An Investigation into the Anti-aging Efficacy of a Serum Containing a Red Mangrove Extract
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Abstract

Research Object—Extracts from the bark of the red mangrove (Rhizophora Mangle) plant have been used in folk medicine for centuries, and numerous studies have demonstrated that the extract possesses antimicrobial, anti-inflammatory, and potent antioxidant activity. Based on these findings, a proprietary extract from the seedlings of the red mangrove was developed and incorporated into a cosmetic formulation and applied topically to determine if it could exhibit any anti-aging efficacy.

Experimental Methods
This study was conducted at AMA Laboratories, Inc., New City, NY, under IRB approval. RIPT: Prior to initiation of anti-aging testing, a (50) person Repeat Insult Patch Test (RIPT) was performed. None of the subjects experienced any adverse reaction. ANTI-AGING: 5 caucasian females, (age 41–53) applied the serum to their face twice daily for 56 days. Elasticity and viscoelastic properties of the skin were measured as a function of flexibility and firmness employing a cutometer. In addition, product effectiveness was evaluated photographically on a subset of 2 subjects via Reverse Photo Engineering.

Results
A statistically significant improvement in mean % elasticity was noted on day 28 and 56 (7.62 % p = 0.003, and 13.58 % p = 0.002, respectively). Also on day 56, the mean % reduction in appearance of wrinkles and fine lines in the subset of 2 subjects was 49.88 %. CONCLUSIONS: The formulation containing red mangrove extract as described above demonstrated increases in skin elasticity and reduction in the appearance of fine lines and wrinkles when applied topically.

Introduction
Rhizophora mangle, more commonly known as the red mangrove, is a woody, salt water-tolerant plant that grows in tropical and subtropical coastal areas throughout the world, especially in the Atlantic basin of the Americas and Caribbean.

Extracts from the bark of the red mangrove (Rhizophora Mangle) plant have been used in folk medicine for centuries, and numerous studies have demonstrated that the extract possesses antimicrobial, anti-inflammatory, and potent antioxidant activity. 1–6 Based on these findings, a proprietary extract from the seedlings of the red mangrove was developed and incorporated into a cosmetic formulation and applied topically to determine if it could exhibit any anti-aging efficacy.
0.2 ml of the test material is dispensed onto the occlusive, hypoallergenic patch.

The patch is then applied directly to the skin of the infrascapular regions of the back, to the right or left of the midline and the subject is dismissed with instructions not to wet or expose the test area to direct sunlight.

After 24 hours the patch is removed by the panelist at home.

This procedure is repeated until a series of nine consecutive 24 hour exposures have been made for every Monday, Wednesday, and Friday for three consecutive weeks.

In the event of an adverse reaction, the area of erythema and edema is measured. The edema is estimated by the evaluation of the skin with respect to the contour of the unaffected normal skin. Reactions are scored just before applications two through nine and the next test date following application nine. In most instances this is approximately 24 hours after patch removal. Clients are notified immediately in the case of adverse reaction and determination is made as to treatment program if necessary.

Subjects are then given a 10–14 day rest period after which a challenge or retest dose is applied once to a previously unexposed test site. The retest dose is equivalent to any one of the original nine exposures. Reactions are scored 24 and 48 hours after application.

Comparison is made between the nine inductive responses and the retest dose.

Skin elasticity and reverse photo engineering

Panel Demographics

Number of subjects enrolled .................... 5
Number of subjects completing study ...... 5
Age Range ............................................ 41–53
Sex .................................................. Female ...................................... 5
Race ............................................... Caucasian ................................ 5

Two subjects selected for additional evaluation via Reverse Photo Engineering:

Number of subjects enrolled .................... 2
Number of subjects completing study ...... 2
Age Range ............................................ 48–53
Sex .................................................. Female ...................................... 2
Race ............................................... Caucasian ................................ 2

Five healthy females were inducted into this study. The demographic data is shown above.

In order to pre-condition the test sites and keep all topical treatments consistent during the study, the panelists were required to abstain from using any self-tanning, anti-aging and moisturizing products, including lotions, creams, gels and nutritional supplements, for a period of at least 72 hours prior to study commencement. On the day of the test panelists reported to the test facility with their faces devoid of topical treatments and were examined by a trained technician.
Upon arrival, panelists were allowed to equilibrate to the ambient environment for 30 minutes prior to measurement. Instrumental analysis was conducted prior to the initial application at baseline and again after 28 and 56 days of use. Detailed, high resolution matched digital photographs were taken at baseline and again after 28 and 56 days of use. Photographs were taken with fixed camera background, distances, angles, settings, lighting, panelist positioning, color bars, white balance, standardized and digitally certified unretouched. Each stage in the progression of the product treatment was graphically documented. This set of photographs thus provided a visual record of the efficacy of the product, on the subjects face.

All participants were instructed to use the test material as a part of their daily routine according to the following instructions:

- Apply a small amount of the test product twice a day all over the face until completely absorbed.

The following distinct noninvasive methods were employed to establish evaluation parameters:

### Skin Elasticity-Cutometer

Cutometer SEM 575 (model 575 Courage + Khazaka) is used to measure skin viscoelastic properties. The measuring principle is based on a suction method. Negative pressure is created in the device, which can be regulated between 20 and 500mbar. Skin is drawn into a calibrated aperture of the probe by negative pressure where skin penetration depth is determined by a non-contact optical measuring system. The optical measuring system consists of a light transmitter and a light recipient, as well as two glass prisms facing each other, which project the light from transmitter to recipient. The light intensity will vary due to the penetration depth of the skin.

### Reverse photo engineering

Exclusively detailed, high resolution before and after digital photographs are taken, with fixed camera background, angles, settings, lighting, panelist positioning, color bars, white balance, standardized and digitally certified unretouched. Photographs are evaluated using image analysis software which allows the evaluation parameter to be captured and quantified. This software also allows wrinkles to be captured and quantified. The size of the area of involvement differs for each test panelist, therefore percent difference is calculated individually and then averaged.

### Statistical Source Data

The source data are Cutometer readings collected prior to application and after 28 and 56 days of use. The data used in the statistical analysis reflect changes from baseline.

### Results

#### RIFT

No adverse reactions of any kind were noted during the course of the study.

#### Skin elasticity (Fig. 1)

Student’s t-test was used in this investigation. This is the test of the null hypothesis that the difference between two responses measured on the same statistical unit has a mean value of zero. In this investigation the changes in wrinkle size (area affected by wrinkle measured in Fig. 2) before and after the treatment were measured. If the treatment is effective, we expect the area affected by wrinkle for many of the patients to be smaller following the treatment. This is often referred to as the paired or repeated measures t-test. Dependent samples (or paired) t-tests typically consist of a sample of matched pairs of similar units, or one group of units that has been tested twice (a repeated measures t-test). Once a value is determined, a p-value can be found using a table of values from Student’s t-distribution. If the calculated p-value is below the threshold chosen for statistical significance {0.05 (5 %)}, then the null hypothesis (Null Hypothesis p>0.05) is rejected in favor of the alternative hypothesis.

Statistical analysis was computed using appropriate Excel statistical software functions, where one function returns the probability associated with a Student’s T-Test and the other returns the t-value of the Student’s t-distribution as a function of the probability and the degrees of freedom.

### Reverse Photo Engineering

Exclusively detailed, high resolution before and after digital photography was taken, with fixed camera background, distances, angles, settings, lighting, panelist positioning, color bars, white balance, standardized and digitally certified unretouched. Each stage in the progression of the treatment regimen was graphically documented and the test area of involvement isolated. Photographs were evaluated using image analysis software which allows the wrinkles to be captured and quantified. The size of the area of involvement dif-

**Fig. 2** Wrinkle related pixels per area of involvement.
fered for each test panelist, therefore percent difference was calculated individually and then averaged.

Conclusions

Based on the RIPT testing, the test serum was considered to be a non-primary irritant and a non-primary sensitizer. Also, within the limits imposed by the conduct and population size of the study described herein, the test material demonstrated increases in skin biological elasticity on the site treated with the product. These increases were statistically significant after 28 and 56 days of use. Improvement in the appearance of fine lines was also demonstrated, however, due to the small sample size, this parameter change was not statistically significant.

References


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