Case Report

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Antibacterial, Antiparasitic and Antifungal Properties of Natural Pyrethrins Obtained from Dalmatian Tansy Used in the Treatment and Care of Sensitive Skin

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ABSTRACT

Determining the susceptibility of pathogens to antibacterial substances is one of the key steps in bacteriological diagnostics that affects the success of both therapeutic therapy and the appropriate selection of cosmetic products. Antibiotic and chemotherapeutic sensitivity tests determine the ability of an antimicrobial agent to inhibit the growth of a microorganism *in vitro.* In modern microbiological laboratories, many different methods of bacterial susceptibility testing are used, but the basic and still widely used in the world is the disk-diffusion method (Kirby-Bauer method). The purpose of the study was carrying out an analysis of the chemical composition and evaluation of selected biological properties of natural pyrethrins. The evaluation of biological activity focused on the antioxidant and antibacterial properties of Dalmatian tansy extract.

Keywords: Demodecosis; Dalmatian Tansy; Almond Acid; pH Value

Introduction

For many years, medicinal plants have been used as traditional medicines in various cultures around the world. Due to the large number of plants with healing potential, research focusing on the analysis of the chemical composition and pharmaceutical and cosmetic properties of medicinal plants as well as on methods enabling quick, screening of the biological activity of plant material deserve special attention. The name Pyrethrum refers to a grass belonging to the genus Tanacetum and more precisely to the family of Asteraceae. Indeed, Pyrethrum is the Tanacetum cinerariifolium, also called *Chrysanthemum* cinerariifolium. It is known as the Dalmatian chrysanthemum, according to its origin in that region of the Balkans, the Dalmatia. The pyrethrum is formed of erected stems of 45-80 cm, and characterized by deeply divided leaves, which are covered on both sides with a dense cottony coating. It is a perennial plant with a daisy-like (Leucanthemum) appearance and white petals as the capitulum is solitaire and surrounded by 2-3 scaly and downy branches. The plant is traditionally used as a natural source of insecticide and so it is economically important in countries where it is cultivated, such as Kenya, Tanzania and Ecuador. Its flowers are pulverized, and the active components called pyrethrins, contained in the seed cases, are extracted and sold as insecticide or repellent component. The flowers are used to extract Tanacetum cinerariifolium. The extract consists of polysaccharides: lignans and sesamine, terpenes and polyphenols:

beta-amyrin, chrysanine, chrysanolide, chrysanthemic acid, cinerins, jasmolines, pyrethrinic acid, pyrethrins, pyrethrol and retrins, amino acids and proteins.

The group of pyrethrins includes six natural chemical compounds with insecticidal activity, obtained from dried flower heads of Dalmatian tansy. They found their original use as ingredients used in the production of chemical plant protection products, as well as insecticides used in closed rooms and preparations used in veterinary medicine. Recently, the beneficial properties of these compounds have started to be used in the production of cosmetics. The discussed compounds show a strong insect-repellent and insecticidal activity. Chemically, pyrethrins are esters of two hydroxy acids (chrysanthemum and pyrethric) with three keto alcohols (pyrethrole, cinerol and jasmolon). Chrysanthemic acid esters with the aforementioned alcohols are: pyrethrin I, cinerin I and jasmoline I (generally pyrethrin I), while esters of pyrethric acid are: pyrethrin II, cinerin II and jasmoline II (generally pyrethrin II) (Figure 1). The insecticidal effect of the powder from dried flowers of *Chrysanthemum* was already known over two to three thousand years ago, in ancient China. In the 19th century, dried and powdered chrysanthemum flowers were used to combat fleas, lice and bedbugs in living quarters. The scope of application was gradually extended to combat flies, cockroaches and mosquitoes that carry various diseases. During World War I, pyrethrum petroleum extract was produced for the first time. Since 1919, extracts based on various solvents and of various purities have found widespread use, primarily for the control of domestic insects (Casida 1980; Maciver et al. 1997). The great advantage of pyrethrins as active substances of insecticides is their quick and strong attack (known as: knock-down effect) and insecticidal action with a simultaneous low toxicity towards warm-blooded organisms and the lack of bio accumulation and rapid biodegradation due to oxidation and photolytic decay (Casida 1980; Maciver et al. 1997).

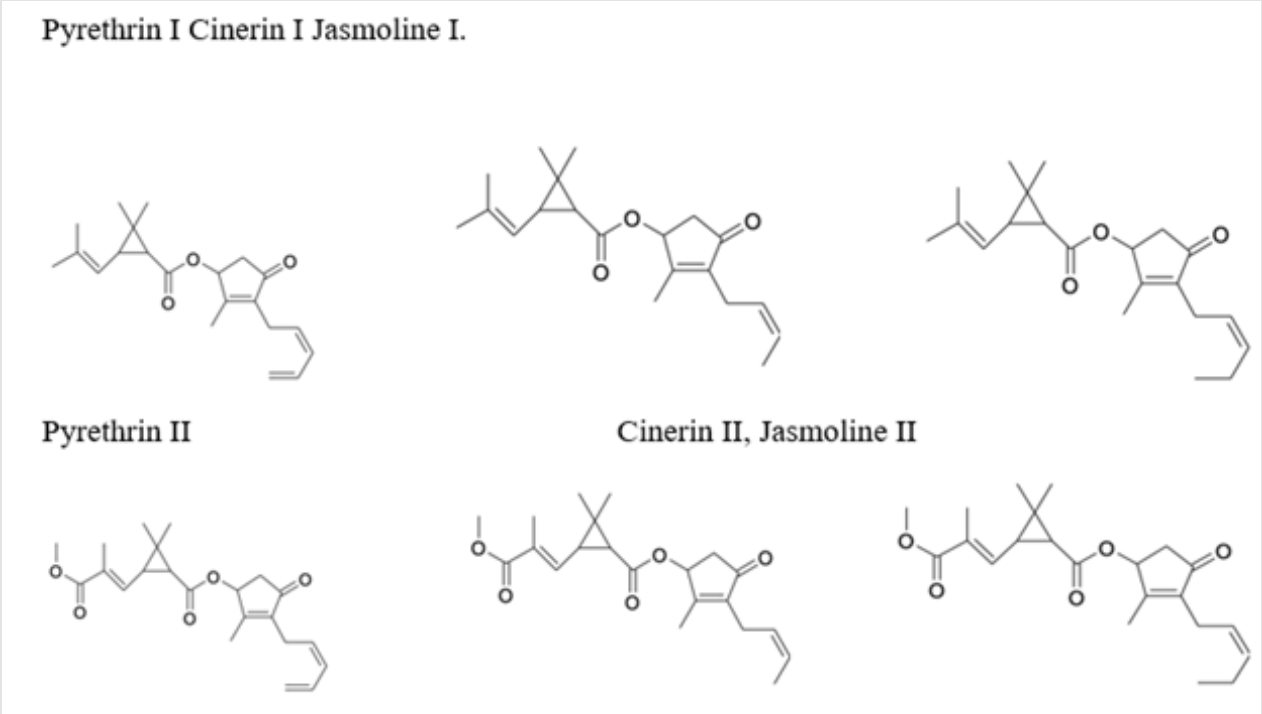

Figure 1: Structural formulas of pyrethrins.

Pyrethrin I and pyrethrin II are believed to be slightly more potent insecticides than the other compounds (Moore 1966; Soloway 1976). Pyrethrins are obtained by extracting plant material. The crude extract (called oleoresin) contains about 20-35% of pyrethrins. It is then subjected to a multi-stage purification process (e.g., naturally occurring fatty acids, waxes and dyes are removed). The purified extract, apart from the increased content of pyrethrins (up to about 60÷70%), is devoid of many natural substances that may cause allergic contact reactions, especially of the skin. After dilution with appropriate hydrocarbons to the standardized, desired concentration of pyrethrins (in the USA most often 45÷55%, in Europe 25%), the product is then sold. Thanks to its bactericidal, insecticidal, fungicidal and antiparasitic properties, Dalmatian tansy extract has found application in therapeutic cosmetology. It is used in cosmetics

dedicated to sensitive skin, especially in people with *demodecosis*. *Demodex* is an arachnid of the mite order, highly specialized and obligatory towards their hosts. Their main food is epidermal cells and components of sebum. They inhabit areas of the facial skin, especially those rich in sebaceous glands, such as the nose, cheeks, forehead and chin. The term *demodecosis* refers to a group of chronic inflammatory dermatoses of the skin, leading to the weakening of the skin-epidermal barrier, caused by toxic and the allergenic effect of metabolites of mites living in humans, such as *Demodex folliculorum* and brevis [1]. The characteristic symptoms of *demodecosis* are alternating inflammations in the form of exacerbations and remissions, possibly related to the *Demodex* development cycle, manifesting disorders typical of rosacea, seborrheic dermatitis (PsA), dry eye syndrome and various forms of dandruff typically located on the face, scalp and eye area (Figure 2).

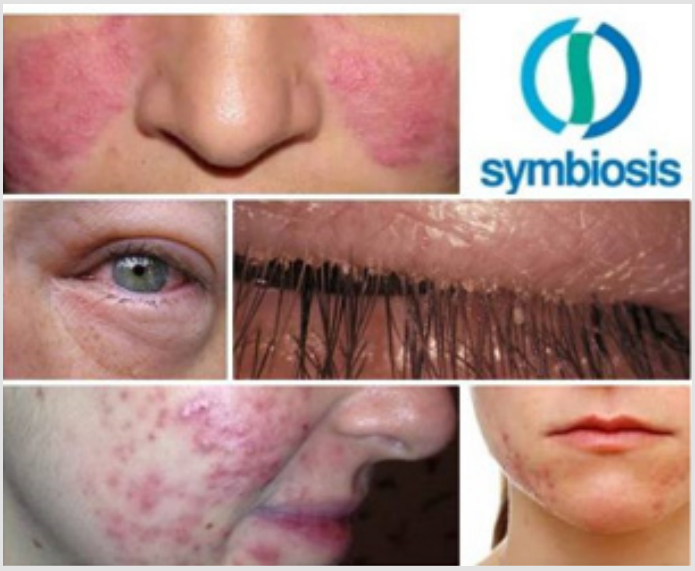

Figure 2: Demodecosis and its symptoms.

Note: (Source: own work, Symbiosis Dermatology Center)

The etiopathogenesis of *demodecosis* is complex and not fully established. Based on her own observations, the author offers a suggestion that the inflammatory changes that accompany *demodecosis* can be compared to the mechanism of atopic dermatitis (AD) caused by contact with house dust mite feces [2]. Erythema, skin sensitivity to factors such as temperature changes, cosmetics intolerance, flaking, dryness, itching, and eczema are typical symptoms of the dysfunction of the epidermal-lipid barrier. In the case of facial skin, where there is a naturally increased activity of the sebaceous glands (the natural habitat of *Demodex* in relation to other parts of the body), the risk of the toxic effects of these mite metabolites increases [3]. This may be favored by environmental conditions in human skin, which result from the disturbance of the natural process of exfoliation and renewal of the epidermis, leading to slower self-cleaning of the sebaceous glands from *Demodex* feces (guanine and protease). The inflammatory reaction, initially manifested by the skin's sensitivity to cosmetics and external factors, is probably associated with a high concentration of guanine and proteases [4]. Guanine, present in mite feces, is a toxic, potent allergen that is likely responsible for immune responses. Cysteine and serine stratum corneum proteases have also been observed in feces of scabies, *Demodex*, as well as house dust mites [5].

The increasing activity and concentration of sebaceous glands may have a direct impact on the coherence of the epidermal-lipid barrier. The association with the increased activity of the stratum corneum proteases and the decreased activity of inhibitors such as cystatin is due to the increase in the alkaline pH of the epidermis and the TEWL index. The interaction of guanine and proteases present in the feces of this parasite may result in an immune response and activation of protease inhibitors, which in turn inhibit the synthesis of NMF and lipids. The disturbance of the integrity of the lipid-epidermal layer, as in AD, is characterized by increased penetration of irritating or allergenic components. Worsening symptoms of skin «sensitivity» to cosmetics may also be the result of the way it is cleansed [6]. Published studies offer analyses of the use of Pyretrin-D series of cosmetics. The present paper presents the antibacterial, antiparasitic and antifungal properties that confirm the effectiveness of the series [7-10].

Case Report

Conducting *In Vitro* Microbiological Tests in the Evaluation of Cosmetics Performance

In the study, the modified Kirby-Bauer method was used in the analysis of the Pyretrin-D series, the leading active ingredient of which is Dalmatian tansy; microorganisms determined included: *Staphylococcus aureus* ATCC® 6538™, *Malassezia furfur* ATCC® 14521™ *Staphylococcus epidermidis* ATCC® 12228™. The Kirby–Bauer method (disk diffusion test) is a qualitative method, based on the diffusion of the active substance contained in a paper disc into a solid substrate. The antimicrobial substance diffuses radially, creating zones with a concentration gradient. Its greatest concentration occurs at the edges of the disc and decreases with the distance from it. The size of the microorganism growth inhibition zone is directly proportional to the degree of its sensitivity to a given substance. The larger the inhibition zone, the more sensitive the microorganism. Depending on the size of the zone and the adopted evaluation criteria, microorganisms are defined as: sensitive and resistant or sensitive, intermediate and resistant. Despite its simplicity, this method requires precise execution and quality control at every stage of the procedure. The introduced modification consisted in applying the tested samples directly to the substrate in order to eliminate differences between different formulations of cosmetics samples in terms of diffusion from paper discs [11-16].

Table 1: Average zone of inhibition of growth of the microorganisms subjected to the test series.

Microorganism / Sample No. / Name	*Staphylococcus aureus* ATCC® 6538 ™	*Malassezia furfur* ATCC® 14521™	*Staphylococcus epidermidis* ATCC® 12228™
	Mean zone of growth inhibition [mm]		
1 / Pyretrin D-foam	25	Not tested	25
2 / Pyretrin D-serum	23	Not tested	19
3 / Pyretrin D-cream	0	Not tested	0
4 / Pyretrin D-tonic	28	Not tested	22
5 / Pyretrin D-shampoo	15	23	Not tested
6 / Pyretrin D-conditioner	0	0	Not tested
7 / Pyretrin D-rub-in liquid	0	0	Not tested
8 / Pyretrin D-trichological peeling	25	24	Not tested

The substrate used was Tryptone Soy Agar (TSA). To qualify the cultures to the range taken into account in the results, the standard criterion for the disc diffusion method was adopted, i.e., obtaining a confluent or postponed colony growth of the tested strain. (Table 1) shows the results of the growth inhibition for the following microorganisms by the test lot. Pyretrin-D face care products were further assessed for antimicrobial properties towards *Cutibacterium* acnes ATCC® 11827™. In order to prepare the inoculum, the bacteria were grown on a solid medium with sheep blood and incubated under anaerobic conditions (37 degrees C, 48 h). Then, the multiplied bacteria were collected from the solid medium with a loop and placed in physiological saline (1 ml). The next stage involved the preparation of the test sample and the control sample (Table 2). Test sample: 50 µl of inoculum/200 µl of a cosmetic sample. Control sample: 50 µl of inoculum/200 µl of saline. Samples were incubated for 30 minutes at room temperature. Then the samples were washed by centrifugation.

Table 2: Preparation of the test and control sample.

Test sample	Control sample
50 µl of inoculum	50 µl of inoculum
200 µl of cosmetic	200 µl of saline

The obtained bacterial pellet was suspended in 100 µl of physiological fluid and plated on solid substrates with sheep blood, and then incubated under anaerobic conditions (37 degrees, 7 days). After completion of growth, the plates were analyzed. The test was replicated 5 times. The results are presented in (Table 3).

Table 3: Test series results.

Sample No.	Sample description	Result
1.	Pyretrin D-foam	no growth
2.	Pyretrin D-serum	no growth
3.	Pyretrin D-cream	growth
4.	Pyretrin D-tonic	no growth
5.	Control sample	growth

Actual *In Vivo* Tests for the Evaluation of Tested Cosmetics Performance

At the Symbiosis Dermatology Center, application research (*in vivo*) on the skin of volunteers was conducted confirming the effectiveness of the applied Pyretrin-D series. The tests were also carried out in real conditions. A group of 30 people underwent Pyretrin-D treatment. The treatment was the first step in the preparation of

the experiment. Home care, on the other hand, was the second stage aimed at eliminating the skin problems faced by the patients. A treatment in the field of therapeutic cosmetology supporting the treatment of *demodecosis* (infection with *Demodex*) was developed by specialists from Centrum Dermatologii Symbiosis Sp. z o. o The study was conducted based on the approval of the Bioethics Committee, resolution number 640/20. Below photos present one of the probants diagnosed with papular eruptions (Figure 3).

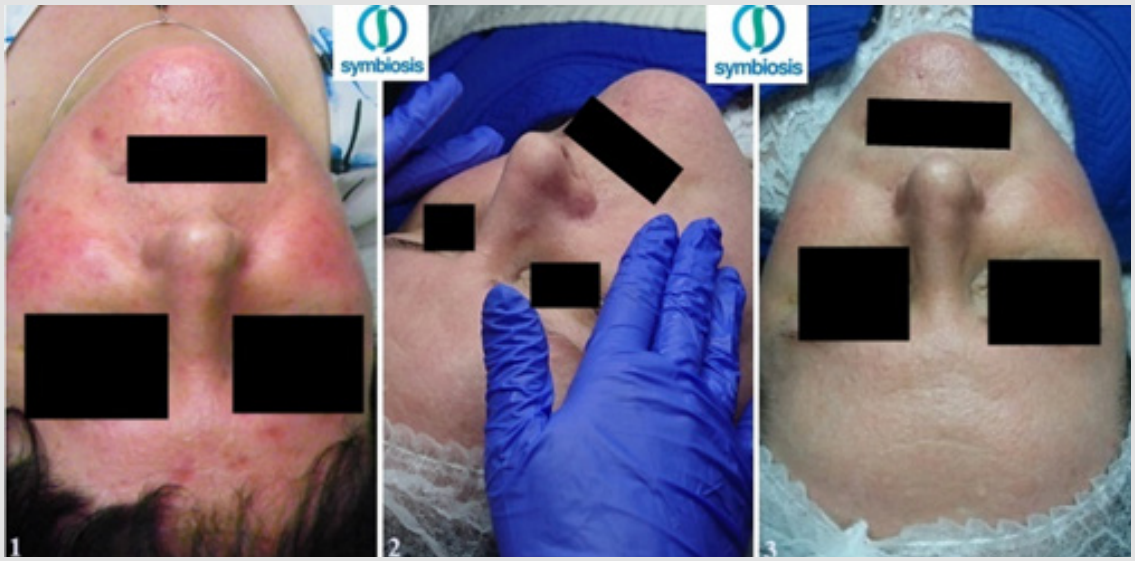

Figure 3: The effects of beauty salon treatments (3 treatments) and home care.

Note: (Symbiosis Dermatology Center, 2017)

Discussion

Conducting *In Vitro* Microbiological Tests in the Evaluation of Cosmetics Performance

As part of the evaluation of the antimicrobial effect of cosmetics on strains of *Staphylococcus aureus* ATCC®6538 ™ and *Malassezia furfur* ATCC® 14521™, in samples 6 and 7 (Pyretrin D-conditioner and Pyretrin D-rub-in liquid, respectively), no antimicrobial activity was observed. A similar observation applies to sample 3 (Pyretrin D-cream), but towards *Staphylococcus aureus* ATCC®6538™ and *Staphylococcus epidermidis* ATCC® 12228™. In the case of the remaining samples, i.e. 1, 2, 4 and 8, the analysis confirmed the antimicrobial activity towards the test strains. The differentiation between the tested samples was also demonstrated in the study of antimicrobial properties towards *Cutibacterium* acnes ATCC® 11827™. Again, sample 3 of Pyretrin D-cream did not have these properties, in contrast to the remaining samples, i.e., 1, 2 and 4.

In Vivo Tests for the Evaluation of Tested Cosmetics Performance

The diagnosed condition was a fairly advanced condition of rosacea caused by secondary bacterial infections which is the most common reason for visiting a dermatologist. The authors of the study observed that this condition requires not only parasiticidal and exfoliating action, but also antibacterial treatment. The combination of hygiene with the use of a series of cosmetics containing tansy extract with exfoliating treatments allows not only the disappearance of symptoms, but also maintaining the effects after the end of antibacterial therapy.

Conclusion

The observed differences in the demonstrated antimicrobial properties should be considered not only in relation to individual extracts or products, but also their use in the cosmetic procedure. In the case of the entire product line, the antimicrobial effect does not have to be a positive property for each of the products of the designed line. For example, the lack of such properties for sample 3, Pyretrin D-cream, may be related to the intended role of the product in cosmetic procedures. The ingredients used to accompany the Dalmatian tansy extract did not support the antimicrobial course of action which affected the activity of the entire product. Similar conclusions can be drawn in the case of the samples of Pyretrin D-conditioner and Pyretrin D-rub-in liquid. The key issue is the pathomechanism of *demodecosis*, which exposes the skin to house

dust mite-like pathogens, i.e., antigens from feces or dead organisms. The route of penetration of these toxic compounds is important. In the case of *demodecosis*, the pathogenesis often cited in the literature is based on the infection of the parasite itself with the *Bacillus oleronius bacterium*. In the opinion of the authors, more important is the presence in the glands of feces and contaminants from the decomposition of dead individuals.

Taking into account the advantages of the proposed beauty salon and home care procedures for sensitive skin caused by *demodecosis*, it is reasonable to establish close cooperation between a dermatologist and a cosmetologist. The current concept of commonly used antibiotic therapy without individually selected hygiene and skin exfoliation seems irrational. In patients who used the Pyretrin-D series, a reduction or complete elimination of inflammation was noticed. Reduction of erythema and closing of the vessels on the nose and cheeks were also found in patients. Improvement in the protective mechanisms of the epidermis was observed in all participants, thanks to which it may be said that inner layers of the skin are better protected. The above prevents infections caused by various microbes. Patients who struggled with *Demodex folliculorum* infections noticed a significant improvement. By eliminating the excrement of this mite, the existing inflammation has improved. All patients noticed that their pores on the surface of the skin were open before the procedure and the application of cosmetics.

Acknowledgment

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Conflicts of Interest

No conflicts of interest to declare. No identifiable patient information has been used however consent has been obtained from the patient for use in publication.

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