THERAPEUTIC EFFECT OF OINTMENT FOR PSORIASIS BASED ON *ACHILLEA MILLEFOLIUM* L., *CALENDULA OFFICINALIS* L. AND *SALVIA OFFICINALIS* L.

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
This study investigates the efficacy of Dermavit cream for treatment of psoriasis, eczema and dermatitis made by Pharmamed d.o.o Travnik for treatment of patients diagnosed with psoriasis. Dermavit cream for treatment of psoriasis, ekczema and dermatitis contains in composition Achillea millefolium extract, Calendula officinalis extract and Salvia officinalis essential oil. Due to the specific composition of extracts contained in Dermavit cream for treatment of psoriasis, eczema and dermatitis, it hydrates and softens the skin, helping it to regenerate and soothe the skin surface. For qualitative analysis of the extracts, UHPLC – MS (Millefolium herba and Calendulae flos) and GC-MS (Salviae folium) were used. According the study results conducted on subjects with diagnosis of psoriasis Dermavit cream for treatment of psoriasis, eczema and dermatitis can be determined to be successful in alleviating psoriasis symptoms. The results are obtained based on a study involving 30 patients with psoriasis, where each of them used the cream for a period of 60 days.

KEYWORDS: psorisasis, Dermavit cream for trearment of psoriasis, eczema and dermatitis, flavonoid components, UHPLC-MS, GC, GC-MS

INTRODUCTION

Psoriasis is a chronic, non-communicable, inflammatory, incurable skin disease characterized by hyperproliferation and abnormal keratinocyte differentiation, inflammatory cell infiltration, and vascular changes (angiogenesis). It is characterized by symmetrical skin lesions with crisp boundaries, red papules and plaques, and generally covered in white and silver scales. The disease's name derives from the Greek term ψωριασις, which combines the words psora (itching) with the suffix -iasis, which means condition or activity [1]. Psoriasis affects around 2% to 3% of the world's population, or 125 million people in total. Therefore, this disease represents a significant public health issue [2].

A wide range of conventional medical therapies have been established for the treatment of psoriasis, from topical therapy (corticosteroids, vitamin D analogues, psoralen, 5-aminolevulinic acid, salicylates, fumaric acid esters, anthralin (dithranol), tacrolimus, retinoids - eg tazarotene) systemic drugs (methotrexate, cyclosporine, retinoids, 6-thioguanine, mycophenolate mofetil, troglitazone, and new biologics, such as adalimumab, alefacept, efalizumab, etanercept, infliximab), to phototherapy, or a combination of all mentioned above [3].

However, most of these therapies cause a number of side effects and have limited effectiveness, leading to skin atrophy, organ toxicity (hepatotoxicity, nephrotoxicity, teratogenicity), carcinogenicity and immunosuppression, which limits their long-term use [4].

In turn, a short-term treatment of psoriasis causes its remission after finishing the treatment or only relieves the patient's condition. Therefore, the invention of new alternative treatments for psoriasis causing fewer side effects would be desirable. It seems that several herbal drugs can meet these requirements and have to be seen as promising new agents for psoriasis treatment. Herbal products are greatly accepted by patients because they are believed to be safer than conventional therapeutics. Moreover, herbal

products present a great structural diversity and multidirectional mechanisms of action, which is not commonly seen in synthetic compounds. Herbal drugs may become an effective treatment for psoriasis, causing lower costs and less side- or toxic effects in comparison to other therapies. Therefore, researchers are still looking for novel herbal products and/or their active constituents, which potentially could be used for the treatment of psoriasis instead of synthetic drugs [5].

However, most studies provide limited information on the effectiveness of topically used herbal products. Therefore, more scientific evidence and documentation is needed to promote the treatment of psoriasis with herbal products.

In this paper, Dermavit cream for psoriasis, eczema, and dermatitis, made by Pharmamed d.o.o. Travnik, further referred to as Dermavit cream, has been investigated. Dermavit cream is part of a line of specialized cosmetics. It's a cream for dry, cracked, dandruff-prone skin, as well as red and inflammatory skin. The line is available in Bosnia and Herzegovina. Phytochemical analysis of plant extract used for cream preparation was performed and the efficacy of Dermavit cream was investigated. A survey of patient with diagnosis of psoriasis was done to assess subjective improvement after using Dermavit cream.

MATERIALS AND METHODS

Extracts from Millefolii herba (*Achillea millefolium* L.) and Calendulae flos (*Calendula officinalis* L.), as well as Salviae officinalis essential oil (*Salvia officinalis* L.), provided by the Pharmamed laboratory, were used for qualitative investigation of potentially active compunds.

The extracts of *A. millefolium* and *C. officinalis* were prepared by maceration according to the Ph. Jug IV [11]. Single extraction of suitably crushed drug was performed at room temperature in a well-closed, light-protected container with 63 percent ethanol in a ratio of 1:5. Shaking and stirring was applied twice a day during maceration lasting 5 days. The macerate was then separated from the drug by squeezing, followed by pressing and left for 2 days in a cool, dark place and then filtered. The obtained extracts were analyzed qualitatively using UHPLC-MS method. The GC method and the GC-MS combination were used to undertake qualitative and quantitative analysis of Salviae officinalis essential oil. Salviae officinalis essential oil was prepared by steam distillation as shown in Figure 1.

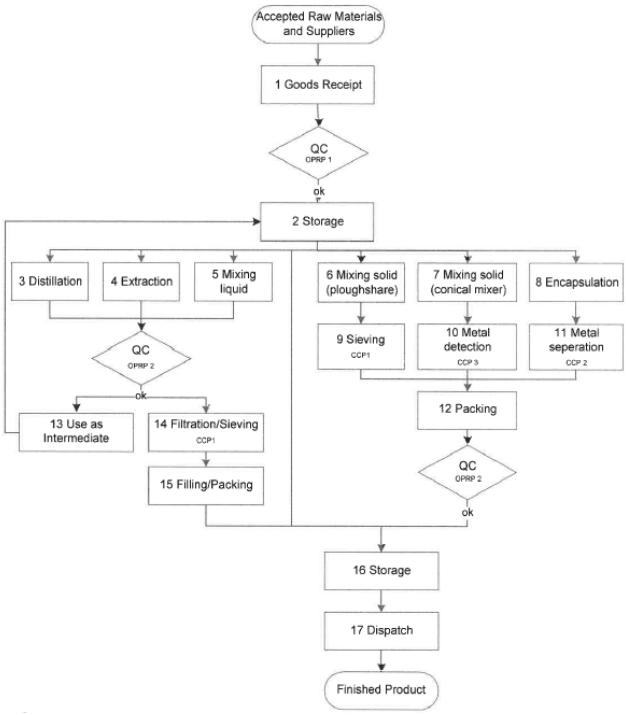

Figure 1. Flow chart production process of *Salviae officinalis* essential oil

ULTRA-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY – UHPLC

The Ultimate 3000 UHPLC system (Thermo Fisher Scientific - San Jose, CA) was equipped with a reversed phase C18 column (Thermo Acclaim; 2.1 mm × 15 cm, 3 μm, 120 Å). Mobile phase A (H_2O/FA, 100:0.01) and mobile phase B (ACN) were degassed prior to their usage. A 66 min binary gradient with flow rate set to 500 μL/min was applied as follows: 0−1 min, 5% mobile phase B; 2− 45 min, 5−95% mobile phase B; 45-55 min, 95% mobile phase B; 56-66 min re-equilibration with 5% mobile phase B). Five microliters of each sample (5mg/mL) were injected followed by a blank injection to ensure proper column washing and equilibration.

MASS SPECTROMETRY - MS

Mass spectrometric detection was performed with a LTQ-XL linear ion trap mass spectrometer (Thermo Fisher Scientific) using the HESI source (300°C heater temperature, 40/10/1 arb. units for the sheath, aux and sweep gases respectively and 3.5kV spray voltage at 275°C capillary temperature) to achieve negative/positive ion mode ionization. MS scans were performed with an m/z range from 150 to 2000. MS/MS scans of the three most abundant ions were achieved through collisional induced dissociation

(CID) with fragmentation at 30% normalized collision energy.

GAS CHROMATOGRAPHY - GC

A Shimadzu GC 2010 Plus gas chromatograph was equipped with Permabond SE54 quartz capillary column (10m x 0.25mm, 0.25m film thickness), and a flame ionizing detector (FID).

The GC apparatus was set to operate in the following manner: the injectors and detectors were kept at 270 °C, while chromatography was carried out in the linear temperature programming mode 50-125°C and at a heating rate of 3° C/min. It took 25 minutes to do the chromatography. Throughout the analysis, the carrier gas was nitrogen (N_2) 5.0 at a constant flow rate of 2.51 ml/min. In a 1:100 split, an essential oil solution in dichloromethane, CH_2Cl_2, was injected.

GAS CHROMATOGRAPHY - MASS SPECTROMETRY – GC-MS

GC-MS analysis of essential oil was performed on a GCMS-QP2010 Shimadzu device equipped with a MN Optima-5 capillary column, Macherey Nagel (50m x 0.25mm, film thickness 0.5μm) connected to a mass spectrometric selective detector (MSD).

The operating mode of the GC-MS apparatus was as follows: Analytical conditions identical to those for GC / FID were applied in the case of GC-MS analysis. Instead of nitrogen, helium (He) was used as the carrier gas at a constant flow rate of 1.14 ml/min throughout the analysis time. The sample was injected using a split/splitless injector at a 1:10 split ratio.

The conditions prevailing in MS are: the temperature of the ion source is 250ºC, and the interface temperature is 270ºC. Mass spectra were recorded for the mass range m/z of 40-500.

Identification was done in accordance with databases and literature.

OBSERVATIONAL CLINICAL STUDY CONDUCTED ON PATIENTS

The effects of Dermavit cream were studied in 30 patients for two months to investigate its efficacy. On a voluntary basis and with respect for privacy, patients were selected according to age, gender and disease's duration. Patients accepted to participate in the study by signing the informed consent form. The study was done in the MGM pharmacy, Zenica branch, at the address Dr. Abdulaziza Aska Borića 23. After 7 days, 15 days, 1 month, and 2 months of using Dermavit cream to treat psoriasis, the changes were photographed and recorded. The parameter like skin flakes, redness and the thickened of the skin were folowed and evaluated with scores from -1 to 2 with patient's dermatologist, who diagnosed the patient with psoriasis. The total withdrawal of symptoms was evaluated with a score of 2, partial withdrawal of symptoms with a score of 1, without effect with a score of 0 and deterioration with a score of -1. Classification of terms is determinated in copperation with dermatologist. For satisfactory improvement, the total number of grades from 4 to 6 was taken, for partial improvement of the total number of grades from 1 to 3, for lack of improvement the total score was 0, and for deterioration the score was less than zero. After 60 days of therapy with Dermavit cream, the patients were given a structured questionary (see Appendix).

The questionary consisted of two parts: the first part contained questions of the patient's demographics, lifestyle and habits, the second part questions about subjective improvement after using Dermavit cream. The answers to questions concerning the effects of the treatment were assessed on a 5-point Likert scale: Totally agree, Somewhat agree, Neither agree or disagree, Somewhat disagree, Totally disagree. The results were processed using google forms.

RESULTS AND DISCUSSION

The review of the literature shows that a great growth has taken place in the worldwide interest in the potential of herbal medicines for the treatment of psoriasis over the last 20 years. Parallel to various synthetic medicines used topically (corticosteroids, vitamin D analogues, retinoids) and systemic (methotrexate, retinoids, cyclosporin), or targeted (biological) therapies (e.g. alefacept, efalizumab, etanercept) also herbal products play an important role as therapeutic agents for psoriasis treatment [7].

There are many advantages of using natural drugs, including patient compliance, less side-effects, easy availability, low-costs, and more than one mode of biochemical action for psoriasis treatment. Therefore, researchers are searching for new herbal products, which have the potential to be an alternative for synthetic drugs in psoriasis therapy [8].

Herbal formulation of Dermavit cream is prepared from two different plant extracts, Calendulae flos extract and Millefolii herba extract and Salviae officinalis essential oil. Calendulae flos is marked by the European medicines Agency for use in the treatment of for skin inflammations, minor wounds and mouth or throat inflammation [9]. Moreover, the intended use does not require medical supervision.

Milefolii herba is recognised as tradional herbal medicinal product for the treatment of small superficial wounds [10]. Salvia officinalis is also maked to be anti-inflammatory agent [11].

Based on our findings, no clinical studies have been reported with preparation that contain combination of plan extract and essential oil.

PHYTOCHEMICAL ANALYSIS

Flavonoid components present in Calendula flos extract (isoquercitrin, rutin, calendoflavoside, isorhamnetin, isorhamnetin-3-O-glucoside, narcissin) and flavonoid components present in Millefolii herba extract (cinarozid, kosmosin, luteolin, apigenin, centaureidin, luteolin-7-O-glukozid, apigenin-7-O-glukozid, salvigenin, eupatilin) were identified by comparing the retention time, and by comparing m/z mass spectrum values with literature published data and databases and are in line with the manufacturer's specifications.

The spectrum of flavonoids rutin from Calendula officinalis esxtract and luteolin from Achilea millefolli extract are presented in Figure 2 and Figure 3.

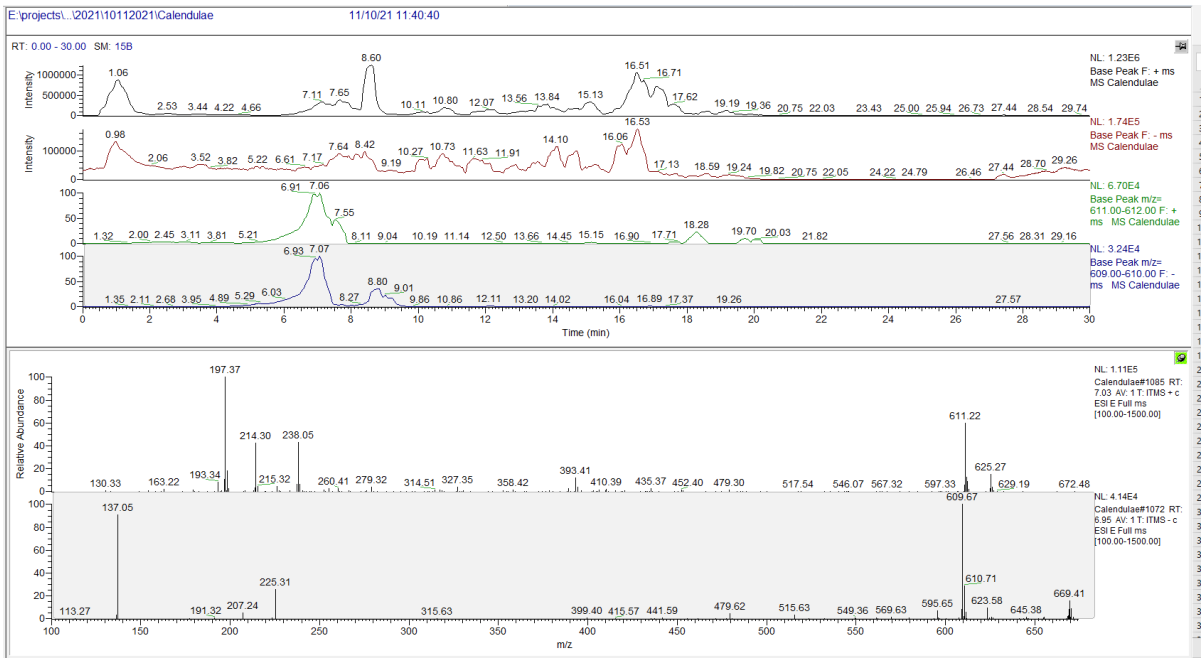

Figure 2. The spectrum of rutin

The observed mass spectrometer shows the dominant deproteinized ion [M-H]¯at m/z 609.67. The spectrum of the base peak shows higher sensitivity in the negative ionization mode. The retention time of the test analyte in the cause is 7.07, and deviates less than 1% from the retention time of the standard solution of 7.1.

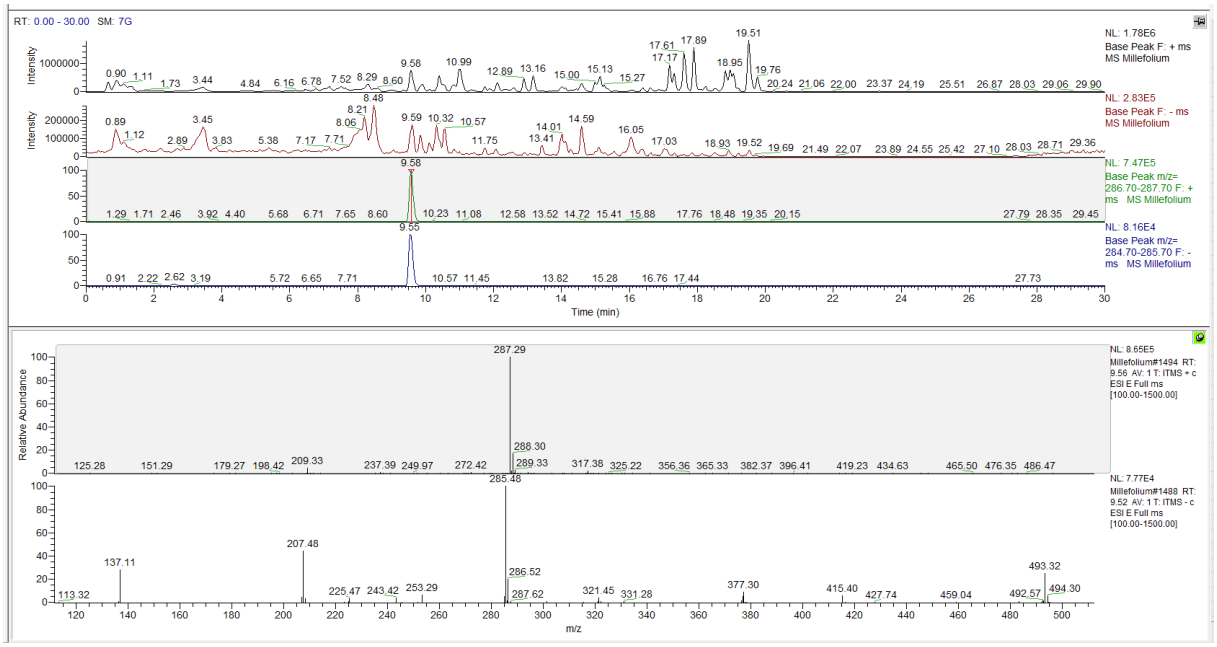

Figure 3. The spectrum of luteolin

The observed mass spectrometer shows a dominant ion at m/z 287.29. The spectrum of the base peak shows higher sensitivity in the positive ionization mode. The retention time of the test analyte in the cause is 9.58 and deviates less than 1% from the retention time of the standard solution which is 9.5.
The chemical profile of Salviae officinalis essential oil obtained by GC analysis is given in Table 1 and the GC chromatogram is shown in Figure 4.

Table 1. Retention time and Area of Salviae officinalis essential oil

Peak	Retention time, tr	Area
1	3.349	123483
2	3.652	154900
3	4.299	59626
4	4.736	13063
5	5.028	1852
6	5.389	2125
7	5.631	27906
8	5.814	299695
9	6.117	1025
10	6.705	17634
11	7.034	1474
12	7.692	9237
13	8.302	675400
14	8.696	147160
15	9.654	485051
16	10.526	83203
17	11.005	. 9419
18	15.451	32370
19	18,216	97853
20	18.294	55619
21	19.069	4144
22	19.253	9393
23	19.343	2653
24	19.421	2807
25	19.497	1176
26	19.537	1682
27	20.228	23548
28	20.665	92756
29	22.014	110868
30	22.751	76093
Total		2623213

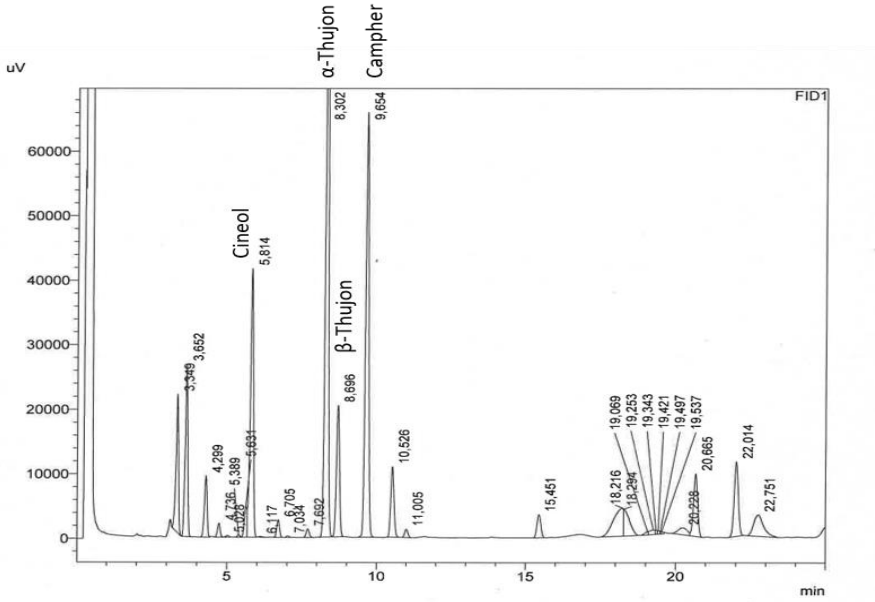

Figure 4. Chromatogram of Salvia officinalis essential oil

Salviae officinalis essential oil has been shown to have 30 different constituents, the highest percentage of α and β-thujone (31.36%), camphor (18.49%), and cinelol (11.42%). Other compounds are present in a smaller percentage (Figure 4).

These result are in line with manufacturer's specifications.

OBSERVATIONAL CLINICAL STUDY CONDUCTED ON PATIENTS

A total of 38 patients who reported having symptoms related to psoriasis were assessed for eligibility criteria, of which 7 were excluded for various reasons (no objective signs of psoriasis, refuse to participate or not eligible for inclusion). A total of 31 patients were eligible for the study, and they randomized in four main groups, according to the disease's duration (Figure 5).

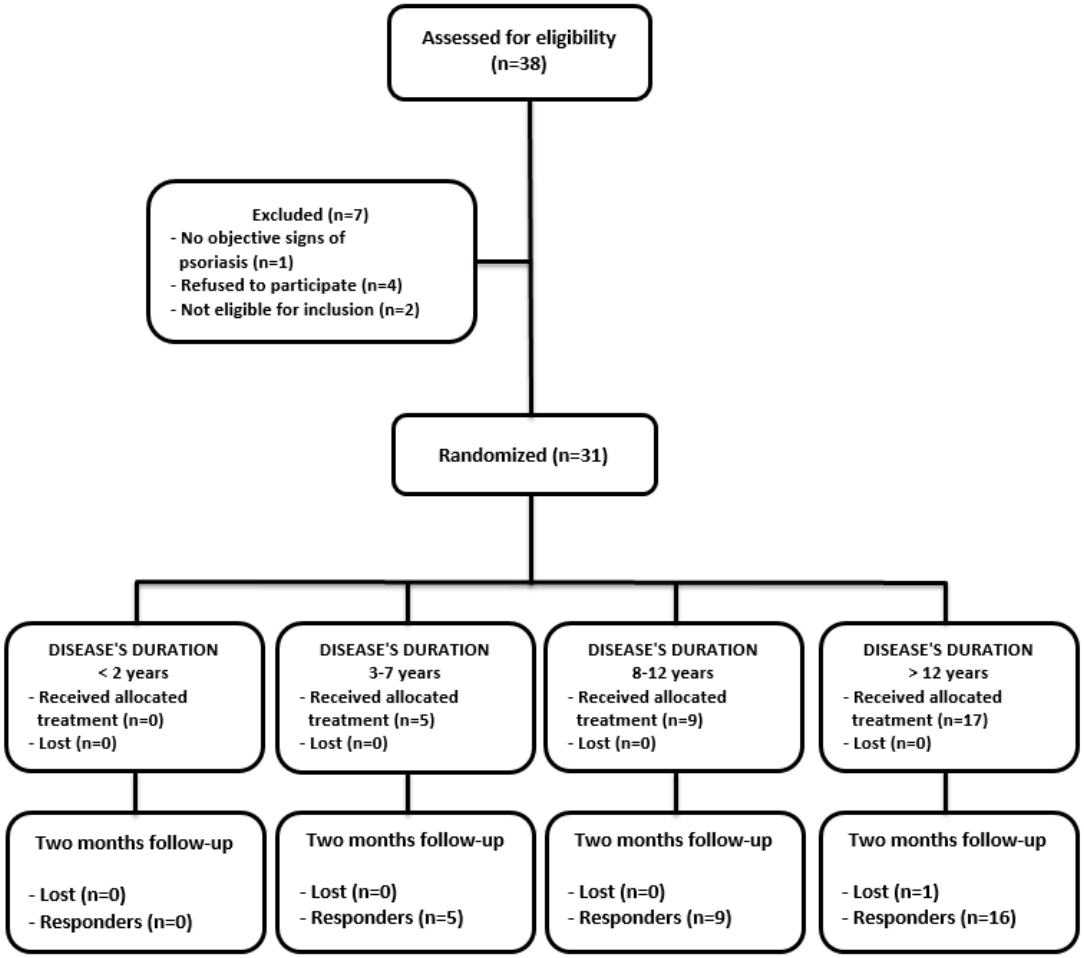

Figure 5. Flow chart of the study

After applying Dermavit cream for sixty days, satisfactory improvement was achieved to the biggest number of patients participating in the study a total of 19 (63.3%), respectively. Partial improvement was achieved on a limited number patients participating in the study, a total of 10 (33.3%), respectively. In the smallest number of patients participating in the study, 1 patient (3.3%), there was no change. There were no cases of psoriasis worsening, after using Dermavit cream. Efficacy of Dermavit cream by age, gender and disease's duration is shown in Table 2.

Table 2. Efficacy of Dermavit cream by age, gender and disease's duration

		EFFICACY OF DERMAVIT CREAM			
		Satisfactory improvement n (%)	Partial improvement n (%)	Lack of improvement n (%)	Deterioration n (%)
GENDER	F	9 (30.0)	4 (13.33)	1 (3.33)	0 (0)
	M	10 (33.3)	6 (20.00)	0 (0)	0 (0)
AGE	< 30 years	3 (10.00)	1 (3.33)	0 (0)	0 (0)
	30–39 years	4 (13.33)	0 (0)	0 (0)	0 (0)
	40-49 years	1 (3.33)	4 (10.00)	1 (3.33)	0 (0)
	50-64 years	7 (23.33)	3 (10.00)	0 (0)	0 (0)
	> 65 years	3 (10.00)	3 (10.00)	0 (0)	0 (0)
DISEASE'S DURATION	< 2 years	0 (0)	0 (0)	0 (0)	0 (0)
	3-7 years	3 (10.00)	1 (3.33)	1 (3.33)	0 (0)
	8-12 years	9 (30.00)	0 (0)	0 (0)	0 (0)
	> 12 years	7 (23.33)	9 (30.00)	0 (0)	0 (0)

Based on the obtained results, no relationship between gender, age, duration of the disease and the effectiveness of the Dermavit cream can be established.

Figure 6 and Figure 7 show examples of satisfactory improvement and partial improvement, respectively.

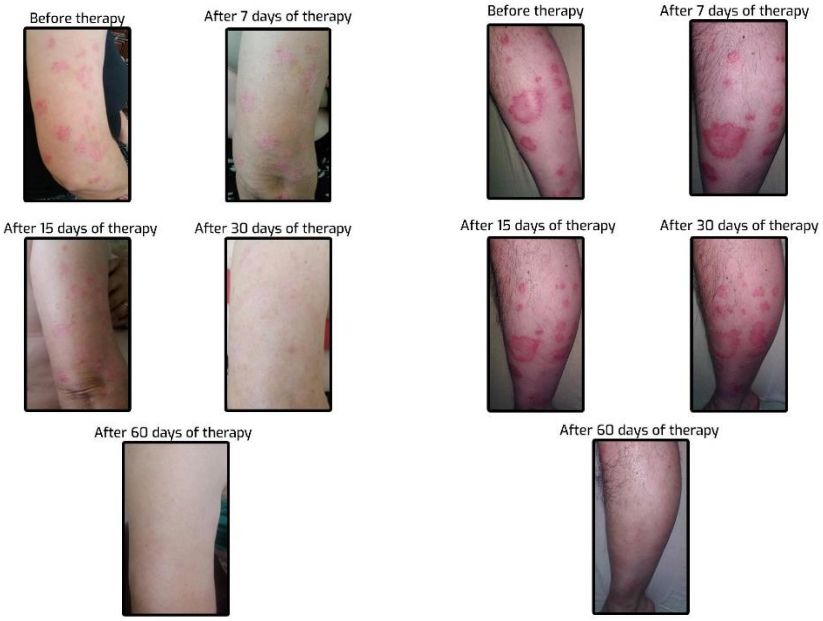

Figure 6. Examples of satisfactory improvement after applying Dermavit cream

Figure 7. Examples of partial improvement after applying Dermavit cream

According to the answers from the questionary, the majority of patients are frequently disturbed by itchy and/or irritated skin or pain, and are frequently concerned about their skin's look. For most patients participating in the study, skin redness, skin thickening, itching and skin peeling were reduced after using Dermavit cream.

CONCLUSION

Herbal products are being increasingly used in the treatment of skin diseases like psoriasis. Unfortunately, most studies provide only limited information about the efficacy and safety of topically used herbal products in the treatment of psoriasis. Therefore, more scientific evidence and

documentation is desired for the promotion of herbal treatment of psoriasis, which must be substantiated by reliable clinical trials. In this paper, Dermavit cream has been investigated.

After applying Dermavit cream for most participants of the study (19/63.3%), a satisfactory improvement was achieved. In this regard, Dermavit cream can be determined to be successful in alleviating psoriasis symptoms.

Furthermore, 90% of patients who used Dermavit cream over a period of 2 months, during the study of the effectiveness of Dermavit cream, totally or somewhat agree with the statement that redness of the skin, thickening of the skin and scales on the skin are reduced. They generally consider Dermavit cream effective in relieving the symptoms of psoriasis, have not noticed any side effects and would recommend them to other psoriasis sufferers.

There are several other products (creams) on the market of Bosnia and Herzegovina for use in psoriasis, such as Psorihtol cream manufactured by Maxivita. All these products are registred as cosmetic products with special purpose. However, unfortunately, this type of research has not been conducted with any of these products, and therefore a comparison is not possible.

REFERENCES

[1] Ritchlin C, F. O. (2007). *Psoriatic and Reactive Arthritis: A Companion to Rheumatology* (1st izd.). Maryland Heights: Mosby.

[2] Nestle FO, K. D. (2009). Psoriasis. *N Engl J Med., 361*, 496–509.

[3] Rahman M, A. K. (2012). Classical to current approach for treatment of psoriasis. *Endocr Metab Immune Disord Drug Targets, 12(3)*, 287-302.

[4] Traub M, M. K. (2007). Psoriasis-pathophysiology, conventional, and alternative approaches to treatment. *Altern Med Rev., 12*, 319–330

[5] Herman A., Herman A.P. (2016). Topically Used Herbal Products for the Treatment of Psoriasis – Mechanism of Action, Drug Delivery, Clinical Studies. Planta Med.; 82(17): 1447-1455

[6] Pharmacopoeia Jugoslavica editio quarta (1984). Belgrade, June 1984.

[7] Lebwohl M, Ting PT, Koo JYM. Psoriasis treatment: traditional therapy. Ann Rheum Dis 2005; 64: 83-86

[8] Jarrett A, Spearman RIG. Psoriasis. In: Taverner D, Trounce J, eds. Histochemistry of the Skin. London: University Press; 1964

[9] European Medicines Agency. Committee on Herbal Medicinal products: European Union herbal monograph on Calendula officinalis L., flos. 27 March 2018 EMA/HMPC/437450/2017. https://www.ema.europa.eu (30.03.2022)

[10] European Medicines Agency. Committee on Herbal Medicinal products: European Union herbal monograph on Achilea milefoilum L., Herba. 12 July 2011 EMA/HMPC/290284/2009. https://www.ema.europa.eu (30.03.2022)

[11] European Medicines Agency. Committee on Herbal Medicinal products: European Union herbal monograph on Salvia officinalis L, folium. 20 September 2016 EMA/HMPC/277152/2015. https://www.ema.europa.eu (30.03.2022)

APPENDIX

Questionary

Topical herbal products in the treatment of psoriasis

1. Age : < 30 years ☐ 30 - 39 years ☐ 40 - 49 yeras ☐
   50 - 64 yeras ☐ ≥ 65 years ☐
2. Sex: m ☐ f ☐
3. Have you been diagnosed with psoriasis? Yes ☐ No ☐
4. Disease's duration:

< 2 years ☐ 3 - 7 years ☐ 3 - 7 years ☐ 8 - 12 years ☐
>12 years ☐

5. How often do you experience itchy skin?
   □ never
   □ rarely
   □ sometimes
   □ often
   □ very often
6. How often are you bothered by the feeling of irritated skin or pain?
   □ never
   □ rarely
   □ sometimes
   □ often
   □ very often
7. How often are you worried about the appearance of your skin?
   □ never
   □ rarely
   □ sometimes
   □ often
   □ very often
8. Does the condition of your skin affect the interaction with other people?
   □ never
   □ rarely
   □ sometimes
   □ often
   □ very often
9. Does the condition of your skin limit the type of clothing you wear?
   □ never
   □ rarely
   □ sometimes
   □ often
   □ very often
10. Does the condition of your skin affect the choice of social activities?
    □ never
    □ rarely
    □ sometimes
    □ often
    □ very often
11. Does your skin condition cause problems at school / work?
    □ never
    □ rarely
    □ sometimes
    □ often
    □ very often
12. Does your skin condition cause relationship / marriage problems?
    □ never
    □ rarely
    □ sometimes
    □ often
    □ very often
13. Have you ever used herbal products in the treatment of diseases? YES ☐ NO ☐
14. Have you ever used Dermavit cream for treatment of psoriasis, eczema and dermatitis? YES ☐ NO ☐
15. How did you start using Dermavit cream for treatment of psoriasis, eczema and dermatitis:
    □ on the recommendation of family / friends
    □ on the recommendation of a physician
    □ on the recommendation of a pharmacist
    □ based on information from the media
    □ other, _____
16. I use Dermavit cream for treatment of psoriasis, eczema and dermatitis regularly and in the way recommended by my doctor / pharmacist: YES □ NO □

17. What are your expectations from the application of Dermavit cream for treatment of psoriasis, eczema and dermatitis:
□ alleviation of symptoms
□ treatment of diseases
□ something else, _____

18. Since i have started to use Dermavit cream for treatment of psoriasis, eczema and dermatitis, my skin redness has decreased:
□ totally agree
□ somewhat agree
□ neither agree, or disagree
□ somewhat disagree
□ totally disagree

19. Since i have started to use Dermavit cream for treatment of psoriasis, eczema and dermatitis I have no thickening on the skin :
□ totally agree
□ somewhat agree
□ neither agree, or disagree
□ somewhat disagree
□ totally disagree

20. Since i have started to use Dermavit cream for treatment of psoriasis, eczema and dermatitis I have no scales on the skin:
□ totally agree
□ somewhat agree
□ neither agree, or disagree
□ somewhat disagree
□ totally disagree

21. Since i have started to use Dermavit cream for treatment of psoriasis, eczema and dermatitis I have no itchy skin :
□ totally agree
□ somewhat agree
□ neither agree, or disagree
□ somewhat disagree
□ totally disagree

22. I generally consider Dermavit cream for treatment of psoriasis, eczema and dermatitis very effective in treatment of psoriasis:
□ totally agree
□ somewhat agree
□ neither agree, or disagree
□ somewhat disagree
□ totally disagree

23. I would recommend Dermavit cream for treatment of psoriasis, eczema and dermatitis to other patients who have not used it before:
□ totally agree
□ somewhat agree
□ neither agree, or disagree
□ somewhat disagree
□ totally disagree

24. I have not noticed any side effects while using Dermavit cream for treatment of psoriasis, eczema and dermatitis:
□ totally agree
□ somewhat agree
□ neither agree, or disagree
□ somewhat disagree
□ totally disagree

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