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Skin irritation test of a microemulsion containing essential oil isolated from *Ocimum basilicum*

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ABSTRACT: Thai basil oils have a demonstrated antimicrobial activity against *Propionibacterium acnes*. An in vitro study revealed that *P. acnes* was more susceptible to *Ocimum basilicum* (sweet basil) oil than other *Ocimum* species. The purpose of this study was to evaluate skin irritation reactions of a 3% *O. basilicum* microemulsion on human subjects. Thirty healthy human subjects were enrolled in a 3-day cumulative irritancy study. A single application, closed patch epicutaneous technique under semi-occlusion condition was used for testing. Four products were tested along side each other. These were a 3% *Ocimum* microemulsion (test product), 3% *O. basilicum* oil in isopropyl myristate (active ingredient), the vehicle alone (placebo), and de-ionized water (control). All products were applied on the upper back of each subject for 1 h, and then removed. After the patch was removed, transepidermal water loss (TEWL), erythema index, scaling, and oedema were evaluated at 0, 1, 48, and 72 h. The results showed no clinically observable irritations in all preparations tested at 1, 48, and 72 h after removal of the patch. The TEWL values remained unchanged from baseline ($P > 0.05$) and erythema indices of all tested formulations were lower than baseline ($P < 0.05$). The TEWL values and erythema indices were not significantly different among all preparations tested. We concluded that a 3% *Ocimum* microemulsion should be considered safe and well tolerated on male human skin.

INTRODUCTION

The *Ocimum* species (including *Ocimum* subgroups *Ocimum basilicum*, *O. sanctum*, and *O. americanum*) are one of the most popular species of herbs and essential oils. For many years these herbs have been used as spices in south-east Asian and Italian cuisine. Furthermore, Thai traditional medicines use these herbs and essential oils because of medical benefits. For example, poultice or salves have been developed from these herbs and essential oils as remedies and treatments for insect bites and ringworm[1].

The essential oils which can be extracted from *Ocimum* leaves are predominantly methyl chavicol, camphor, 1,8-cineole, and α-bergamotene[2–4]. In vitro studies demonstrate a strong inhibitory effect of essential oil extracted from *O. basilicum* (sweet basil) against some pathological bacteria including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Propionibacterium acnes*[4,5].

P. acnes infection is a major cause of acne vulgaris and there is an increasing bacterial resistance to its treatment. Discovery of new topical antibacterial products, especially herbal products, is an attractive idea. However, proven clinical efficacy is needed to warrant their benefit. In vitro data have shown that among several *Ocimum* species, *P. acnes* was highly susceptible to *O. basilicum* oil and the oil is stable at a concentration of 3.0% v/v microemulsion[4].

Prior to proceeding with any clinical trials, safety data must be collected to assure human safety. Animal studies have demonstrated that *Ocimum* oil products are safe in animals with LD_{50} and LC_{50} values in mice and brine shrimp larva being 957 mg/ml and 9.9 µg/ml, respectively[6]. Essential oils extracted from *O. basilicum* appear to be the least toxic extract among different *Ocimum* species. However, *Ocimum* oil products may cause skin irritation either from the essential oil itself or from other ingredients[7]. Basil products tested on human subjects for contact dermatitis using the patch test have given results that appeared to be negative[8].

No evidence to date has indicated if a 3% *Ocimum* product would cause any skin reaction. A study of 2% and 5% *O. gratissimum* oil in polysorbate 80, 50% ethanol, cetomacrogol blend, and petrolatum base have been performed. In these studies, no irritation

was observed in 2% *Ocimum* formulations, while 5% *O. gratissimum* oil in 50% v/v ethanol and petrolatum base caused skin irritation with mild burning, skin-darkening effect, and pruritus[9].

The aim of our study was to perform the skin irritation test of a microemulsion that contains 3% *O. basilicum* oil using a single application closed patch epicutaneous test with the semi-occlusion method[10].

MATERIALS AND METHODS

The study product: *O. basilicum* oil

The sweet basil oil was manufactured and procured from Thai-China Flavours and Fragrances Industry Company. Sweet basil was grown in Khon Kaen, NE Thailand and harvested in the summer season. The composition of the major components of sweet basil oil was determined by gas chromatography-mass spectrometry (Agilent Technologies, UK). The dominant materials in sweet basil oil were found to be methyl chavicol (93.0%), α-bergamotene (2.2%), and 1,8-cineole (1.0%).

Preparation of the microemulsion

The microemulsions of sweet basil oil were formulated by mixing them with isopropyl myristate (oil phase). The oil phase was added to the mixture of the water phase consisting of polysorbate 80, 1,2-propylene glycol (cosolvent), and de-ionized water. Hydroxyethyl cellulose in an amount of 0.5% w/v was then added to the premixed microemulsions to improve the viscosity. The final systems were mixed with a magnetic stirrer until homogeneous dispersions were obtained. Placebo products were composed of isopropyl myristate, tween 80, 1,2-propylene glycol, de-ionized water, hydroxyethyl cellulose, and paraben concentration.

Characteristics of the tested products and the microbial contamination test

Test and placebo products were prepared using the same inactive ingredients without the active ingredient of the test product (3% sweet basil oil). The test product had a clear white colour while the placebo product was turbid white. Both products retained their smooth texture throughout the study period. Both test and placebo products were pH 5.5. The products were tested for microbial contamination using spread plate technique according to the Thai Herbal Pharmacopeia (2000) regulations prior to the trial. The results of test confirmed both products (placebo and test products) were negative for all bacteria tested.

Study design

The study was a single blind, fixed area for application, controlled trial conducted at Cosmetics and Natural Product Research Centre, Naresuan University, Phitsanulok, Thailand. The protocol was reviewed and approved by the institutional review board of Naresuan University (protocol number: 50 02 04 0028; year: 2006). All subjects provided a written informed consent before enrolling.

Four products, namely, (I) 3% *O. basilicum* oil in isopropyl myristate (active ingredient), (II) 3% *Ocimum basilicum* oil in microemulsion (tested product), (III) microemulsion without *O. basilicum* oil (placebo), and (IV) de-ionized water (negative control) were tested using the single application closed patch test method[10] to assess irritating contact dermatitis reactions. The tested formulations were applied on the upper back of each subject in 4 small areas. Each test area was separated from each other by 3 cm, and then covered with a 2.5 cm^2 semi-occlusive patch (Blenderm tape and webril cotton) for 1 h. The patch was then removed and webril cotton was used to remove the tested products from the skin. Irritation reactions were assessed before and at 1, 48, and 72 h after patch removal. During the study period, subjects were allowed to shower provided that no soap, detergent, or cosmetic product was applied to the upper back.

The evaluation of skin irritation was determined by objective assessment using a Tewameter (TM 210 Courage+Kazaka) and expressed in transepidermal water loss (TEWL, values in g/m^2h) and a Mexameter (MX 18 Courage+Kazaka, Cologne, Germany, for skin redness, i.e., erythema index). All subjects had to rest for at least 30 min in a room with optimum conditions (20 ± 5 °C and 40–60% air humidity). Visual clinical assessments (checking for erythema, scaling, and oedema) were evaluated by a dermatologist.

Subjects

Eligible subjects were healthy Thai males aged between 13 and 35 years. Exclusion criteria included the use of any interfering topical or systemic therapy (such as steroids, retinoids, hydroquinone, or benzoyl peroxide applied within 2–4 weeks before the study) that could affect the tested results. Subjects who had a blister or wound on their upper back, history of eczema or psoriasis within six months, history of skin cancer, or history of hyperallergic reaction to sweet basil or ingredients of this product were excluded.

Statistical analysis

The data analysis was completed using a statistical program. Mean, SD, and frequency were used to describe the subjects' demographic data at baseline. The differences in mean TEWL values and erythema indices at 1, 48, and 72 h of study were compared with baseline (0 h) using a paired t-test at a significance level of 0.05. In addition, the differences in mean TEWL values and erythema indices of the active ingredient (I), tested product (II), and the placebo (III) were compared with de-ionized water (negative control) (IV) using an independent t-test at a significant level of 0.05.

RESULTS

Thirty healthy Thai male subjects with the mean age, weight, and height of 18 ± 3 years, 60.6 ± 9.9 kg, 167 ± 5 cm, respectively, were enrolled in this study. Vital signs at screening were normal in each subject. There was no dropout or loss to follow-up during the study. Clinical evaluation using visual scoring assessed by a dermatologist revealed no erythema, scaling, nor oedema in all subjects at 1, 48, and 72 h after the patch removal.

For the objective assessment parameters, most of the TEWL values remained unchanged from the baseline, with an exception of the values at 48 h post patch removal when the values were significantly reduced from the baseline. The erythema indices of all testing formulations were also lower than baseline ($P < 0.05$). Furthermore, when compared among the preparations tested, TEWL values and erythema indices were not significantly different among all preparations ($P > 0.05$) (Table 1).

Table 1 Results of all measurement at baseline (0 h), 1 h, 48 h, and 72 h.

Period	Preparation			
	De-ionized water	Active ingredient	Placebo	Tested product
TEWL values (g/m^{2}h) ($n = 30$):				
0 h	10.0 ± 2.4	11.7 ± 3.4	10.9 ± 3.3	12.6 ± 6.5
1 h	11.6 ± 7.8	12.7 ± 4.0	11.8 ± 5.3	13.3 ± 8.4
48 h	8.0 ± 2.1[a]	8.8 ± 4.0[a]	8.2 ± 3.2[a]	8.6 ± 5.0[a]
72 h	11.7 ± 6.6	11.5 ± 6.1	11.3 ± 8.5	15 ± 12
Erythema index ($n = 30$):				
0 h	323 ± 73	304 ± 69	305 ± 69	300 ± 67
1 h	302 ± 68[a]	287 ± 65[a]	290 ± 69[a]	289 ± 70[a]
48 h	288 ± 70[a]	278 ± 69[a]	279 ± 65[a]	277 ± 65[a]
72 h	298 ± 88[a]	275 ± 72[a]	280 ± 71[a]	277 ± 67[a]

[a] $P < 0.05$ when compared with baseline (0 h).

DISCUSSION

Any TEWL value lower than 70 g/m^{2}h is considered safe[11]. As demonstrated in the results of our study, all of TEWL values were under 70 g/m^{2}h suggesting that all ingredients in our product did not cause any skin irritation.

Although the mean TEWL values at 48 h were significantly lower than that of the baseline, none were greater than 70 g/m^{2}h. We suspected that the particular occurrences might have been caused by several factors, such as the subjects' physical activity and environmental conditions including air humidity, room temperature, or airflow. Erythema indices measured at 1, 48, and 72 h showed no erythema formation, which is consistent with the visual evaluation by the dermatologist. Among the tested products, TEWL values and erythema indices were not significantly different. These results illustrated that the test product, placebo, and active ingredient seemed to be as safe as de-ionized water (negative control).

The products of *Ocimum* essential oils have been previously tested at two concentrations; 2% and 5% for skin irritation. The 5% product has been found to irritate human skin, while the 2% was safe[9]. Our results have confirmed that the 3% *Ocimum* oil microemulsion appeared to be safe and did not cause any irritation reaction on human skin.

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