 5 AAE/L range and the correlation coefficient was 0.99642.

To measure the antioxidant capacity, 1 mL of sample (AD and EO solution, AM diluted 250 times with ethanol and AR diluted 1000 times with distilled water) were added in 25 mL calibrated flasks, then 5 mL DPPH 1.268 mM in methanol, filled up to the mark with methanol and let in the dark, to the room temperature for 45 minutes before the absorbance registration at 530 nm versus methanol.

CONCLUSIONS

The studied wild plant release in their extracts a very complex composition of bioactive compounds with beneficial effects on the skin, difficult to analyse and identify in short time.

Rapid tests as pH, ORP, total phenols or DPPH Radical Scavenging test could globally evaluate the antioxidant activity.

Two calibration curves for DPPH test have been developed using as standards gallic acid and ascorbic acid. The proposed results interpretation for antioxidant capacity evaluation using DPPH test seems to be simpler and more efficient for the plant extracts for cosmetic use.

From all studied natural wild plant extracts, essential oils have the highest ORP and polyphenolic compounds, significantly higher than in alcoholic macerates, aqueous distillates and aqueous residues.

Antioxidant capacities expressed as mg GAE/mL and mg AAE/mL increase in order AD<EO<AM<AR.

Roumanian plant species represent a good source for those cosmetic industries who are seeking to create pure and natural cosmetics, in limited quantities.

Taking into account the results of previous studies of other researchers as well, we consider that the ORP measurement of natural mixtures could provide objective, cheap and reliable information about antioxidant activity of complex samples.

REFERENCES

1. U. Cornelli, *Clin. Dermatol*, **2009**, *27*, 175 – 194.
2. Lu Hui, Li He, Lu Huan, Li Xiao Lan and Zhou Ai Guo, *Afr. J. Microbiol. Res*., **2010,** *4*, 309 - 313.
3. R. Prusinowska and K. B. Śmigielski, *Herba Pol*., **2014**, *60*, 56 - 66.
4. X.- L. Zhang, Y. - S. Guo, C. –H. Wang, G. – Q. Li, J. – J. Xu, H. – Y. Chung, W. – C. Ye, Y. – L. Li and G. – C. Wang, *Food Chemistry*, **2014**, *152*, 300 – 306.
5. H. Y. Gong, W. H. Liu, G. Y. LV and Xiaoying Zhou, *Rev. Bras. Farmacogn.,* **2014,** *24*, 25 - 32.
6. J. P. Kamdem, A. Adeniran, A. Augusti Boligon, C. Vargas Klimaczewski, O. Olalekan Elekofehinti, W. Hassan, M. Ibrahim, E. Pansera Waczuk, D. Francine Meinerz and M. Linde Athayd, *Ind. Crops Prod.,* **2013**, *51*, 26 – 34.
7. A. Arceusz and M. Wesolowski, *J. Pharm. Biomed. Anal,*. **2013,** *83*, 215 – 220.
8. S. Rehman, R. Latief, K. A. Bhat, M. A. Khuroo, A. S. Shawl and S. Chandra, *Arabian J. Chem.* **2013,** doi:10.1016/j.arabjc.2013.09.015

9. A. de Sousa Barros, S. Maia de Morais, P. A. Travassos Ferreira, Í. G. Pinto Vieira, A. Aragão Craveiro, R. Oliveira dos Santos Fontenelle, J. E. Silva Alencar de Menezes, F. W. Ferreira da Silva and H. Araújo de Sousa, *Ind. Crops Prod.,* **2015,** *76,* 557 – 564.
10. D. N. Dai, T. D. Thang, E. E. Emmanuel, O. O. Abdulkabir and I. A. Ogunwande, *Am. J. Ess. Oils Nat. Prod.*, **2015**, *2*, 12 - 16.
11. I. B. El Hadj Ali, M. Chaouachi, R. Bahri, I. Chaieb, M. Boussaïd and F. Harzallah-Skhiri, *Ind. Crops Prod*., **2015**, *77*, 631 – 639.
12. S. Santoyo, L. Jaime, M. R. García-Risco, M. Lopez-Hazas and G. Reglero, *Ind. Crops Prod.,* **2014** *52*, 475 – 480.
13. M. Oroian and I. Escriche, *Food Res. Int.,* **2015**, *74*, 10 – 36.
14. L. Barros, L. Cabrita, M. Vilas Boas, A. M. Carvalho and I. C.F.R. Ferreira, *Food Chem.,* **2011**, *127,* 1600 – 1608.
15. P. K. Mukherjee, N. Maity, N. K. Nema and B. K Sarkar, *Phytomedicine,* **2011**, *19*, 64 – 73.
16. E. Chirilă, E. Oancea and I. A. Oancea, *Ovidius Univ. An. Chem.*, **2014,** *25*, 75 - 80.