

EVALUATION OF THE EFFICACY AND CHARACTERIZATION OF AN ANTI-ACNE CREAM CONTAINING HERBAL EXTRACTS

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Abstract

The main objectives of the present study were to formulate and to evaluate an anti-acne cream based on alcoholic extracts of *Thymus vulgaris*, *Rosa centifolia*, *Rosmarinus officinalis* and *Saponaria officinalis* and to evaluate the efficacy and the sensorial characteristics of the emulsion. Firstly, the *in vitro* antimicrobial activity of the herbal extract was evaluated by agar-well diffusing method. *Staphylococcus aureus* ATCC 12602, *Escherichia coli* ATCC 29998 and *Candida albicans* ATCC 10231 were selected as test microorganisms. In the second part of the study, the efficacy of an oil-in-water cream containing ethanolic extracts and non-comedogenic ingredients was established in a prospective non-randomized study on twelve volunteers using the cosmetic product twice daily. The activity of sebaceous glands on the mid forehead, cheeks and chin was assessed by measuring the sebum level with the Sebumeter[®] device (Courage Khazaka). The subjective characteristics of the cream base were evaluated by the volunteers, following a 1 to 10 scale and reported by filling an evaluation questionnaire. The obtained results were statistically significant and revealed the efficacy of the tested cream, which reduced the seborrhoea after 4 weeks of cream application. The product was well tolerated and appreciated by the volunteers regarding its spreadability, penetration ability and lack of stickiness.

Rezumat

Obiectivele principale ale prezentului studiu au constat în formularea și evaluarea unei creme antiacneice pe bază de extracte alcoolice de *Thymus vulgaris*, *Rosa centifolia*, *Rosmarinus officinalis* și *Saponaria officinalis* precum și în evaluarea eficacității și a caracteristicilor senzoriale ale emulsiei. În primul rând a fost evaluată *in vitro* activitatea antimicrobiană a extractelor vegetale prin metoda difuzimetrică. Au fost selectate ca microorganisme test: *Staphylococcus aureus* ATCC 12602, *Escherichia coli* ATCC 29998 și *Candida albicans* ATCC 10231. În a doua parte a studiului, a fost evaluată eficacitatea unei emulsii U/A conținând extractele etanolice menționate anterior și ingrediente non-comedogene printr-un studiu prospectiv non-randomizat pe doisprezece voluntari care au utilizat produsul cosmetic de două ori pe zi. Activitatea glandelor sebacee de la nivelul frunții, pomeților și bărbiei a fost evaluată prin măsurarea nivelului de sebum cu ajutorul dispozitivului Sebumeter[®] (Courage Khazaka). Caracteristicile subiective ale bazei de cremă au fost evaluate de către voluntari pe o scară de la 1 la 10 și raportate prin completarea unui chestionar de evaluare. Rezultatele obținute au fost statistic semnificative și au evidențiat eficacitatea cremei testate de a reduce seboreea după 4 săptămâni de aplicare a cremei. Produsul a fost bine tolerat și apreciat de voluntari în ceea ce privește capacitatea de etalare, penetrarea în piele și lipsa de adezivitate.

Keywords: acne, O/W emulsion, herbal extracts

Introduction

Acne vulgaris is one of the most common dermatological diseases, caused by a combination of pathogenic factors including follicular hyperkeratosis, seborrhoea and alteration of the skin lipids, proliferation of *Propionibacterium acnes* (*P. acnes*) and inflammatory phenomena [5, 10, 12, 14]. Microcomedones represent pathological structures considered primary precursors in acne. Accumulation of sebum and keratin elicited by androgen-mediated stimulation causes follicular obstruction which then leads to the development of

microcomedones. The lipid-rich environment is considered a good substrate for the proliferation of anaerobic Gram positive microorganism, *P. acnes*. It produces lipases, hydrolases and proteases which are responsible for sebum decomposition and releasing of fatty acids. These stimulate an inflammatory response, with a preponderance of the cytokines IL-1 α , IL- β and TNF- α , which is thought to activate hyperkeratinisation, cell adhesion and follicular obstruction. The presence of upregulated CD3-positive and CD4-positive T-cells in microcomedones and the increased number of macrophages in lesions indicate that acne represents a

primary inflammatory disease. This inflammation manifests through the presence of acneiform lesions such as papules, pustules, nodules, and cysts [5, 8, 10, 12].

The management of acne requires understanding four main factors of the pathophysiology: disease duration, disease severity, past response to treatment and skin colour [4, 10, 24].

As the seborrhoea is one of the pathogenic factors in the physiopathology of acne, diminishing the sebum production is one major purpose in its treatment [11, 25]. Another important goal of the anti-acne treatment is represented by the inhibition of proliferation of *P. acnes*, involved in the development of inflammatory acne.

Despite of numerous products available in present, the acne treatment involves multiple challenges like antibiotic resistance, high costs treatments, inability of anti-acne drugs incorporated in conventional vehicles to reach pilosebaceous follicle and to release the active substance at therapeutic levels. The absence of an appropriate animal model to develop new delivery systems for anti-acne drugs represents another limitation of the acne therapy.

Over the last 20 years, in the context of increasing resistance to existing anti-microbial agents and numerous side effects reported, the interest for natural compounds have increased. The medicinal plants present a great potential in the development of new anti-acne products considering antibacterial, anti-inflammatory, antioxidant and antiandrogen effects of the phytochemical compounds [5, 20].

Phytochemical analysis of *Thymus vulgaris* (*Lamiaceae*) indicates the presence of flavonoids, phenols, thymol, carvacrol and eugenol. The antioxidant, antibacterial and antifungal effects of *Thymus vulgaris* have been reported in extensive clinical studies therefore this plant species is considered valuable in phytoterapy [2, 15, 16, 22, 33, 34]. *Rosa centifolia* (*Rosaceae*) is a rose species which contains mainly phenyl ethanol, geranyl acetate, geraniol, linalool, benzyl alcohol, benzaldehyde, nerol and citronellyl acetate. It has been demonstrated that *Rosa centifolia* extract possesses significant anti-microbial and antioxidant activity [17, 29]. *Rosmarinus officinalis*, a member of the *Lamiaceae* family represents a large source of di- and tri-terpenoids, flavonoids and phenolic acids. Rosmarinic acid, carnosic acid and carnosol represent the main antioxidant compounds. Rosemary biologic activity is represented also by a strong antimicrobial effect [1, 18, 21, 32].

Saponaria officinalis is a plant belonging to the *Caryophyllaceae* family that is used for its antioxidant, antibacterial and antiproliferative activity. The main constituents are bisdesmodic saponins, quillaic acid, flavonoids and phenolic compounds [23, 30].

The main objectives of the present study were the ingredients selection and preparation of the non-comedogenic oil in water emulsion containing alcoholic extracts of *Thymus vulgaris*, *Rosa centifolia*, *Rosmarinus officinalis*, *Saponaria officinalis* and the most suitable substances selected as ingredients for the cosmetic cream base, the *in vitro* testing of the ethanolic extracts of *Thymus vulgaris*, *Rosa centifolia*, *Rosmarinus officinalis* and *Saponaria officinalis* for antibacterial/antifungal effects, the *in vivo* testing for anti-acne effect of the cosmetic formulation which contains the natural extracts mentioned above and the assessment of several cosmetic characteristics, tolerance and efficacy of the cream by sensorial evaluation.

Materials and Methods

Emulsion preparation

Three samples of anti-acne O/W cream with different compositions were prepared with non-comedogenic ingredients and the minimum amount of fatty base (12%) (Table I). Vegetal extracts were incorporated in the cream base in variable quantities of 2-3%. The formula with the most suitable rheological and physical properties (colour, creaming and liquefaction) was selected for *in vivo* testing.

Table I
Ingredients of the cream base

Ingredients	Amount
Caprylic/Capric Triglycerides	12%
Cetylstearyl Alcohol	
Glycerol stearate	
Sodium laurylsulfate	1-2%
Glycerol	4-8%
Carbopol 976	0.1-0.5%
Triethanolamine	0.05- 0.2%
Kaolin	2%
Zinc oxide	
Talcum	
Methylparaben:propylparaben (3:1)	0.1%
Water	to 100%

The lipophilic surfactants: caprylic/capric triglycerides, cetylstearyl alcohol and glyceryl stearate were supplied by Farmec Company Romania. The triethanolamine, sodium laurylsulfate, glycerol, zinc oxide, talcum, kaolin and carbopol were supplied by Vitamar Romania. The other materials used were the following: methylparaben, propylparaben and distilled water (Ph. Eur.). All substances and reagents used were of pharmaceutical purity.

The O/W emulsion was prepared by heating separately the oil phase and the aqueous phase containing the hydrophilic surfactant. The surfactant, sodium laurylsulfate was dissolved in warm water at 50°C and then glycerol was added in

the aqueous phase. The oil phase was added in the aqueous phase under continuous stirring at high temperature at $50^{\circ}\text{C} \pm 2^{\circ}\text{C}$, for different time intervals. Carbopol gel was prepared by dispersing the powder in water, neutralized with triethanolamine and then added to the cream under continuously stirring at 500 rpm.

In vitro test of antimicrobial activity of the vegetal extracts

Antibacterial/antifungal susceptibility was determined using the agar-well diffusing method according to the methodology established by the Clinical Laboratory Standards Institute CLSI [35]. Three different strains were tested: *Staphylococcus aureus* ATCC 12602, *Escherichia coli* ATCC 29998, and *Candida albicans* ATCC 10231. *S. aureus* was chosen because of the implication in the pathology of severe acne by superinfection of the affected areas. Briefly, the method consists on wells made in the agar medium and filled with the extracts [31]. Bacterial inoculum was prepared using direct-colony suspension diluted, in order to obtain a standardized size inoculum of approximately 1.5×10^8 CFU/mL (colony-forming unit/mL). Then, the bacterial inoculum was evenly spread across the surface of a suitable solid culture media. *S. aureus* and *E. coli* strains were tested on Mueller-Hinton agar; *C. albicans* was tested on Sabouraud agar [6]. Following incubation at 37°C overnight, the inhibition zones surrounding the wells were measured. The solvent used was also tested in order to evaluate a potential antimicrobial activity.

In vivo determination of sebum level

Subjects

Twelve healthy volunteers, all females, aged between 19 - 23 years were selected to participate in the study. The sebum levels were determined at several areas on the face: forehead cheeks and chin (the "T zone"). Inclusion criteria were: age (between 18 - 30 years), phototype (II to V), skin type (oily or combined) predisposed to acne. Exclusion criteria: age under 18 years, severe acne, hormonal treatment for acne or other medical conditions, extreme skin sensitivity. All subjects signed an informed consent before enrolling into the study.

Study protocol

During the study each volunteer followed the same face cleaning routine: twice per day, morning and evening, with the same product and clear guidance regarding the quantity used how to apply and remove it. None other cosmetic product was used on the tested areas.

First, the skin tolerance of the cream was evaluated before study using a "patch-test" held in place for 24 hours. Treatment sites were assessed before and after the application of the test material. A negative control was used to facilitate the evaluation. The quantification of the skin irritation is given through

a numeric scale (0 – none, 1 – erythema, 2 – oedema, 3 – desquamation, 4 – vesicles). The final result is presented as an average of the quotations obtained for each volunteer. The instrumental measurements for skin lipid levels were performed before treatment, 2 hours and then after 1, 2, 3 and 4 weeks after cream application.

Sebumetric analysis

The most important skin parameter which is involved in acne is the sebum level. Biometrically, the sebum level was assessed using the Sebumeter[®] (Courage Khazaka, Germany), which measures the skin surface lipids. The measuring device is represented by a non-invasive cartridge with a mat tape which is brought in contact with the skin. The mat tape becomes transparent in relation with the activity of the sebaceous glands. The transparency of the tape is measured by a photocell reporting the area in μg of sebum/cm².

The percentage changes of sebum level were calculated using the formula:

$$\% \text{change} = [(T0 - Tf)/Tf] \times 100,$$

where: T0 – baseline values, Tf – value obtained at the end of the study [19].

Statistical analysis

The analysis was performed by relating the data of the cosmetic product treated sites to the corresponding starting value. The obtained data were analysed, calculating the mean and standard deviation for the quantitative variables of every group and the proportions for the qualitative variables. The difference of means before and after 28 days was tested using a *t*-test for paired samples. A *p* value < 0.05 was considered significant.

Sensory analysis of tested product

Sensory analysis represents a valuable tool in qualifying consumer perception regarding a cosmetic product. The volunteers were asked to fill out a questionnaire evaluating the sensory properties of the tested product from their point of view. They received the formulation and the instructions to evaluate the odour, spreadability, penetration, after-feel, stickiness and oiliness following a 1 to 10 scale (1 minimum and 10 maximum of a characteristic). In order to facilitate the evaluation process, samples representing the borders (value 1 and value 10 for each characteristic) of the scale were made available to the volunteers.

Sensory attributes were evaluated between two fingers and rubbed into the skin, on the dorsal side of the hand. Spreadability was evaluated as the ease of spreading the cosmetic emulsion over a given surface (2 cm²) and the penetration degree was appreciated as the speed of absorption into the skin. Each sample was characterized also regarding after-feel (the feel perceived after topical application of

the cosmetic product), the stickiness of the cosmetic product (the work necessary to overcome the attractive forces between the surfaces of the two fingers joined together by a layer of cosmetic emulsion) and the oiliness (the greasy feel perceived after cosmetic product application).

Results and Discussion

In vitro test of antimicrobial activity of the vegetal extracts

Below, we summarize the inhibition pattern of microorganisms tested. In Figure 1 it is depicted the qualitative screening of the antimicrobial activity of the tested vegetal extracts.

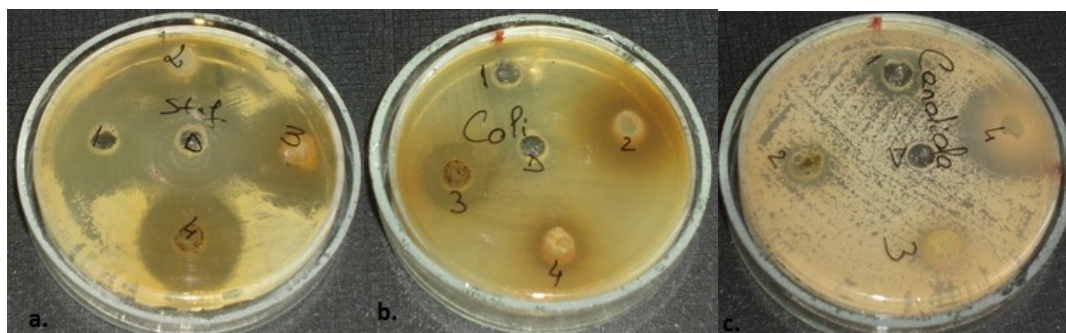


Figure 1.

The qualitative screening results of the antimicrobial activity on the investigated compounds exemplified for: a) *S. aureus* ATCC 12602; b) *E. coli* ATCC 29998; c) *C. albicans* ATCC 1023; (1 - *Saponaria* tincture; 2 - *Rosa* Tincture; 3 - *Thymi* tincture; 4 - *Rosmarinus* Tincture; 5 - tincture solvent)

Table II

Antibacterial activity of the extracts by agar-well diffusion method

Entry	Tincture	<i>S. aureus</i> inhibition area (mm)	<i>E. coli</i> inhibition area (mm)	<i>C. albicans</i> inhibition area (mm)
1	<i>Saponaria</i>	20	17	15
2	<i>Rosa</i>	25	17	no inhibition
3	<i>Thymi</i>	27	20	no inhibition
4	<i>Rosmarinus</i>	27	no inhibition	25
5	Solvent	25	no inhibition	no inhibition

The results showed a good antibacterial effect against *S. aureus* for *Thymus* and *Rosmarinus* tinctures, while *Saponaria* tincture had no antibacterial effect; it was weaker than the solvent used to prepare the tincture. The reason for this particular pattern remains unclear. We noticed an important antibacterial effect against *E. coli* for *Thymus*, *Saponaria* and *Rosa* tinctures, while *Rosmarinus* tincture had no effect. The *Rosmarinus* tincture, however, had a better antifungal effect than *Saponaria* tincture. The data suggest some predictable patterns of susceptibility for some mixed skin superficial infections. The *Rosmarinus* tincture could be considered as a valid option for mixed infections due to some Gram-positive bacteria and yeast – *S. aureus* and *C. albicans*. The *Saponaria* tincture could be effective when *E. coli* and *C. albicans* are isolated, but our results are not accurate enough to be extrapolated to other species, mainly *P. acnes*. An extensive metagenomic study regarding association of commensal skin species with *acne vulgaris*, revealed important differences at strains level and genomic level of a the dominant microorganism of the skin microbiome – *P. acnes*

[13]. Testing of *P. acnes* strains was not included because of limited procedures available in our laboratory. Although the results cannot be generalized to other microorganisms, the present data showed an attractive alternative of herbal extracts in the treatment of superficial skin infections.

These antibacterial and antifungal effects also contribute to the preservation of the formulated product. Adding to the cosmetic formula the appropriate concentration of natural extracts with antimicrobial activity may allow reducing the quantity of synthetic preservatives as methylparaben and propylparaben used to ensure the antimicrobial stability of the product.

Emulsion formula overview

In this formulation, the vegetal extracts were incorporated in an O/W cream base as antioxidant and antimicrobial agents. *Thymus vulgaris* extract provides an anti-acne effect due the high concentration of thymol, a substance widely used as antiseptic, by inhibiting the proliferation of *P. acnes*. Other active substances from *Thymus vulgaris* extract like carvacrol, eugenol, p-cimene

also possess antibacterial, antifungal and antioxidant properties. *Rosa centifolia* extract also represents a source of antioxidants due to the polyphenols presence. *Rosmarinus officinalis* and *Saponaria officinalis* extracts present antioxidant activity which could be helpful in supporting antioxidant defence mechanisms in *acne vulgaris*. Recent studies revealed the role of free radicals and antioxidant enzymes in the pathophysiology of *acne vulgaris* and that the patients with *acne vulgaris* are under systemic and cutaneous oxidative stress induced by reactive oxygen species [7, 28]. Sebum characteristics are altered in *acne vulgaris* with lower concentrations of linoleic acid and higher levels of squalene peroxide. The role of linoleic acid to inhibit the activity of oxygen free radicals is disrupted in patients with acne, while the antioxidant enzymes activity is intensified [28]. *P. acnes* plays an important role in engendering of reactive oxygen species which leads to an increased secretion of inflammatory cytokines (IL-1 α , β -defensin-2, IL-8). *P. acnes* produces chemotactic factors for neutrophils which generate a release of inflammatory factors like lysozyme enzymes leading to the destruction of the follicular wall as a result of the phagocytosis. In the inflammatory tissue, reactive oxygen species alter DNA and/or membrane lipids causing cell damage at the inflammation site [3, 7, 26, 27, 28].

Based on these observations, the four vegetal extracts incorporated in the cosmetic cream act synergistically in reducing inflammatory reaction mediated by oxidative stress in addition to their antimicrobial activity. Moreover, the other ingredients of the cosmetic cream were chosen for the most suitable properties in acne patients. In this formulation, triethanolamine was used to neutralize carbopol in order to achieve maximum viscosity. Kaolin, zinc oxide and talcum were used as adsorbent powders to ameliorate the oily aspect of the skin. Glycerin was incorporated in the cream formula as effective humectant to increase *stratum corneum* hydration. When it is used as a moisturizer, a humectant agent is usually associated with occlusive compounds,

in our case caprylic/ capric triglycerides and glycerol stearate [9]. Due to occlusive properties of fatty compounds, it was used a minimum percentage of caprylic/capric triglycerides, cetylstearyl alcohol and glyceryl stearate as oily phase. The fatty compounds included in the formulation exert a minimum comedogenic effect.

In vivo determination of sebum level

Tolerance test

The product was very well tolerated by all volunteers. The average irritant score of the product was 0.00; none of the tested volunteers developed a reaction after the product application. Thus, this product may be considered as non-irritant regarding its primary skin tolerance.

Determination of sebum level

Among all physiopathological factors, sebum secretion is directly connected with the severity of acne. Moreover, the increased sebum level represents an appropriate environment for *P. acnes* proliferation in the follicles [19].

The percent of the changes in skin sebum level, at the end of the study compared with baseline values were 75.66% μg sebum/ cm^2 for forehead area, 65.32% μg sebum/ cm^2 for cheek area and 78.36% μg sebum/ cm^2 for chin area.

The results of the clinical study indicated that the application of the herbal cream leads to a significant reduction of sebum level. According to these results, the sebum level in forehead area was reduced from 226.6 ± 24.7 $\mu\text{g}/\text{cm}^2$ to 129 ± 40.7 $\mu\text{g}/\text{cm}^2$ after 28 days of cream application ($p = 0.000004$). For cheek area the sebum level decreases from 196.9 ± 52.1 $\mu\text{g}/\text{cm}^2$ to 119.1 ± 55.9 $\mu\text{g}/\text{cm}^2$ ($p = 0.004761$), while for the chin area the reduction was from 204.4 ± 35.8 $\mu\text{g}/\text{cm}^2$ to 114.6 ± 46.0 $\mu\text{g}/\text{cm}^2$ ($p = 0.000124$). The statistical analysis revealed a significant decrease in the average sebum level after 28 days of treatment for all three zones and all tested subjects, showing a good efficiency of the tested cream formula (Figure 2). The results were presented as mean \pm standard deviation.

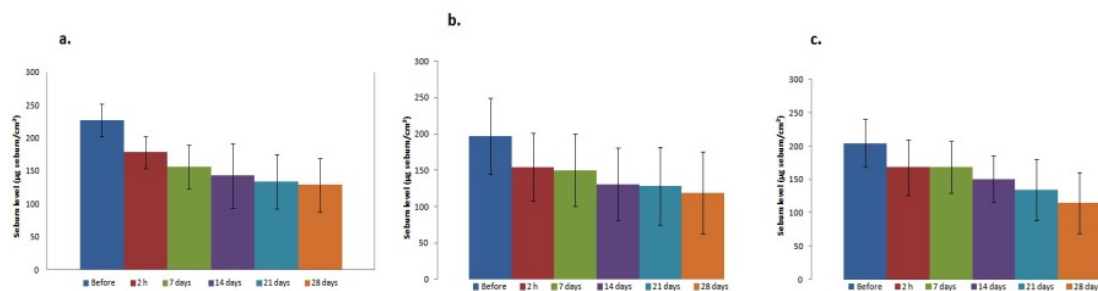


Figure 2.

Relative change in the sebum secretion rate after 28 days, mean values \pm SD, $n = 12$
(a. forehead area, b. cheek area, c. chin area)

Sensory analysis of tested product

Figure 3 presents the result of the volunteers' appreciation for each established characteristic of the tested product. As we can observe, all volunteers considered that the tested product has a pleasant smell, due to the vegetal extracts, has a good spreadability and a good ability of skin penetration. The volunteers appreciated also that the product was not sticky, but the after feel was not very good due to the degree of oiliness felt after application. Using these observations, the formulated cream may be recommended as moisturizer cream for oily skin during the winter season or in case of weakened skin by over-drying acne treatments.

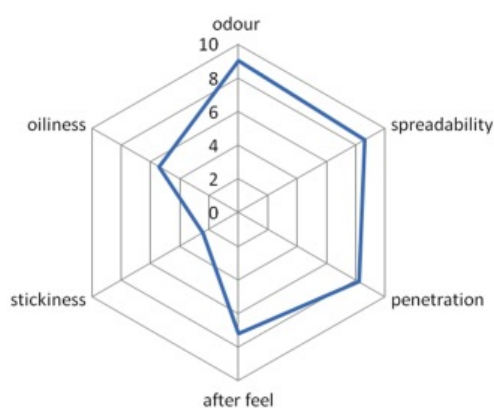


Figure 3.

Sensory analysis for tested product according to media of the volunteers' appreciation

Conclusions

An anti-acne product containing four vegetal extracts was formulated. By combining the effects of these extracts, antibacterial, antifungal and antioxidant effects were obtained, useful in acne prone skin care.

A significant reduction of the sebum level was found after 4 weeks of treatment with the tested formula. The product was well tolerated and appreciated by the consumers regarding its odour, spreadability, penetration ability and lack of stickiness. A reducing of oiliness feel after application recommended in order to increase the patients' compliance when using this product, as it must be used for a long period of time.

It can be concluded that the tested cream showed excellent cosmetic properties and very good efficacy in decreasing the sebum secretion levels and it can be helpful for the treatment of skin conditions accompanied by acne prone skin.

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