

RED DRY SKIN™

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1.0 Short Summary

About psoriasis

Psoriatic Form Conditions are skin disorders which can be defined by three main conditions:

- Presence of chronic inflammation (*mediated by T cells*)
- Autoimmune disease (*increased levels of Cytokines are detected*)
- Accelerated proliferation of dermal cells (*up to 24 times or more*)

In psoriasis, an activated immune system triggers the skin to reproduce every three to four days, building up on the outer layers (epidermis and keratin). The epidermis thickens, blood flow increases and reddens the skin, and silver-gray scales cover it. Psoriasis can be itchy and sore.

In general, doctors treat psoriasis in three steps:

Step 1: Medications applied to the skin (topical therapy);

Topical steroids, Tar compounds, Vitamin D3, Retinoids, salicylic acid.

Step 2: Treatments that use **light (phototherapy)**;

Sunshine (climatotherapy), Ultraviolet therapy (UVA and UVB), PUVA (Psoralen plus UVA),

Step 3: Medications given as a pill or injection (systemic therapy);

Cyclosporine (Cyclosporine works by suppressing the immune system in a way that slows the build-up of dead skin cells).

Patients and doctors are not satisfied with these medications, especially due to their severe side effects and partial efficacy.

Our research strategy

Against this background, we focused our preclinical research on finding plant extracts active on three aspects:

- a) Reducing dermal proliferative rate (cytostatic) during biological process (not necrotic).
- b) Regulation of the levels of cytokines production..
- c) Anti-inflammatory.

The most important 25 medicinal plant species, traditionally used by the Arab population to treat psoriasis, were identified and selected. Each plant was extracted using different solvents and tested in unique biological experimental systems as needed.

Novel product:

A combination of 4 herbs which are highly effective in synergistic and multi-level healing of psoriasis and related symptoms such as red, dry, scaly irritated skin.

Active Constituents.

Combination of extracts of specific plants of the plant species *Nigella sativa*; *Eruca sativa*; *Citrus limonum*; *Hypericum perforatum*.

Indications.

- Psoriasis (Mild to Moderate Severity).
- Inflammatory Skin Disorders.
- Dery Scaly Eczema.

Actions.

- Cytostatic.
- Normalizing immune responses in skin
- Anti-inflammatory.
- Soothing Emollient.

Mechanisms.

Natural products on the market are generally anti-inflammatory herbs, which are minimally effective and act on one aspect of the problem. This product is a synergistic combination which is highly and uniquely effective. It combines proven anti-inflammatory effects against the symptoms of irritation and redness, proven treatment of local immune reactions and cytokines, proven cytostatic effects against excessive cell proliferation, and skin restorative properties.

Research.

The herbal active ingredients have been demonstrated in skin cell and tissue culture at the Hebrew University's Skin Biochemistry Laboratory to be highly effective cytostatic agents with minimum cytotoxicity and possess a controlling effect of cytokines production. Studies and published scientific evidence indicates activity on cytokines and supports anti-inflammatory effects of the active ingredients at several steps in the inflammatory process.

Proprietary Position

The herbal active ingredients and herbal combination is currently undergoing the patent process. The specific uses, plant parts, varieties and processing methods are unique and proprietary.

Regulatory Status.

Three herbs classified as foods under food regulations. Fourth, *Hypericum*, classified as Food Supplement in USA, and on General sale List for open sale for topical use in UK.

Safety.

All herbs are on the INCI list of ingredients approved for cosmetic use in the EU. Phase I clinical safety tests on 12 volunteers have been carried out at a leading local medical centre with no evidence whatsoever of adverse effects during daily application for 21 days.

Informal clinical Trials.

Patient observation trials on over 17 patients have demonstrated significant remissions. A Phase II clinical trial protocol has been prepared.

2.0 Preclinical Data.

Methods and materials:

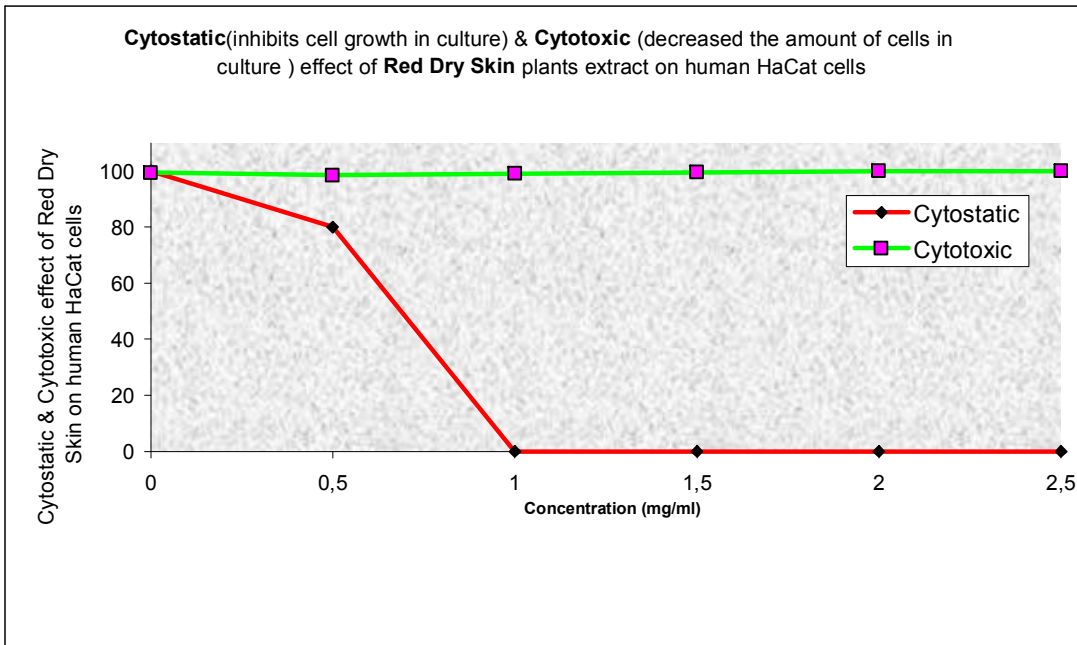
2.a. Screening of inhibitory substance from traditional plants

The study was conducted by the Myers Skin Biochemistry laboratory in the Hebrew university, life sciences institute, Jerusalem, Israel, under the supervision of Prof. Yourm Milner. Each result represents an average of 5 repeated experiments.

The most important 25 medicinal plant species, traditionally used by the Arab population, were identified and selected. In order to find the potential plant extract which could be used as a cytostatic agent in psoriasis and other dermatoses, we have used cell cultures of keratinocytes cell line, HaCat and organ culture of skin. Several concentrations of aqueous ethanol: water (1:1 v/v) and hexane extracts of variety of traditional plants known to have an effect on skin were applied to the skin and cell cultures (~50% confluent). Following 48 hr of proliferation in 37°C in proper medium the cells were fixed and evaluated for their number on plate relative to control cultures without any extract. The cytostatic doses (IC₅₀) leading to 50% cell proliferation inhibition were evaluated from inhibition curves (cell number vs. concentration of plant extract) where the 50% reduction in cell number is considered as IC₅₀. Similarly, cytotoxic effects of these materials were evaluated by incubation of various concentrations of the plants extracts with confluent cell cultures and following by 48 hrs incubation at 37°C. The cell numbers still remaining on the plate were evaluated. CD₅₀ is cytotoxic concentration leading to reduction of 50% of the cells number in culturing relation to non treated controls.

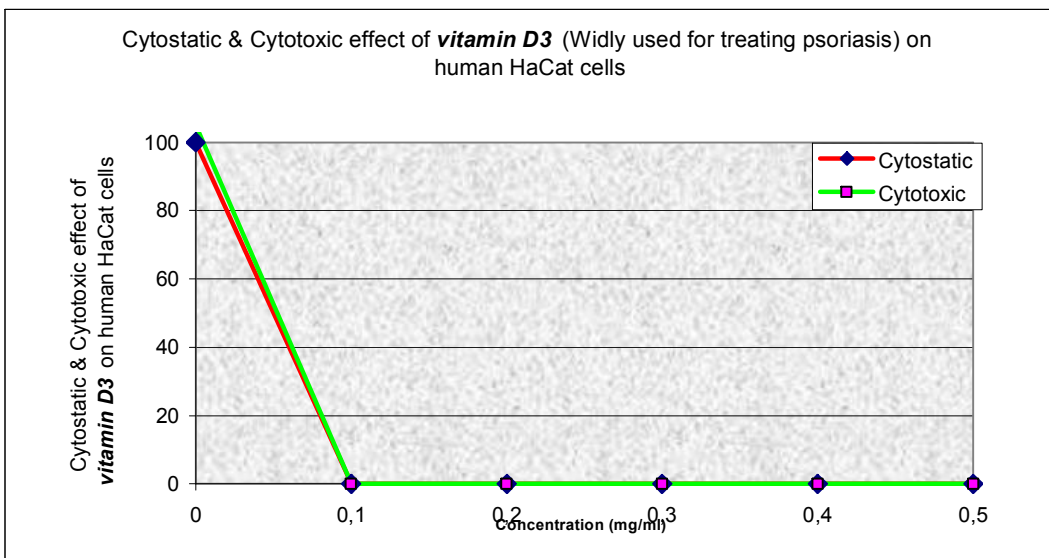
As for organ culture studies we have used organ culture of pieces of whole skin incubated at 37°C for 16 hrs with various concentrations of plant extract, the effect of the plant extract on epidermal cell proliferation was evaluated by measuring ³H-Thymidine incorporation into DNA in the epidermis. The cytostatic effect was evaluated by determining the inhibitory concentration IC_{50†} leading to 50% reduction in the incorporation of ³H-Thymidine into epidermal cells.

Figure 1 A.



The graph 1A shows a Remarkable inhibition of cell proliferation without cytotoxic effect

Figure 1 B:



This fig. (1B) shows that Vitamin D3, which is known to be an effective treatment for psoriasis, exhibits no concentration differences between cytototoxic and cytototoxic effects (extremely cytototoxic).

Clarifications.

1) For cytototoxic and cytototoxic experiments we used HACAT (Human source) cell line. Where the plant extract was Ethanol-Water (50%-50%) in **figure 1 A**. The Y axis (in fig. 1A and 1B) represent the values of dividing cells (proliferating cells), then the triangular line of data means that our extract caused decreasing of the cell dividing number. In other words the slope of the graph shows a strong cytototoxic effect.

The graph with dotted symbols represents the cytototoxic activity. The results show that the extract is not cytototoxic and did not affect the basal condition (cell number) when it had been added. Fig 1B shows that vitamin D3 has remarkable cytototoxic effect, In the same time has a strong cytototoxic effect .

2-b. Test on immune system by monitoring the secretion of cytokines by primary isolated T-cells, in vitro.

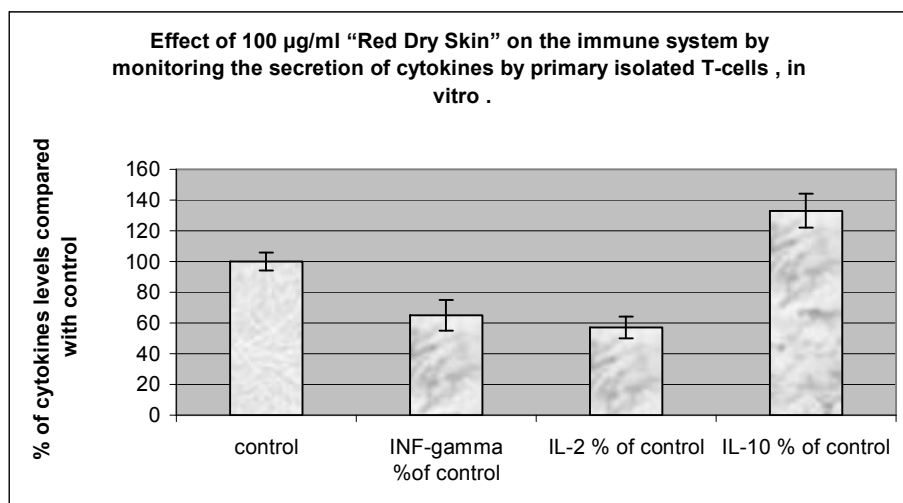
The study was conducted by the Regional Research and Development Center – The Galilee Society, Israel, under the supervision of Dr. Bashar Saad.

Data given represents the mean \pm standard deviations of three independent experiments carried out in triplicates

Psoriasis is a T-cell-mediated inflammatory disease in humans. The pathogenesis of psoriasis is linked to activation of several types of leukocytes that control cellular immunity and to a T-cell-dependent inflammatory process in skin that accelerates the growth of epidermal and vascular cells in psoriasis lesions. Critical steps in immunologic activation include Langerhans cell maturation (activation), T-cell activation, differentiation and expansion of type 1 T cells, selective trafficking of activated T cells to skin, and induction of an inflammatory cytokine and chemokine cascade in skin lesions (Krueger, 2002). Therefore, the effects of plant extracts on the secretion of cytokines by primary isolated T-cells were examined in or group. The production of IL-2, IFN-g, and of IL-10 was measured after a 24-hour treatment of the T-cells with various concentrations of plant extract. The concentrations of IL-2, IFN-g, and of IL-10 in supernatants of cultured T-cells were assessed with commercially available kits following the manufacturer's instructions. IL-2 and IFN-g concentrations were found to be significantly decreased after treatment with concentrations higher than 100 microgram/ml. IL-10 levels in the supernatant were significantly increased after treatment.

Therefore, we conclude that down regulation of the Th1-derived IL-2, IFN-g, and up regulation the Th2-derived IL-10 are responsible for the observed anti-psoriatic activity of our plant extracts. These cytokines are known to affect both Langerhans cell maturation and keratinocyte proliferation and differentiation. Our plant extract may affect the paracrine effects of either T cell - keratinocytes or T-cells - Langerhans cells.

Fig 2



We conclude, from the above results, that down regulation (decreasing) of the Th1-derived IL-2, INF-gamma, and up regulation (increasing) the Th2-derived IL-10 are responsible for the observed anti-psoriatic activity of our plant extracts. {IL-10 is involved in terminating inflammatory actions, while IL-2 and INF-gamma are involved in activating immune response}

References:

1. Krueger JG, (2002) The immunologic basis for the treatment of psoriasis with new biologic agents. J Am Acad Dermatol. 46:1-23.

2. c. Nitric Oxide Determination (NO) – Test for inflammation detection in co-cultures of cells:

In vitro cell culture:

Cells: Human hepatoplastoma cell line HepG2 that retains differentiated parenchymal functions of normal hepatocytes and can be grown indefinitely, thus permitting long-term studies to be performed. The cells from HepG2 cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) with a high glucose content (4.5 g/l) supplemented with 10% vol/vol inactivated foetal calf serum, 1% nonessential amino acids, 1% glutamine, 100 U/mL penicillin, and 10µg/ml streptomycin. Human monocytes cell line THP1 and mouse macrophages cell line J774 were maintained in the same DMEM as for HepG2 cells. All three cell lines were maintained in a humidified atmosphere of 95% O₂ – 5% CO₂ at 37°C. The culture medium of the cell lines was changed twice a week. At 70 – 80% confluence, cells were trypsinized and plated in microtiter dishes. 24h after cell seeding, cells were exposed to various concentrations of the plant extracts in fresh serum-free medium.

In vitro cell culture: In the next phase of our experiments, co-cultures of hepatocytes and macrophages were created using three-dimensional foam structures (DegraPol-foam) as cell carriers (Saad et al.,2003). Both, hepatocytes and macrophages were found to adhere, proliferate and preserve their specific phenotype when cultured on DegraPol-foam. Cells from the hepatocyte cell line HepG2, from the macrophage cell line J774, and from the human monocyte cell line THP-1 were used. The viability of the cells was assessed by the trypan blue exclusion test and cells with more than 85% viability were used.

The *in vitro* test was performed as follows:

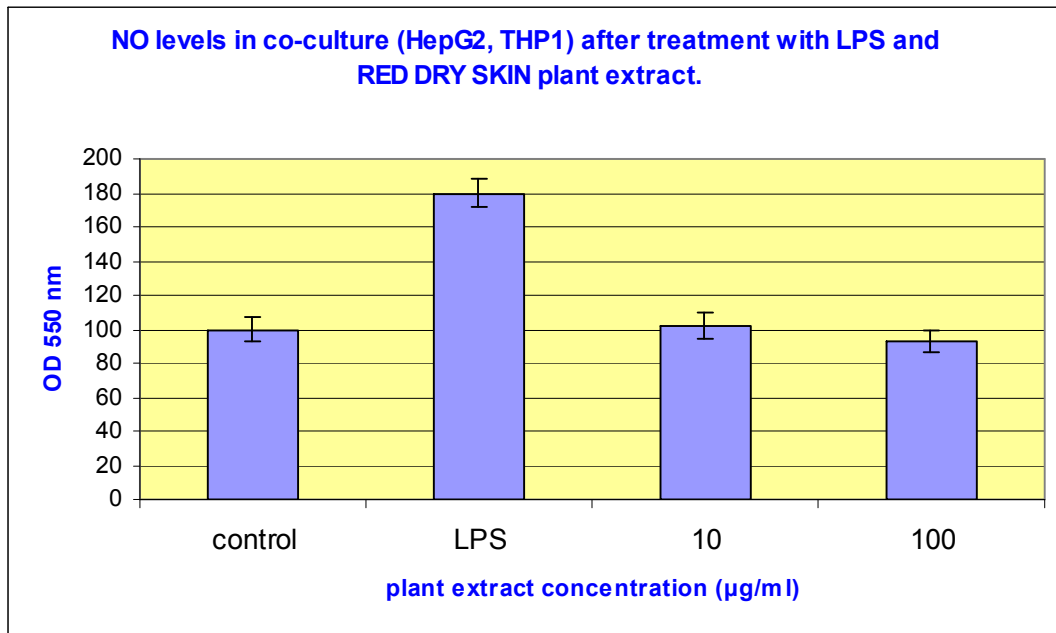
(1) Collagen type I coated DegraPol-foam discs of 14 mm diameter and 300 µm in thickness was placed in the bottom of each well of a 6-well tissue-culture plate. (2) Hepatocytes and macrophages/monocytes were seeded on DegraPol-foam at a density of 3x10⁶ cells/cm² and 5x10⁵ cells/cm² in 1 ml culture medium, respectively. (3) Co-cultures were maintained at 37°C and 5% CO₂ for 4h. (4) Five ml of fresh medium were added. (5) At 24h, the medium was exchanged with the same culture medium containing 10µg Lipopolysaccharide/ml and various concentrations of potential plant extracts diluted with fresh medium. (6) After 24h and 48h of treatment, NO measurement was carried out as follow.

Nitric Oxide Determination (NO) – Test for inflammation detection in co-cultures of cells:

Nitrite determination was done on 50 µl aliquots of sample mixed with 200 µl of the Griess reagent (Ding et al., 1988). The absorbance was read at

540nm after 10 min of reaction and NO₂⁻ concentration was determined with reference to a standard curve using concentrations from 1 to 250µM sodium nitrite in culture medium.

LPS-induced NO production by the Hepatocytes and/or monocytes from the THP-1 cell line.



The graph shows a significant reduction of NO levels after treating the pre activated co-culture (due to exposure to LPS) with 10 µg/ml of RED DRY SKIN™ extract.

References:

1. Saad B, Abu-Hijleh G, Suter UW. Polymer biocompatibility assessment by cell culture techniques In Arshady R (Ed.). *The PMB Series Volume 1: Introduction Polymeric Biomaterials* 2003; The Citus Books pp. 263–99
2. Ding, A.H.; Nathan, C.F.; and Stuehr, D.J. (1988) *J. Immunol.* 141:2407

3.0 Clinical Data on *RED DRY SKIN*TM.

Background.

The new anti-psoriasis preparation based on Arabic medicinal plants was tried in a clinical observation study with 17 patients over period of three months. The study was carried out in Northern Israel by Antaki Center in the years 2002-3.

Patients: There were 17 patients from ages 8- 62. They included all stages of severity, one group with light psoriasis, in one or two places only, another group with intermediate symptoms, at several sites, and a third group of patients with severe psoriasis covering most of the body. All patients came after a period of treatment with conventional medicine, which they felt no longer, gave them benefit. All were from the North of Israel.

Preparation: The preparation was a proprietary anti-psoriasis cream containing concentrated extract of 4 herbs, as active principles, in a conventional cream inert base.

Treatment: The patients came after diagnosis and treatment in conventional medicine. All the patients had already received treatment lasting from one year to 5 years. They were seen and assessed initially by the study team and prescribed the preparation, which they applied topically twice a day over the psoriasis lesions. There were no internal or additional treatments. They were then called for observation after 2 weeks, and then every two weeks thereafter. At each observation, their lesions were checked and their symptoms such as irritation, inflammation etc. were recorded.

Results: Five younger patients from ages 14 to 30 with light to intermediate psoriasis responded relatively quickly and reported considerable relief within the first period of 2 weeks. Within 2 weeks, they all reported reduced flakiness, redness, and irritation. Within 4 weeks, 3 of them had no more symptoms and were left only with signs of past-healed lesions. Two of the patients continued for one month more after which the lesions had virtually gone, leaving only signs of healed lesions. (Two of them attacked by psoriasis for two years, one for 4 years and two for 6 years. This entire group was treated with conventional drugs.)

Five older patients from ages 32 until 49 who generally suffered from intermediate to severe psoriasis responded more slowly. They received treatment over a three-month period. They all reported a steady reduction in the symptoms and relief, over a three-month period. At the end of the period, the lesions were very much smaller and less disturbing. However over the period new lesions gradually appeared which were also treated and also gradually reduced in size and severity. Continuous treatment after the 3-month period stopped further spreading and the occurrence of new lesions. (Three of them had psoriasis for 6-7 years; two of them had psoriasis for 10 years. All were treated with conventional drugs.)

During the entire trial period of 6 months, 4 of the patients showed recurrences after the 3-month treatment period. They received further treatment, which stopped the spreading of the new lesions. 6 patients have had no further occurrences during the 6-month follow up period.

Seven patients included in this trial were treated with a cream prepared as described in the examples above. The age of the seven patients ranged from 8-62 years, and all had the diagnosis of psoriasis established by a physician. All patients applied the crème twice daily, and all patients developed a favourable clinical response within the first week after initiation of treatment. Among the young patients (8, 13, 15 years old) (This group was treated by conventional drug, and all of them had light to intermediate psoriasis for 2-3 years) treatment was continued for a month, and none of these patients developed any recurrence for a year following this treatment.

In 2 patients (35, 39 years old) (One of them had intermediate psoriasis for 9 years and the second had severe psoriasis for 12 years, both were treated by conventional drugs) treatment was continued for two months, and another 2 patients (42, 62 years old) (Both had severe psoriasis; one for 12 years and the second for 15 years and they used all kinds of conventional drugs) were treated for three months. After these periods of treatment the psoriasis lesions had completely disappeared. However within one year 3 of the patients developed new lesions, which in all three patients were successfully treated by repeated application of the crème. No adverse events were demonstrated in any of the patients.

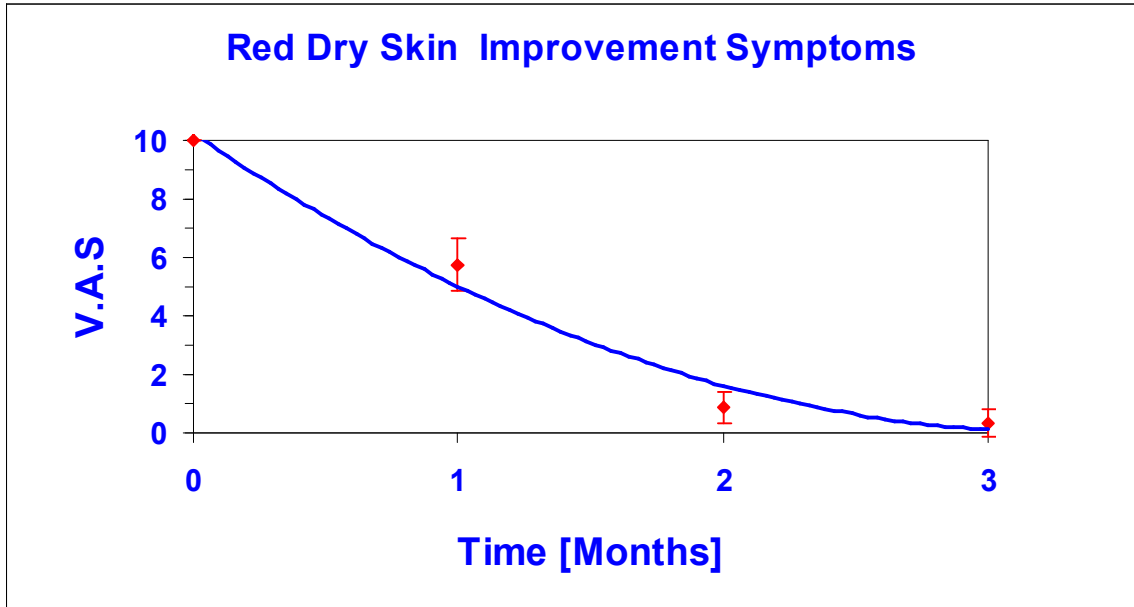
Conclusion

The results showed significant and in some cases dramatic improvement of psoriasis lesions over a relatively short period, depending on severity. Younger patients with less severe disease responded more quickly and were less likely to report recurrences. Older patients with more severe symptoms responded very well, with a major reduction in the lesions, but it took longer and there was more likelihood of recurrences. Overall this new remedy is of considerable potential interest and merits further formal clinical study.

Data:

(Scale: 0 - *no improvement*, 10 - *complete recovery*)

Improvement after 3 months	Improvement after 2 months	Improvement after 1 month	Period of illness (years)	Severity	sex/Age	Name
10	10	7	6	Intermediate	F/30	Mk
10	10	10	6	Intermediate	M/27	RS
10	10	6	4	teIntermedia	F/19	NH
10	10	10	2	Light	M/15	SK
10	10	8	2	Light	M/14	AS
8	8	5	10	Intermediate to severe	M/49	SS
9	7	4	10	Severe	f/41	MH
10	9	5	6	Intermediate	F/39	RN
10	8	4	7	Severe	m/32	KF
8	7	5	7	Intermediate to severe	M/34	OK
10	9	6	12	Intermediate	M/62	RJ
	10	7	9	Intermediate	F/39	AR
9	7	6	7	Severe	M/42	MM
10	10	8	6	Intermediate	F/35	LD
10	10	8	3	Light	M/15	TM
10	10	7	3	Intermediate	M/13	ZE
10	10	10	2	Light	F/8	VS



Improvement of symptoms by means of Visual Analogue Scale according to patient perception
“10” heavy symptoms, “0” no symptoms

Before



After



4.0 Opinion and Survey, monographs (*Eruca*, *Nigella*, *Hypericum*).

By Dr. Stephen Fulder, Phd.

Users direction:

Apply on psoriasis twice a day.

A unique cream for treating psoriasis based upon a scientific formula enriched with anti-inflammatory, anti-septic and cytostatic compounds.

INGREDIENTS	%
<i>Aqua</i>	To 100%
<i>Isopropyl myristate</i>	7
<i>Cetyl alcohol</i>	5.4
<i>Lanolin alcohol</i>	4.8
<i>Cera alba</i>	4.5
<i>Cetyl palmitate</i>	3.3
<i>Hypericum perforatum</i>	3
<i>Eruca sativa</i>	3
<i>Nigella sativa</i>	3
<i>Glyceryl stearate</i>	2.9
<i>Sodium lauryl sulfate</i>	0.5
<i>Citrus limonum</i>	1.5
<i>Phenoxyethanol</i>	0.7
<i>Imidazolidinyl urea</i>	0.2
<i>BHT</i>	0.2

The four herbs used in the cream are:

***Eruca sativa* seeds** (Common English names: Rocket, Rocket salad) Latin. Synonyms: *Brassica sativa*, *Hesperis matronalis*, *Eruca vesicalis*)

***Nigella sativa* seeds** (Common English names: Black cumin)

***Citrus limonum* or *Citrus medica* dried fruit** (Common English names: lemon, lime, limene, ethrog etc. Latin synonyms: *Citrus limon*, *Citrus limonus*, *Citrus aurantifolia*, *Citrus lumia*, *Citrus acida*)

***Hypericum triquetrifolium* and/or *hypericum perforatum* leaves** (no common name)

Scientific Data:

***Eruca sativa*.**

Eruca sativa is known generally as a food, in which the leaves are eaten as part of salads. It has been known as a garden vegetable since Bible Times, and there are many records of its household usage from the Hellenistic period onwards(II). Reference 2 is the major review of this plant. It has collected the major uses throughout history and classifies them. These uses are: for eye infections (antibacterial action), increasing fertility and sperm production, as deodorant when eaten internally or when sprinkling ground seeds under the arms and as an aid to digestion and kidney function.

Maimonedes and Ibn Wahsiyya are quoted as stating that the ground seeds when mixed in a cream and spread on the face can be used for acne. There is a classic review of the uses of this plant which illustrates its wide application in traditional medicine(II).

There are several reports in the literature that *Eruca sativa* has a weak antimicrobial effects. It has been tested against a range of bacteria, yeasts, crustaceans and insect larvae and has been found to be active at high concentrations(IV, V). There has been an attempt to check this plant for cytotoxic and potential antitumor effects. It had no antitumor effects in mice, but the chloroform extract did have some cytotoxic effects in cell culture at doses of 12 micrograms/ml, while the ethanolic extract was inactive at much higher doses. The implications of this are unclear(VI). Antimitotic effects, that is, cytostatic effects against cell division, have also been studied, and the results were negative(VII).

Eruca sativa Monograph

The final product undergoes the following tests

Assay – Linoleic acid , additional internal peaks

Foreign matter EP (2.8.2)

Microbiology EP 5.14 4-B - Maximum 10^4

Relative density EP (2.2.5)

Ethanol EP (2.9.10)

Methanol and Propanol EP (2.8.16)

Analytical Procedures

The plant was analysed by HPLC .

Reagents: Acetonitrile : Buffer Phosphate 90:10

Buffer phosphate: - Phosphoric acid Analytical grade: Water distilled 2: 1000.

All reagents used – HPLC grade.

Sample Preparation: - 50 grams milled plant was extracted with 200 ml ethyl alcohol 50% for 1.5 hours at a temperature of 70 °C. Then filtered through a fine filter. The residue was again extracted using another 200 ml Ethyl alcohol 50% and filtered.

The filtrates were mixed together for HPLC analysis.

Standard Preparation: Linoleic acid 3mg/ml – Fluka 62230

Chromatographic Conditions

HPLC type: HP 1090 Diode array

Column: Phenomenex Luna C18 ; 250 X 4.6 mm

Column Temperature: 40 °C

Mobile Phase: isocratic

Acetonitrile: Buffer Phosphate 9:1

Flow Rate: 1.0 ml/min

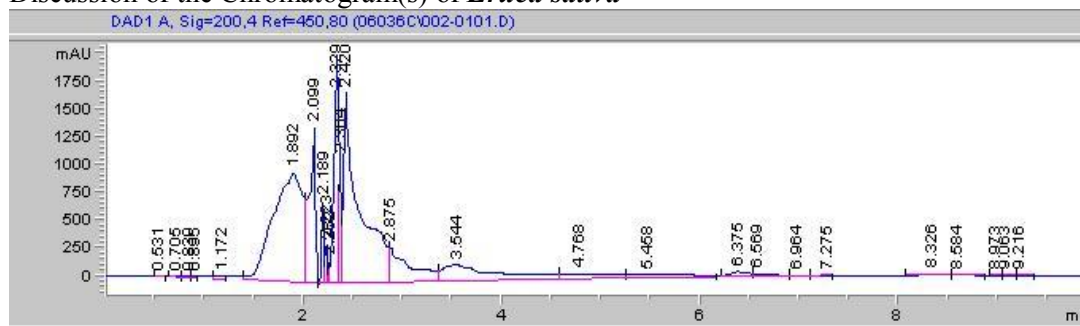
Detection: 210 nm and Diode array

Injection Volume: 20µl.

Run Time: 10 minutes

Peaks Identification: Retention time and UV/ Vis spectrum

Discussion of the Chromatogram(s) of *Eruca sativa*

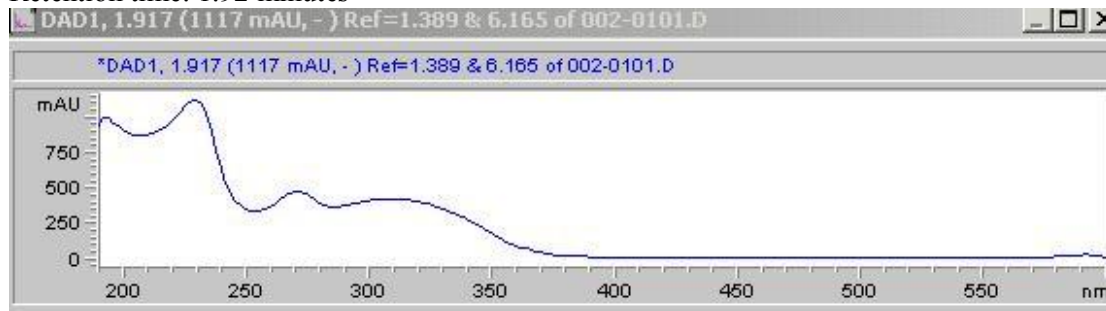


The peaks to be used as a finger prints are:

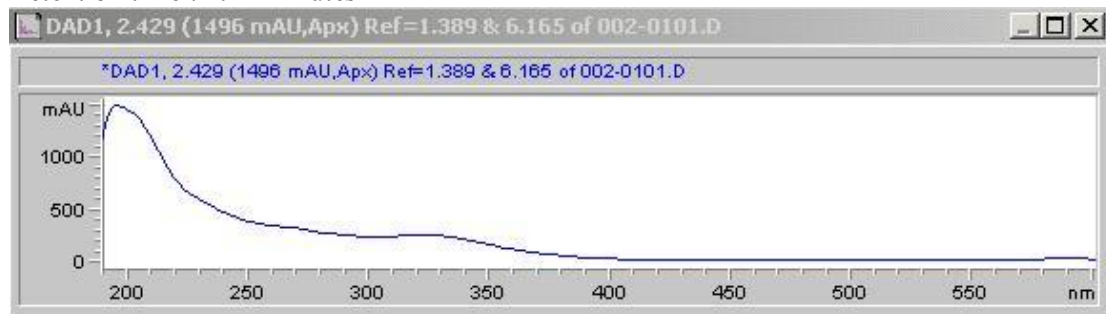
Retention time
1.92
2.42
8.39

Spectrophotometric Assay.
UV Vis spectrums of main peaks

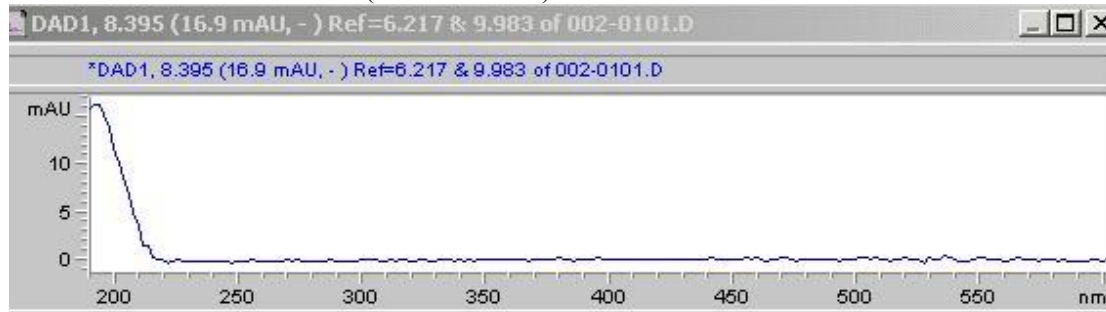
Retention time: 1.92 minutes



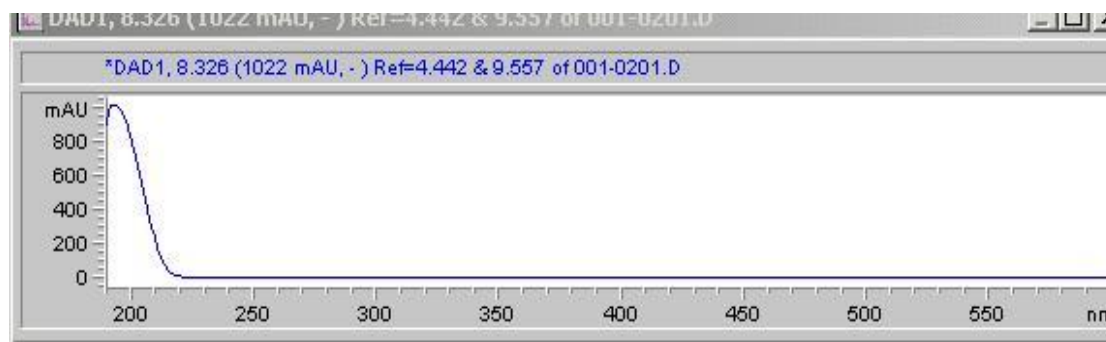
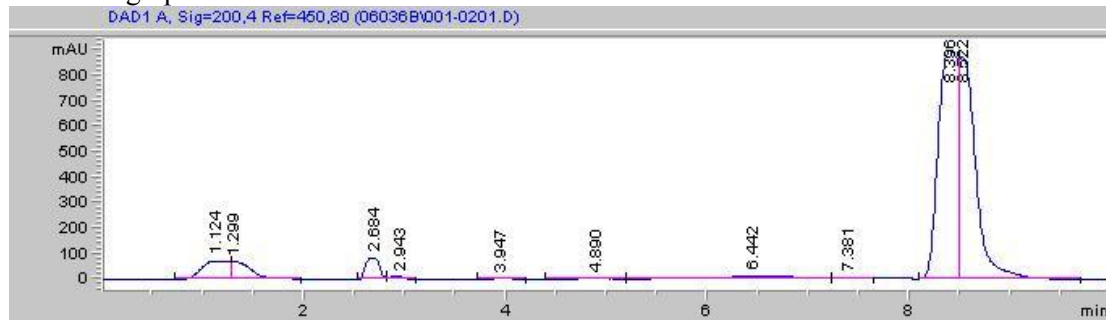
Retention time : 2.42 minutes



Retention time : 8.39 minutes (**Linoleic acid**)



Chromatograph of standard **Linoleic acid** retention 8.39



Citrus Medica/Limonum/Acida.

This is a variety of dried lemon or lime, derived from Iran, and available throughout the Arabic countries. It can be regarded as similar to the lemon in biological and therapeutic effects. However the preparation used here of the dried fruit is unusual. The traditional and scientific sources always refer to lemon juice, oil or rind, of fresh lemon. Herbal sources list a very large number of uses of lemon, almost all of them internal. Externally, it has cosmetic uses, including assistance in removing spots and cellulitis. It is regarded as an astringent that can be used in sunburn, itching (see ref. 5). There is evidence that it contains antioxidants that could explain some of the relief in such case(VIII). In addition, lemon and all the citrus family contain citrus flavonoids in the peels, which can have anti-inflammatory, cancer-preventive and antihistamine effects.

These include, for example, a stimulation by auraptene, a flavonoid of one of the orange species, of a number of immunological factors including macrophage and lymphocyte functions(IX). Such effects could in a general way reduce the inflammatory symptoms of any inflammatory skin condition, including eczema or psoriasis(X). There is a study on the successful treatment of psoriasis by juice of the related citrus, grapefruits(XI). There is plenty of evidence that citrus species contain flavonoids which can prevent cells from too much proliferation(XII). Citrus species in general all seem to have components that can have cytostatic or antiproliferative effects on cells in culture(XIII). Cytotoxic effects on *Citrus medica* have been tested, and it was clear that it has no cytotoxic effects(XIV). However the antiproliferative effects can be useful in the treatment of psoriasis which is a disease of skin thickening.

Nigella sativa.

Nigella, or black cumin, is well-known as a spice and folk medicine throughout the Middle East and Asia. It bears no botanical or other relationship to cumin seed. Traditional herb books describe the plant as helping against inflammation, causing sweating and helping the menstruation process. The seeds are used to aid digestion, and to support the liver. In Indian medicine there is a mention of

Nigella sativa in skin treatments such as eczema and pityriasis(XV). It is known to have a distinct anti-inflammatory effect, which would tend to make it symptomatic for all kinds of internal and external inflammations, such as eczema and psoriasis. The effect is not general, on all aspects of the immune response, but tends to be on macrophage rather than lymphocyte stimulation(XVI). The anti-inflammatory active ingredients are known, and are regarded as thymoquinone(XVII), as well as derivatives and relatives of this compound(XVIII). These compounds are found in the oily fraction.

It may account for the fact that the oil has long been known in folk medicine to provide symptomatic relief for skin inflammations, including psoriasis, which is why it can be bought today as a soap and in various folk preparations of the seeds. The oil is similar to other omega-3 oils in its anti-inflammatory effect. An interesting patent has been filed which does indicate that psoriasis and inflammatory skin conditions can be treated by ingesting large quantities of oil internally in a manner similar to for example evening primrose oil(XIX). Another area that has been researched is in cancer prevention.

The methanol extract has been found to be cytotoxic to a number of types of tumor cells in tissue culture(XX). There are several studies showing that *Nigella* has an anticarcinogenic effect when given alongside, before or after chemical carcinogens(XXI,XXII). There is evidence too that *Nigella* has an attenuating effect on the toxic adverse effects of anticancer drugs, and therefore has been suggested as an adjuvant during cancer treatment(XXIII). These studies support its use in psoriasis, which is characterized by excessive cell growth of the skin epithelia. Besides this, *Nigella* has antibacterial, antihepatotoxic and hypoglycemic effects. An important new review of this plant has confirmed potent anti-inflammatory, anti-oxidant, hepatoprotective, antimicrobial and some immune supporting effects, a range similar to that found with sources of omega-3 oils(XXIV).

Nigella sativa monograph

The final product undergoes the following tests
Assay – Linoleic acid , additional internal peaks
Foreign matter EP (2.8.2)
Microbiology EP 5.14 4-B - Maximum 10^4
Relative density EP (2.2.5)
Ethanol EP (2.9.10)
Methanol and Propanol EP (2.8.16)

Analytical Procedures

The extract was analyzed by HPLC and Spectroscopy for some of the peaks

Reagents: Acetonitrile: Buffer Phosphate 9:1

Buffer phosphate: - Phosphoric acid Analytical grade: Water distilled 2: 1000.

All reagents used – HPLC grade.

Sample Preparation: - 100 grams milled plant was extracted with 300 ml Ethyl alcohol 50% for 1.5 hours at a temperature of 70 °C. Then filtered through a fine filter. The residue was again extracted using another 300 ml Ethyl alcohol 50% and filtered.

The filtrates were mixed together for HPLC analysis.

Standard Preparation: Linoleic acid from Fluka 62230 - 3mg/ml

Chromatographic Conditions

HPLC type: HP 1090 Diode array

Column: Phenomenex Luna C18 ; 250 X 4.6 mm

Column Temperature: 40 °C

Mobile Phase: isocratic

Acetonitrile: Buffer Phosphate 9:1

Flow Rate: 1.0 ml/min

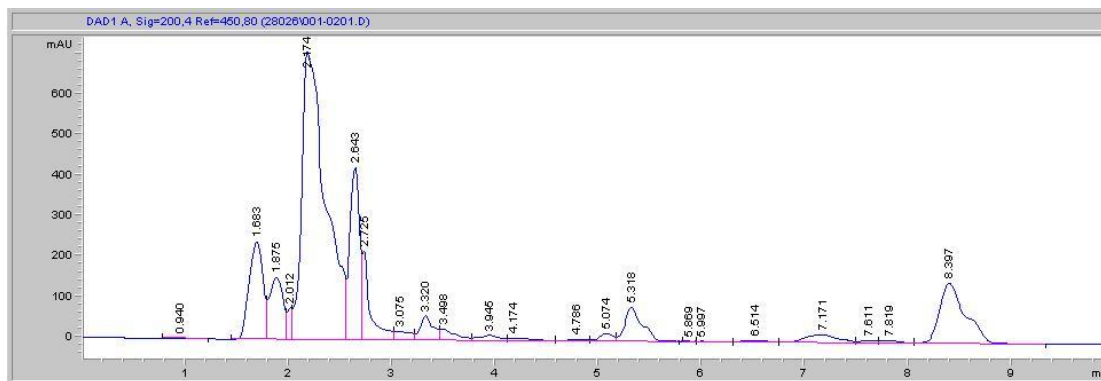
Detection: 210 nm and Diode array

Injection Volume: 5µl.

Run Time: 10 minutes

Peaks Identification: Retention time and UV/ Vis spectrum

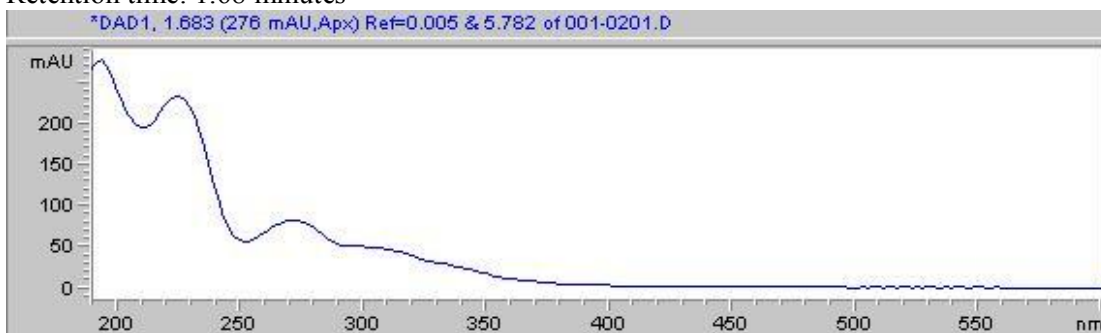
Discussion of the Chromatogram(s)



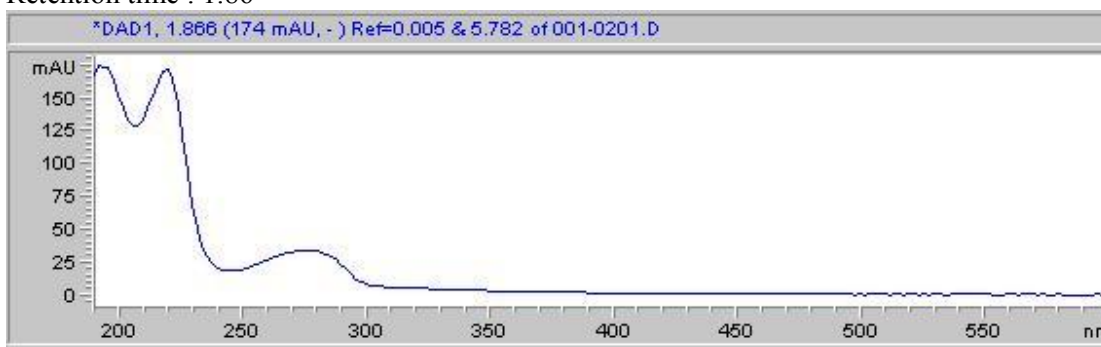
The peaks to be used as a finger prints are:

Retention time
1.68
2.17
3.94
8.38

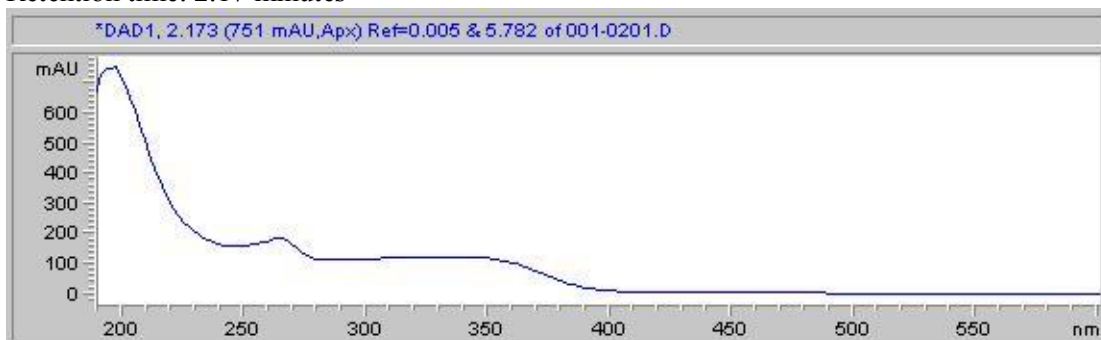
Spectrophotometric Assay.
UV Vis spectrums of main peaks
Retention time: 1.68 minutes



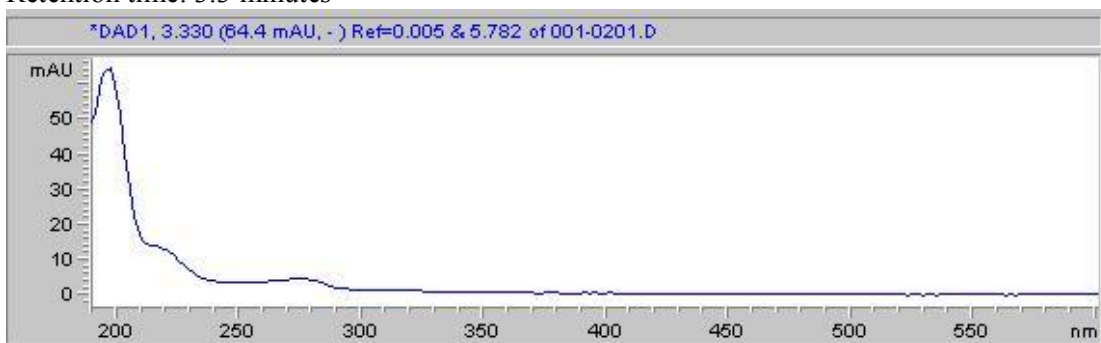
Retention time : 1.86



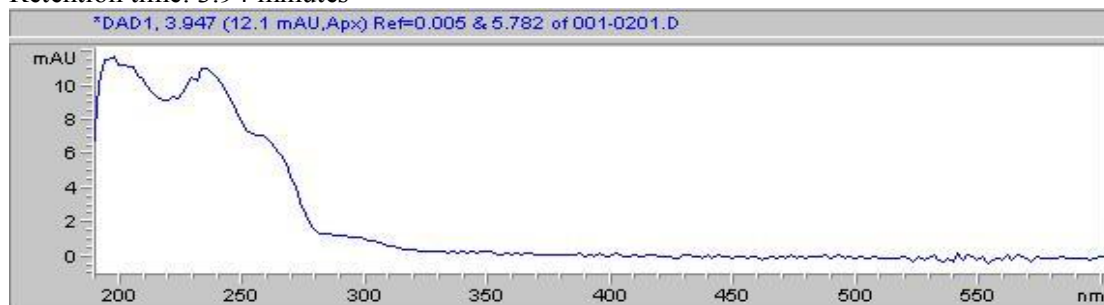
Retention time: 2.17 minutes



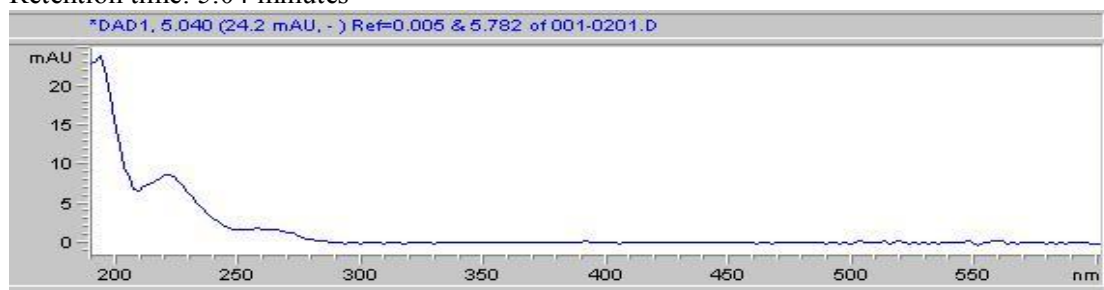
Retention time: 3.3 minutes



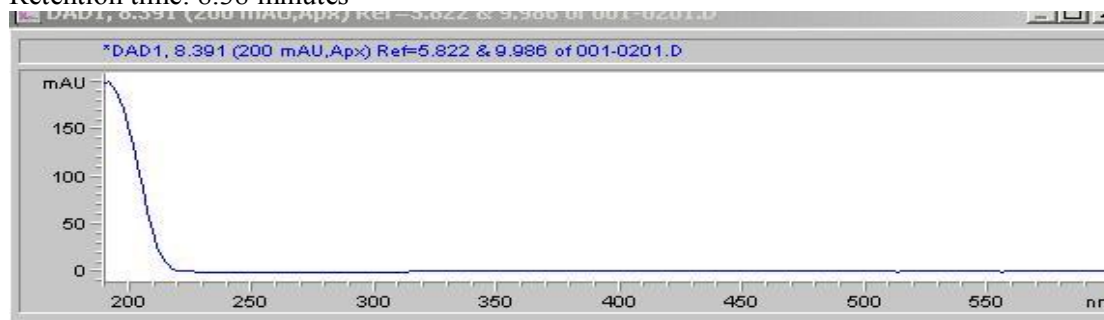
Retention time: 3.94 minutes



Retention time: 5.04 minutes

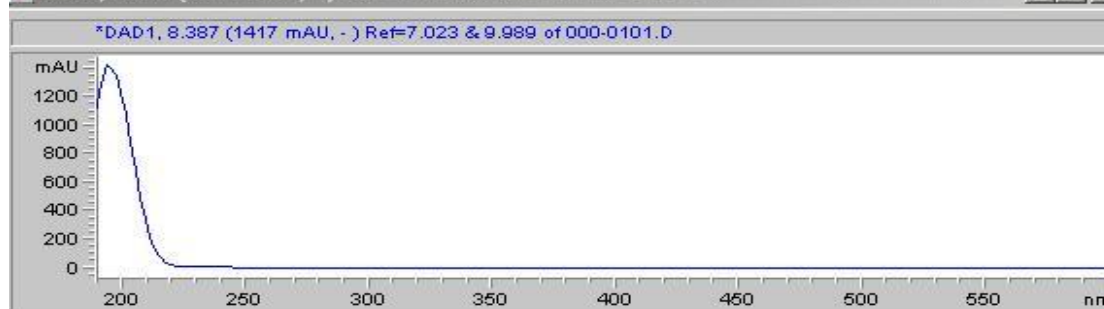
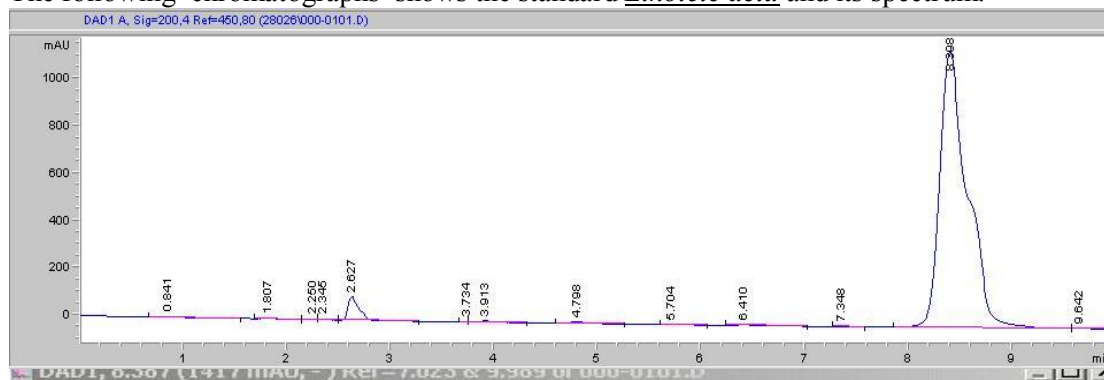


Retention time: 8.38 minutes



Further the peak at 8.38 minutes was identified as *Linoleic acid*

The following chromatographs shows the standard *Linoleic acid* and its spectrum.



Hypericum triquetrifolium.

There is very little mention of this species in the literature, either in the traditional literature such as the classical herb books, or in the scientific literature. The species is rarely investigated, most effort being devoted to *Hypericum perforatum*, St. John's Wort. There is a mention of possible cytotoxic effects in cell culture(XXV).

There is a study from Turkey on antinociceptive activity of methanol extracts of *Hypericum triquetrifolium* in animals. Some effect was observed in reducing possible pain reactions. In this paper, *Hypericum triquetrifolium* is used as an example of *Hypericum* species which might have similar effects to *H. perforatum*, which has antidepressive and also possible antinociceptive effects.

The paper mentions that both species have been "ethnomedically used in different parts of Turkey for their sedative, antihelminthic and antiseptic effects(XXVI)."

Hypericum perforatum is another distinct species of *Hypericum*, and is known to have dermatological applications. In a patent, and also in books of pharmacological agents(XXVII), *Hypericum triquetrifolium* is shown to contain hypericin and pseudohypericin, which are also the supposed active ingredients of *Hypericum perforatum*. *Hypericum perforatum* extracts and hypericin and hypericin derivatives are known to be phototoxic, and can have light-activated cytostatic effects which are similar to, but weaker than, psoralens, which are used in treatment of psoriasis (together with UV light)(XXVIII). The photosensitizing effects of hypericin are seen as possible importance in cytotoxic effects for which there is some evidence in vitro(XXIX).

There is evidence that hypericin, a constituent of both *Hypericum* species mentioned, is an anti-inflammatory agent and therefore could have a general effect in relief of some of the inflammatory symptoms of psoriasis. In one study, hypericin is mentioned as reducing cytokine expression(XXXI), which would reduce inflammatory responses. Anti-inflammatory effects are one of the reported uses of *Hypericum* in the Kommission E monographs and the American Herbal

Monograph for St. John's Wort (*Hypericum perforatum*)(XXXII). In addition, *Hypericum* species have been demonstrated to support wound healing(XXXIII), and this might be expected to aid in psoriasis. In a new study, topical applications of this species has been shown to have an anti-inflammatory effect on activated T cells in association with skin cells, indicating again, a general use for anti-inflammatory skin conditions(XXXIV), such as psoriasis. However, nowhere in the scientific or traditional literature is either species tested as a specific antipsoriatic remedy.

Hypericum monograph

The final product undergoes the following tests

Assay – Rutin, additional internal peaks
Foreign matter EP (2.8.2)
Microbiology EP 5.14 4-B - Maximum 10^4
Relative density EP (2.2.5)
Ethanol EP (2.9.10)
Methanol and Propanol EP (2.8.16)

Analytical Procedures

The plant was analyzed by HPLC, Spectroscopy and TLC
Reagents: Acetonitrile: Buffer Phosphate 15:85
Buffer phosphate: - Phosphoric acid Analytical grade: Water distilled 2: 1000.
All reagents used – HPLC grade.

Sample Preparation: - 100 grams milled plant was extracted with 1000 ml Ethyl alcohol 50% for 1.5 hours at a temperature of 70 °C. Then filtered through a fine filter. The residue was again extracted using another 800 ml Ethyl alcohol 50% and filtered.

The filtrates were mixed together for HPLC analysis.

Standard Preparation: Rutin 1mg/ml – Sigma R5143

Chromatographic Conditions

HPLC type: HP 1090 Diode array

Column: Kromasil 60 -5CN 250 X 4.6 mm

Column Temperature: 40 °C

Mobile Phase: isocratic

Acetonitrile : Buffer Phosphate 15:85

Flow Rate: 1.0 ml/min

Detection: 254nm and Diode array

Injection Volume: 10µl.

Run Time: 20 minutes

Peaks Identification: Retention time and UV/ Vis spectrum

TLC Conditions

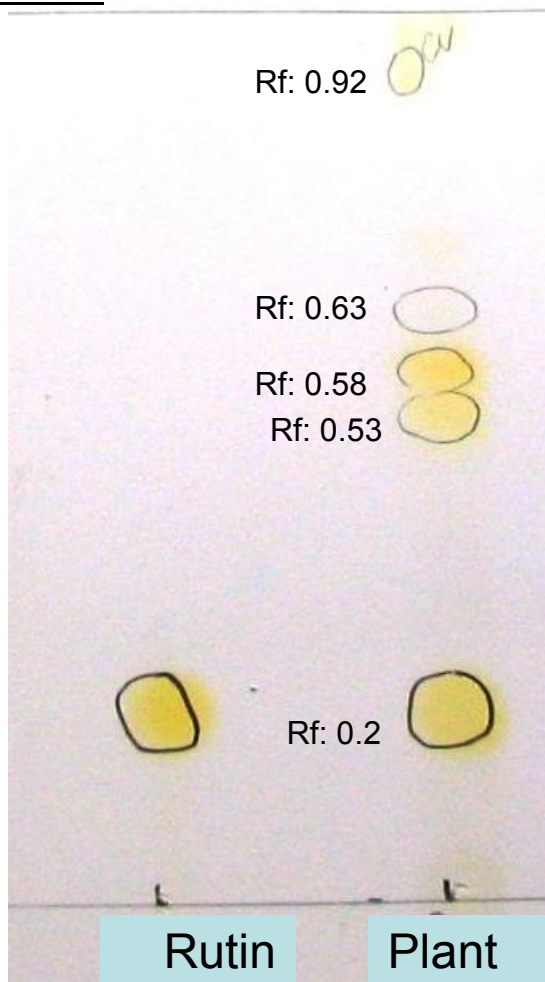
TLC Plates: - 20x20 polygram sil G/UV 254

Mobile Phase: Formic acid: Water: Ethyl acetate **6:9:90**

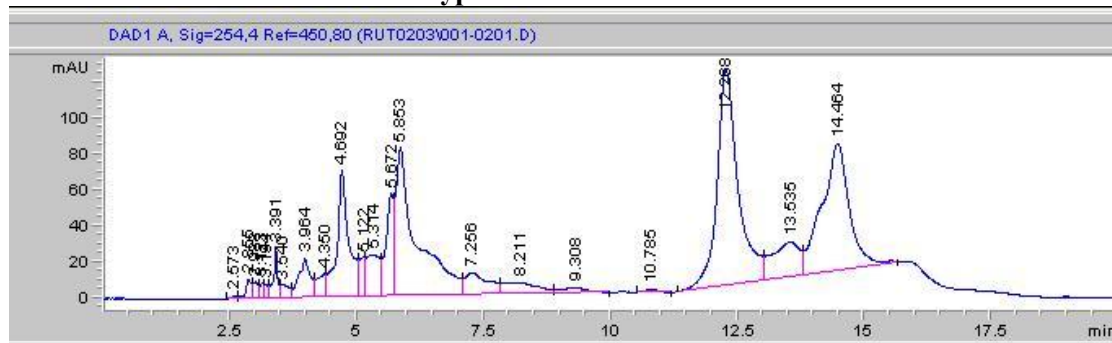
Path – 10 cm

Standard: - Rutin

Discussion of the Chromatogram(s)



Main Peaks and retention times of **Hypericum**:

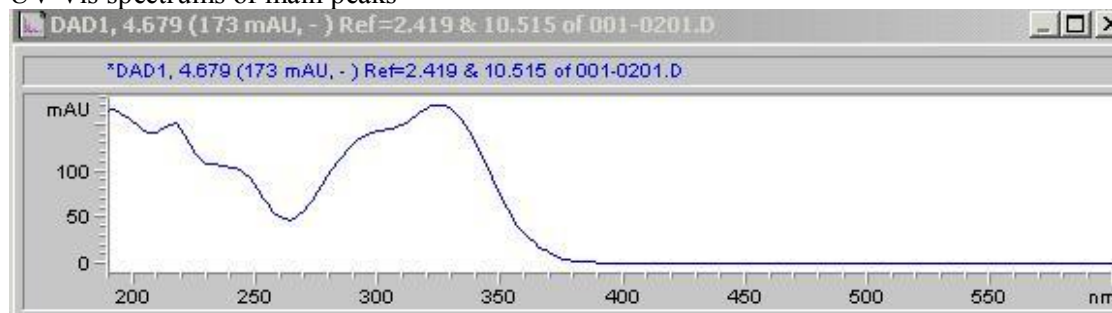


The peaks to be used as a finger prints are:

Retention time
4.68
12.0
12.2

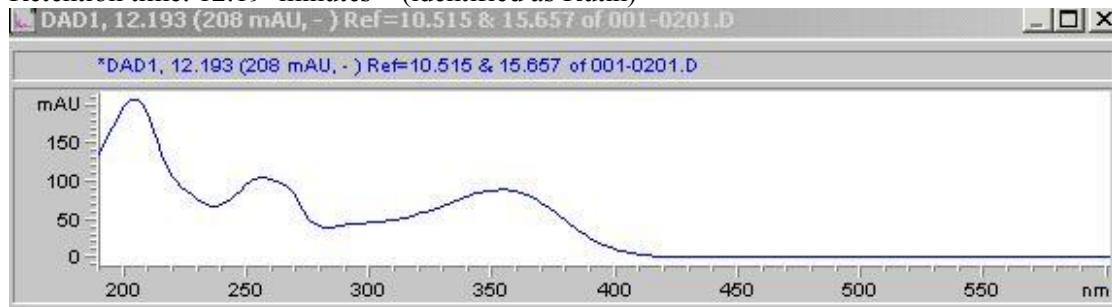
Spectrophotometric Assay.

UV Vis spectrums of main peaks

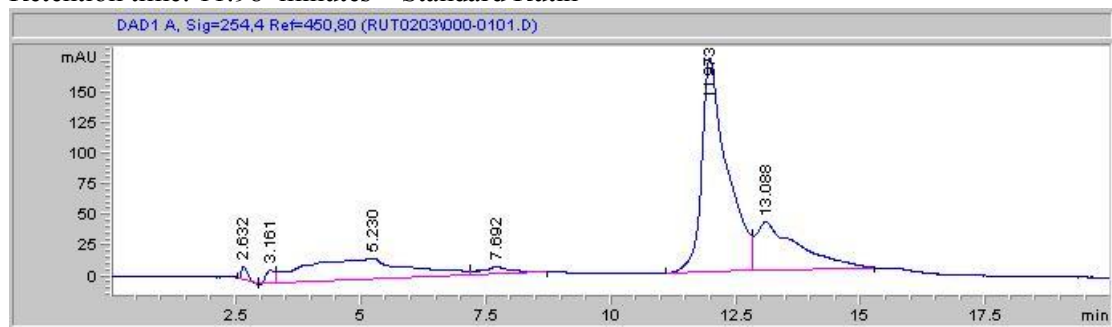


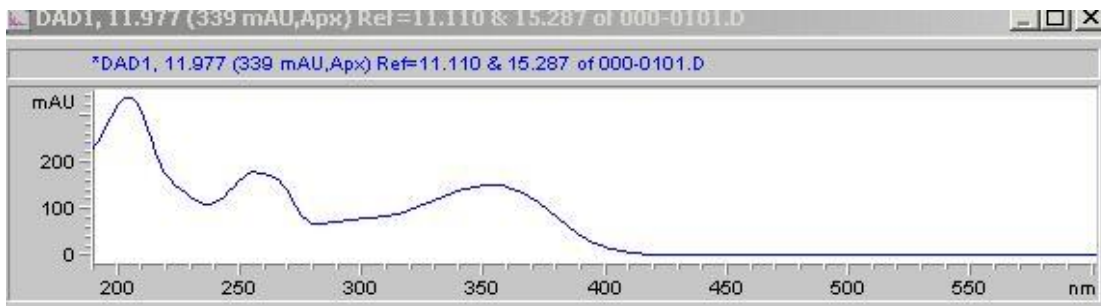
Retention time: 4.68 minutes - **Peak HA**

Retention time: 12.19 minutes – (identified as Rutin)



Retention time: 11.98 minutes – Standard Rutin





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