


ORIGINAL CONTRIBUTION

Anti-aging and brightening effects of a topical treatment containing vitamin C, vitamin E, and raspberry leaf cell culture extract: A split-face, randomized controlled trial

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Funding information

This study was funded by Max Biocare Pty Ltd, Australia.

The copyright line for this article was changed on January 31, 2020 after original online publication

Abstract

Background: Skin aging has many manifestations such as wrinkles, uneven skin tone, and dryness. Both intrinsic and extrinsic factors, especially ultraviolet light-induced oxidative radicals, contribute to the etiology of aging. Human skin requires both water- and lipid-soluble nutrient components, including hydrophilic and lipophilic antioxidants. Vitamins C and E have important protective effects in the aging process and require exogenous supply. Raspberry leaf extracts contain botanical actives that have the potential to hydrating and moisturizing skin. Topical products with these ingredients may therefore combine to provide improved anti-aging effects over single ingredients.

Objectives: To evaluate the anti-aging and brightening effects of an encapsulated serum containing vitamin C (20% w/w), vitamin E, and European raspberry (*Rubus idaeus*) leaf cell culture extract.

Methods: Fifty female volunteers aged 30-65 years were allocated one capsule of serum for topical application on one side of the face for 2 months, in addition to self-use of facial skin products. Both test (treated) and contralateral (untreated) sides were dermatologically assessed after 4 and 8 weeks. Skin color (melanin index), elasticity, radiance, moisture, and water evaporation were measured by Mexameter MX18[®], Cutometer[®], Glossometer GL200[®], Corneometer CM825[®], and Tewameter TM300[®] instruments, respectively (Courage + Khazaka Electronic GmbH). Skin microtopography parameters, smoothness (SEsm), roughness (SEr), scaliness (SEsc), and wrinkles (SEw), were measured by Visioscan[®] VC98 USB (Courage + Khazaka Electronic GmbH), and gross lifting effects were measured by VECTRA[®] H1 (Canfield Scientific), and adverse reactions and satisfaction were also assessed.

Results: Skin color, elasticity, and radiance were significantly improved. The smoothness, scaliness, and wrinkles were also revealed significant improvement. Mild adverse reactions were tingling and tightness.

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Conclusions: The vitamin C, vitamin E, and raspberry leaf cell culture extract serum has anti-aging and brightening effects of skin.

KEYWORDS

antioxidant, raspberry leaf cell culture extract, skin aging, skin radiance, vitamin C, vitamin E

1 | INTRODUCTION

Facial skin aging is one of the most prevalent cosmetic concerns to women. The many noticeable manifestations such as wrinkles, sagging, uneven skin tone, and dull and dry skin can significantly impact self-esteem and social relations.¹ Both intrinsic and extrinsic factors are involved in skin aging; however, photoaging is an important cumulative factor.² Nowadays, there are many modalities that can improve the condition of aging skin and address the underlying mechanisms of cellular and molecular damage. Treatment with topical antioxidants represents a noninvasive approach that treats the underlying mechanisms of cellular and molecular damage from oxidative free radicals. Other agents include topical cell growth regulators and retinoids, which are used alongside the more invasive procedures such as chemical peeling, ablative and nonablative laser photo-rejuvenation, radiofrequency (RF) therapy, dermal fillers, and botulinum toxin.³

Human skin has both water- and lipid-soluble compartments with their respective bioactive antioxidants. Many of these, including vitamins C and E, are not able to be produced endogenously and so must be provided externally or through diet. L-ascorbic acid is the most abundant antioxidant in the skin and is the biologically active form of vitamin C. The maximum concentration of topical L-ascorbic acid for percutaneous absorption is 20%.⁴ Vitamin C has many anti-aging skin effects, not only as a potent antioxidant and mediator of photo-damage and melanogenesis, but also through its roles in supporting collagen biosynthesis and stability, which provide renewal and anti-inflammatory effects.⁵⁻⁷ The lipid-soluble properties of vitamin E, which supports skin levels of tocopherols, allow it to pass down to the deepest layers of the stratum corneum via sebaceous gland secretions, to reside within cell membranes and protect them from oxidative stress.⁸ Furthermore, vitamin E can reduce hyperpigmentation induced by ultraviolet light.⁹

Vitamins C and E work synergistically in quenching free radicals. Vitamin C regenerates the oxidized form of vitamin E to its reduced form. Topical use of 15% L-ascorbic acid combined with 1% alpha-tocopherol has been shown to provide significantly more protection against sunburn cell formation compared with either L-ascorbic acid or 1% alpha-tocopherol alone.¹⁰

Raspberry (*Rubus idaeus*) leaf cell culture extract has many potent antioxidants able to protect cells from oxidative stress. The extract also has important lipid component of epidermal hydrolipidic film, which can protect skin from transepidermal water loss and induce the expression of genes involved in molecular pathways that

support skin hydration and moisturization, such as hyaluronic acid synthesis.^{11,12}

To provide maximal anti-aging and brightening effects to facial skin, topical products may combine more than one active ingredient in one formulation. The aim of this study was to evaluate the synergistic anti-aging and brightening effects of topical vitamin C, vitamin E, and raspberry leaf cell culture extract on aging facial skin in women. There have been no previous published studies that have assessed the synergistic effect of these three compounds in an Asian population.

2 | MATERIALS AND METHODS

The study was conducted in a group of 50 Thai women with Fitzpatrick skin type III or IV, mean age 47 years, recruited through advertising and electronic media. Women showing the presence of facial wrinkles, skin sagging, skin dryness, or uneven skin color were included in the study. Subjects were excluded if they were under a physician's care or were taking any medication that might interfere with the test results; were using nonsteroidal anti-inflammatory drugs; had a history of hypersensitivity to cosmetics; showed signs of skin infection or inflammation; or suffered from diseases that would increase the risk associated with participation or that would interfere with the results. Women who were pregnant and lactating or intending to become pregnant during the study period were also excluded. Further exclusion criteria related to skin treatments included the use of oral retinoids, dermal fillers, RF therapy, micro-focused ultrasound therapy (in the previous year); botulinum toxin injections (in the previous 8 months); had laser resurfacing (in the previous 6 weeks); or chemical peels or dermabrasion (in the previous 2 weeks). Subjects were asked to abstain from changing any facial skin products for at least 2 weeks prior to the study and nutritional supplements for at least 1 month prior to the study.

The treatment serum (Rejuvenating Capsules, Antioxidant and Collagen Booster Serum; supplied by Belmarama, a Division of Max Biocare Pty Ltd.; manufactured by Catalent Inc) contained the ascorbic acid 20%, tocopheryl acetate 1%, and *Rubus idaeus* leaf cell culture 0.0005%. Subjects were advised to apply one capsule to the random side of the face every night for 8 weeks. The contralateral side of the face was left untreated (control side). Facial skin products based on participants' daily self-use were allowed on both sides, and per their usual routine.

Skin parameters were measured at baseline and after 4 and 8 weeks of test product use by the dermatologist in a single-blinded

fashion. Subjects were required to rinse their face thoroughly with a neutral lotion and acclimatized to the ambient environment for at least 15 minutes before measurements.

In vivo skin parameters were evaluated as following methods:

- Cutometer[®] dual MPA 580 (Courage + Khazaka Electronic GmbH) to determine skin color using melanin index parameter (Mexameter MX18[®]), elasticity using R2 parameter (Cutometer[®]), radiance using gloss DSC value parameter (Glossmeter GL200[®]), moisture (Corneometer CM825[®]), and transepidermal water loss, TEWL, (Tewameter TM300[®]) on both upper cheeks in well-defined measurement locations.
- Visioscan[®] VC98 USB (Courage + Khazaka Electronic GmbH) to determine skin microtopography using the SELS (Surface Evaluation of the Living Skin) parameters—smoothness (SEsm), roughness (SEr), scaliness (SEsc), and wrinkles (SEw) on both outer corner of the eyes in well-defined measurement locations.
- VECTRA[®] H1 (Canfield Scientific) to determine gross/visual lifting effect by measuring the distance between the upper and lower part of the face (from the hairline to the lower limit of the face corresponding to the mandible) in two points on both sides: medial and lateral canthus.

In addition, after 4 and 8 weeks of product use, the volunteers were assessed the adverse reactions by the physician and were asked to do satisfaction questionnaire regarding the anti-aging effects and the serum itself.

Statistical analysis was performed using GraphPad Prism v8.3.0 and SPSS v26. The Shapiro-Wilk test was used to determine the normality of the data distribution. The repeated measures ANOVA with Bonferroni correction and Friedman's multiple comparisons test was used to assess changes of normally distributed variables and non-normally distributed groups, respectively. Sample comparisons for TEWL were performed using the Kruskal-Wallis rank sum test, as the data were unable to be adjusted for multiple comparisons. Differences between treatment and non-treatment groups were considered biologically significant for *P*-values of .05 or less.

This study was approved by the Ethical Review Committee for Human Research, College of Integrative Medicine, Dhurakij Pundit University (003/62EX), and research protocols were conducted in agreement with the Declaration of Helsinki on human subjects. All subjects were informed and gave their consent before enrollment (clinical trial registration: TCTR20190418004, www.clinicaltrials.in.th).

3 | RESULTS

Skin was found to be significantly lighter in color and higher in elasticity on the treated side, compared to the untreated side, after 8 weeks of product use (Figure 1A,B), whereas skin radiance improved significantly after both 4 and 8 weeks of use (Figure 1C). No significant changes in skin moisture level ($P > .9999$) or TEWL

($P > .9999$) between treatment or control sides were observed (Table 1).

Skin topography assessments revealed statistically significant improvements in skin smoothness, scaliness, and wrinkles after both 4 and 8 weeks of use (Figure 2A-C). However, skin roughness was higher on the treated side compared to the untreated side on the 8th week (Figure 2D). There was no significant improvement on gross lifting effect ($P > .9999$, Table 1).

The serum was well tolerated after application. No adverse reaction in the form of redness, swelling, dryness, desquamation, itching, or burning was observed. Eight percent of subjects reported mild tightness ($n = 4$) in the 4th week, which decreased to four percent ($n = 2$) in the 8th week. Thirty-eight (76%) subjects had mild and transient tingling (mostly <1 minute) in the 4th week and reduced to twenty-five (50%) in the 8th week.

4 | DISCUSSION

Skin aging, one of the most concerned problem in women, can be noticed as wrinkles, sagging, uneven skin tone, and dull or dry skin.¹ The causes of skin aging can be categorized into intrinsic/chronological and extrinsic factors. Extrinsic factors especially ultraviolet are superimposed on intrinsic factors and account for most age-associated changes in skin appearance. Ultraviolet irradiation causes the aging skin by producing free radicals and reactive oxygen species (ROS), which interferes collagen synthesis, degrades collagen and elastin, and damages lipid component of membranes leading to ceramide and arachidonic acid release causing more water loss and more inflammation, respectively.¹³ There was previous study showed that topical antioxidants reduce oxidative damages, the cause of aging skin.^{8-10,12,14,15} To provide maximum anti-aging effect to the skin, topical products may combine various ingredients. The present evaluation revealed improvements in skin aging, pigment appearance, and skin integrity that were in agreement with the synergistic, anti-aging effects of topical vitamin C, vitamin E, and raspberry leaf cell culture extract on Asian skin types.

The product was found to have a measurable reduction in melanin index. Vitamin C is known to decrease melanin production⁵ and topical vitamin E is effective in reducing UV-induced hyperpigmentation,⁹ with the combination of both being more pronounced.¹⁰ An in vivo study by Espinal-Perez et al¹⁶ found that topical L-ascorbic acid (5%) decreased the melanin index after 3 months of treatment, whereas a study by Xu et al¹⁷ involving Chinese women found that topical L-ascorbic acid (23.8%) combined with a chemical penetration enhancer with iontophoresis improved skin lightness from the second week. This study confirmed the whitening effect of the serum. There was improvement in the melanin index at the 8th week after serum use which was earlier than the effect seen by Espinal-Perez et al, but later than that of Xu et al. Because of different parameters used, we cannot directly compare the results. But, the reason might be that the former used lower concentration of ascorbic acid while the latter used iontophoresis for enhancing penetration of vitamin C into the skin.

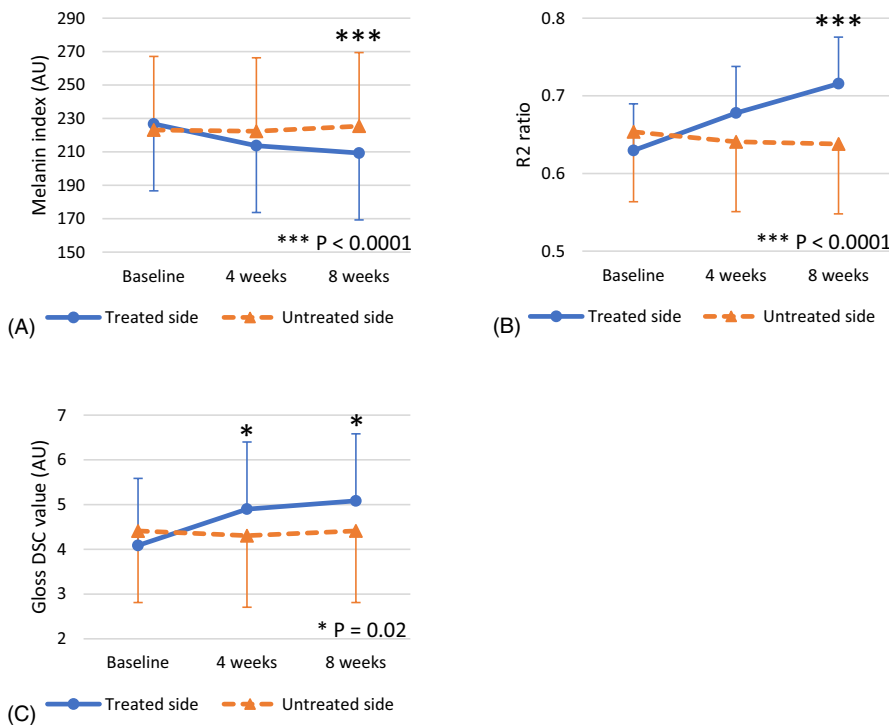


FIGURE 1 Mean melanin index (A), R2 ratio (B), and gloss DSC value (C) at baseline, 4, and 8 wk. P -value < .05, determined as significant value

TABLE 1 The results of skin parameters

Parameters	Treated side			Untreated side			P1	P2
	Baseline	4 wk	8 wk	Baseline	4 wk	8 wk		
R2 ratio	0.63 ± 0.06	0.68 ± 0.07	0.72 ± 0.06	0.65 ± 0.10	0.64 ± 0.10	0.64 ± 0.10	.07	<.0001
Melanin index	226.7 ± 42.4	213.7 ± 39.4	209.3 ± 41.2	223.1 ± 42.6	222.3 ± 44.9	225.4 ± 46.6	.557	<.0001
Gloss DSC value	4.09 ± 1.52	4.90 ± 1.65	5.08 ± 1.61	4.41 ± 1.58	4.31 ± 1.49	4.41 ± 1.71	.0221	.0201
Moisture level	73.04 ± 9.35	74.33 ± 8.46	73.77 ± 9.75	72.09 ± 9.29	73.77 ± 8.13	73.62 ± 10.06	>.9999	>.9999
TEWL (g/h/m ²)	11.48 ± 2.38	12.45 ± 2.05	11.79 ± 2.15	11.10 ± 2.04	12.87 ± 2.09	11.66 ± 2.45	>.9999	>.9999
SELS parameters								
SEsm	178.7 ± 28.2	160.9 ± 24.2	154.4 ± 23.8	171.3 ± 30.4	173.0 ± 34.5	171.8 ± 31.3	.0233	<.0001
SEr	2.98 ± 1.02	2.51 ± 0.80	2.27 ± 0.78	2.69 ± 0.90	2.74 ± 0.93	2.82 ± 0.92	.206	<.0001
SEsc	0.67 ± 0.11	0.58 ± 0.12	0.55 ± 0.11	0.67 ± 0.13	0.66 ± 0.12	0.68 ± 0.13	.0317	<.0001
SEw	67.96 ± 15.64	58.46 ± 13.41	57.21 ± 12.79	64.82 ± 14.66	65.59 ± 14.93	66.39 ± 14.77	.001	<.0001
Gross lifting effect								
Medial canthus	164.5 ± 7.4	164.7 ± 7.1	164.7 ± 6.6	164.7 ± 7.3	164.8 ± 7.2	164.6 ± 6.7	>.9999	>.9999
Lateral canthus	153.9 ± 7.3	154.1 ± 7.4	153.3 ± 6.5	153.6 ± 7.3	153.7 ± 7.2	153.4 ± 6.2	>.9999	>.9999

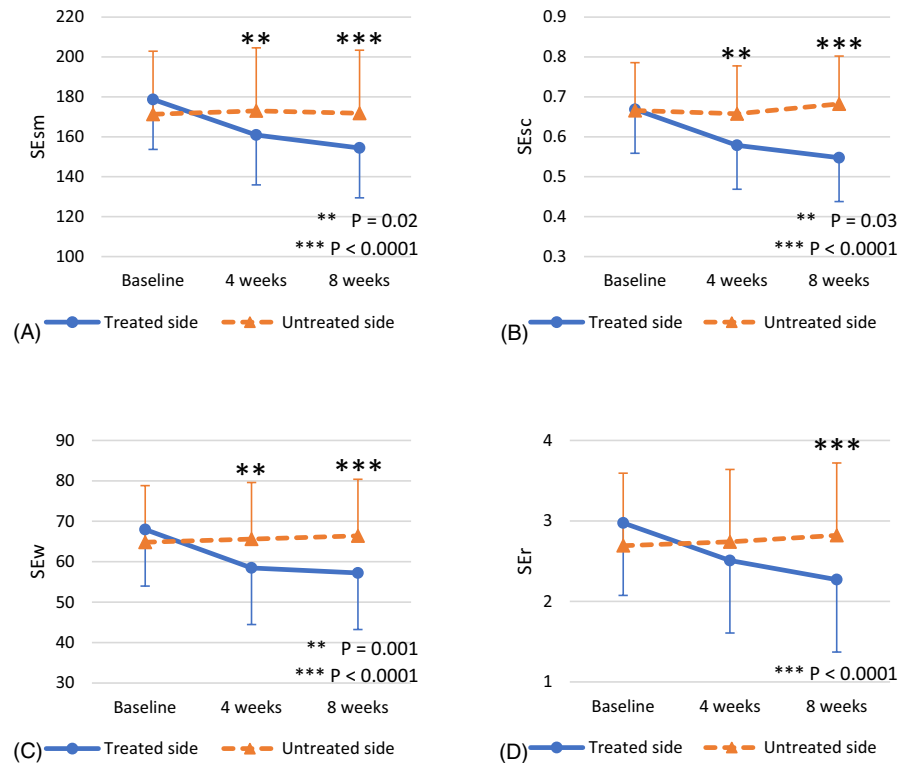
Abbreviations: P1, P -value at 4 wk (comparison to control); P2, P -value at 8 wk (comparison to control); P value < .05 considered significant; R2 ratio, skin elasticity; SEr, skin roughness; SEsc, skin scaliness; SEsm, skin smoothness; SEw, wrinkles; TEWL, transepidermal water loss (g/h/m²).

The present study found increased R2 ratio, representing increased skin elasticity, which is in accordance with other studies.^{12,14,18,19} Three active ingredients of the serum are antioxidants, so to some degree, this serum can protect collagen and elastin degradation from generated reactive oxygen species, and therefore, the increased elasticity may represent a preservation effect. Furthermore, vitamin C is also necessary for collagen biosynthesis and stability,^{4,6} and raspberry (*Rubus idaeus*) leaf cell culture extract can induce the expression of genes involved in collagen and elastin.¹² All of these effects together improve skin elasticity. Both the lightening effect and the elasticity

effect of the serum may have also combined to improve the surface integrity of skin and its ability to reflect white light, which would explain the increase in the gloss DSC value.

We observed no significant improvement of skin moisture or TEWL. This is consistent with the in vivo study by Xu et al and Campos et al^{17,20} This might have been due to the acidity of ascorbic acid, which may have caused some shedding of epidermal cells and loss of skin surface moisture. Tito et al¹² found that an oil-soluble extract of *Rubus idaeus* leaves enhanced skin moisture measured by Corneometer CM850[®] but the concentration of the extract was

FIGURE 2 Mean SEsm (A), SEsc (B), SEw (C), and SEr (D) values at baseline, 4, and 8 wk. SEsm and SEr values are inversely proportional to skin smoothness and skin roughness, respectively. *P*-value < .05, determined as significant value



much higher than that in our study. Moreover, Tito et al¹² studied in women with dry to very dry skin who applied the extract twice a day, whereas the subjects in our study had sufficiently moisturized skin (due to additional self-use of standard topical products), live in a tropical climate with high humidity, and applied the serum once a day.

Skin topography parameters of skin smoothness, scaliness, and wrinkles in our study showed significant improvements. These are in agreement with the skin elasticity and DSC gloss values. However, skin roughness gave the opposite result, which was unexpected. A possible explanation for this might be that the SEr parameter is calculated from the number of pixels which have a smaller gray level than the threshold of SEr, average number of wrinkles, and width and height of the histogram of gray level distribution. SEr is inversely proportional to number of wrinkles but directly proportional to the number of pixels which have a smaller gray level than the threshold. So, it might be that the elasticity improvement had more effect than the number of wrinkles, such as a tightening effect. However, the elasticity improvement of skin was not sufficient to see a gross contour lifting effect.

In relation to tolerability, the present study showed that the adverse effects of the serum were relatively mild and more tolerable after 1 month. Regarding this limitation, this study may be yet be inferred to middle-aged Thai or Asian women with Fitzpatrick skin type III or IV.

5 | CONCLUSIONS

This present study demonstrated that an encapsulated serum containing vitamin C, vitamin E, and raspberry leaf cell culture extract

can improve most signs of aging skin, namely skin darkening, elasticity, radiance, smoothness, scaliness, and wrinkles. However, the elasticity improvement was not sufficient to cause gross lifting effects. Furthermore, the serum was well tolerated.


ACKNOWLEDGMENT

We would like to express appreciation to all volunteers, who kindly gave excellent cooperation. Special thanks to Ms Phassara Klamsawat, Ms Phonsuk Yamlexnoi, and Mr Panyawat Wongjaruwat for their assistance in recruiting subjects and managing the database.

CONFLICT OF INTERESTS

No conflict of interests has been declared.

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How to cite this article: Rattanawiatpong P, Wanitphakdeedecha R, Bumrungrert A, Maiprasert M. Anti-aging and brightening effects of a topical treatment containing vitamin C, vitamin E, and raspberry leaf cell culture extract: A split-face, randomized controlled trial. *J Cosmet Dermatol*. 2020;19:671–676. <https://doi.org/10.1111/jocd.13305>