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Potential of native Thai aromatic plant extracts in antiwrinkle body creams

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Synopsis

Antioxidant activities of 10 essential oils and 10 absolutes extracted from Thai aromatic plants were evaluated and compared to thyme oil, trolox, quercetin, and kaempferol by two independent assays: the 2, 2-diphenyl-1-1-picrylhydrazyl (DPPH$^{\bullet}$) radical scavenging assay and the thiobarbituric acid reactive species (TBARS) assay for lipid peroxidation. We found that four essential oils including ginger oil (*Zingiber officinale* Roscoe), Wan-sao-long leaf oil (*Amomum uliginosum* Koen), lemongrass oil (*Cymbopogon citratus*), holy basil oil (*Ocimum sanctum* L.), and the absolute of dwarf ylang-ylang [*Cananga odorata* Hook. f. & Thomson var. *fruticosa* (Craib) J. Sinclair] exhibited high antioxidant activity in both DPPH and TBARS assays and possessed satisfactory fragrance properties. These were then combined into an essential oil blend (EOB) and retested for antioxidant activity. The EOB also exhibited high antioxidant activity in the above assays. It was then incorporated into a stable cream base as EOB body cream. The EOB body cream was found to be best able under storage in stress conditions and presented significantly higher antioxidant activity than its' cream base both before and after stability testing. The effect of EOB body cream on skin surface topography was evaluated in 29 healthy volunteers using the Skin Visiometer® (SV 600 FW, CK Electronic GmbH, Germany). Three parameters, R*a*, R*z* (roughness), and surface, were analyzed. After 4 weeks of application, the EOB body cream showed significant reductions in surface and R*z* compared with before treatment ($p < 0.05$, paired *t*-test), and with untreated and placebo treatment ($p < 0.05$, Duncan test). These results indicate that the essential oils and absolutes from Thai plants may serve as potential sources of natural antioxidants for spa and cosmetic products designed to prevent or treat signs of skin aging.

INTRODUCTION

The recent resurgence of "back to nature" themes and consumer preference for natural health and beauty solutions have refocused attention on the healing power of indigenous herbs and plants—a key component in the ancient Thai tradition of natural healing. Thailand has great diversity of herbs and spices and continues to provide a rich assortment of natural ingredients for exotic herbal preparations. Many of the rejuvenating

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treatments offered in modern spas have evolved from these ancient recipes. There is a clear consensus that, spa treatments may go beyond superficial pampering. Holistic healing is central to the spa concept.

Antioxidant compounds have been found in numerous plant materials including seeds, oils, grains, vegetables, fruits, leaves, bark, roots, and waxes. Most of the plant antioxidants fall into the category of polyphenols or flavonoids. Essential oils and plant extracts are good sources of natural antioxidants. A number of studies on antioxidant activities of essential oils and plant extracts from some aromatic plants have been reported such as ylang-ylang (1,2), turmeric (3,4), lemongrass (1,5), and ginger extracts (1,6) Confirm all citation. Currently, the spa business is growing rapidly around the world. As spa operators feel the pressure of mounting competition and strive to differentiate to survive and excel, an increasing number of Thai spas are developing their own lines of product that capture the exotic qualities of Thai herbs. This has contributed to a renewal of traditional Thai herbal recipes long prized for the healing potency of the natural ingredients from which they are made. Creating a new line of antiaging spa products from Thai aromatic plants through scientific principles is thus an important challenge.

The aging process encompasses progressive physiological changes in an organism that lead to senescence, the decline of biological functions, and the organism's ability to adapt to metabolic stress with time (7). There are many theories that explain the aging process. Since the "free radical theory of aging" was formulated by Dr. Denham Harman in 1956, free radicals have been widely accepted to contribute to aging. This theory postulates that aging is accelerated by free radical reactions associated with environmental influences, disease, failures of the antioxidant defense, and the intrinsic aging process. Free radicals are highly reactive chemical species carrying an unpaired electron in their outer orbit. They abstract electrons from the surrounding molecules (lipids, proteins, and DNA) to complete their own electron structure, thus inducing molecular and cellular damage. Such reactions are strongly implicated in the development of pathogenesis of chronic diseases as atherosclerosis, diabetes, rheumatoid arthritis, skin and neurodegenerative disorders, cancer, as well as in the aging process. As reported at the *3rd Monte Carlo Anti-Aging Conference*™ in September 2002, the intrinsic (genetically determined) and the extrinsic [ultraviolet (UV)- and toxic exposure–mediated] skin aging processes overlap and are strongly related to an increased generation of free radicals in the skin. The skin photoaging process, in the presence of natural sunlight or artificial UV sources, happens continuously and leads in time to dryness, deep wrinkles, sagging, loss of elasticity, mottled pigmentation, and telangiectasia (8). Use of antioxidants such as α-tocopherol, ascorbic acid, and polyphenol compounds may be an effective way to slow down skin aging (9,10). Essential oils and aromatic plant extracts are good sources of natural antioxidants (1–3,5,6). The goal of this study was to develop extracts selected from Thai aromatic plants that exhibited high antioxidant activity with satisfactory fragrance properties into a prototype product for antiaging body treatments.

MATERIALS AND METHODS

CHEMICALS AND PLANT MATERIALS

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-thiobarbituric acid 98% (TBA), t-octylphenoxypolyethoxyethanol (Triton X-100), and butylated hydroxytoluene (BHT) were

purchased from Sigma-Aldrich Inc (St. Louis, MO), Cholesterol from lanolin ($C_{27}H_{46}O$) was purchased from Flukachemie GmbH., (Tokyo, Japan). Phosphatidylcholine (Epikuron 200) was purchased from Degussa, (Frankfurt, Germany). The 2,2´-azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Pure Chemical Industries, (Osaka, Japan). Other chemicals and solvents were of analytical grade. All the plants used were cultivated and collected from Chiang Mai Province, Thailand.

PREPARATION OF THE EXTRACTS

The seven kinds of rhizomes of Zingiberaceous plants [*Zingiber cassumunar* Roxb., *Curcuma domestica* Val., C. *mangga* Val. & Zijp., *Z. officinale* L., *Alpinia galanga* (L.) Swartz, Z. *ottensii* Val. and *Curcuma* sp.], leaves petiole of *Cymbopogon citratus*, and leaves of *Ocimum sanctum* collected in northern provinces of Thailand were hydrodistilled for 5 h using a Clevenger-type apparatus. The obtained volatile oils were dried over anhydrous sodium sulfate. The petals or whole flowers of 10 plants [*Mammea siamensis* Kosterm., *Michelia alba*, *Plumeria alba*, *C. odorata* (Lam.) Hook. f. & Thomson var. *fruticosa* (Craib) J. Sinclair, *Millingtonia hortensis* L., *Alangium salviifolium* subsp. *hexapetalum* Wang., *Alstonia scholaris* (L.) R. Br., *Gardenia augusta*, *Saraca thaipingensis* Cantley ex King, and *Quisqualis indica* L.] collected in northern provinces of Thailand were extracted with petroleum ether for 24 h. After filtering and evaporating to dryness in a vacuum, the concretes obtained were treated with ethanol (EtOH) to produce absolutes. All of the volatile oils and absolutes were stored in the dark at 4°C until tested.

DETERMINATION OF ANTIOXIDANT ACTIVITIES

Antioxidant activities of all extracts were determined by two different methods, scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and lipid peroxidation [thiobarbituric acid reactive substance (TBARs)].

Determination of antioxidant activity with DPPH radical scavenging method. In this assay, the stable free radical DPPH, which has a strong absorption at 520 nm, reacts with antioxidants and produces colorless 2,2-diphenyl-1-picrylhydrazine independently of enzymatic activities (7). Dilution series of test compounds, dissolved in EtOH, were performed in sterile disposable microplates, using freshly prepared 167 µM $DPPH^{\bullet}$/EtOH solutions, 180 µl. Trolox, quercetin, and thyme oil served as reference antioxidants. The samples were tested in dilutions ranging from 0.5 to 500 mg/ml with a final volume of 200 µl for all of the assays. The results were determined after 30 min of reaction time to analyze antiradical activities. The disappearance of the free radical $DPPH^{\bullet}$ was measured spectrophotometrically at 520 nm with a microplate reader. The percentage inhibition was calculated by the equation: % inhibition = $(A_{control} - A_{sample}/A_{control}) \times 100$ where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of test compound. Extract concentration providing 50% inhibition was calculated from the graph plotted inhibition percentage against extract concentration.

Determination of antioxidant activity with TBARs method. A modified TBARS assay was used to measure the antioxidant activity of the extracts in terms of inhibition on lipid peroxidation (8–10). A liposome suspension, consisting of cholesterol (0.25 g),

phosphatidylcholine (0.03 g), and 20 ml of 0.2 M potassium phosphate buffer (pH 7.2), was prepared in a sonicator. The essential oil or absolute in EtOH (100 µl), was mixed with a mixture of the sonicated solution (600 µg/ml) and AAPH (0.07 M, 60 µl). The resulting mixture was incubated at 50°C. After incubation, the solution (80 µl) was mixed with 0.2% BHT (24 µl), 3% Triton-X (100 µl), 20% acetic acid (500 µl), and 0.6% TBA (250 µl). Then the mixture was heated for 30 min, cooled to room temperature and absorbance was measured at 540 nm with a microplate reader. The percentage inhibition was calculated same as in DPPH assay. Extract concentration providing 50% inhibition was calculated from the plot of inhibition percentage against its concentration. Trolox, quercetin, and thyme oil that are known antioxidants served as positive controls.

GAS CHROMATOGRAPHY ANALYSIS OF THE ESSENTIAL OILS AND FLOWER ABSOLUTES

Essential oils 0.5 % v/w in ethanol and flower absolutes were characterized by gas chromatography–mass spectrometry (GC-MS) using a Shimadzu model GCMS-QP2010 Plus (Kyoto, Japan) with mass-selective detector with electron impact ionization. The samples were separated using a DB-5 MS capillary column (5% phenylmethylpolysiloxane, 30 m × 0.25 mm, 0.25 µm film thickness) Helium was used as carrier gas. Injector temperature 200°C, split ratio 1:50. Column temperature was 90°C, ramp to 130°C with the rate of 5°C/min, ramp to 133°C with the rate of 1°C/min for 3 min, ramp to 134°C with the rate of 0.5°C/min for 3 min, ramp to 136°C with the rate of 0.5°C/min for 8 min, and then ramp to 230°C/min with the rate 15°C/min for 5 min.

Chemical constituents were characterized by matching mass spectra with reference compounds mass spectra in WILLEY 7 and NIST 2005 Library.

PREPARING OF ESSENTIAL OIL BLEND

Essential oils and absolutes with good antioxidant activity were selected for odor evaluation via fragrance by 21 volunteers. Each volunteer smelled the samples and scored the odor from 1 (least like) to 5 (most like). The essential oils and absolutes that presented high odor satisfaction were blended into the essential oil blend (EOB).

FORMULATION AND STABILITY TEST OF CREAM BASE AND EOB BODY CREAM

A cream base containing 45.6% of oil phase (jojoba oil, avocado oil, vitamin E acetate, cyclomethicone, glyceryl stearate, stearic acid, stearyl alcohol, isopropyl myristate, BHT, and ceteareth-25) and 55.4% of water phase (propylene glycol, triethanolamine, purified water, sodium metabisulfite, and preservative) was formulated. Texture smoothness, color, odor, spreadability, feel on skin, and pH were observed at the starting point and after stability testing under storage at eight cycles of heating and cooling (alternation of storage conditions from 50°C for 48 h to 4°C for 48 h as one cycle). Creaming and cracking were also observed. The EOB (1.5% w/w) was then incorporated into the stable cream base and its stability was also determined as described above.

CLINICAL EVALUATION OF WRINKLE-REDUCING CAPACITY OF EOB BODY CREAM

A double-blinded, placebo-controlled clinical study was performed on 29 healthy females aged 20–60 years. All subjects were allergy free for 1 week and had not used steroids for 4 weeks before study enrollment. Subjects who were pregnant, lactating, dieting, smoking, or have a history of excessive alcohol consumption were excluded. Other skin treatments on the test area were prohibited. In addition, the treated areas were protected against strong sun light and UV exposure during the study that lasted 28 days. The treatment room was controlled at 25° ± 1°C and 40–60% relative humidity during the measurements.

All enrolled subjects signed a written consent form approved by the ethical committee of Chiang Mai University before enrollment. Subjects were asked not to apply any products onto the test areas 3 days before starting the study. The EOB was proposed for body massage creams in spa treatments, therefore the test area was on the volar forearm. Volunteers were requested to apply the products twice daily on the forearm, 2 inches below and above the elbow and wrist, respectively, for 4 weeks. Approximately 0.2 g of the EOB body cream or the control cream base was applied to the forearms with right–left balance. A portion of each forearm was left untreated to serve as an additional control. The test areas were cleaned with mild soap and rinsed with water and towel wiped to dryness. The subjects then equilibrated in the waiting room for 30 min before skin measurements were carried out. Effects on skin condition were evaluated using the Skin Visiometer® SV 600 for analysis of the skin surface profile with three parameters [roughness (R*a*, R*z*) and surface] at the three test sites (untreated site, N; active-cream site, A; placebo-cream site, B). Skin moisture was evaluated with the Corneometer® (CK Electronic GmbH, Germany) at the same tested sites. Paired *t*-tests were used to examine changes in R*a*, R*z*, and surface values as well as skin moisture content (before and after each treatment). Efficacy was assessed at baseline (D0) and for 28 days (D28). The percentage efficiency values of all parameters were calculated by the following equation: (value at measuring point − value at initial point) × 100/ value at initial point. The data were subjected to a two-way analysis of variance and the significance of the differences between means was determined by Duncan's multiple range test ($p < 0.05$) using SPSS software version 17.0. for Windows (SPSS Inc., Chicago, IL). After testing was finished, all volunteers were asked to fill out a questionnaire on product satisfaction.

RESULTS AND DISCUSSION

The essential oils obtained were colorless to pale yellow liquids and yielded 0.16–0.87%, whereas the absolutes obtained were slightly viscous, pale yellow to dark brown in color with a characteristic fragrance and yielded 0.06–0.47%.

DETERMINATION OF ANTIOXIDANT ACTIVITIES

The DPPH radical scavenging activities of the essential oils and absolutes compared with reference antioxidants are shown in Table I. For essential oils, holy basil oil (*O. sanctum* L.) exhibited the highest antioxidant activity with IC_{50} of 0.6294 mg/ml followed by Phlai oil (*Z. cassumunar* Roxb., IC_{50} = 1.0599 mg/ml), ginger oil (*Z. officinale*, IC_{50} = 4.385 mg/ml), and Wan-sao-long root oil (*A. uliginosum*, IC_{50} = 5.2725 mg/ml). Holy basil oil

Table I
Antioxidant Activities of the Essential Oils and Absolutes From Some Thai Aromatic Plants and Reference Standards

Test samples	DPPH (IC_{50}, mg/ml)	TBARs (IC_{50}, mg/ml)
A. uliginosum, leaf	6.9438	1.4000
Z. cassumunar	1.0599	1.7017
A. uliginosum, root	5.2725	1.4814
Curcuma longa	24.1751	1.7071
Z. ottensis	30.1142	2.1419
A. galanga	24.7664	2.5125
O. sanctum	0.6294	1.9388
Curcuma aromatica	24.6113	1.9158
Z. officinale	4.3852	2.1094
C. citratus	26.7727	1.0665
C. mangga	26.1832	2.2993
C. odorata	1.4264	ND
A. salviifolium subsp. *hexapetalum*	1.6765	ND
M. hortensis	1.6139	ND
M. siamensis	0.3271	ND
Gardenia jasminoides	5.3689	ND
A. scholaris	3.1725	ND
M. alba	0.7155	ND
P. alba	1.0766	ND
S. thaipingensis	5.9394	ND
Q. indica	8.1580	ND
Quercetin	0.0059	0.0383
Trolox	0.0105	0.4014
Kaempferol	0.0113	0.1212
Thyme oil	1.0002	0.2303

ND: Not determined.

showed higher antioxidant activity than thyme oil (IC_{50} = 1.0002 mg/ml). For absolutes, saraphi (*M. siamensis* Kosterm.) exhibited the highest antioxidant activity with IC_{50} of 0.3271 mg/ml followed by white chempaka (*M. alba*, IC_{50} = 0.7155 mg/ml) and temple tree (*P. alba*, IC_{50} = 1.0766 mg/ml). Among all extracts, saraphi, white chempaka, and holy basil oil showed higher antioxidant activity than thyme oil. The absolute of saraphi exhibited the highest antioxidant activity, whereas Phai-dam oil (*Z. ottensii*) exhibited the lowest antioxidant activity with IC_{50} of 30.1142 mg/ml. These results strongly suggest that the main radical scavenging activity from these aromatic flowers does not arise merely from their essential oil components but rather from other phenolics such as flavonoids and anthocyanins (11).

The antioxidant activity of essential oils and reference compounds determined by the TBARS assay is also shown in Table I. Lemongrass oil presented the highest inhibition of peroxidation with IC_{50} of 1.0665 mg/ml followed by Wan-sao-long leaf oil

(IC_{50} = 1.4000 mg/ml), Wan-sao-long root oil (IC_{50} = 1.4814 mg/ml), and Phlai oil (IC_{50} = 1.7017 mg/ml), but all of the oils exhibited antioxidant activity lower than quercetin (IC_{50} = 0.0383 mg/ml), kaempferol (IC_{50} = 0.1212 mg/ml), thyme oil (IC_{50} = 0.2303 mg/ml), and trolox (IC_{50} = 0.4014 mg/ml).

For absolutes, at 15 mg/ml, the absolute of dwarf ylang-ylang showed the highest inhibition of peroxidation (58.81%) followed by Rangoon creeper (*Q. indica* L., 58.16%) and white champaka (*M. alba*, 53.46%), but all of the absolutes exhibited lower antioxidant activity than the reference antioxidants as mentioned above.

Comparison of antioxidant capacities of essential oils and absolutes determined by two different methods, DPPH and TBARS, did not show a relationship between the results. It is possible that these activities resulted from different mechanisms. As previously described (2,12), the use of different methods and concentrations is necessary in antioxidant activity assessment. This study shows that a single assay may not be sufficient to estimate the antioxidant activity of a test compound. The combination of two methods, applied in this study, was a good choice to investigate the antioxidant activity of essential oils and absolutes.

The DPPH assay is faster than the TBARS assay and it can be helpful for investigation of novel antioxidants to obtain preliminary information on radical scavenging abilities (13,14). This assay is simple, rapid, and needs only a UV–visible (UV-VIS) spectrophotometer to perform, which probably explains its widespread use in antioxidant screening. Moreover it is sensitive and requires a small amount of sample (12). This method is considered to be mainly based on an electron transfer reaction, and hydrogen atom abstraction is a marginal reaction pathway (15). The TBARS method is sensitive to *in vitro* lipid peroxidation (16). Lipid peroxidation is one of the important causes of skin aging. Both methods, DPPH and TBARS allow testing of both lipophilic and hydrophilic substances. The results of the antioxidant power measurement depend on the chosen method, concentration, as well as the nature and physicochemical properties of the studied antioxidants (12). This study confirms that the same antioxidant samples exhibited different antioxidant values depending on the concentration and method.

GC ANALYSIS OF THE ESSENTIAL OILS AND FLOWER ABSOLUTES

Chemical constituents of the essential oil were characterized by GC-MS with direct injection, whereas headspace-SPME-GC-MS was used for absolutes. Chemical groups of essential oils and flower absolute were mostly monoterpenes and sesquiterpenes. Besides aromatic components, flower absolutes also consisted of fatty acid. Holy basil oil showed the highest antioxidant activity in the DPPH assay. The volatile components of holy basil oil were methyl eugenol, β-elemene, α-humulene, and germacrene D. Lemongrass oil, Wan-sao-long oil, and absolute from ylang-ylang gave potent activity for TBARS assay. The components found in lemongrass oil were citral, myrcene, limonene, citronellal, geraniol, and geranyl acetate, whereas Wan-sao-long oil consisted of *p*-(1-butenyl) anisole as a major constituent. The highest antioxidant activity of flower absolutes was found with ylang-ylang, composed of *p*-methyl anisole, geranyl acetate, *trans*-caryophyllene, germacrene D, and benzyl benzoate. The terpenoid compounds and fatty acids in flower absolutes may promote antioxidant activities. Essential oils and aromatic extracts are complex

natural mixture in which their biological activities sometimes are the result of synergism (12).

ANTIOXIDANT ACTIVITY OF EOB

Ginger oil, Wan-sao-long leaf oil, lemongrass oil, holy basil oil, and absolute of dwarf ylang-ylang were selected to blend into EOB based on their antioxidant activity and odor satisfaction (data not shown). The EOB was also evaluated for antioxidant activity by the DPPH and TBARS assay. It showed high antioxidant activity with IC_{50} of 6.3487 and 10.9215 mg/ml by DPPH and TBARS assays, respectively.

ANTIOXIDANT ACTIVITY OF THE EOB BODY CREAM

The antioxidant activity of EOB body cream was determined by both the DPPH and TBARS assays. Freshly prepared EOB body cream exhibited scavenging effect by the DPPH assay of 43.30 ± 1.61% at a concentration of 20 mg/ml. The % inhibition of cream base was 33.06 ± 1.14 (Figure 1).

In the TBARS assay, the EOB body cream showed a better inhibitory effect than the cream base at all concentrations. The IC_{50} of EOB body cream was significantly lower than cream base (IC_{50} = 4.24 ± 0.05 mg/ml and 4.93 ± 0.04 mg/ml, respectively, $p < 0.05$) as shown in Figure 2. After heating-cooling cycling, both creams were re-evaluated. The scavenging abilities of EOB body cream for the DPPH showed % inhibition of 41.52 ± 2.67 mg/ml at a concentration of 20 mg/ml, which was higher than the cream base (% inhibition of 35.56 ± 0.52 mg/ml, $p < 0.05$). The EOB body cream also exhibited a higher inhibitory effect on lipid peroxidation than cream base (IC_{50} = 3.98 ± 0.05 mg/ml and 4.71 ± 0.27 mg/ml, respectively, $p < 0.05$). This indicates that the antioxidant activity of EOB body cream was significantly higher than cream base both before and after stability testing. Moreover, it was stable in the accelerated test (no significant difference, $p < 0.05$).

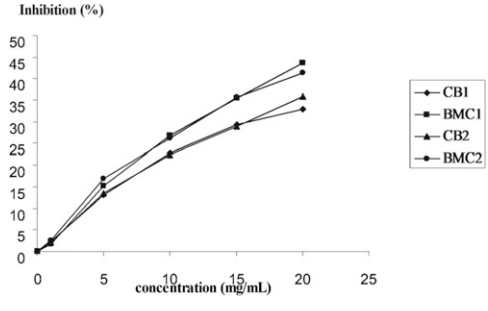

Figure 1. Inhibitory effect of cream base and EOB body cream measured by the DPPH assay, before and after heating-cooling cycling test. (CB1: Cream base before stability test, CB2: cream base after stability test, BMC1: EOB body cream before stability test, BMC2: EOB body cream after stability test).

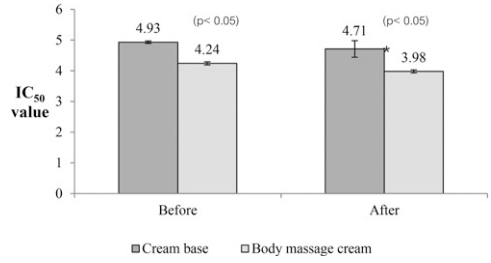

Figure 2. IC_{50} value (mg/ml) of cream base and EOB cream (body massage cream) on TBARS assay for lipid peroxidation before and after heating-cooling cycling test.

FORMULATION AND STABILITY TEST OF EOB BODY CREAM

The freshly prepared EOB body cream and cream base were also examined for their physical stabilities against heating-cooling cycling. Both freshly prepared and after-stability testing, the EOB body cream showed good appearance with a pale yellow color, smooth and homogeneous texture, and its pH was only slightly changed. No cracking or creaming was observed. The product had good spreadability on the skin, as shown in Table II.

CLINICAL EVALUATION OF WRINKLE-REDUCING CAPACITY OF EOB BODY CREAM

Photodamaged skin is characterized by fine and coarse wrinkling, rough texture, shallow color, and uneven pigmentation. Measuring effects on skin surface texture and wrinkles is one way to investigate the efficacy of "antiaging" products.

The effect of the EOB body cream on skin surface texture was evaluated in a 4-week clinical study with 29 human subjects using the Skin Visiometer® (17). Three parameters were analyzed (R*a*, R*z*, and surface). Paired sample tests were used to examine changes in values, before and after each treatment (untreated, treated, and placebo). Posttreatment,

Table II
Physical Properties of EOB Body Cream Before and After Stability Testing

	EOB body cream	
Topics	Before	After
pH	7.11	7.10
Physical properties		
Color	Pale yellow	Pale yellow
Texture	Smooth and homogeneous	Smooth and homogeneous
Consistency	Tender	Tender
Odor	Unique fragrant odor	Unique fragrant odor (no change)
Spreadability	Very good	Very good
Feel on skin	Soft	Soft
	Enriched skin glossy	Enriched skin glossy

Table III
Effect on the Skin Roughness Parameters, R*a*, R*z*, and Surface, from the Three Treatments

	Roughness parameters		
Topics	Surface	R*a*	R*z*
Untreated area, N			
Before	6.20 ± 0.80	14.88 ± 2.54	63.63 ± 9.81
After	6.02 ± 0.72	13.88 ± 2.28	59.76 ± 8.33
Placebo area, B			
Before	5.92 ± 0.73	14.29 ± 2.65	60.92 ± 9.40
After	5.78 ± 0.72	13.38 ± 2.60	57.34 ± 9.37
Treated area, A			
Before	5.98 ± 0.80	14.44 ± 2.50	61.89 ± 10.37
After	5.69 ± 0.61	13.32 ± 2.30	55.79 ± 8.63*

*Significantly different at $p < 0.05$.

the difference between untreated, treated, and placebo treatment was analyzed by Duncan's multiple range test ($p < 0.05$). All 29 subjects completed the clinical trial.

Results as mean values of the 29 volunteers are represented in Table III and Figure 3. After 4 weeks, the application of EOB body cream led to a significant improvement in

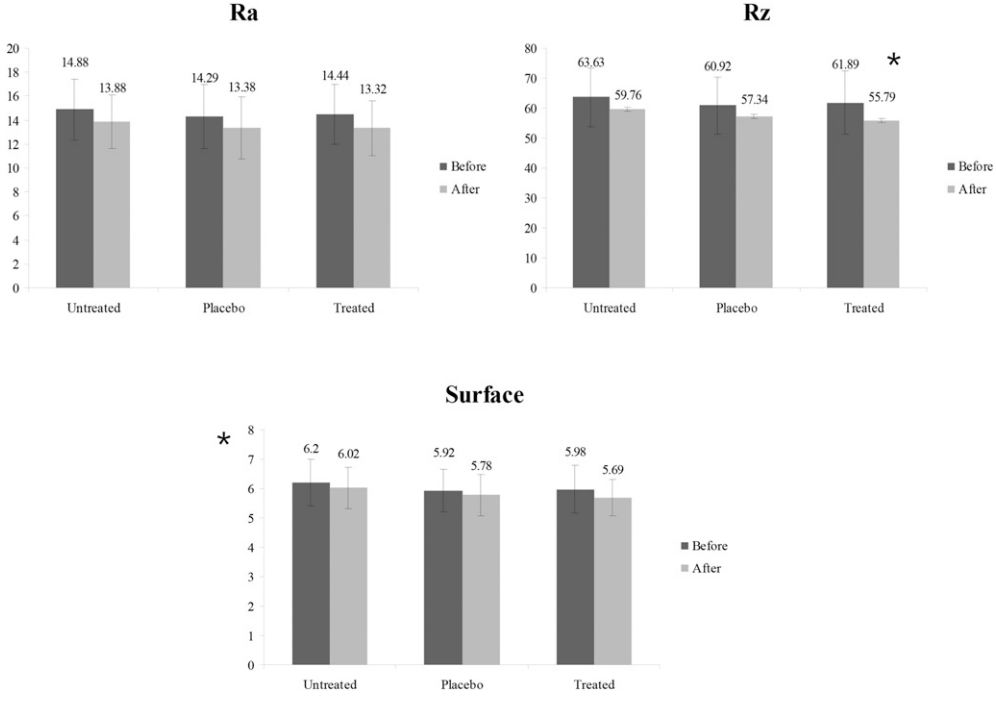

Figure 3. Mean values of the skin roughness parameters, R*a*, R*z*, and surface, before and after 4 weeks application of cream base and EOB body cream (untreated, placebo, and treated) in 29 healthy volunteers. *= Significantly different at $p < 0.05$.

surface texture especially in terms of R*z* ($p < 0.05$), while changes in the untreated and placebo area were not statistically significant (Figure 3). This indicates that the EOB body cream led to reduction of skin roughness ($\Delta Rz = -6.1$, the average maximum difference between the highest peaks and deepest valleys in the surface profile, which defines skin roughness) (17–19).

The posttreatment % efficiency values on reducing the skin roughness parameters, R*a*, R*z*, and surface, were also calculated by the equation: % efficiency value = (mean $\text{value}_{\text{after}}$ − mean $\text{value}_{\text{before}}$/mean $\text{value}_{\text{before}}$) × 100, as shown in Table IV. Statistical analysis of the posttreatment, % efficiency data showed a significant difference between the effect of EOB body cream and both its vehicle (placebo cream) and untreated on R*z* and surface (Table IV). This result indicates that the EOB body cream has the capability to reduce skin roughness, and comparison to placebo cream indicates that the wrinkle-reducing efficacy of the EOB body cream is due to the antioxidant capacity of the EOB. Roughness of forearm skin surface has been shown to increase with age, (20–21) implying that the EOB cream had an antiaging effect on the skin surface.

The Corneometer® was used to assess relative changes in skin hydration through skin capacitance measurements (22). The results revealed that skin hydration was significantly increased by both cream base (placebo cream) and EOB body cream compared to the untreated area as shown in Figure 4. This implies that the moisturizing effect can be attributed to the emollients and moisturizers in the cream base.

The level of subject's satisfaction is often assessed by asking subjects to rate how much they like a cream overall, using a five-point Likert scale. The satisfaction results are shown in Table V and ranged from "like extremely" to "very much" for softness of cream, spreadability, cream gloss, and softness of skin. Overall, the satisfaction with the EOB body cream was high (77.2% ranged from "like extremely" to "very much"). A total of 70% of panelists felt relaxed after using this cream and no skin irritation occurred in them. More than 85% of the panelists would like to reuse this cream because they found that their skin was enriched with moisture and looked young and healthy (data not shown).

Table IV
The % Efficiency Values Calculated from the Mean Values of the Skin Roughness, R*a*, R*z*, and Surface After 4 Weeks Application of Cream Base, EOB Cream (Untreated, Placebo, and Treated) in 29 Healthy Volunteers

	% efficiency values		
Topics	Surface	R*a*	R*z*
Untreated area, N	−2.90	−6.72	−6.08
Placebo area, B	−2.36	−6.37	−5.88
Treated area, A	−4.85*	−7.76	−9.86*

*Significantly different from groups B and N at $p < 0.05$.

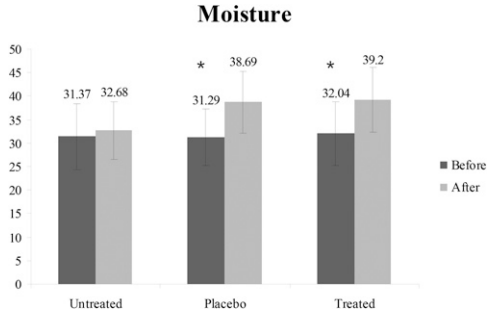

Figure 4. Corneometer readings (arbitrary units) and % efficiency values after 4 weeks of application of cream base, EOB body cream (untreated, placebo, and treated) in 29 healthy volunteers.*Significantly different at $p < 0.05$.

CONCLUSION

Four selected essential oils and absolutes from Thai aromatic plants that exhibited high antioxidant activity and acceptable odor were blended into the EOB. Use of this blend in a moisturizing body cream produced a cosmetically acceptable product with statistically significant wrinkle-reducing efficacy in a 4-week clinical study. In conclusion, our results strongly indicate that the essential oils and absolutes from Thai aromatic plants can serve as a potential source of natural antioxidants for spa and cosmetic products designed to treat and prevent signs of skin aging.

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Table V
The Percentage of Satisfaction on EOB Body Cream in the Panelists

Topic	Like extremely	Very much	Nether like nor dislike	Like slightly	Dislike extremely
Satisfaction before use (%)					
1. Cream texture	20.0	53.3	24.0	2.67	0
2. Odor	7.58	42.4	45.5	3.03	1.56
3. Color	19.5	57.1	23.4	0	0
Satisfaction after use (%)					
1. Softness of cream	25	65	7.5	2.5	0
2. Spreadability	44.3	40.5	15.2	0	0
3. Cream glossy	36.6	48.8	14.6	0	0
4. Softness of skin	25.3	55.7	19	0	0
5. Odor	7.81	37.5	42.2	12.5	0
6. Overall satisfaction	31.6	45.6	22.8	0	0

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