ANTI SPOT™

List of Contents

1.0 Short Summary of ANTI SPOT™.

About Acne

Acne is a chronic inflammatory disease unique to human sebaceous glands, following remarkable increasing of sebum secretion as a result of hormonal changes, which lead to sebum blockage and bacterial growing. The disease usually affects the face, upper back, or chest, causing lesions of different grades, from non-inflammatory comedones (plugs), to inflammatory papules, pustules and nodulo-cystic lesions. Acne vulgaris affects 70%-90% of adolescents and usually resolves spontaneously before age 25, but in some cases it may begin later and persist for many years. While it lasts acne is a great source of discomfort and psychological stress to patients, and in severe cases it can leave permanent scars (pits) in affected skin.

Our research strategy

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

a) Reducing of sebum secretion from human cultured sebaceous glands.
b) Bacteriostatic / bactericides.
c) Anti-inflammatory.

A large screening to identify the most important plants traditionally used for treating acne (around 33 plants species) was conducted by our group. Each plant was extracted using different solvents and tested in a unique biological experimental system (human Sebaceous glands), anaerobic bacteria system and nitric oxide test using co-cultures of cells from the human hepatocyte cell line (HepG2) and cells from the human monocyte cell line (THP1), to evaluate their effects on sebaceous secretion, antimicrobial and anti-inflammatory effects, respectively.

Building novel product:

Our anti acne product is based on a combination of the most effective of three plant extracts, in which *Inula helenium* extract (50% alcohol/ 50% water- See fig1) and 50% alcohol based extract of *Saponaria* (see fig 2) showed very strong inhibition of sebum secretion. *Saponaria* had showed remarkable antimicrobial effect (1.2 times more than the antibiotic reference), where ANTI SPOT™ extract derived highly anti-inflammatory effects. In the other hand, lemon peel oil is widely known as antiseptic and anti-inflammatory agent. We found that the mixture of the three extracts have a positive synergetic effect in treating acne.

ANTI SPOT™:

A combination of 3 herbs which immediately and safely stops sebum production without excessive skin drying. In addition it has skin supporting, antiseptic and anti-inflammatory properties.

Active Constituents.

A specific mixture of three herbs: *Inula helenium*, *Saponaria officinalis*, *Citrus limonum*.

Indications.

- Mild Acne vulgaris, especially in adolescence.
- Oily skin.
- Spots, pimples, blackheads.

Actions.

- Stops excessive sebum production.

- Antiseptic effects.
- Anti-inflammatory effects.

Mechanism

Current herbal products on the market only have a single effect. This effect is usually astringent and antiseptic, which does not treat the basis of the problem. This herbal combination has unique synergistic effects which dramatically halts sebum production from sebum glands, and thus treats the core of the problem. It does so without excessive skin drying, in contrast to other anti-acne products. This is combined with mildly antiseptic and anti-inflammatory activity. Indeed some of the herbal constituents add to skin moisture, softness and texture.

Research

Antaki has tested the herbs in a sophisticated *in vitro* organ culture system which accurately measures sebum production from sebum glands. The herbs quickly stop excessive sebum and oil production at very low doses in a non-toxic manner. They also possess strong antimicrobial and anti-inflammatory effects. Published scientific research also shows the herbs to have skin moisturizing and significant anti-inflammatory activity.

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

The herbs are classed as food supplements or foods and are on the General Sales List in the United Kingdom. *Citrus limonum* and *Inula* are also covered by various herbal monographs, including German Health Ministry Commission E.

Safety

The herbs are on the INCI List of approved cosmetic ingredients for sale in the EU. There is no evidence of toxicity or adverse effects.

2.0 Preclinical Data.

2.a..The effect of traditional medicinal plant extracts on sebum production in sebaceous glands organ culture - summary

Materials and methods

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 8 repeated experiments.

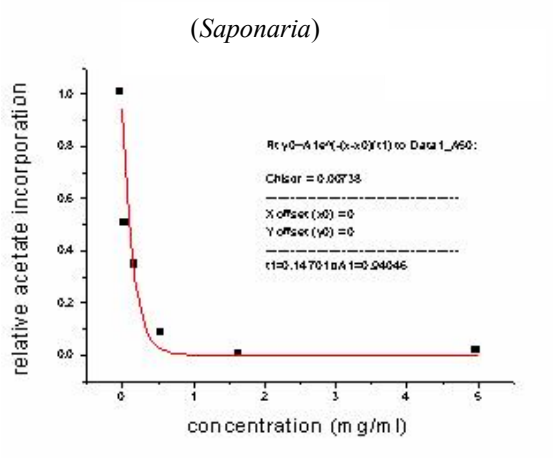
Stock solutions of dried plant extracts were prepared at 3-5 mg/ml in growth medium (F12-DMEM plus growth factors [1-3]) and sterile-filtering (0.2 µm Amicon disposable filters). Azelaic acid (AZA), 200 mM in ethanol, and saturated 13-*cis*-retinoic acid (RET) in ethanol (estimated ~ 15 mg/ml, 50 mM) were prepared as well in one experiment to serve as positive controls.
Sebaceous glands were obtained by microsurgery (if possible along with entire pilosebaceous units) from breast reduction skin. Two to ten glands were used for testing the effect of extracts at different concentrations as described for the particular experiments.

The glands were placed on sterilized PVDF membranes for attachment and subsequent handling which we found to be an improvement to an accepted procedure [2,3]. After a recovery period of ~9-12 hours [3] extracts were added to the growth medium in serial dilutions of the stock solution After incubation for another 9-12 hr with the plant extracts a dose of radioactive acetate was added to the media to give a 1-5 µCi /ml final radioactivity count and the glands and skin samples were incubated for an additional 9-12 hr. At the end of this incubation period the glands were thoroughly washed with 0.2 mM non-radioactive acetate in PBS and lipids were extracted by the Bligh and Dyer lipid extraction procedure [4]. The extractions were repeated once to ensure maximal yield. The fractions of the two lipid extracts were combined and an aliquot of each was counted in a scintillation counter to obtain the amount of radioactive acetate incorporated into total lipids.

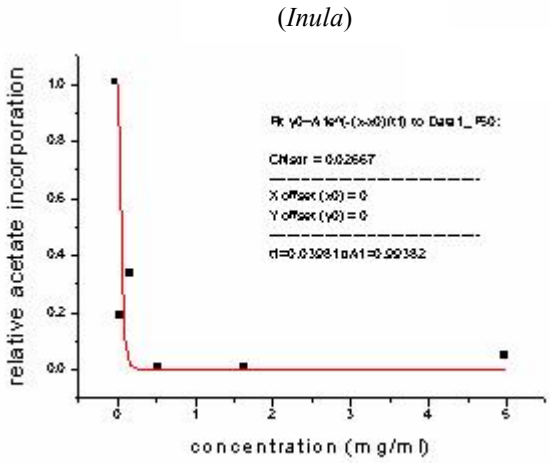
Retinoic acid was tested, as positive control, using an initial concentration of 0.075 mg/ml ($2.5 * 10^{-4}$ M) and four 1:3 serial dilutions thereafter.

Results:

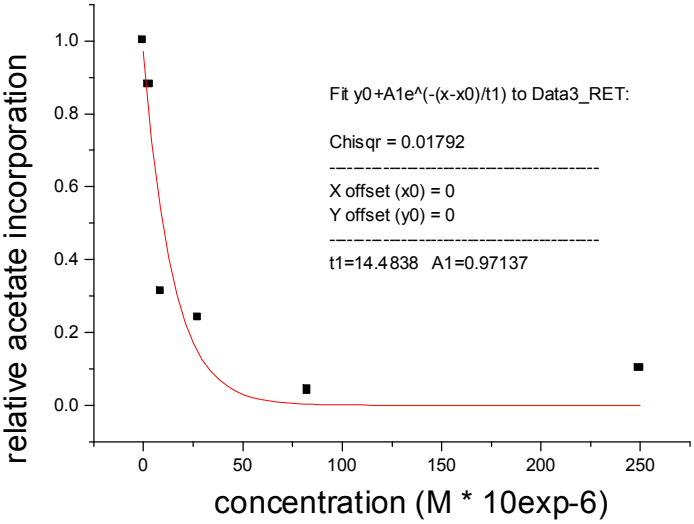
(*Saponaria*)



(*Inula*)



The results above show remarkable inhibitory effect of Inula and Saponaria extracts on sebaceous glands secretion (the glands were obtained by microsurgery from breast reduction skin).



The above results show inhibitory effect of retinoic acid on Sebaceous glands secretion.(the glands were obtained by microsurgery from breast reduction skin).

Results:

Retinoic acid, is mainly used to treat Acne, was used as a positive control. The results showed a significant inhibition of sebaceous glands secretion at pharmacological concentration and reach almost a complete inhibition. At the same time the two plant extracts (*Saponaria* and *Inula*) achieved the same effect but at higher concentration. The advantage of using plant extract is well known compared with Retinoic acid which has serious adverse effects.

References

1. Rheinwald JG & Green H, 1975, Serial cultivation of strains of human epidermal kerationcytes: the formation of keratinizing colonies from single cells, Cell, 6; 331-334.
2. Downie MMT & Kealey T, 1998, Lipogenesis in the human sebaceous gland: Glycogen and glycerophosphate are substrates for the synthesis of sebum lipids, J Invest Derm, 111; 199-205. (c.f. using polycarbonate filters)
3. Kealey T, 1990, Effects of retinoids on human sebaceous glands isolated by shearing, Methods Enzymol, 90; 338-345.
4. Bligh EG , Dyer WJ, 1959, A rapid method of total lipid extraction and purification, Canad. J of Biochem and physiol, 37(8); 911-917.

2. b. Antimicrobial activity screening methods:

These tests were carried out at microbiological labs at An-najah university-Nablus – Palestinian authority by Prof. Mohammed Shtaya. Each result represents an average of 3 repeated experiments.
Test Micro-organisms: include bacterial and candidal strains and were obtained from The American Type Culture Collection, ATCC (Table 1.).

Table 1. Test micro-organisms

Reference Strains	**ATCC**	**No.**
Propionibacterium acnes *	ATCC	6919
	ATCC	6921
Escherichia coli	ATCC	25922
Proteus vulgaris	ATCC	13315
Pseudomonas aeruginosa	ATCC	27853
Staphylococcus aureus	ATCC	25923

*Tests carried out under anaerobic conditions in Gaspak jars.

Disk Diffusion Method
Application of Extracts on Sterile Disks: disks of 6 mm diameter were prepared from Whatman filter paper no.1, placed in glass Petri dishes and autoclaved for 15 min. Twenty-five microliters of the required extract were added to each sterile disk, and disks were dried under a laminar flow sterile bench. The final content of each disk was 5 mg of extract.

Preparation of inocula:
Part of an isolated bacterial colony was transferred into a 5-ml Muller-Hinton broth (MHB) tube for aerobic bacteria, or into 25 ml reinforced clostridium medium (RCM) for *Propionibacterium acnes,* and the tube was incubated for 4-18 hours (for aerobic bacteria), or incubated anaerobically in Gaspak jars for 48 hours, at 37 ^{o}C. The growth turbidity in the broth was adjusted by further incubation or dilution with sterile physiological saline, after comparison with that of a MacFarland nephelometer tube no. 0.5 (10^8 cfu/ml) using a spectrophotometer at 625 nm (optical density 0.08-0.1). An inoculum of 10^6 cfu/ml of bacterial suspension was prepared by diluting 0.1 ml of the prepared bacterial broth culture with 9.9 ml sterile saline.

Susceptibility Test
Using a sterile cotton applicator, 10^8 cfu / ml of bacterial suspension was swabbed on the surface of Muller-Hinton agar (MHA) plates for aerobic bacteria, or blood agar (with sheep blood 5-7%) (BASB) medium plates. The selected extract discs (mg/disc) were then distributed evenly on the surface of the seeded agar plate. The specific reference antibiotics discs (10 mg/disc) were placed onto the agar plate beside the extract discs (Table 2). Three replicate plates were used for each test. The MHA plates were incubated upside down at 37ºC for 18 hours. The BASB plates were incubated upside down anaerobically in Gaspak jars at 37ºC for 48 hours. The inhibition zone around each disc was then measured using transparent ruler (Table 3).

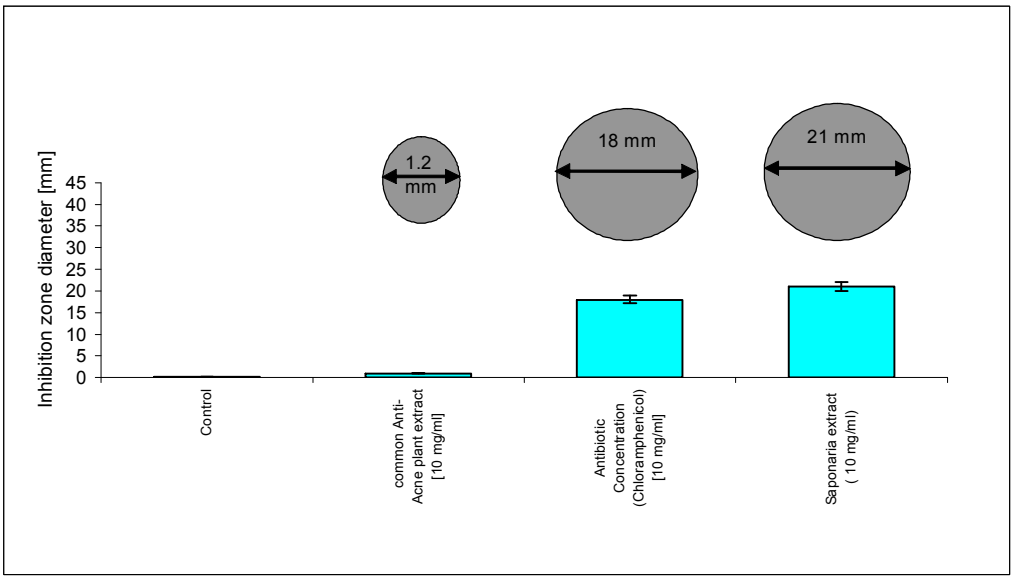
Table 2. Reference antibiotics used in susceptibility tests.

Susceptible Strains	Reference Antibiotic	Concentration
Anaerobic bacteria		

Propionbacterium acnes *	Chloramphenicol	10 mg/disc
Gram negative bacteria		
Escherichia coli	Ampicillin	10 mg/disc
Proteus vulgaris	Gentamicin	10 mg/disc
Pseudomonas aeroginosa	Gentamicin	10 mg/disc
Gram positive bacteria		
Staphylococcus aureus	Penicillin G	10 mg/disc

*Tests were carried under anaerobic conditions.

Antimicrobial susceptibility testing of *Saponaria* extract against *Propionibacterium acnes*, compared with Chloramphenicol and common Anti-Acne plants:



Saponaria extract showed in vitro antibacterial activity against P. acnes 1.2 times higher than that of the reference antibiotic.

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% O_2 – 5% CO_2 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.

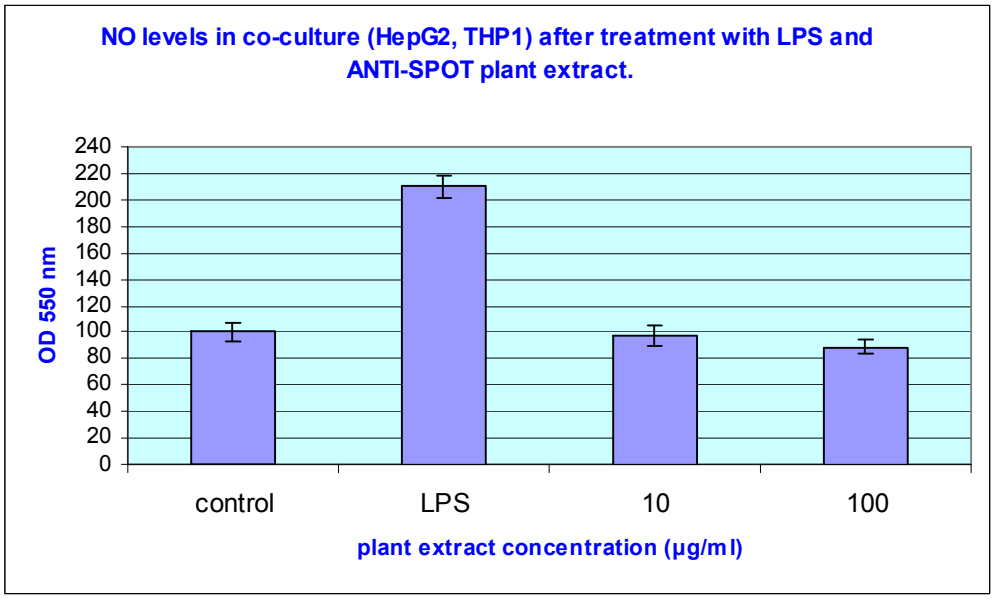
In vitro cell culture: In the next phase of our experiments, co-cultures of hepatocytes and macrophages were created using three-dimensional foam structures (DegraPol-foam) as cell carriers (Saad et al., 2003). Both, hepatocytes and macrophages were found to adhere, proliferate and preserve their specific 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 $3x10^6$ cells/cm2 and $5x10^5$ cells/cm2 in 1 ml culture medium, respectively. (3) Co-cultures were maintained at 37°C and 5% CO_2 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 NO_2^- 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 ANTI SPOT™ 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.

This study was carried out by Antaki Center on 16 patients (Ages 14-28) in the northern region of Israel in the years 2002-2003. The test period for patients longed from two-four weeks.

Results: 7 patients reported that the Acne almost disappeared after a week of using the cream, and remained with signs that disappeared after the third week.

Four patients reported that the Acne disappeared after two weeks and the signs remained after the forth week.

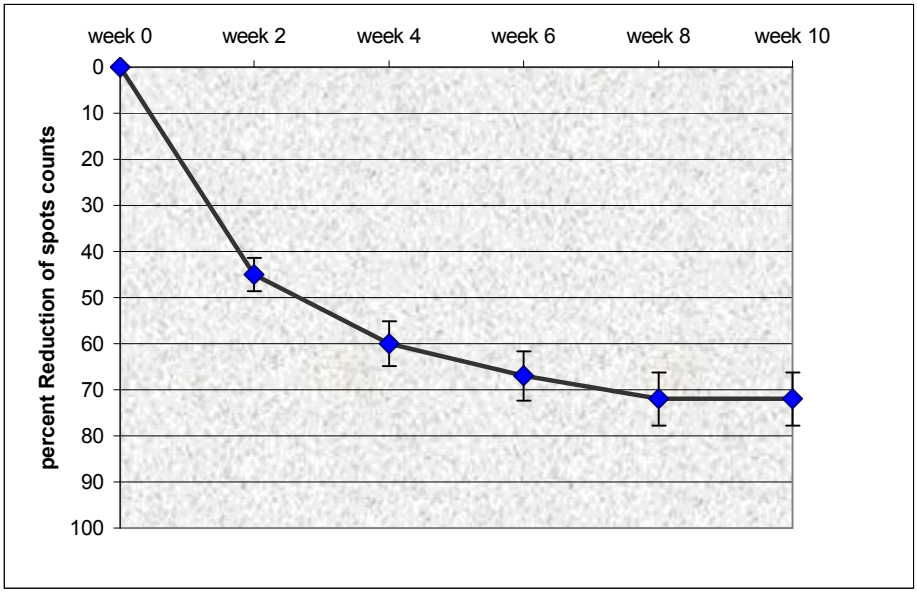
Three patients felt significant improvements, however reported that the size of Acne minimized but did not disappeared totally.

Two patients reported that the cream improved little bit their situation but the continued to suffer from the Acne as they did before use.

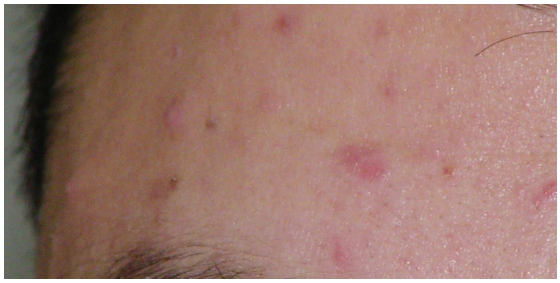
(Scale: 0 - *no improvement*, 10 - *complete recovery*)

Patient No	Age (years)/sex (f/m)	Severity:	Improvement during 1st week	Improvement during 2nd week	Improvement 3rd week	Period of illness; years
1	17/m	Intermediate	7	9	10	1
2	27/f	Light	8	9	10	3
3	25/f	Light	8	10	10	4
4	18/m	Intermediate	7	10	10	4
5	14/m	Light	8	10	10	11months
6	19/f	Intermediate	6	8	10	3
7	20/f	Intermediate	7	10	10	5
8	26/m	Severe	4	7	10	6
9	25/f	Severe	2	4	4	4
10	27/f	Intermediate	7	9	10	5
11	16/m	Severe	6	8	10	2
12	21/f	Light	10	10	10	4
13	24/f	Severe	4	4	4	8
14	19/f	Severe	4	6	8	5
15	23/f	Intermediate	6	8	10	5
16	15/m	Severe	6	7	8	1

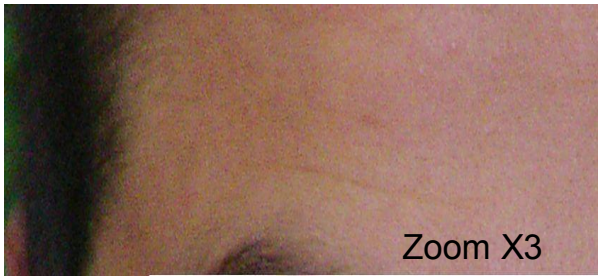
Clinical trials summarizing the effect of ANTI SPOT™ on inflammatory spots.



The graph describes the results of clinical trials on patients with inflammatory spots using percent reduction of spots counts.



Before treatment



After the treatment

The above pictures showing the efficacy of 8 weeks treatment with ANTI SPOT™ cream twice/day.

4.0 Opinion and Survey; monographs

By Dr. Stephen Fulder, Phd.

User's direction:

Apply on acne twice a day.

A unique cream based on a scientific formula, cosmetic compositions comprising herb extracts for reducing sebum production and treating acne which contains anti-inflammatory and anti bacterial (anaerobic) active fractions from three plants.

The three plants are *Citrus limonum*, *Inula helenium*, *Saponaria officinalis*.

INGREDIENTS	**%**
Aqua	To 100
Cetyl alcohol	6.5
Triticum vulgare = wheat Germ oil	4
Isopropyl myristate	4
Propylene glycol	3
Saponaria officinalis	3
Inula helenium	3
Glyceryl stearate	2.9
Stearic acid	2.4
Citrus limonum	1
Triethanolamine	0.75
Phenoxyethanol	0.7
DMDM hydantoin	0.5
BHT = Butylated Hydroxy Toluene	0.1

Scientific data:

Citrus medica/limonum/acida.

Identity:

Latin name: *Citrus medica, Citrus limonum*
Common names: Citrus, Lemon, Limene
Arabic names: Laymoon Hamid

Characteristics

- *Family:* Rutaceae
- *Seasonality:* Fruits in the autumn
- *Life Form:* Perennial tree

o This is a variety of dried lemon or lime, derived from Iran, and available throughout the Arabic countries.

o It can be regarded as similar to the lemon in biological and therapeutic effects. However the preparation used here of the dried fruit is equivalent to dried lemon peel, which is rather different to the traditional use of lemon juice, oil or rind of fresh lemon.

o Indigenous to India, now cultivated in the Mediterranean area and worldwide subtropical regions.[1]

o *Varieties and Ecotypes:* There are two varieties, one is black after drying and comes from Persia, another is white after drying and comes from India. The differences and varieties and exact names of the varieties are not yet known.

o Flowers are arranged singly or in short, sparsely-flowered racemes, hermaphrodite or functionally male. The petals are purplish-suffused on the outer surface. There are 25 to 40 stamens in coherent groups.[2]

o The fruit is 6.5 to 12.5 cm large, 8- to 10-locular, yellow when ripe, oblong or ovoid, with a broad, low, mamilliform projection at the apex. The rind is somewhat rough to smooth and the pulp is acid.[3]
It is a small tree whose twigs is angular when young and soon become rounded, glabrous, with stout auxiliary spines. The leaves are broadly elliptical, acute, serrate or crenate.[4]

Traditional Use

Internally it is used to reduce stomach and systemic acidity, and to help stimulate the liver and remove bile as well as small stones from the bile gland.
o It has a healing antiseptic property, which can stop bacterial growth.
o Externally, it has cosmetic uses, including assistance in removing spots and cellulitis, and can help in skin whitening.
o It is regarded as a soothing antioxidant and anti-inflammatory remedy that can be used in sunburn and itching.
o It has a strong astringent effect, which makes it useful in problems like acne and haemorrhoids.
o It can combat oily skin, inflammation and external irritations.
o The lemon proved itself in the past to be the best remedy for scurvy.[5]
o Lemon juice was also recommended as a drink for fever, as a remedy for acute rheumatism and as an antidote to intoxicants, particularly opium.[6]
o Other recommended applications were and still remain colds, sunburn, and as a quinine substitute for malaria or to reduce body temperature with typhus.[7]

Arabic medicine

o Traditional Arabic medicine mentioned several general uses for the "medical Lemon", such as removal of scars, hyperpigmentation, wrinkled skin, infected wounds and freckles.
o Ibn-Rasul refers the plant to infected wounds and skin allergies.
o Daud Al-Antaki extends the range of uses to include hyperpigmentation, freckles and "glow" (BAHAK)[8].

Evidence of use in the EU

o Lemon is one of the most universally used of medicinal foods.
o The herb is being used in Italy in cases of intestinal astringent, depurative, anti influenza and aperitive.[9]

Chemistry

o The dried peel contains some essential oils of lemon, such as limonene, 1,8, cimeole, beta and gamma pinene, terpinene, and citral, and is rich in flavonoids, such as hesperidin, as well as phenolic fruit acids such as ascorbic and coumarinic acid. It also contains pectin.

Safety

o Only one report has been found, after extensive searches, covering this exact species. It shows that extact of dried aerial parts, similar to our material has an intraperitoneal LD50 dose of more than 1 gram/kg[10].
o However as this is regarded as a variety of lemon (*Citrus limonum*) the assumption is that it is as safe as lemons for human use.
o No adverse effects have been found. Lemon is classified as a food by regulatory authorities.
o Lemon oil has been found to cause photoxicity in rare cases. This is due to furocoumarin derivatives, namely oxypeucedanin and bergapten, which are likely to be present in *Citrus medica*[11].

Efficacy:

Pharmacology studies

- 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, stimulation by auraptene, a flavonoid of one of the orange species, of a number of immunological factors including macrophage and lymphocyte functions[12]. Such effects could in a general way reduce the inflammatory symptoms of any inflammatory skin condition, including haemorrhoids[13].
- There is a study, for example of the treatment of psoriasis, a typical inflammatory skin condition involving irritation similar to haemorrhoids, by juice of grapefruits[14]. However this is relatively distant from lemon.
- There is evidence that citrus peel, oil, etc. contain antioxidants that could explain some of the relief in such cases[15].
- Lemon oil in particular seems to have a strong anti-oxidant effect[16]. *Citrus* species in general all seem to have components that can have cytostatic or antiproliferative effects on cells in culture[17].
- *Citrus limonum* has antifungal and bacteriostatic effects which allow its use in skin treatment of fungal and other infections[18].

Inula helenium.

Identity

Latin name: *Inula helenium.* L.
Common names: Inula leaves.
Arabic names: Tayun.

Characteristics

- Close species to (*Inula viscosa.*)
- Woody plants up to 0.5 m tall, which resemble miniature shrubs.[19]
- Found in Israel including the Palestinian autonomy and the Golan Heights, as well as in the Jordanian Kingdom and Sinai.[20]

Traditional Use

- *Inula* is used by local people against infections and inflammations, including treatment for fevers.
- It has been used to calm external skin irritations and heal wounds.
- The roots are used against cough and catarrh, as an antiseptic and expectorant, which loosens phlegm and supports mucus membranes.

Arabic medicine

- Reported use in the Middle East for muscle relaxation and infertility.[21]

o Being used in Israel for female sterility, and to treat rheumatic pains, broken bone disorder, soften bones, hard skin, warts on foot, general tonic, muscle spasms, open wounds and local paralysis. [22]

Evidence of use in the EU

- The similar European plant, elecampane, is GRAS and on open sale.
- Ethnobotanical research has shown that it is used in Italy as an antiseptic, and for stopping bleeding and closing wounds[23]
- Patents have been taken out covering antifungal effects (WO 9920109) and potential antiviral effects (IL 80329) of extracts or active ingredients from the plants.
- CAS No. 84012-20-4 EINECS No.: 281-666-1

Indications

- Infections, wounds, inflammations and dermatoses externally, cough and catarrh, internally.

Chemistry

- The plant has small amounts of essential oils in the leaves when fresh.
- There are flavonoids[24] such as rhamnocitrin, which are anti-inflammatory, and the same report has demonstrated a biologically active glycosyl analogue of diacylglycerol.[25]
- The strongly active antifungal compounds appear to be sesquiterpene lactones[26]. The leaves appear to be rich in sesquiterpenes[27].
- There are also interesting triterpenoids[28].

Safety

- The toxicity is very low. The large dose of 3 gm/kg of a methanolic extract given I.P. showed no ill effects even in long term dosing[29], and a similar dose of 2.5 gm/kg also showed no adverse effects by ingestion[30].

Efficacy:

Pharmacology studies

o There is very clear evidence of anti-inflammatory effects. For example, a group of azulene compounds, similar to those found in chamomile, was isolated from the leaves and found to be the active component that reduces fever in experimental animals[31].

o At a dose of 250-500 mg./kg, a 10% decoction of the leaves of Inula had a significant anti-inflammatory effect on the carageenan paw oedema test[32].

o The methanolic extract, but not more non-polar solvents, would seem to have the greatest anti-inflammatory effects in mice ear inflammation test[33].

o Activity against bacteria and fungi is also clear. Alcoholic and water extracts of above ground parts showed significant activity against some bacteria, and especially against the yeast *Candida albicans,* more than many other local plants which are sometimes claimed to have such effects[34].

o A study has shown that this plant has powerful antifungal effects against dermatophytes, and the activity resides on the sesquiterpene lactone fraction[35].

Clinical studies

o There are no known clinical studies on this species. However a close species, *Inula racemosa*, has been tested in patients with ischemic heart disease, with significant improvements in cardiac rhythms and performance[36]

Monographs:

o This species is similar to elecampane (*Inula helenium*) which is represented in various herbal monographs, and in Martindale.

o CAS No. 84012-20-4 EINECS No.: 281-666-1

Inula monograph

(created with the cooperation of Karmat micro-encapsulation Ltd)

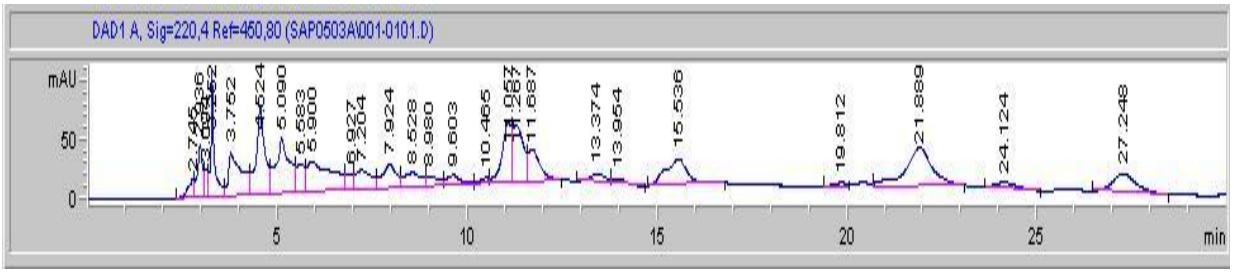
The final *Inula* Extract undergoes the following tests.
Assay –internal peaks
Foregin matter EP (2.8.2)
Microbiology EP 5.14 4-B - Maximum 10^4
Relative density EP (2.2.5)
Ethanol EP (2.9.10)
Methanol and Propanol EP (2.8.16)

Analytical Procedures

The plant was analysed by HPLC .
Reagents: Acetonitrile : Buffer Phosphate 20:80
Buffer phosphate : - Phosphoric acid Analytical grade: Water distilled 2: 1000.
All reagents used – HPLC grade.
Sample Preparation: - 50 grams milled plant was extracted with 350 ml Ethyl alcohol 50% for 1.5 hours at a temperature of 70 ^{o}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.

Chromatographic Conditions

HPLC type: HP 1090 Diode array
Column: Kromasil 60 -5CN 250 X 4.6 mm
Column Temperature: 40 ^{o}C
Mobile Phase: isocratic
Acetonitrile: Buffer Phosphate 20:80
Flow Rate: 1.0 ml/min
Detection: 220/270 nm and Diode array
Injection Volume: 20µl.
Run Time: 30 minutes
Peaks Identification: Retention time and UV/ Vis spectrum
Discussion of the Chromatogram(s)

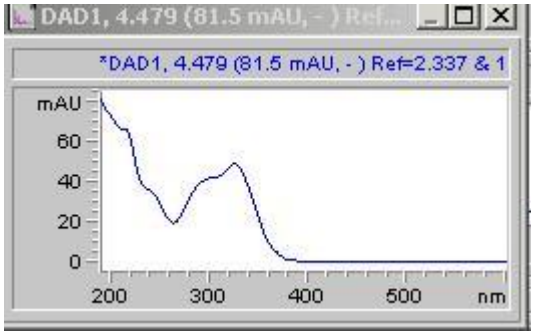


The peaks to be used as a finger prints are:

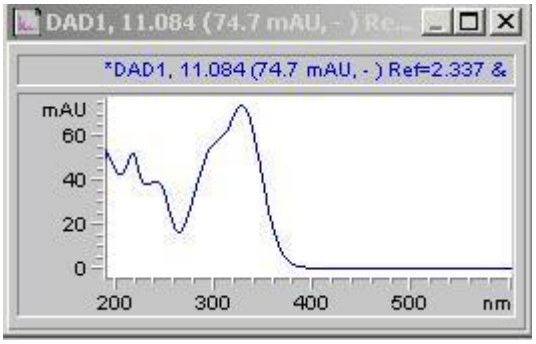
Retention time
4.48
11.08
11.69
21.9

Spectrophotometric Assay.
UV Vis spectrums of main peaks

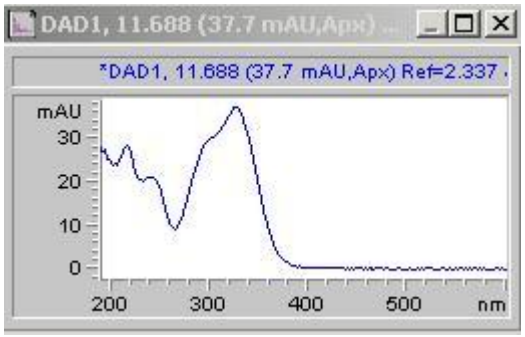
Retention time: 4.48 minutes



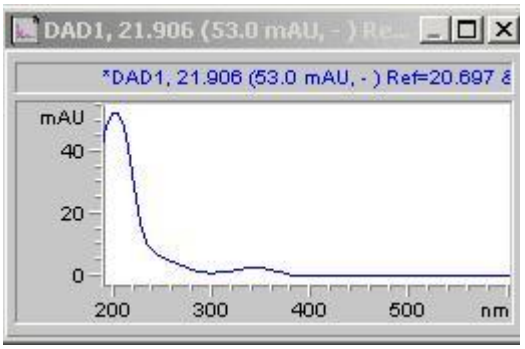
Retention time : 11.08 minutes



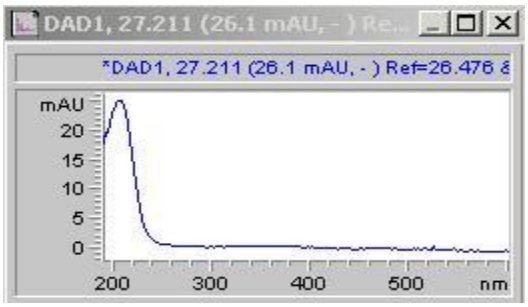
Retention time: 11.69 minutes



Retention time 21.9 minutes



Retention time: 27.2 minutes



Saponaria, Soap Wort (*Saponaria officinalis* L.)

Soapwort is mentioned in the traditional Arabic literature as helpful in skin conditions generally, such as itching, and eruptions on the skin arising from internal infections although acne is not specifically mentioned in a large number of texts that have been checked. Acne is mentioned in one herbal text as an example of a condition for which it would be helpful to use Soapwort as a skin wash. Traditionally it is used to wash and clean the skin in a gentle, non-irritating way. This herb has other effects such as reducing mucous production, catarrh and coughing. Soapwort is widely used in the European and Middle Eastern herbal tradition, mostly as a mild expectorant for chest problems.

A mild anti-inflammatory effect in rats has been found when extract was injected, but the doses used were not stated. Saponins in Soapwort may have an adjuvant activity, stimulating the immune response to specific antigens such as viral antigen

Identity

Latin name: *Saponaria officinalis L.*
Common names: Saponaria, Soap Wort, Bouncin bet, Fuller's herb, Sweet Betty, Wild sweet William, Crow soap.

Characteristics

- Family: Caryophyllaceae.
- Native of Europe, Middle East.
- A stout herbaceous perennial with a stem growing up to 4-5 feet high. Leaves lanceolate, slightly elliptical. Large pink flowers with no odour with a bitter and slightly sweet taste.[37]

Traditional Use

- Used traditionally to treat skin diseases. It is used in skin in conditions such as itching, and eruptions on the skin arising from internal infections. Acne is mentioned in one herbal text as an example of a condition for which it would be helpful to use Soapwort as a skin wash[38].
- Traditionally it is used to wash and clean the skin in a gentle, non-irritating way, and remove fat and dirt build-up without causing a rebound action increasing sebum production after washing. It acts with other herbs to allow passage of herbal ingredients through the skin.
- It has other uses such as reducing mucous production, catarrh and coughing.
- Martindale claims that saponaria has been used as an expectorant and diuretic.[39]
- The plant has proved to be very useful in jaundice and other visceral obstructions. It is a valuable remedy for rheumatism or cutaneous troubles.[40]

Arabic medicine

- Middle Eastern ethnopharmacology reports that the roots are used in acne.[41]

Evidence of use in the EU

- Soapwort is widely used in the European and Middle Eastern herbal tradition, mostly as a mild expectorant for chest problems.
- Soapwort root is sold in Europe as one of the constituents of Turkish Halva.
- Has a German Commission E monograph, confirming sale in Europe
- CAS: 84775-97-3. EINECS No.283-921-2

Indications

- The German Commission E defines indication of catarrhs of the upper respiratory tract.[42] In addition, indications of skin cleansing and dermatoses.

Chemistry

o The active ingredients of the plant are assumed to be saponins, because they are present at a high level, up to 5% of dry weight, giving extracts of the plant an obvious soapy and foamy nature, and giving rise to the plant's name.

o A number of triterpenoid saponins have been isolated from Soapwort and termed saponariosides[43], which may be similar to the quillaic acid saponins found in the *Quillaia saponaria* tree bark[44].

Safety:

Side Effects

o In rare cases, stomach irritation.[45]

o This plant has the lowest therapeutic index that is the gap between toxic and effective doses. It is regarded as non-toxic in normal doses. There is no accurate data on toxicity.

o It has been found to cause gastrointestinal upsets when a concentrated saponin-containing extract is ingested, although the doses were not clear[46]. Only one case of human poisoning with Soapwort is recorded in the literature, dating from 1922[47].

o The acute and chronic toxicity of a commercial preparation from Soapwort has been studied in experimental animals. The plant had a significant toxicity on injection by the i.p. route, but a very low toxicity by any other route. No adverse effects were found after one or three months treatment with a high dose either topically or by oral consumption[48]. This is similar to the toxicity recorded for other saponin-containing plants which can be toxic when given by injection (causing hemolysis) but are non-toxic for topical use

o A Bulgarian language paper on toxicity of Saponaria shows that there is potential toxicity with i.p. delivery, but absolutely no toxicity by oral or topical routes, both acute and chronic. This relates to a *Saponaria* product called Distin-Gel[49].

o There has been one published case report of a toxic effect in a human from the oral administration of high doses of *Saponaria*. But this was dated 1922, and since then there has been no reported cases[50].

o Ingestion of very high doses by grazing animals can cause potential digestive symptoms[51].

Efficacy:

Pharmacology studies

o *In vitro* studies have found cytotoxic effects and growth inhibition in cells in culture, which have been suggested as pharmacologically relevant[52]. However this is more likely to be a general toxic effect on cells *in vitro*.

o A mild anti-inflammatory effect in rats has been found when extract was injected ip, but the doses used were not stated[53].

o There is a great deal of literature on an immunotoxic glycoprotein called saporin, which is used in molecular biology, but this has little relevance to the clinical use of plant extracts. Saponins in Soapwort may have an adjuvant activity, stimulating the immune response to specific antigens such as viral antigens[54].

Clinical studies

Monographs

o German Commission E Monograph

o Martindale

o CAS: 84775-97-3. EINECS No.283-921-2

Saponaria monograph
(created with the cooperation of Karmat micro-encapsulation Ltd)

The final product undergoes the following tests

Foregin 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 20:80
Buffer phosphate : - Phosphoric acid Analytical grade: Water distilled 2: 1000.
All reagents used – HPLC grade.

Sample Preparation: - 50 grams milled plant was extracted with 350 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.

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 20:80

Flow Rate: 1.0 ml/min

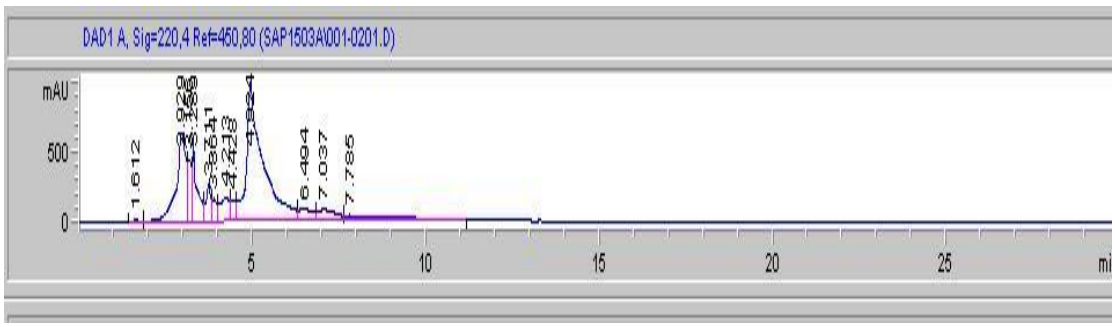
Detection: 220/270 nm and Diode array

Injection Volume: 10µl.

Run Time: 30 minutes

Peaks Identification: Retention time and UV/ Vis spectrum

Discussion of the Chromatogram(s)



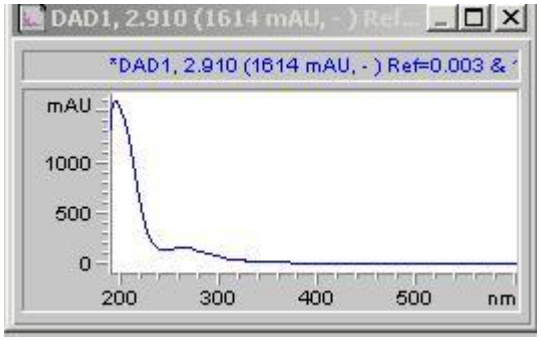
The peaks to be used as a finger prints are:

Retention time
2.91
4.94
7.01

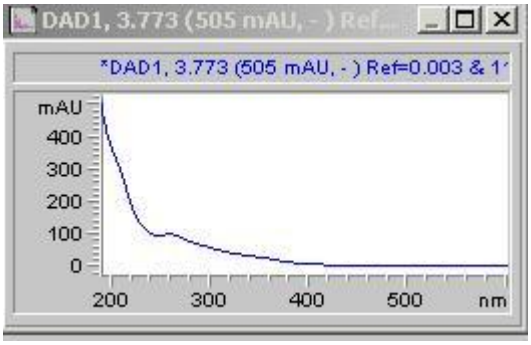
Spectrophotometric Assay:

UV Vis spectrums of main peaks

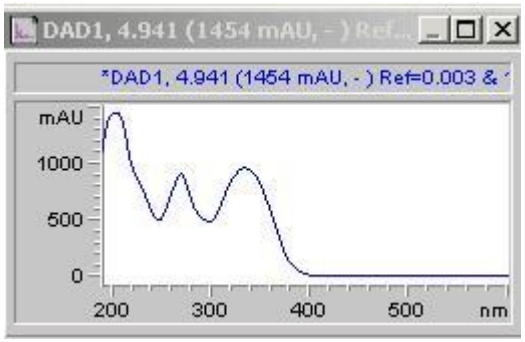
Retention time: 2.91 minutes



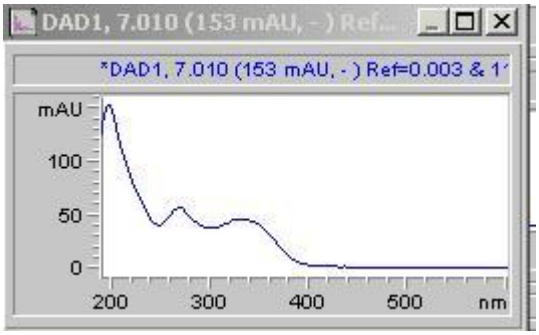
Retention time : 3.77 minutes



Retention time 4.94 minutes



Retention time: 7.01 minutes



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