



NOVEL TOPICAL FORMULATIONS BASED ON *O. BASILICUM* AND *T. PRATENSE*: ANTIOXIDANT, ANTIMICROBIAL, AND ANTI-INFLAMMATORY EFFECT

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ABSTRACT

This study aimed to highlight the importance of phytochemical-based formulations in the treatment of wounds with different etiologies, avoiding the possible side effects generated by conventional treatments. We present the preparation method of novel topical formulations (cream, ointment, and hydrogel) based on mixed extracts of *Ocimum basilicum* and *Trifolium pratense* along with evidence of their antimicrobial and antioxidant effect *in vitro*. The anti-inflammatory and wound healing properties of the new formulations were evaluated against different chronic inflammatory processes by presenting three clinical cases of chronic skin diseases. The clinical cases presented in our study revealed good outcomes of the cream formulation in the case of dyshidrotic eczema, ointment formulation in the case of hypertrophic scar, and hydrogel formulation in the case of psoriasis lesions. The best effect was noticed after 6 days of topical application of hydrogel on psoriatic plaques, resulting in a significant reduction of erythematous plaques.

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Introduction

Wounds of different etiologies possess all the characteristics of a common wound, such as the regulation of protease levels, elevated pro-inflammatory cytokines, persistent and elevated reactive oxygen species (ROS), the presence of senescent fibroblasts, prolonged infection, while stem cells are dysfunctional and insufficient [1]. The etiology of skin diseases can vary widely, from environmental factors such as UV radiation and pathogens to bacteria or fungi, genetic factors, and autoimmune disorders. The wound healing process involves several precise physiological phases: homeostasis, inflammation, proliferation, and remodeling.

The epidermal cells of the skin possess the activity of continuous self-renewal and replacement of dead cells. It has been estimated that the renewal time of the human epidermis is on average 40 to 56 days. The capacity for cell renewal declines rapidly with age, but this process can be slowed down or even reversed by some phytochemicals, which protect the skin by quenching free radicals and reducing inflammation through the inhibition of NF-κB [2, 3].

Among the phytochemical agents, phenolic compounds constitute a group of secondary metabolites that have important functions in plants. In addition to the beneficial effects on the host, phenolic metabolites (polyphenols) possess several biological properties that positively influence health status.

Various pieces of evidence suggest that the body can benefit from the properties of plant phenolic compounds obtained either through diet or by direct application on the skin, as they can alleviate symptoms and inhibit the development of various pathological changes in the skin. Due to their natural origin and low toxicity, phenolic compounds are a promising tool in eliminating the causes and effects of skin aging, skin diseases, and the breaking process of the skin, in various types of wounds

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and burns [4].

The premises of our study are based on previous results obtained by *in vitro* studies (dermal fibroblast cultures) and *in vivo* tests (animal model) using a polyphenol-rich mixture of *Ocimum Basilicum* and *Trifolium Pratense* extracts, with effective antioxidant, antimicrobial and pro-inflammatory activities, playing an important role in the recruitment of specific cells to the site of inflammation and accelerating the entire chain of reactions involved in the healing process. In addition, these polyphenolic compounds were proven to promote the proliferation and migration of human dermal fibroblasts [5, 6]. Unquestionably, the properties of the polyphenols contained in these phytochemicals make them excellent candidates for infection control and cutaneous healing promotion.

The available literature offers many examples of phytochemicals with high activity in terms of wound repair and skin regeneration. A study carried out on *Populus nigra* buds extract showed a favorable activity *in vitro*, in a model of normal human dermal fibroblasts (NHDF), based on the presence of the following compounds: six phenolic acids (caffeic, p-coumaric, isoferulic, di-O-methylcaffeic, cinnamic), three flavonoids (pinocembrin, pinobanksin, and its derivative) and salicin. The identified compounds showed strong antioxidant activity. Curcumin, another widely studied polyphenol compound, which is extracted from turmeric, possesses significant anti-inflammatory, antioxidation, and antibacterial effects, playing a crucial role in wound healing through the formation of granulation tissue, collagen deposition, tissue remodeling, and wound contraction [7]. In mice, the administration of curcumin at the dosage of 100 mg/kg body weight revealed the induction of vascularization [8], while clinical trials have demonstrated that curcuma extract can be safely administered also to patients [2].

The activity of polyphenols is even more significant in the case of antibiotic-resistant strains due to their defense mechanism against phagocytes, and the activity of polymorphonuclear leukocytes. It is well known that methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, enterococci resistant to glycopeptide antibiotics and vancomycin, β -lactam, and macrolide-resistant pneumococci are the most common bacterial strains present at the site of infected wounds. If some bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) are colonized and reproduced in the wound tissue, a significant delay in the healing process is observed [9].

In recent years, more than 90% of staphylococci, pneumococci, and enterococci species isolated from severe infections be resistant to antibiotics, and hence, the demand for alternative antibacterial products is increasing. Phenolic compounds can be extensively used in the treatment of different bacterial infections without causing a simultaneous toxic effect on tissues, and on the other hand, are cost-effective treatment options [10, 11]. The antibacterial mechanisms of phenols are considered to be related to the inhibition of the nuclear factor-kappa B (NF- κ B) pathway, but also other signaling pathways, including transforming growth factor-beta (TGF- β) and mitogen-activated protein kinase pathway are considered [2, 12, 13].

The effectiveness of different phytochemicals varies, as they play a particular role in the different phases of wound healing. Biological activities, topical absorption, performance, and sensorial factors of phenolic compounds could be improved by developing novel therapeutic formulations, such as high-performance carriers of phenolic compounds, acting as a direct method of applying the active substance to the irritated skin while minimizing the adverse effects caused by the conventional medication (for example allergic reactions).

Acute and chronic injuries possess complex healing mechanisms, and current therapeutic approaches do not fully meet the demands of a dynamic everyday life. Therefore, the development of natural products-based formulations is highly necessary for addressing difficult-healing wounds.

In this context, this work aimed to elaborate novel topical formulations based on mixed extracts of *Ocimum basilicum* and *Tifolium pratense* (OB+TP) and to demonstrate their antimicrobial and antioxidant effect *in vitro*. Moreover, the anti-inflammatory effect and wound healing properties of the new formulations were evaluated against different chronic inflammatory processes by presenting 3 clinical cases (dyshidrotic eczema, hypertrophic scar, and psoriasis lesions).

Materials and Methods

Preparation of Ocimum basilicum and Trifolium pratense Extracts

The aerial parts of *Trifolium pratense* and *Ocimum basilicum* were used for the extract preparation, as previously reported [6, 14, 15]. Briefly, the vegetal material was dried at 50 °C until the weight remained unchanged, then crushed resulting in a fine powder containing a ratio of 1:1 (w/w) *Ocimum basilicum* and *Trifolium pratense*. A 70% hydroalcoholic solution (1:10 g/v) was added to the powder and kept for 24 hours in the dark, under continuous agitation. The hydroalcoholic extract was evaporated under vacuum using a rotary evaporator and then subjected to lyophilization (lyophilizer Christ Alpha 1-2 LDplus).

Antioxidant Capacity by Electrochemical Method

The reagents ascorbic acid, K₂HPO₄, and KH₂PO₄ were purchased from Sigma Aldrich and were used as received. The electrochemical measurements were carried out on a potentiostat (PGSTAT 128N Autolab, Metrohm, Belgium) equipped with Nova 2.1.2 software. A conventional three-electrode electrochemical cell was employed using a glassy carbon electrode (GCE, 3 mm diameter, BAS) as a working electrode, a Pt wire as a counter electrode, and Ag/AgCl as a reference electrode. A diamond paste (2 μ m) was applied for GCE polishing and then rinsed with distilled water and ethanol.

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed for the electrochemical characterization of the extract using the following parameters: for CV: start potential 0V, upper vortex potential +1.5V, lower vortex potential

-0.5V, stop potential 0V, 1 scan; for DPV: start potential -0.5V, stop potential +1.5V, step 0.01V, modulation amplitude 0.05V, modulation time 0.05s, interval time 0.1s. The ascorbic acid calibration curve was also measured by DPV.

Antimicrobial Activity by Kirby Bauer Diffusion Method

Two Gram-positive and one Gram-negative strains were used: *Staphylococcus aureus* (+) - ATCC 25923 LOT 01302502, BBD 06.2021, *Streptococcus pyogenes* (+) ATCC 19615 LOT 02001702, BBD 06.2021, *Pseudomonas aeruginosa* (-) - ATCC 25668 LOT 06500109, BBD 12.2021. Three samples containing different concentrations of the mixture of vegetal extracts were tested: sample 1 (S1) - 50 µl extract mixture; sample 2 (S2) - 100 µl extract mixture; sample 3 (S3) - 200 µl extract mixture.

As a control, 70% alcohol (the solvent employed for extraction) was used: control 1 (C1) - 50 µL alcohol, control 2 (C2) - 100 µL alcohol, control 3 (C3) - 200 µL alcohol.

Also, antibiotics used as positive controls were: Ciprofloxacin (for all three bacterial species), Azithromycin (for *S. aureus*, *S. pyogenes*), Cefoxitin (for *S. aureus*), Doxycycline (for *S. aureus*, *S. pyogenes*), Clindamycin (for *S. aureus*, *S. pyogenes*), Gentamicin (for *P. aeruginosa*), Nitrofurantoin (for *P. aeruginosa*), Meropenem and Ceftriaxone (for *P. aeruginosa*).

The microorganisms were incubated overnight in the broth (Mueller-Hinton) and compared to the 0.5 McFarland Turbidity standard. The Agar medium was used because it does not inhibit sulfonamides and ensures the reproducibility and pH of the medium.

The bacterial suspensions were prepared using a sterile inoculation loop by touching four or five isolated colonies of the microorganism, then being suspended in 2 ml of 0.9% sterile saline solution. The next step was to vortex the saline tube to create a smooth suspension. Later, the turbidity of this suspension was adjusted to a standard of 0.5 McFarland. The suspension was applied 15 minutes after preparation. A paper disc with a diameter of 6 mm was used, each disc being infused with the above-described amount of sample solutions (S1-S3). Similarly, the controls were infused with 70% alcohol respectively C1-C3 solutions, and then manually applied to the surface of the agar dishes inoculated with microorganisms.

The plates were incubated at 37°C for 24 hours before reading the results. The antibacterial activity was assessed by measuring the diameter of the inhibition zones, including disc size (6 mm), using a metric ruler. All tests were performed in triplicate.

Preparation and Characterization of the Novel Topical Formulations

Preparation of Different Therapeutic Formulations

Three types of bases have been selected to obtain the new formulations: 1) simple ointment- for its emollient effect (Formula A); 2) emulsion -for its absorbent properties (Formula B); and 3) hydroalcoholic gel-for hydration effect (Formula C). In this way, the formulations can be applied to various cutaneous lesions (**Table 1**).

Table 1. The formulas of the bases used in the study.

Components	Formula A Ointment (g)	Formula B Cream (g)	Formula C Gel (g)
Aqua distillata	-	75.90	78.00
Adeps lanae anhydricus	50	-	-
Vaselinum album	50	-	-
Alcoholum cetyl stearyliscus	-	10	-
Cera	-	2.5	-
Sodium lauryl sulfate	-	1.5	-
Triethanolamine	-	-	1
Alcoholum etilicum	-	-	10
Glycerine	-	10	10
Carbopol 940	-	-	1
p-OH-methyl benzoate	-	0.1	-

The final formulations were obtained by incorporating the freeze-dried extract of OB+TP (1:1 w/w) in 90 grams of each base (A, B, and C) resulting in three novel products containing a 5% extract mixture.

To be suitable for medical practice, the pharmaco-technical properties of the new formulations were evaluated (organoleptic control, pH evaluation, rheological characteristics).

Rheological Behavior

To describe the rheological behavior of the novel formulations, the viscosity variation was determined according to the frictional force in a time interval. Rheological parameters were determined using a Brookfield rotational rheometer (Brookfield CT3) at a spindle rotation speed of 2 rpm (spindle no. 64) with the following parameters: trigger - 6 g, distance - 15.00 mm, and speed: 0.50 mm/s.

Knowing the effects of different ointment formulations and preparation technology on their physical and mechanical properties, these properties can influence the ease of application on the skin, bio-adhesion, therapeutic activity, and the bioavailability of incorporated active compounds.

pH Measurement

To determine whether the pH of novel formulations is suitable for skin application, the Inolab 7310 pH-meter was employed at 20°C, using a SenTix 81 Plus electrode. The pH values are tabulated in **Table 2**, along with the organoleptic features and viscosity value of each formulation.

Selection of Patients and Clinical Case Presentation

We have selected three cases showing chronic inflammations in different pathologies: 1) Dyshidrotic palmar eczema, 2) Hypertrophic scar, and 3) Psoriasis lesions. The patients were registered in the Department of Dermatology and Venereology, Salonta Municipal Hospital, Romania, between July and August 2021, and selected as they followed the recommended therapeutic indications.

The study was carried out following the ethical regulations based on the Decision of the Research Ethics Commission No. 03/CEFMF/30.06.2021 (emitted by the Research Ethics Committee of the Faculty of Medicine and Pharmacy, University of Oradea) and the informed consent of the patients. The novel topical formulations (cream, hydrogel, and ointment) containing a 5% mixture of OB+TP as the active ingredient, were applied twice a day, for 7 days in the case of keloid scar (cream) and psoriasis (hydrogel), and 9 days in the case of dyshidrotic eczema (cream). The outcomes of the treatment were evaluated taking into account that the patients did not follow an adjuvant medical treatment.

Results and Discussion

Antioxidant Capacity of OB+TP Extract Mixture Evaluated by Electrochemical Method

The electrochemical methods have recently gained a lot of interest due to their increasing applications in various fields including biomedical, nanotechnology, food, and environmental sectors [11, 16-18].

Concerning the determination of antioxidant activity, the electrochemical methods present some advantages in comparison with the conventional spectrophotometric method: it is simple, cost-effective, fast, portable, and more sensitive. Electrochemical biosensors based on enzymes (tyrosinase, laccase, peroxidase) and DNA have been developed for determining the antioxidant activity of natural compounds, using different techniques such as cyclic voltammetry (CV), square-wave voltammetry (SWV), and differential pulse voltammetry (DPV) [19, 20]. Other electrochemical sensors (without biorecognition element) have been applied for the detection of non-enzymatic antioxidants (especially polyphenols), many of them being modified with nanomaterials [19, 21, 22].

The electrochemical behavior of the mixt extract OB+TP analyzed by DPV is presented in **Figure 1a** indicating two oxidation peaks at around 0.250 V and 0.900 V, respectively. CV technique was also performed at different scan rates (from 10 mV/s to 300 mV/s) (**Figure 1b**) revealing the redox signals corresponding to the bioactive compounds in the extracts: an intensive anodic peak (starting from 75 mV/s a second lower peak appeared) and a small cathodic peak.

The increase in the scan rate is accompanied by the increase of the current intensity and potential shifting (towards more positive values for oxidation and negative values for reduction).

The ΔE_p varied between 400 mV and 600 mV resulting in an irreversible redox process. The oxidation and reduction intensity linearly varies with the square root of the scan rate ($y = 0.845 \cdot X + 2.5136$, $R^2 = 0.983$) indicating an electrochemical process controlled by the diffusion of the bioactive compounds from the solution towards the electrode surface.

The antioxidant capacity of the extract mixture OB+TP was expressed as ascorbic acid equivalent antioxidant capacity (AEAC) using the calibration curve of ascorbic acid (concentrations were in the range $10^{-5}M$ - $5 \cdot 10^{-3}M$, with the equation $y = 0.1836 + 6537.5428 \cdot X$, $R^2 = 0.998$). The mixture OB+TP presented a calculated AEAC of 17.36 mg/g with an RSD of 2.05%.

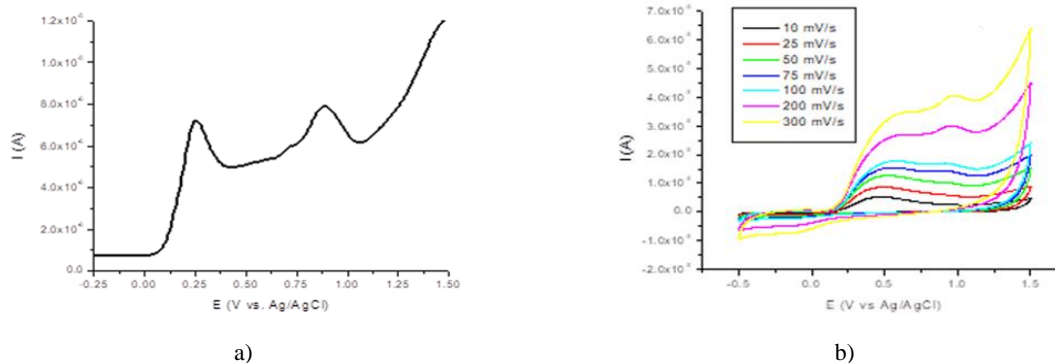


Figure 1. a) DPV of the mixt extract OB+TP (dilution 1:10 in PBS pH 7.4); b) CVs of the mixt extract (dilution 1:5 in PBS pH 7.4) at different scan rate.

The two anodic peaks recorded by the DPV technique can be attributed to the oxidation process of the catechol groups from polyphenols and flavonoids existing in considerable amounts in plant extracts [6, 21, 22]. The high values for the ΔE_p obtained by the CV technique indicate an irreversible redox process of the constituents. CV also revealed a linear variation of the oxidation and reduction peak intensities with the square root of the scan rate, suggesting a diffusion-controlled process. The antioxidant capacity of the mixture OB+TP was calculated considering ascorbic acid as equivalent (1.736 mg/ml). This value was higher compared to other studies: Rezzoug *et al.* obtained 3.657 $\mu\text{g/ml}$ for the ethanolic extracts of *O. basilicum* and *T. algeriensis*, meanwhile, Edemhanria *et al.* obtained 17.78 $\mu\text{g/ml}$ and 17.32 $\mu\text{g/ml}$ for the ethanol extracts of basil leaves [23, 24]. As previously demonstrated, the antioxidant capacity given by the polyphenolic compounds is influenced by the harvesting time, the extraction method (solvent used), and the analytical method applied for determination [25-30].

Antimicrobial Activity of the Mixt Extract OB+TP

The diameter of the inhibition zone against selected microorganisms compared to the control (antibiotics) and 70% ethanol, respectively, are presented in **Table 2**. Three different concentrations of the mixt extract (S1, S2, S3) and ethanol (C1, C2, C3) were employed.

Table 2. Antimicrobial activity of the mixt extract OB+TP compared to antibiotics and 70% alcohol as a control against selected gram-positive and gram-negative bacteria.

Bacteria	Antibiotic	CONTROL (70% Alcohol)	Mixture OB+TP
	(Inhibition zone mm \pm standard deviation)		
<i>Pseudomonas aeruginosa</i>	Ciprofloxacin 26 \pm 0.5	C1 15 \pm 0.2	S1 15 \pm 0.1
	Nitrofurantoin 15 \pm 0.4	C2 15 \pm 0.1	S2 18 \pm 0.11
	Gentamicin 16 \pm 0.12	C3 18 \pm 0.4	S3 20 \pm 0.5
	Ampicillin + Sulbactam 18 \pm 0.154		
<i>Streptococcus pyogenes</i>	Ciprofloxacin 24 \pm 0.5	C1 9 \pm 0.5	S1 20 \pm 0.14
	Azithromycin 25 \pm 0.15	C2 14 \pm 0.14	S2 20 \pm 0.11
	Oxacillin 21 \pm 0.22	C3 26 \pm 0.12	S3 30 \pm 0.4
	Doxycycline 28 \pm 0.22		
<i>Staphylococcus aureus</i>	Clindamycin 24 \pm 0.41		
	Ciprofloxacin 26 \pm 0.45	C1 7 \pm 0.5	S1 8 \pm 0.45
	Azithromycin 21 \pm 0.21	C2 13 \pm 0.4	S2 15 \pm 0.2
	Cefoxitin 29 \pm 0.4	C3 27 \pm 0.01	S3 30 \pm 0.13
	Doxycycline 25 \pm 0.55		
	Clindamycin 29 \pm 0.12		

One can observe that OB+TB extract in concentrations of 100 μl and 200 μl possesses a weaker antimicrobial activity compared to the antibiotic (Ciprofloxacin), but stronger than the control (70% alcohol) at the same concentrations, in the case of *Pseudomonas aeruginosa*.

Concerning *Streptococcus pyogenes*, all three tested concentrations (S1-S3) exhibited a stronger antimicrobial activity compared to the control (70% alcohol). Moreover, the concentration of 200 μl was more effective than the strongest antibiotic (Doxycycline).

Concerning *Staphylococcus aureus*, a similar stronger activity was observed for all three samples compared to the control (70% alcohol), while compared to the antibiotics (Cefoxitin and Clindamycin), the concentration of 200 μl (S3) presented a higher inhibition area.

Analyzing the previously reported data in the literature, we noticed a more effective antimicrobial activity of *Ocimum basilicum* extract against *Staphylococcus aureus* (34.00 mm \pm 0.31 for 25 μL sample/discs), reported by Shafique *et al.*, [31], while other authors reported a lower activity (11.0mm \pm 0.6; 16mm \pm 0.5 for 20 μL sample/discs and 16.0mm \pm 0.05 for a concentration of 10.53 \pm 0.17 μg TPC/60 μL /disk, respectively).

In contradiction with these results, the antimicrobial assay of alcoholic extract of *Trifolium pratense* studied by Vlasisavljevic *et al.* didn't show any significant activity towards *P. aeruginosa*, while a weaker effect was revealed against *Staphylococcus aureus* (7.4 \pm 0.64 mm) [32, 33].

General Characterization of the Bases Used for the Preparation of Topical Formulations

Table 3 shows the rheological parameters (viscosity), pH, and organoleptic properties of the three bases used to prepare the new formulations for topical application. As can be noticed, the pH value is close to the natural pH value of the skin, being suitable for the development of new formulations (ointment, cream, or gel) with good bioavailability and non-irritating [34, 35]. The overall characteristics are favorable, allowing adequate stretching on the skin surface and conferring a uniform and adherent appearance.

Table 3. Characteristics of the bases used for the preparation of topical formulations.

Formula types	Organoleptic control			pH	Viscosity (mPas)
	Appearance	Color	Odor		
Formula (A) Ointment	Homogeneous	green-brown	Characteristic	6,8	256
Formula (B) Cream	Homogeneous	green-brown	Characteristic	6,5	245
Formula (C) Gel	Homogeneous	green-brown	Characteristic	6.5	225

Clinical Case Presentation

Dyshidrotic Palmar Eczema

We will present the case of a 48-year-old patient, who presented to our outpatient service with erythematous-vesicular lesions, intensely pruritic, with deep and firm vesicles with clear contents located on the bilateral palmar level. The patient accuses the occurrence of injuries in the context of working conditions in an environment with increased humidity. The lesions have a recurrent character, with the patient reporting their occurrence for several years with periods of exacerbation depending on the workplace humidity.

Dyshidrotic eczema is a chronic, recurrent dermatosis that occurs at the palmoplantar level, characterized by the appearance of blisters and even firm blisters of various sizes, with intense itching. The vesicles are formed as a result of the process of epidermal spongiosis, their resistant character is given by the thickness of the epidermal tissue characteristic of palms and plants [36, 37].

The strongly pruritic vesicles will initially contain clear fluid, but which tends to become purulent and superinfected.

Following the anamnesis and the clinical appearance of the lesions, we established the diagnosis of dyshidrotic eczema. The patient was recommended to use the cream formulation containing *Ocimum basilicum* and *Trifolium pratense* extract twice a day, in the morning and the evening, re-evaluating the patient after 4 days and 9 days.

During the treatment, a visible improvement of the lesions was observed, with the remission of blisters and local itching and the appearance of scaling characteristic of the healing phase of eczema (**Figure 2**).

Upon applying the cream twice a day, the patient's degree of tolerance was very good, the patient did not report any observed side effects. The advantages of using the cream formulations based on *Ocimum basilicum* and *Trifolium pratense* extracts rely on avoiding the corticosteroids and the subsequent side effects (atrophy, purpura, and other systemic reactions) [38].



Figure 2. Clinical aspects of the case - Dyshidrotic eczema, highlighting visible improvement of the lesions following treatment with a cream formulation based on *Ocimum basilicum* and *Trifolium pratense* extract.

Hypertrophic Scar

We will present the case of a 73-year-old female patient with a hypertrophic scar on the right shoulder, caused by an injury with loss of continuity 5 years ago. The patient complains of local itching, sometimes a burning sensation, and pain to the touch following physical effort accompanied by physical limitation due to the sensation of local irritation.

Hypertrophic scars and keloids are two forms of abnormal wound healing. Both are characterized by local proliferation of fibroblasts and excessive production of collagen as the body's response to a discontinuous injury.

However, their clinical and histopathological features differ. The keloid is raised from the surface of the skin and extends beyond the edges of the solution of continuity into healthy skin, often with pincer-shaped lesions, like the claws of a crab. Hypertrophic scars remain at the level of the continuity solution without invading the healthy tissue.

Compared to healthy tissue, both these lesions have increased cellularity, increased vascularity, and much more developed connective tissue, may present with itching or pain, and may even inhibit the mobility of adjacent tissues. Their color can vary from pink, to purple (early lesions), the color of healthy or hypo-hyperpigmented skin compared to healthy tissue, both these lesions have increased cellularity, increased vascularity, and much more developed connective tissue, and may present with itching or pain, and may even inhibit the mobility of adjacent tissues. Their color can vary from pink to purple (early lesions), to the color of healthy or hypo-hyperpigmented skin [39].

When evaluating the scar, it was noticed a smooth surface, pale pink, is firm to the touch, presenting a depressed central area due to the periodic injection of local corticosteroids to flatten and minimize local symptoms, as well as to improve aesthetics. The size was approximately 7 cm long and 3 cm wide.

The patient was advised to apply the ointment formulation based on *Ocimum basilicum* and *Trifolium pratense* extracts twice a day, for one week.

After 7 days, we noticed an improvement in terms of local pruritus, as well as better hydration of the scarred skin, which led to a slight decrease in tension at the site of the wounded area compared to the adjacent tissue.

Concerning the size, firmness, and aesthetics, no local change was observed (**Figure 3**). The patient did not report any side effects after using the ointment, no local allergic phenomena, color changes, local thinning of the skin, tingling, itching, local erythema, secondary skin infections, or acne.

By using the ointment based on *Ocimum basilicum* and *Trifolium pratense* extract, the corticosteroids application was avoided, while offering better hydration of the skin, with no undesirable side effects such as thinning of the skin, appearance of telangiectasias, pigmentation disorders, or skin sensitivity.

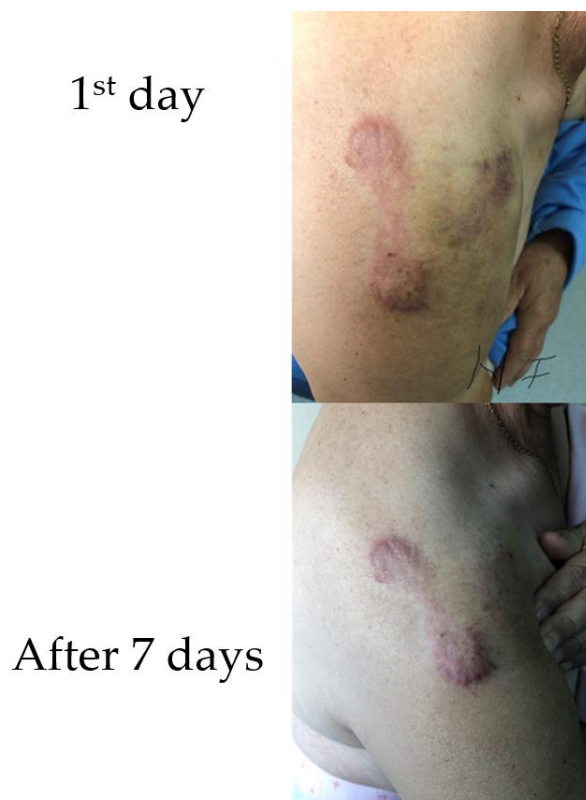


Figure 3. Clinical aspects of the case - Hypertrophic scar showing a slight improvement in local pruritus following the treatment with the ointment based on *Ocimum basilicum* and *Trifolium pratense* extracts.

Psoriasis Lesions

We present a case of a male patient, aged 56 referred from the outpatient clinic to our dermatology department with well-defined erythematous scaly annular plaques, scattered on his trunk abdomen, and elbows.

Psoriasis is a skin disorder driven by the immune system, especially involving T cells. Normally, T cells help protect the body against infection and disease. In the case of psoriasis, T cells are put into action by mistake and become so active that they trigger other immune responses, which lead to inflammation and rapid turnover of skin cells [40].

Upon clinical examination, we noticed erythematous medium-large plaques on the abdomen, chest, and elbows with silvery scaling, with a positive Auspitz sign, PASI score 7 (psoriasis area severity index), indicating medium erythema, scale, and induration. PASI is a measure of overall psoriasis severity and coverage assessing body surface area, erythema, and scaling.

The patient denied any current treatment for his condition. Medical history revealed that the patient had suffered from psoriasis for the past 8 years, and treated his flare-ups with local corticosteroids.

We started local treatment with the hydrogel formulation based on *Ocimum basilicum* and *Trifolium pratense* extract twice a day, in the morning (after the shower) and the evening before sleep. We noticed an improvement in patient condition starting with day 3 of treatment, but more obvious after 6 days of treatment (**Figure 4**).



Figure 4. Clinical aspects of the case – Psoriasis lesions showing a slight improvement in the local area following the treatment with hydrogel based on a mixture of *Ocimum basilicum* and *Trifolium pratense* extracts.

The erythrodermic plaques were visibly diminished with reduced silvery scaling, and the skin stopped being itchy. The PASI score decreased to 4 after six days of treatment with the hydrogel based on *Ocimum basilicum* and *Trifolium pratense* extract. Based on our previous studies carried out on the same extracts we can also state that the ethanolic extract of *Trifolium pratense* of the following biologically active compounds: catechin, syringic acid, cinnamic acid, chlorogenic acid, caffeic acid, ferulic acid, and rutin; meanwhile, the following were present in the ethanolic extract of *Ocimum basilicum*: syringic acid, cinnamic acid, chlorogenic acid, caffeic acid, ferulic acid, and rutin [6].

Caffeic, chlorogenic and ferulic acids have been shown to possess wound healing activities [14].

These substances also function as free radical scavengers, and phenolic compounds could be potentially effective in the treatment of various skin conditions, including signs of aging, acute and chronic skin diseases, and injuries [41]. Numerous studies have highlighted the strong biological activity of polyphenols, especially in dermal cell cultures, a fact also confirmed by our study on an animal model (rats) [5].

Plant phenols have a significant potential to inhibit or even reverse signs of aging such as wrinkles or signs hyperpigmentation, therefore they are also promising molecules for the development of new cosmetic formulations [42].

Most often, conventional treatments used to treat dermatological diseases can cause irritation, phototoxicity, hypersensitivity reaction, organ toxicity, antibiotic resistance, immunosuppression, and malignancy of the process, therefore natural remedies are an important alternative [30, 43-46].

Conclusion

As alternatives to conventional treatments for chronic inflammatory dermatological diseases, we propose novel formulations based on natural compounds extracted from *Ocimum basilicum* and *Trifolium pratense*. The antioxidant properties of the mixed extract OB+TP were evaluated by an electrochemical method using ascorbic acid as equivalent highlighting the involvement of catechol groups from polyphenols and flavonoids, the effect being more evident compared to similar studies reported in the literature.

The antimicrobial tests carried out by the Kirby-Bauer method using different concentrations, demonstrated the efficiency of OB+TP mixed extract against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes*, the best results being noticed for the concentration of 200 µl. The effect was more intense against Gram (+) than Gram (-) germs. The strong

antioxidant and antimicrobial activity of the mixed extract was further exploited to develop novel therapeutic formulations designed for the treatment of acute and chronic wounds susceptible to superinfection and/or antibiotic resistance. Hence, novel formulations (ointment, cream, and hydrogel) were prepared and applied in 3 clinical cases: dyshidrotic palmar eczema, hypertrophic scar, and psoriasis lesions. The clinical cases presented in our study revealed good outcomes of the cream formulation in the case of Dyshidrotic eczema, ointment formulation in the case of Hypertrophic scar, and hydrogel formulation in the case of Psoriasis lesions. The best effect was noticed after 6 days of topical application of hydrogel on psoriatic plaques, resulting in a significant reduction of erythematous plaques, reduction of adherent pearly white scales, and local itching.

Our results highlight the importance of phytochemical-based formulations in the treatment of chronic wounds with different etiologies, avoiding the possible side effects generated by conventional treatment with corticosteroids such as allergic skin reactions, secondary skin infections, skin thinning, skin discoloration, itching, or stretch marks.

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Ethics statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the University of Oradea, Faculty of Medicine and Pharmacy (protocol code CEFMF/03, date of approval 30 June 2021). Informed consent was obtained from all subjects involved in the study.

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