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PHYSICAL-CHEMICAL AND MICROBIOLOGICAL ANALYSIS OF ORIGINAL PLANT-SOURCE EXTRACTS USED IN COSMETICS

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In this paper four plant-source cosmetic waters and seven essential oils have been subjected to various physical-chemical analysis and their antibacterial properties have been evaluated by specific microbiological test. The cosmetic waters were obtained from Hippophae rhamnoides, Betula pendula, and the essential oils from Picea abies, Lavandula officinalis, Lavandula angustifolia, Mentha aquatica, Origanum vulgare, Thymus serpillum and Melissa officinalis. Due to the interest for dermato-cosmetic use, pH, oxidation-reduction potential, density, and antimicrobial response on methicillin-resistant Staphylococcus aureus, Klebsiella and Escherichia coli have been measured and compared for all these plant extracts.

Keywords: plant-source cosmetic waters, essential oils, pH, oxidation-reduction potential, antimicrobial activity

1. Introduction

Plants continue to provide a dynamic research in the field of non-invasive drugs discovery [1], since phytochemicals become new materials of novel bioactive compounds [2]. The terpenoids constitute the largest class of products and many interesting compounds have been used in the industrial sector of flavors, fragrances, spices, and are also used in perfumery and cosmetics. Many terpenoids also have biological activities and are consequently used for therapeutic purposes [3]. There have been studies that show these organic compounds have anticarcinogenic potential and offer diverse health-promoting effects due to their antioxidant attributes [4-6]. The antioxidants are species which

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protect the organisms from radical-induced oxidative stress [7]. The antioxidant activity of phenolics is mainly due to their redox properties. This allows them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [7-9]. A study on the phenolic content of *Hippophae rhamnoides* (sea buckthorn) has been previously developed using Folin Ciocâlteu method and showing a high total phenolic content (TPC) of 0.236 mg/GAE/dry net [10]. Other physical-chemical studies on sea buckthorn extracts has been reported for the use in the treatment of skin pigment spots [11]. Their results lead to the conclusion that sea buckthorn contains organic compounds with antioxidant and regenerative properties. Due to their antioxidant benefits for the human body, plants are included in many pharmaceutical and dermato-cosmetic products. Therefore, cosmetic facial waters and essential oils obtained from different plant species are a major source for pure and natural skincare therapies. Therapeutic essential oils are also considered to be one of the potential sources for the screening of anticancer, antimicrobial, antioxidant, and free radical scavenging agents [12-14].

In this paper four plant-source cosmetic waters and seven essential oils have been subjected to various physical-chemical analysis and their antibacterial properties have been evaluated by specific microbiological test. The cosmetic waters were obtained from sea buckthorn (*Hippophae rhamnoides*) and birch (*Betula pendula*) sprouts, and the original essential oils obtained from: spruce (*Picea abies*), lavender (*Lavandula officinalis*), wild-lavender (*Lavandula angustifolia*), wild-mint (*Mentha aquatica*), wild-oregano (*Origanum vulgare*), thyme (*Thymus serpillum*) and melissa (*Melissa officinalis*). The extracts were examined following the pH, oxidation-reduction potential (ORP), density, and antimicrobial activity.

2. Materials and methods

2.1 Plant material

Sea buckthorn fruits and lavender were carefully harvested from Constanța County, Romania, while birch sprouts, spruce resin, wild-lavender, wild-mint, wild-oregano thyme and melissa from the wild area of Buzău County, Romania, in 2016. After manual elimination of all physical impurities, the plants were stored in a room dedicated to raw materials, at 17-22ºC, on nets at the Careless Beauty Romania laboratory.

2.2. Chemicals

Distilled water was purchased from Viomed Romania. The solvent (*vegetal dermocosmetic deca-solvent*, A-2018-00286 registered patent) was purchased from Careless Beauty Organic Cosmetics Romania. Istrate-Meitert microbiological culture media, ADCL (deoxycholate citrate agar) and AABTL (blue agar) were obtained from MLT Distribution. Ceftriaxone, biseptol, ceftazidime, nitrofurantoin, ceftazidime, nalixidic acid, chloramphenicol, tobramycin, netilmicin, ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin were purchased from MIT Distribution, Romania and TODY Company, Romania.

2.3. Working methods

A regular Neoclevenger procedure was used for the hydrodistillation of the organic compounds that are sensitive to heat by steaming the plant materials.

The cosmetic waters were obtained using 1 kg of plant material and 4L distilled water (1:4 ratio) in each hydrodistillation process. Each plant material (sea buckthorn fruits, seeds, peel and birch sprouts) was subjected to hydrodistillation for approximately three hours at different temperatures (90-110°C). The waters were collected and stored properly in dark glass vessels for cooling.

The essential oils were obtained using 1 kg of each plant and 4 L of vegetal dermocosmetic deca-solvent (1:4 ratios). The plant material used was spruce resin, lavender, wild-lavender, wild-mint, wild-oregano, thyme and melissa flowers. 2 mL of essential oil resulted from each hidrodistillation. The oils were collected and stored properly in dark glass bottles.

The microbiological study was performed using Istrate-Meitert microbiological culture media: ADCL (deoxycholate citrat agar) and AABTL (blue agar).

2.4. Instrumentation

The hydrodistillation process was performed on a manufactured Neoclevenger system equipped with a 5L extraction vessel. Density was measured with Anton Paar SVM 3000 thermostat device. pH and oxidation—reduction potential (ORP) was measured with Waterproof pH 310 Meter, Oakton, SUA with a combined electrode.

2.5. Physical-chemical analysis

The physical-chemical analysis followed the pH, potential of oxidation-reduction and density of the cosmetic waters obtained from different parts of two plant species: sea buckthorn and birch. The sea buckthorn waters were obtained individually from the fruits, seeds and peel. The birch water was obtained from the sprouts.

2.6. Microbiological analysis

The microbiological analysis study was performed following the antibacterial activity of the original essential oils on the following bacterial germs: methicillin-resistant Staphylococcus aureus, *Klebsiella* and *Escherichia coli*. The results were read at 4-8-12-24 hour intervals on Petri dishes in agar medium, and they were compared with samples treated with selected antibiotics in similar conditions. Mean values of triplicate experiments have been given.

3. Results and discussions

The pH values of the four original plant-source cosmetic waters and potential of oxidation-reduction values are shown in Table 1. Density values are shown in Table 2.The results for the microbiological analysis are shown in Table 3, which summarizes the results obtained from the main images shown in Figures 1-4.

The pH values of the four original plant-source cosmetic waters (Table 1) are weak acidic (in the range 3.37 - 5.08). This acidic pH is given by the plant-source composition which ensures the long-term stability of these cosmetic waters, without any synthetic additives. The results pinpointed the favorable pH of these cosmetic products with topical application when dealing with a dermatological skin treatment. Skin pH is acidic (4–6), while the body's internal environment is more alkaline (between 7 and 9). Due to the high degree of absorption through the skin, the composition of the cosmetic waters has a major influence on the entire organism. When using weak acidic plant-source skin care products, with pH values of 3 – 5, similar to our pH skin-surface, it is expected a high degree of absorption of these products, and a long-term maintenance of an optimum skin pH, which is beneficial.

Oxidation-reduction potentials are linked to the antioxidant capacity of the analyzed products and to their pH values. The method for the determination of oxidation-reduction potential values in plant-source samples is very fast, and the results are obtained in about 20 seconds. In the plant-source extracts, usually a low value of pH is associated with a high oxidation-reduction potential value [15]. In the analyzed cosmetic waters, oxidation-reduction potential values ranged between 122.6 mV - 190.2 mV (Table 1), being higher than the potential of the regular industrial cosmetics (90-100 mV).

Density of the four cosmetic waters ranged between 0.8813 - 0.9238 g/cm^3 (Table 2), being dependent of the part of the plant used and of the plant source. For instance, the density of all sea buckthorn cosmetic waters is higher than that of the birch sprouts water. Also, density values in sea buckthorn waters vary in the following order: peels > seeds > fruits.

The pH, oxidation-reduction potential and density values can be explained by composition of the extracts. For example, sea buckthorn is mainly rich in Vitamin C, beta-caroten, and many organic acids (malic, citric, tartaric, succinic) [16]. Birch is rich is fructose, sucrose, minerals and betulinic acid [17]. The essential oils obtained from spruce resin, lavender, oregano, thyme and melissa have a high content of terpenoids and phytosterols such as oleanolic acid, betasitosterol and ursolic acid; polyphenols such as rutin, quercetin, kaempferol and gallic acid [18].

Table 1

pHs and oxidation-reduction potentials of the plant-source cosmetic waters obtained from sea buckthorn and birch sprouts

Crt.	Plant-source cosmetic water	pH	ORP, mV
1	Sea buckthorn fruits water	4.64	122.6
2	Sea buckthorn seeds water	5.08	128.0
3	Sea buckthorn peels water	3.37	190.2
4	Birch sprouts water	4.20	144.6

Table 2

Density values (g/cm^3) of the plant-source cosmetic waters obtained from sea buckthorn and birch sprouts

Crt.	Plant-source cosmetic water	Density at 20°C
1	Sea buckthorn fruits water	0.8813
2	Sea buckthorn seeds water	0.9180
3	Sea buckthorn peels water	0.9238
4	Birch sprouts water	0.9064

Table 3 shows that the original essential oils present an inhibitory activity on Methicillin-resistant *Staphylococcus aureus* in the following order: thyme < wild-lavender < spruce resin < lavender ~ wild-mint < melissa.

Table 3

Microbiological activity (mm) on Escherichia coli, Klebsiella and Methicillin-resistant Staphylococcus aureus of seven essential oils

Crt.	Essential oil	*Escherichia coli*	*Klebsiella*		*Methicillin-resistant Staphylococcus aureus*	
1	Spruce resin	-	-		18	
2	Lavender	16	10	Ceftriaxone	17	Chloramphenicol
3	Wild-lavender	14	15	Biseptol	20	Tobramycin
4	Wild-mint	**19**	10	Ceftazidime, Nitrofurantoin	17	Biseptol
5	Wild-oregano	-	11	Ceftazidime, Nitrofurantoin	16	Chloramphenicol
6	Thyme	-	**16**	Nalixidic acid	**35**	Netilmicin Ciprofloxacin Ofloxacin Levofloxacin Moxifloxacin
7	Melissa	-	-		15	Biseptol

The antimicrobial activity of the essential oils who presented an inhibitory activity on *Klebsiella* is shown as following: thyme < wild-lavender < wild-oregano < lavender ~ wild-mint. The antimicrobial activity of the essential oils who presented an inhibitory activity on *Escherichia coli* is shown as following: wild-mint< lavender < wild lavender. Consequently, all essential oils extracted from these plant species can be used in dermato-cosmetics.

As concerns the microbiological activity of lavender oils on Methicillin-resistant *Staphylococcus aureus* and *Klebsiella* it can be also mentioned that the wild plant has higher values than the cultivated one.

The antimicrobial activity was evaluated from the diameter (mm) of the spot obtained after 4-8-12-24 hour intervals in agar medium, and the values for 24 hours were retained and compared with samples treated with selected antibiotics in similar conditions.

Fig. 1. Antimicrobial activity (24 h) of spruce resin, wild-oregano and melissa essential oils on Methicillin-resistant *Staphylococcus aureus*

Fig. 2. Antimicrobial activity (24 h)of wild-mint(top) and wild-oregano (down)essential oils on Methicillin-resistant *Staphylococcus aureus*

Fig. 3. Antimicrobial activity (24 h) of wild-lavender (top) and lavender(down) essential oils on *Klebsiella*

Fig. 4. Antimicrobial activity (24 h) of wild-mint (left), lavender (right), wild-lavender(top) essential oils on *Escherichia coli*

4. Conclusions

This study indicates that pH, oxidation-reduction potential, density and antimicrobial capacity are good for an efficient plant-source cosmetic treatment. The plant-source extracts can be highly recommended for topical applications, especially in cosmetic products used when dealing with dermatological issues. Likewise, this study strongly pinpointed the pharmacological potential of these plants: birch, spruce, wild-lavender, wild-mint, wild-oregano, thyme and melissa. A special mention can be done for the wild plants which present sometimes higher microbiological activity that the cultivated ones, which underlines the therapeutic effects expected from the wild plant species harvested from the Romanian Carpathian area that can be used in cosmetics.

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