

## The obtaining and the technique for evaluation of some natural extracts used in cosmetics

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### Abstract

Up to a certain period, the essential purpose of cosmetology was beautifying women. Makeup made her face more attractive by concealing imperfections. It should be considered that skin is an organ of the body, and like other organs, it reflects the physiological health. In this experimental research, there were obtained different alcoholic extracts from calendula and rosehip using three procedures: extraction by Soxhlet apparatus, extraction by simple refluxing, and maturation. The herbs extracts were incorporated into a base cream for an easier application on the skin. There were used SKH1 mice and their skin parameters were evaluated by noninvasive methods. The creams are good enough for human application, but it must care to the allergenic character of rosehip extracts.

**Keywords:** natural extract, cosmetics, marigold, rosehip, Soxhlet

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### 1. Introduction

At the end of the nineteenth century, dermatologists began to use chemical substances for smoothing facial wrinkles and scars. An example is phenol, which is used to treat acne, followed by trichloroacetic acid, and alpha-hydroxy acids respectively in 1990 [1]. Various celebrities were referred to the role and importance of beauty in every woman's life. The most famous physicians of antiquity, the Greek Hippocrates, the Romans Galen and Celse were interested in cosmetics. They prescribed rules of hygienic skin care and even some treatments for unsightly dermatosis [2]. In Ancient Greece, too much use in cosmetic products prompted Solomon and Socrates to stand up against these abuses. Decay of the Roman Empire led to a long decay period for care cosmetics. Only in the Renaissance the concern for beauty reappears, revealing always, new cosmetics [3].

Keeping the skin in a perfect health status and in physiological balance is the subject of Cosmetics, defined as a discipline that aims to beautify the body in order to keep the skin freshness and her appearance. "Cosmetics" is also an art that corrects imperfections and the techniques, which are used, give shine and highlight some features and characteristics.

Biochemistry researches, surgery, and physiotherapy development remove many physical imperfections. Aesthetic concerns are now an important part of modern dermatology, physicians currently inquiring aesthetics and cosmetology. Since the barrier between normal healthy skin and some unsightly dermatosis is not precisely defined, various skin abnormalities affecting equally concern both the doctor and the esthetician beautician. Their support and concern everyone for their care will help preserve the health and beauty [4].

The pathway till presently cosmetic substances and to the scientific use of what nature provides us was a very long trip for the humanity. Knowledge of appropriate chemicals to beautify and recipes and then had to be mixed them are among the oldest achievements of the human mind [5].

Empirical procedures for obtaining cosmetics and hygiene products have long been obsolete, now the formulations are based on knowledge of morphology, histology, physiology, and pathology of the skin, on the use of a large number of raw materials and the progress made in technology applied to obtain them. The usage of scientific techniques allowed the optimization of appearance, stability, the safety, and efficacy of cosmetics [6].

The herbs must be harvested at certain times in order to obtain the curative effect. To keep active all the herbs are dried thoroughly and store in cardboard boxes or cloth bags in dry places [7].

The organic syntheses reached the industrial production of a large number of active ingredients. In some cases these synthetic compounds have replaced lesser or greater the herbal product (e.g. most vitamins). In other cases, the use of natural products continue to be economically profitable, than getting either synthetically active principles, for example, most alkaloids. Use of the herbs may be preferred to pure chemical and therapeutic standpoint. In this case the crop is preferred for complex action provided by synergism of different compounds [8].

Both medicinal and aromatic herbs are part of the so-called utility herbs category means that these herbs have a particular purpose for humanity [9].

Marigold or *Calendula officinalis* from *Compositae/Asteraceae* family (Figure 1) is an annual herbaceous, cultivated or spontaneous, showing a root around 20 cm long, an erect and branched stem, around 20 cm high. It has grouped and orange flowers. It blooms from June to September [10].

Marigold is found throughout the country, with moderate claims against light and moisture; it vegetates well on all soil types. The flowers are the used organ of the herbs [12].



Figure 1. Marigold or *Calendula officinalis* [11]

Besides large amounts of carotenoids, marigold contains a saponin whose sapogenin is the oleanolic acid ( $C_{30}H_{48}O_3$ ), a pentacyclic triterpene. In addition it also contains volatile oils, flavonoids, flavone glycosides, bitter substances, malic acid, vitamin C, proteins, and esters of fatty acids [13]. Marigold extracts are used in cosmetics, toiletries and pharmaceutical preparations due calming and vasoprotector effects (improves peripheral circulation, reduces redness), antiseptic, healing and decongestants. Extracts in butylene glycol and glycerol (aqueous solution) are used in concentrations ranging from 2-7% in cosmetic preparations used as beach products aimed irritated and chapped skin [14].

Rosehip or *Rosa canina L.* from *Rosaceae* family (Figure 2) is a shrub that has a branch root in the soil. Its branched stems form a shrub with thorny branches. It presents pink or white flowers which are solitary or grouped, 2-3 in a blossom. Rosehip blooms in June. The fruits are enclosed in a red receptacle [9]. It is spread near fences, roads, forest glades, deciduous forests, rarely in coniferous forests from lowlands to an altitude of 1700 m. The fruits (*Fructus Cynosbati*) are harvested in August-September [11]. Its active ingredients are: sugar, malic acid, citric acid, pectin, tannins, volatile oils, lecithin, dextrin, vanillin, minerals as  $K^+$ ,  $Ca^{2+}$ ,  $Fe^{3+}$ ,  $Mg^{2+}$ , vitamin C [7]; it presents an astringent restructured, and toning action.



Figure 2. Rosehip or *Rosa canina L.* [15]

## 2. Materials and methods

### 2.1. Extracts obtaining

The obtaining of natural herbal extracts is the first and most important step in this experimental study. There were chosen three different variants: (i) extraction with Soxhlet apparatus at 78 °C for 2 hours, (ii) normal refluxing at 78 °C for 60 min., and (iii) maceration at room temperature for 7 days.

Table 1. Samples codes and descriptions

Code	Raw materials	M.U.	Quantity
	<i>Technique</i>		
MS	Marigold	g	5
	Ethanol (96%)	ml	40
	Distilled water	ml	10
	<i>Soxhlet</i>		
RS	Rosehip	g	5
	Ethanol (96%)	ml	40
	Distilled water	ml	10
	<i>Soxhlet</i>		
MR	Marigold	g	5
	Ethanol (96%)	ml	40
	Distilled water	ml	10
	<i>Refluxing</i>		
RR	Rosehip	g	5
	Ethanol (96%)	ml	40
	Distilled water	ml	10
	<i>Refluxing</i>		

MM	Marigold	g	5
	Ethanol (96%)	ml	40
	Distilled water	ml	10
	<i>Maceration</i>		
RM	Rosehip	g	5
	Ethanol (96%)	ml	40
	Distilled water	ml	10
	<i>Maceration</i>		

### 2.2. Creams preparation technique

Based on these extracts, it was obtained a series of pharmaceutical formulations (semisolid creams).

Composition:

I. Cetostearyl alcohol	_____	8.0 g
Cremophor A6	_____	1.9 g
Cremophor A25	_____	1.9 g
Liquid paraffin	_____	13.0 g
Parabens	_____	0.2 g
II. Water	_____	67.7 g
III. Propylene glycol	_____	7.0 g
Extract	_____	0.3 g

The mixture (I) and water (II) are heated separately at 80 °C. Then the water (II) is added over mixture (I) under vigorous stirring (1000 rpm). The mixture (III) is heated, then mixed with the product obtained previously (mixture from I+II) and it is continued the stirring. The final mixture is slightly cooled until is reached the room temperature; it is packaged, labeled - name of the sample and production date.

### 2.3. Animals protocol

The hairless mouse SKH1 is very often used in skin research [16, 17]. Female mice (ten weeks old) were maintained during the experiments in National Institute of Animal Health (NIAH) recommended conditions: 12 hours light-dark cycles, food and water *ad libidum*, temperature 24±2 °C, humidity above 55%. The creams were applied on mice skin for 15 weeks (0.3 mL / application). There were used 14 mice: group MS (2 mice, treated with cream based on MS extract), group RS (2 mice, treated with cream based on RS extract), group MR (2 mice, treated with cream based on MR extract), group RR (2 mice, treated with cream based on RR extract), group MM (2 mice, treated with cream based on MM extract), group RM (2 mice, treated with cream based on RM extract) and group 0 (2 mice as blank treated with cream without any extract). The mice were shaved weekly, and the applications and measurements were done twice a week.

After applications, each determination was performed within 30 minutes. All the measurements on the mice skin were carried out with a Multiprobe Adapter System (MPA5) from Courage-Khazaka, Germany.

### 3. Results and Discussions

Marigold based creams were yellowish, homogeneous, low odor, pleasant. Solubility at 25 °C: does not dissolve in water, hardly soluble in methanol, ethanol, acetone. pH =  $6.6 \pm 0.1$ . Conservation: does not require the addition of preservatives - stable above 90%. Viscosity - decreases with increasing temperature, relatively stable between pH = 3-10, and constant between pH = 5-8. Rosehip based creams were white, homogeneous, strong-smelling, pleasant. Solubility at 25 °C: does not dissolve in water, hardly soluble in methanol, ethanol, acetone. pH =  $6.3 \pm 0.3$ . Conservation: does not require the addition of preservatives - stable above 90%. Viscosity - decreases with increasing temperature, relatively stable between pH = 4-9, and constant between pH = 6-8.

In the case of the control group treated with base cream without active substances (group 0), TEWL values (recorded as differences between a measured value and the average value which was measured on the same mouse) indicate a very slight increase which is specific for dermatocosmetic treatments with fatty cream.

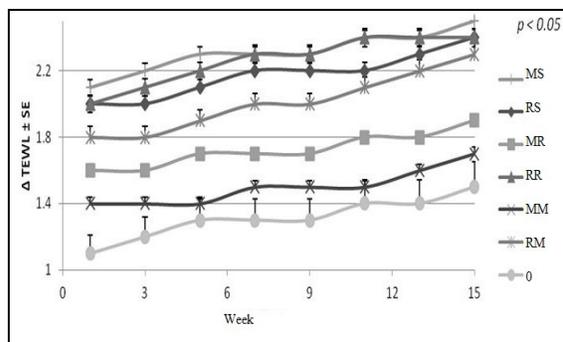


Figure 3. Evolution of TEWL differences

In the case of the groups where it were applied creams with extracts/active ingredients, transdermal water loss increases were more pronounced, but not to such a degree as to be considered alterations of the skin or substances with harmful effect, prohibited for skin application (Figure 3).

Measured pH values fluctuate slightly (Figure 4), in a range of values from 0.1 to 0.4. There is a slight increase in the differences (positive or negative), but could not be correlated with the treatment.

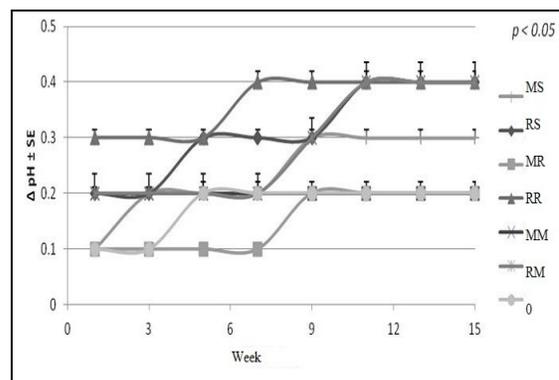


Figure 4. Evolution of pH differences

There were not observed mice skin sebum differences in many studies, but in this case, sebum values (recorded as differences between a measured value and the average value which was measured on the same mouse) indicate a uniform increase for each mouse due to the increased fat content of the creams (Figure 5).

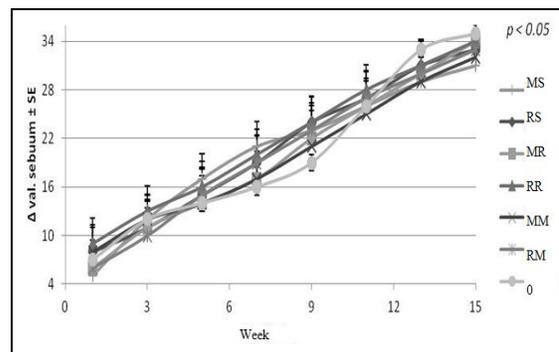


Figure 5. Evolution of sebum differences

Values of melanin, the skin pigment (recorded as the differences between a measured value and the average value which was measured on the same mouse) were uniformly upward trend, but could not be correlated with the type of treatment. In general, there is a slight linear increase melanin content in the skin over skin testing (Figure 6).

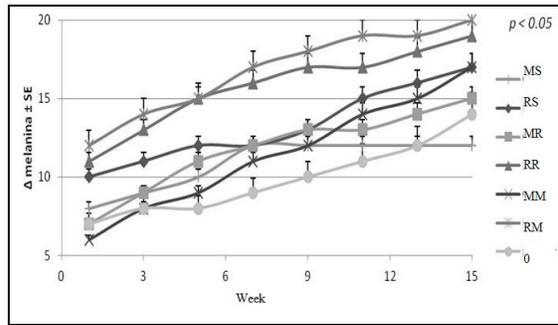


Figure 6. Evolution of melanin differences

In the case of erythema measurements, there were significant differences between the two types of samples (calendula based cream, and rosehip based cream). Thus, after nine weeks of application-evaluation, in the case of rosehip based creams, the differences in erythema recorded values exceeded 20 units, while for calendula based creams the differences were less than 15 units (Figure 7). At the end of experiment, after 15 weeks of application-evaluation, in the case of rosehip based creams, erythema reached 35 units while for calendula based creams, the differences were generally around 15 units. Thus, it can be concluded that rosehip based creams cause allergic reactions which increase skin hemoglobin level.

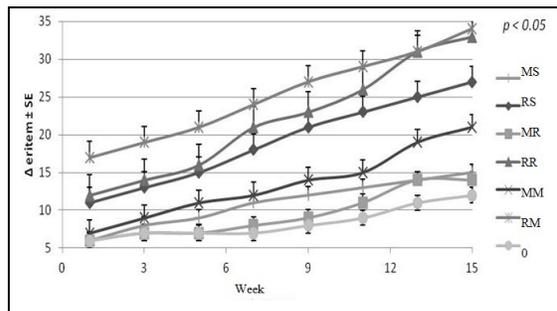


Figure 7. Evolution of erythema differences

In the case of corneometry measurements (Figure 8), which show the level of *Stratum corneum* hydration, there were recorded downward trends in all groups. Thus, the applying of these creams slightly decreases the *Stratum corneum* hydration, but the recorded differences were in the range of 0.6 to 1 unit specific for a treatment that does not harm normal skin and cannot be considered aggressive treatment or a specific toxic compound.

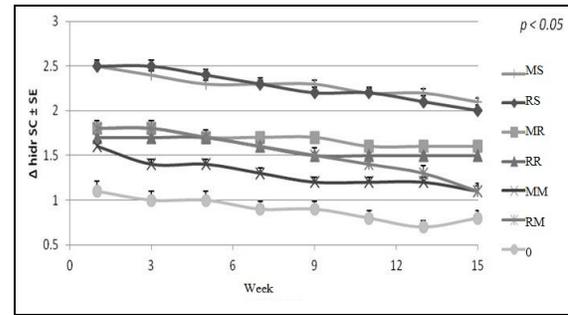


Figure 8. Evolution of *Stratum corneum* hydration differences

#### 4. Conclusion

From a therapeutic standpoint, the role of active principles can be played by a single substance or by a complex of substances. Active principle or principles can be derived, respectively isolated from plant product and used as pure chemicals. The aim of this study was to obtain alcoholic extracts of calendula and rosehip, using laboratory techniques frequently encountered in organic chemistry and also to get creams and to analyze its toxicological character. All the obtained creams were good enough in order to be used as cosmetics, but it must care to the allergenic character of rosehip extracts.

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#### Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

#### References

1. History of Dermatology. <http://www.dermatology-center.com/resources/history.cfm>. Last accessed: November 5th, 2012.
2. Balaban, A.T.; Banciu, N.; Pogany, I. *Aplicații ale metodelor fizice în chimia organică*, Ed. Stiintifica și Enciclopedică, Bucharest, **1983**.
3. Bojor, O.; Popescu, O. *Miracolele terapeutice ale plantelor*, Mica Enciclopedie de Fitoterapie, Ed. Edimpex Speranța SRL, Bucharest, **1993**.
4. Choromańska, A.; Saczko, J.; Kulbacka, J.; Skolucka, N.; Majkowski, M. The potential role of photodynamic therapy in the treatment of malignant melanoma-an in vitro study. *Adv Clin Exp Med*. **2012**, 21(2), 179-185.

5. Becker, H.; Berger, W.; Domschke, G.; Fanghänel, E.; Faust, J.; Fischer, M.; Gentz, F.; Gewald, K.; Gluch, R.; Mayer, R.; Müller, K.; Pavel, D.; Schmidt, H.; Schollberg, K.; Schwetlick, K.; Seiler, E.; Zeppenfeld, G. *Organicum - Chimie organica practica*, Bucuresti, **1982**.
6. Ciucanu, I. *Metode fizico-chimice in analiza chimica instrumentala*, Ed. Mirton, Timisoara, **1996**.
7. Ciulei, F.; Grigorescu, E.; Stanescu, U. *Plante medicinale, Fitochimie și Fitoterapie*, Ed. Medicala, Bucharest, **1993**.
8. Coiciu, E.; Racz, G. *Plante medicinale si aromatice*, vol. I, Ed. R.P.R., Bucharest, **1962**.
9. Cosmovici, L.; Zisu, L. *Caleidoscop cosmetic*, Ed. Medicala, Bucharest, **1988**.
10. Krier, H. *Medicina naturista - Miracolul plantelor*, Ed. Helicon, Timisoara, **1997**.
11. Elisa, gradina mea de vis. <http://t0.gstatic.com/images?q=tbn:ANd9GcQZXQomUu0y6HDcERfzcP1OxG0TXbfakgIa9GvV9nqfJOINcVTB&t=1>. Last accessed: November 5th, 2012.
12. Marotti, M.; Piccoglia, R.; Galletti, C. *Herba Hing*, **1989**, 28(1-2), 37-44.
13. Merica, E.; Lungu, L. *Geologia produselor cosmetice*, Ed. Corson, Iasi, **2000**.
14. Mocanu, S.; Raducanu, D. *Plante medicinale in terapeutica*, Ed. Militara, Bucharest, **1983**.
15. Arbusti. <http://ecomuntiimacinului.files.wordpress.com/2011/03/macesul-rosa-canina3.jpg>. Last accessed: November 5th, 2012.
16. Benavides, F.; Oberyszyn, T.M.; VanBuskirk, A.M.; Reeve, V.E.; Kusewitt, D.F. The hairless mouse in skin research. *J Dermatol Sci*. **2009**, 53(1), 10-18, doi: [10.1016/j.jdermsci.2008.08.012](https://doi.org/10.1016/j.jdermsci.2008.08.012)
17. Rogers, F.A.; Hu, R.H.; Milstone, L.M. Local Delivery of Gene-Modifying Triplex-Forming Molecules to the Epidermis. *J Invest Dermatol*. **2012**, Sep 27. doi: [10.1038/jid.2012.351](https://doi.org/10.1038/jid.2012.351).