

SYNOPSIS

EFFECTIVENESS AND SKIN TOLERABILITY OF AQUANOVA SOLUBILISATE FROM *RESEDA LUTEOLA L.* IN THE PATCH TEST AND IN THE UV ERYTHEMA TEST



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INTEGRATIVE DERMATOPHARMACY, NATURAL COMPOUND RESEARCH AND PHOTODERMATOLOGY

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Introduction

Plants and their extracts have been used in dermatology for centuries. In part their application is based on personal experience, traditions and inherited methods, but also, particularly in recent times, on scientific knowledge. During the last years phytotherapy has gained importance as a complementary therapy for skin disorders, but also the use of plant extracts in cosmetics is playing an increasing role. In particular for those patients who depend on long-term medication for the treatment of inflammatory skin diseases (e.g. corticosteroids), topical preparations based on plants (creams, ointments) may represent an alternative. Compared to corticosteroids topical plant extracts usually display less side effects and in addition facilitate user-administered medication with non-prescription and freely accessible products.

Reseda luteola L. is a plant whose main active ingredient, luteolin, has demonstrated an anti-inflammatory effect in numerous studies. Despite the experimental data on the anti-inflammatory effectiveness of luteolin, there are no reports in the international literature on the effectiveness of *Reseda luteola* L. in humans.

For this reason the dermatopharmacy group at the Department of Dermatology in Freiburg felt compelled to look for ways of manufacturing and solubilising *Reseda* extract and to design a study in which the anti-inflammatory effect could be tested in an internationally established and validated test model, the UVB erythema test. The UVB erythema test is an established and suitable method of testing the anti-inflammatory potency of test substances in healthy subjects.

Objectives

In previous clinical investigations the dermatopharmacy group could not confirm the effectiveness of *Reseda* extract in the UV erythema test despite of its potent anti-inflammatory potential *in vitro*. It was suspected that the cause of this was an insufficient solubilisation and penetration of the extract. Therefore, a 10% solubilisate of the *Reseda* extract was produced by Aquanova AG, Darmstadt, which facilitated a continuously adjustable, complete solution in water. The objective of the study presented here was to clinically test the anti-inflammatory effect of this *Reseda* extract solubilisate produced by Aquanova AG, Darmstadt in different concentrations in the UV erythema test. The test preparations were applied to defined test areas on the back of the test persons. After 30

minutes of occlusive application, the test fields were subjected to a defined, individually adapted UVB radiation dose in order to cause a local inflammatory reaction ("erythema" or "reddening of the skin"). The effectiveness of the test preparations to inhibit the UV-induced erythema was examined 24 hours later based on the reduction of the skin reddening caused by the UVB. The erythema was quantified by photometric measurement using a Mexameter. In parallel the same approach was used on skin that was not irradiated in order to test for any possible skin-irritating effect of the solubilisates.

Study design

Ten healthy subjects aged between 24 and 50 years were included in the study; five subjects were male and five female. Subjects with dark tanned or extremely fair skin and test persons with pathological light sensitivity were excluded. The consumption of anti-inflammatory or immunosuppressing medication represented a further exclusion criterion, as did the participation in another study during the last four weeks. The study was carried out in late autumn to exclude tanning due to summer sunbathing. The test persons were requested to refrain from swimming, sporting, sauna, or sunbed tanning during the study. The study was approved by ethics commission of the University of Freiburg. All subjects that participated in the study gave their written informed consent.

Materials

The test *Reseda* solubilisate was produced by the company Aquanova by processing a standardised dry *Reseda* extract (powder) with polysorbate. The concentration of the *Reseda* extract in the solubilisate was 10% (w/w). Using a HPLC method developed by the dermatopharmacy group Freiburg in cooperation with an analytical laboratory, it could be shown that the solubilised *Reseda* flavonoids are completely incorporated in the solubilisate, i.e. complete solubilisation is obtained (data not shown). Various concentrations of the extract were obtained from the solubilisate by diluting with bidistilled water. The application of various concentrations of the *Reseda* solubilisate allowed the determination of the anti-inflammatory threshold dose. The following table shows the dilution series of the *Reseda* solubilisate (left column) with the corresponding amount of contained *Reseda* extract (right column).

<i>Reseda</i> solubilisate : bidistilled water	Final concentrations of the <i>Reseda</i> extract
1:4	2.5%
1:8	1.25%
1:16	0.625%
1:32	0.312%
1:64	0.155%
1:128	0.075%

Table 1: Dilution series of the 10% *Reseda* solubilisate.

UV erythema test

Day 1

On the first day the individual light sensitivity of the respective test person was determined by means of graded exposure to UVB. For the test a UVB light source and a light scale was used which consisted of a template with six windows (Fig. 1) which were closed after defined time intervals by an automatic timer. Thus the skin areas were subjected to irradiation periods of various lengths and of increasing UVB doses. UVB irradiation by artificial light sources is a simple and widespread method of creating skin reddening. Apart from a mild itching, a stretching sensation or soreness, other side effects are not to be expected.



Fig. 1: Positioned light scale.

Day 2

24 hours after irradiation the erythema intensity (the intensity of the skin reddening) of the test areas was visually assessed and the individually required UVB dose was calculated. With the aid of a leather template (Fig. 2) test areas were marked on the back and the background erythema was measured using a photometer (Mexameter) which facilitates the objective and quick determination of the skin redness. Thus the reference values for the skin areas without radiation and without treatment were determined.

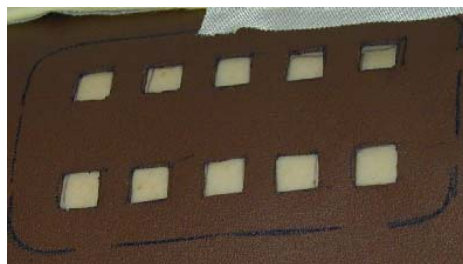


Fig. 2: Artificial leather template and marked test fields.

The diluted *Reseda* solubilisate preparations were applied using a pipette (50 µl) to small filter papers in patch test chambers and stuck onto the test fields with special plasters. The following illustration (Fig. 3) shows their arrangement on the back of the test person.

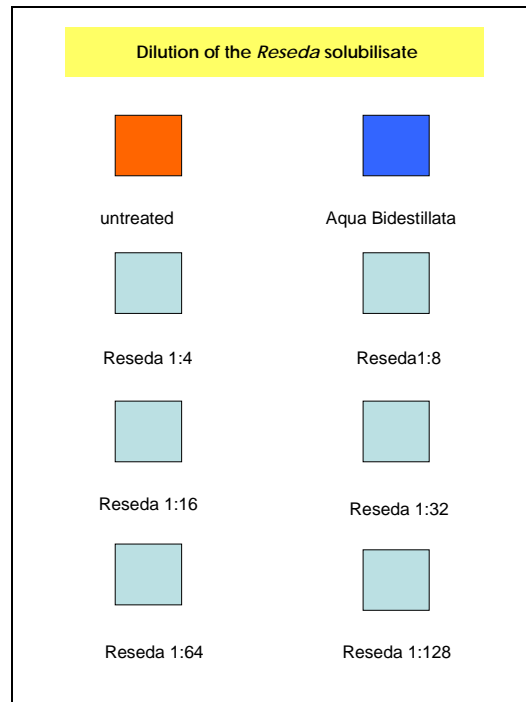


Fig. 3: Arrangement of the test areas on the back of the test person.

After 30 minutes of occlusive application to allow the penetration of the test substances, the plaster was carefully removed and the test areas were irradiated with the individually defined UVB dose (1.5 minimal erythema doses, MED). On the other half of the back the same substances were applied without UV irradiation.

Day 3

On the third day of the study or 48 hours after irradiation the erythema of the test areas was measured with the photometer (Mexameter). From the data obtained from the reading it was possible to draw conclusions about the effectiveness of the test preparations (Figs. 4 and 5).



Fig. 4 : A test person's back.

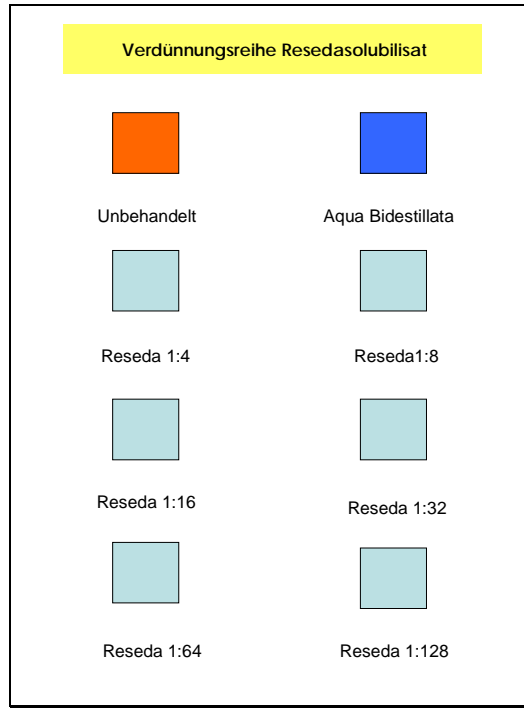


Fig. 5: Treatment of the test areas.

On the non irradiated site the test areas were examined with regard to possible skin irritations due to the solubilisate.

Results

As can be seen from in Fig. 4, some fields, i.e. those treated with the *Reseda* solubilisates, show a reduction of the UV-induced erythema. This was confirmed with the complete evaluation of all test persons. The following figure (Fig. 6) shows the comparison of the erythema index (erythema intensity) of the test areas treated with the test preparations compared to the two reference areas. The 2.5% and the 1.25% concentration of the *Reseda* extract display the strongest inhibition of the skin erythema.

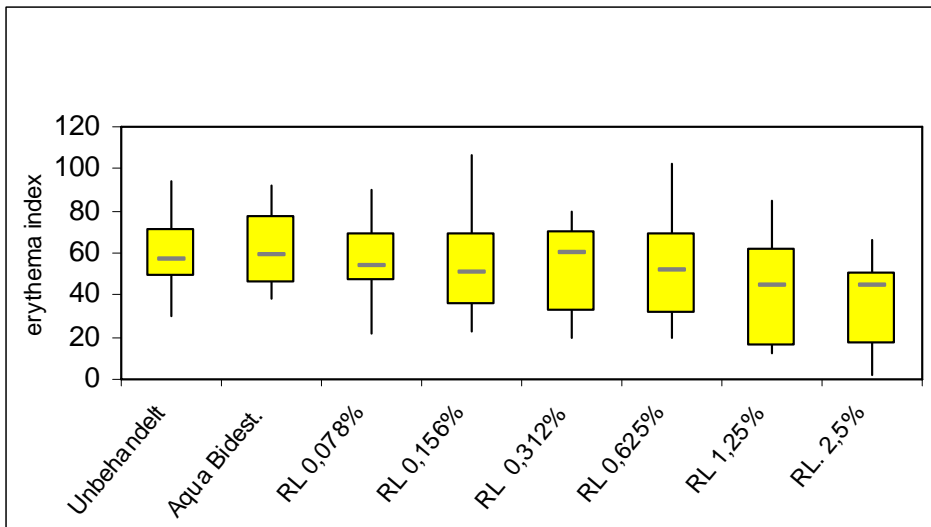


Fig. 6: Illustration of the erythema index on the skin irradiated with 1.5 times MED.

The test substances were also applied to the skin without irradiation. There was not any skin irritation, i.e. an increase in the skin reddening due to the application of the *Reseda* solubilisate (Fig. 7).

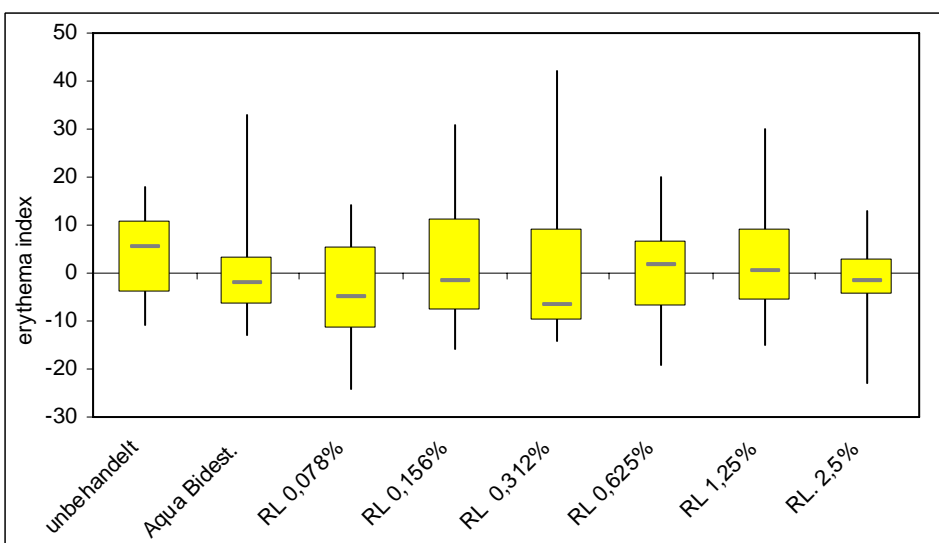


Fig. 7: Illustration of the erythema index on the skin which was not irradiated.

Conclusion and discussion

After various unsuccessful efforts to incorporate the *Reseda* extract in topical vehicles it was possible for the first time to dissolve the *Reseda* extract, which has a poor solubility in various solvents, by using a solubilisate from Aquanova AG, Darmstadt. In a clinical test with ten healthy test persons a dose-dependent inhibition of the UV-induced inflammation by the *Reseda* solubilisate could be demonstrated. The dose-dependent inhibition of the UV-induced erythema illustrates that the active compounds are not only released from the micelles, but also penetrate the upper layers of the skin.

The skin tolerability of the *Reseda* solubilisate on the non-irradiated site was excellent. An increase in the skin reddening did not occur with any of the solubilisate concentrations used. The effect was comparable to that of the solvent, i.e. distilled water.

In summary, the data from the pilot study on Aquanova solubilisates on the skin permit for the first time the following statements to be made:

1. Through the Aquanova solubilisate technology also plant extracts which are difficult to dissolve can be completely and stably rendered soluble and diluted continuously with water.
2. The solubilised active substances are released when applied to the skin and penetrate into the upper skin layers, which is clearly proven by the dose-dependent biological effects.
3. The tolerability of the solubilisates on the skin is excellent up to the tested concentration of 2.5% v/v.

Aquanova solubilisates thus exhibit excellent prerequisites as carriers of active substances for dermatological vehicles. However, the compatibility of the solubilisates to various vehicles must be examined. This is also the case for any new topical formulation. From the present study it can be concluded that Aquanova solubilisates are particularly suitable for incorporation into the aqueous phase of a topical formulation.

The release and penetration of care substances and active substances for the care of the skin depend on many factors such as the pH value, polarity of the solvent and interactions with accompanying substances of the vehicle. Therefore, the selection of an unsuitable vehicle in the worst case can completely prevent the release of an active substance. Furthermore, interactions between the vehicle and the incorporated active substances can result in chemical inactivation of the active substance. Finally, the type of "packaging" of the active substance is of decisive importance for the stability of the formulation, for the release from the vehicle and above all for the penetration into deeper skin layers. The successful development of dermatological external formulations is therefore dependent on a complicated interaction between the special features of the stratum corneum, the composition of the vehicle, and the "packaging" of the applied care and active substances.

It should be noted that the epidermis principally represents a penetration barrier which is normally impermeable for molecules. The free incorporation of care and active substances in the vehicle, but also the incorporation in conventional carriers such as liposomes can do little to change this fact.

The product micelles of Aquanova solubilisates represent an interesting new approach to the solution of the penetration problem. In contrast to liposomes and microemulsions that cannot provide particle diameters of less than 100 nm, the product micelles from Aquanova produce clearly smaller size units, which even be smaller than 30 nm. In contrast to liposomes, which are often unstable in acids and which therefore separate in an acid medium, Aquanova solubilisates are stable in acids; they are completely and irreversibly water-soluble and it has been shown that they lead to a stronger and faster penetration of care and active substances into the upper skin layers. This has been impressively demonstrated for various formulations with Aquanova solubilisates. Penetration studies with vitamin E solubilisates, retinol solubilisates and coenzyme Q10 solubilisates revealed excellent penetration up to the basal layers of the stratum corneum for all formulations (research report from Prof. Gehring, 2003).

Also, the present investigation with solubilised *Reseda* extract confirm the successful solubilisation and release of flavonoid complexes to the skin. The tolerability of the solubilisate on the skin is excellent.

Particularly with the processing of plant extracts and active substances, the Aquanova solubilisates represent a very promising approach. The solubilisation of sensitive active substances facilitates the design of innovative and effective care and therapy concepts.