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Original research article

Comparative effectiveness of oral astaxanthin given daily *vs.* every other day on skin elasticity: randomized, double-blind cohort research

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ABSTRACT

Astaxanthin is an excellent antioxidant known as the "king of antioxidants". Many clinical studies have shown that astaxanthin helps delay skin ageing. Astaxanthin also has a long half-life, so astaxanthin does not necessarily have to be taken daily. However, there is no clinical study to prove this idea. Thus, 49 healthy Thai females were recruited and treated with astaxanthin at 4 mg daily or astaxanthin at 4 mg every other day for eight weeks. All volunteers were tested for the following four skin properties: elasticity, moisture, TEWL, and melanin index by using a Cutometer, Corneometer, TEWAmeter and Mexameter. The satisfaction of the volunteers was also assessed at the first visit and eight weeks after taking astaxanthin. Both groups showed significant improvements in skin elasticity after eight weeks, and there were no differences in the increase in skin elasticity of either group. Skin moisture was improved in the group taking astaxanthin daily ($p = 0.003$) and showed a tendency for improvement in the group taking astaxanthin every other day ($p=0.052$). TEWL and the melanin index were not statistically improved in either group. These results demonstrate that taking astaxanthin every other day could significantly improve skin elasticity, with no difference compared to taking astaxanthin daily. Furthermore, taking astaxanthin every other day tended to enhance skin moisture content.

Keywords: astaxanthin, antioxidant, elasticity, moisture, TEWL

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1. Introduction

Astaxanthin is a ketocarotenoid that was originally extracted from lobsters by Kuhn and Sorensen.[1] It is widely distributed, especially in seafood sources including salmon, shrimp, crayfish, and microalgae. *Haematococcus pluvialis*, a green microalga, is considered the primary natural source for human consumption.[2] Astaxanthin is an excellent antioxidant known as the "king of antioxidants".[3] It has many mechanisms for preventing skin aging, such as antioxidant, anti-inflammatory, and immune-enhancing effects. Astaxanthin can also reduce skin damage. For example, in vitro, astaxanthin suppresses cell damage caused by free radicals and the induction of MMP-1 in the skin after UV irradiation.[4] It can also increase collagen content through inhibition of MMP-1 and MMP-3 expression in human dermal fibroblasts.[5] During wound healing, astaxanthin is an effective compound for accelerating wound healing in full-thickness dermal wounds in mice. Astaxanthin has shown significantly increased expression of wound healing biological markers such as collagen type Iα1 and basic fibroblast growth factor (bFGF) .[6] Moreover, astaxanthin is reported to improve the DNA repair capacity of cells exposed to UV radiation. Astaxanthin inhibits UV-induced DNA damage and increases the expression of oxidative stress-responsive enzymes.[7]

Several clinical studies have reported the effects of astaxanthin in wrinkle reduction, photo- aging inhibition, suppression of melanin synthesis and decreased hyperpigmentation.[2,7,8] In the skin, astaxanthin has been shown to improve skin elasticity, texture, and moisture content.[9-12] Concerning the pharmacokinetic properties of astaxanthin, it is absorbed via passive diffusion in the intestinal cells. The maximum concentrations of natural astaxanthin derived from microalgae *Haematococcus pluvialis* are observed mainly between 7 hours and 21 hours after intake and range from 0.055 to 1.3 mg/L.[2] In a study by Okada et al, the elimination half-life of astaxanthin was reported to be about 24 hours when taken before food and about 30 hours when taken after food. The AUC for astaxanthin in the after-meal group was found to be 2.4 times higher than in the before-meal group. Smoking was found to decrease bioavailability.[13] Astaxanthin has a long half-life, so it does not necessarily need to be taken daily. However, there is no clinical study to prove this idea.

In the present study, therefore, we evaluated the effects of dietary supplementation with astaxanthin given daily vs. every other day on skin deterioration. We set skin elasticity as the primary outcome and other skin conditions, including moisture, TEWL and melanin index, as secondary outcomes. In addition, subjective skin conditions and safety evaluation were also assessed.

2. Materials and Methods

2.1 Study design, randomization and blinding

We performed a randomized, double-blind, cohort design comparison trial to evaluate the effects of oral astaxanthin given daily vs. every other day on skin elasticity, skin moisture, TEWL and melanin index in healthy Thai females for eight weeks. This study was approved by the Institutional Review Board of Dhurakij Pundit University (EC approved no. 014/63EX), and written informed consent was obtained from all subjects participating in the trial. In this study, the participants were divided by the block randomization method (block size of 4) into experimental and control groups in which the participants, practitioners and clinicians were blinded. The control group received astaxanthin divided into small bags containing one tablet per bag with the date of intake written on the front of each one. The experimental group received the above-mentioned astaxanthin alternate with one placebo tablet per bag by writing the date of intake on the front of each one. The participants took one tablet after breakfast once a day for eight weeks. All participants

were tested to measure the following four properties of the skin: elasticity, moisture, TEWL, and melanin index by using Cutometer, Corneometer, TEWAmeter and Mexameter. The researchers also assessed the satisfaction of the participants at the first visit and at eight weeks after taking astaxanthin. Allocation was concealed until all participants had finished the tests.

2.2 Study Participants

A total of 51 healthy Thai females aged 30-45 years were enrolled in the study. This study consisted of a supplementation period for eight weeks from March to May 2021. Participants with the following criteria were included: (1) age from 30 to 45 years and BMI between 18.5 and 29.9 at the time informed consent was provided; (2) no work in the sun; (3) ability to visit the administrative facility on every test day. Participants with the following criteria were excluded from the study: (1) menopausal women; (2) behavior or use of oral medications or injections affecting the absorption of astaxanthin, such as smoking; (3) history of allergies to any component of trial foods, such as soybean oil; (4) skin disease such as allergic rashes, inflammation or acne at the site to be tested; (5) intake of medicines, food or dietary supplements affecting skin conditions such as isotretinoin or tranexamic acid during the testing; (6) failure to take the supplement for more than 2 consecutive days or missing more than 4 doses within a 2-week period; (7) use of new cosmetics and creams during the experiment or discontinuation of old products; (8) botulinum toxin injection at the test site within 6 months before the test; (9) injection of fillers or stem cells in the area to be tested within 1 year before the test; (10) HIFU within 6 months before the trial; (11) ulthera or thermage within 12 months before the trial; (12) pregnancy or lactation when the informed consent was provided, or intention to become pregnant during the trial; (13) request for withdrawal from the study.

2.3 Supplement Formulation

The dietary supplement was administered in the form of 1 capsule containing 4 mg astaxanthin in a dark green capsule (size = No. 0). The placebo was administered in the form of 1 capsule containing maltrodextrin instead of astaxanthin in a dark green capsule (size = No. 0). There were no differences in the external appearance of the dietary supplement and the placebo. For the astaxanthin capsule, we used natural astaxanthin derived from *Haematococcus pluvialis* (Astax®, Mega Lifesciences Co., Ltd., Samutprakarn, Thailand).

2.4 Measurement Parameters

1. Elasticity: The skin on the outer corner of the left eye (1 cm laterally from the lateral canthus) was measured by using the Cutometer® MPA 580 (Courage+ Khazaka electronic GmbH, Cologne, Germany).

2. Moisture: The skin on the right side of the forehead (2 cm above the right eyebrow at the mid pupillary line) was measured by using the Corneometer® (Courage+Khazaka electronic GmbH, Cologne, Germany).

3. TEWL: The skin on the inside of the left ear pole (2 cm inside of inferior auricular root) was measured by using the TEWAmeter® (Courage+Khazaka electronic GmbH, Cologne, Germany).

4. Melanin Index: The skin on the left side of the forehead (2 cm above the left eyebrow at the mid pupillary line) was measured by using the Mexameter® (Courage+Khazaka electronic GmbH, Cologne, Germany).

5. The patients' evaluation of their skin and side effects (questionnaires) by using patient satisfaction scores where 0 meant no change/ dissatisfaction and 10 meant significant change/high satisfaction.

2.5 Statistical Analyses

In this comparative study, the effects of oral astaxanthin given daily *vs.* every other day on skin elasticity and moisture content were analyzed by paired t-test for matched pairs. The differences between groups for oral astaxanthin given

daily and every other day on the skin were analyzed by an independent t-test. This methodology was chosen for the data following a normal distribution. In TEWL and melanin index, the Wilcoxon test for matched pairs was used. The differences between groups for oral astaxanthin given daily and every other day were analyzed by the Mann-Whitney U test. This methodology was chosen for the data that did not quite follow a normal distribution.

3. Results

Fifty-one participants were included in our study. These participants were assigned to the group administered astaxanthin every other day (treatment group) (n=25) or to the group administered astaxanthin daily (control group) (n=26). One participant in the treatment group and one participant in the control group were excluded from the analysis due to not taking dietary supplements continuously. Finally, 24 participants in the treatment group (mean age 36.13±4.29, mean BMI= 23.26±2.79) and 25 participants in the control group (mean age 36.24±4.5, mean BMI= 22.62±3.23) were analyzed. Baseline values were not significantly different between groups (Table 1).

Table 1. Baseline demographics and baseline values of skin parameters.

	Astaxanthin EOD (n=24) $\bar{x}$**±SD**	**Astaxanthin OD (n=25)** $\bar{x}$**±SD**	***p*-value[a]**
Age (yrs.)	36.13±4.29	36.24±4.5	0.46
BMI	23.26±2.79	22.62±3.23	0.23
Elasticity, R2	0.6130±0.1105	0.6415±0.0882	0.16
Moisture	63.96±13.44	60.74±9.44	0.17
TEWL	18.57±5.81	17.4±3.63	0.2
Melanin index	312.17±61.13	297±56.8	0.43

TEWL, transepidermal water loss. [a]By independent t-test. $\bar{x}$, mean.

3.1 Elasticity

In the treatment group, elasticity (R2) of the skin was significantly improved from 0.6130±0.1105 to 0.7942±0.1105, $p<0.001$, at week 8. In the control group, elasticity (R2) of the skin was significantly improved from 0.6415±0.0882 to 0.7901±0.0696, $p<0.001$, at week 8. A similar improvement was observed, with no statistically significant differences found between the two groups (Fig.1, Table 2).

3.2 Moisture Content

In the treatment group, the moisture content of the skin increased from 63.96±13.44 to 67.46±10.82, $p=0.052$, but no statistically significant differences were found at week 8. However, a tendency to improve skin moisture content was observed. In the control group, the moisture content of the skin significantly improved from 60.74±9.44 to 65.26±11.24, $p=0.003$, at week 8 (Fig. 2, Table 3).

3.3 TEWL

In the treatment group, TEWL increased from 18.57±5.81 to 18.87±9.12, $p=0.079$, with no statistically significant differences found at week 8. In the control group, TEWL increased from 17.4±3.63 to 18.63±5.5, $p=0.071$, with no statistically

significant differences found at week 8 (Fig. 3, Table 4).

3.4 Melanin Index

In the treatment group, the melanin index decreased from 312. 17±61. 13 to 302.67±60.72, p=0.34, with no statistically significant differences found at week 8. In the control group, the melanin index increased from 297±56. 8 to 307±59. 64, p= 0. 2, with no statistically significant differences found at week 8 (Fig. 4, Table 5).

3.5 Skin Condition Evaluated by the Subjects' Self-Assessment and Clinical Safety

Participant satisfaction scores were determined by self-assessment at week 8 and came out positively (Fig. 5, 6). Treatment was well-tolerated, and no subjective adverse events were reported during the 8-week trial period.

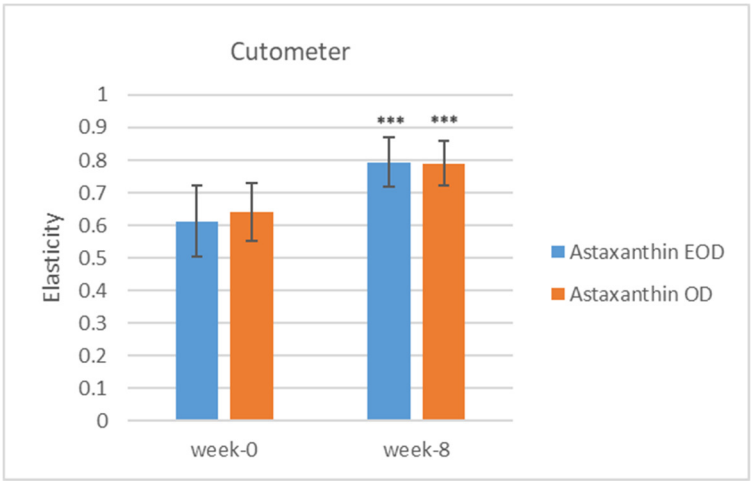

Fig.1. Skin elasticity for astaxanthin administered daily or every other day.
Notes: *** p<0.001 *vs.* pre-treatment.

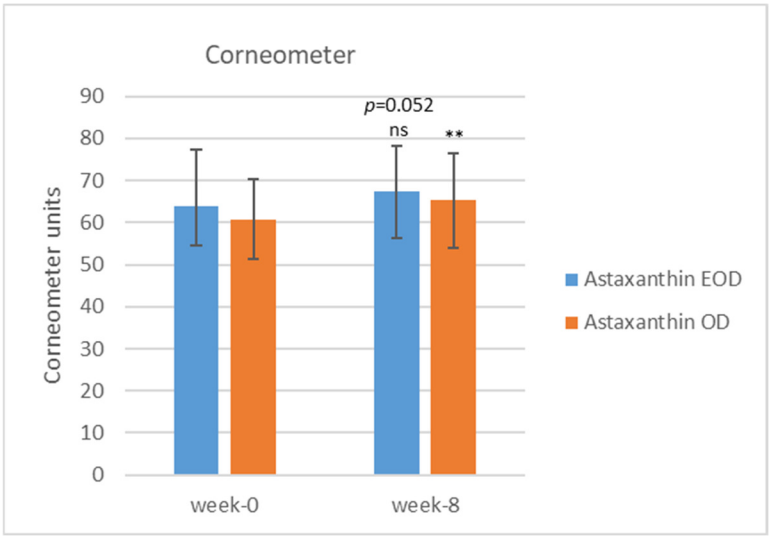

Fig. 2. Moisture content for astaxanthin administered daily or every other day.
Notes: ** p=0.003 *vs*. pre-treatment. ns, not significant at p>0.05.

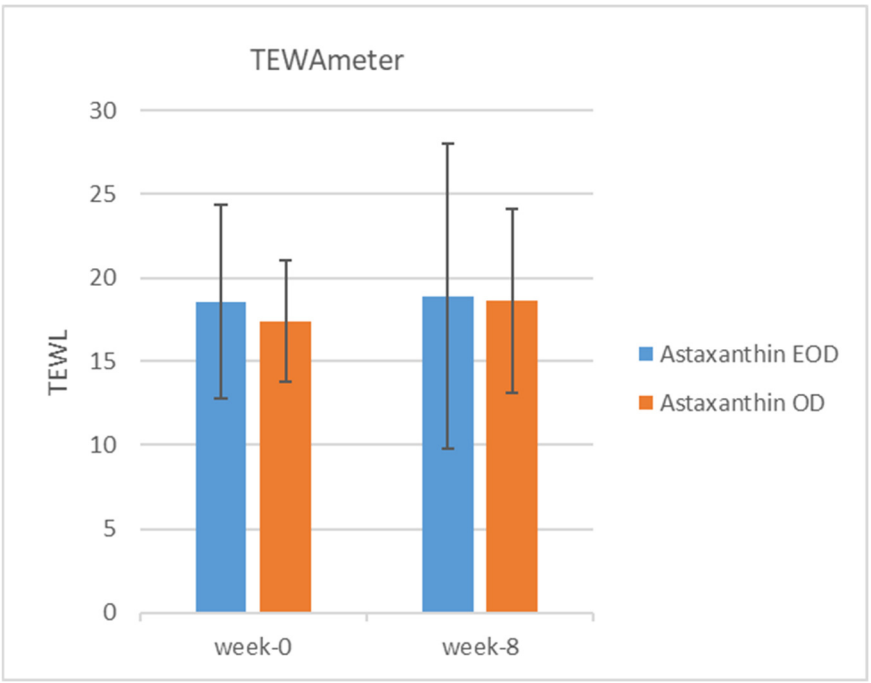

Fig. 3. TEWL for astaxanthin administered daily or every other day**.**

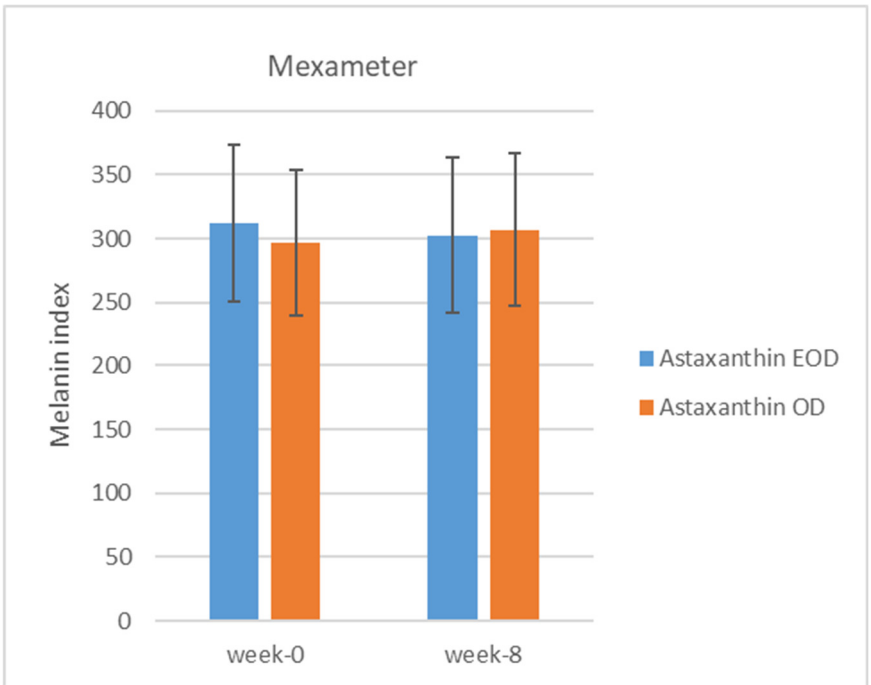

Fig. 4. Melanin index for astaxanthin administered daily or every other day**.**

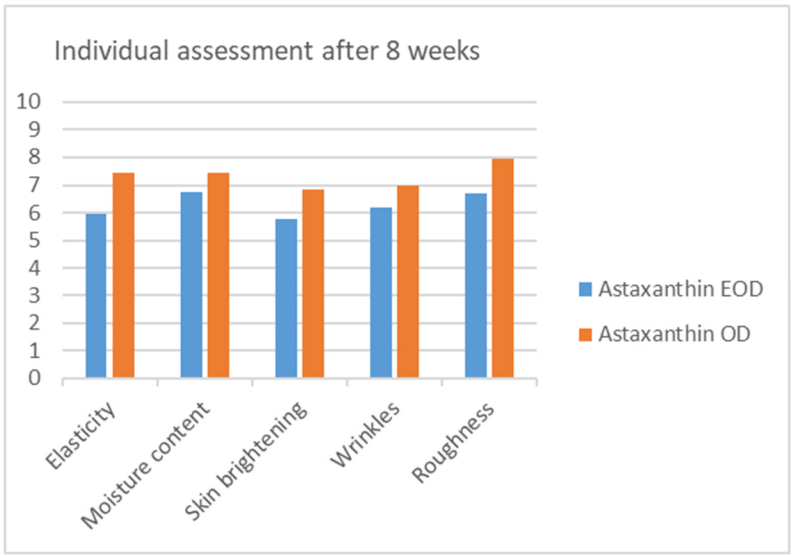

Fig. 5. Subjective Skin Conditions: elasticity, moisture content, brightening, wrinkles and roughness all improved with administration of astaxanthin daily or every other day.

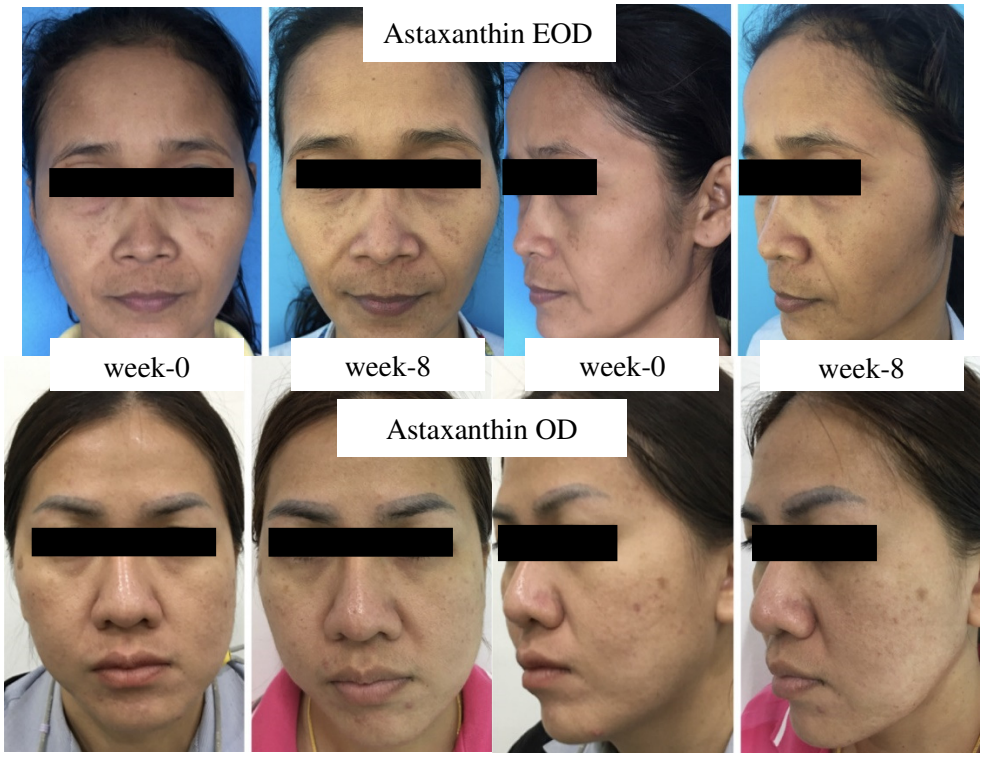

Fig. 6. Skin surface photographs.

Table 2. Mean, mean difference and standard deviation (SD) of skin elasticity at weeks 0 and 8 of treatment with astaxanthin in the groups administered every other day or daily.

Elasticity	**Astaxanthin EOD (n=24)** $\overline{x}$**±SD**	**Astaxanthin OD (n=25)** $\overline{x}$**±SD**	***p*-value[a]**
Pre-treatment	0.6130±0.1105	0.6415±0.0882	0.161
After 8 weeks	0.7942±0.1105	0.7901±0.0696	
$\overline{D}$±SD	0.1813±0.1003	0.1487±0.0639	0.09
p-value[b]	<0.001***	<0.001***	

Notes**:** [a]By independent t**-**test**.** [b]By paired t**-**test**.**
$\overline{x}$, mean**.** $\overline{D}$, mean difference**.**

Table 3. Mean, mean difference and standard deviation (SD) of moisture content at weeks 0 and 8 of treatment with astaxanthin in the groups administered every other day or daily.

Moisture	**Astaxanthin EOD (n=24)** $\overline{x}$**±SD**	**Astaxanthin OD (n=25)** $\overline{x}$**±SD**	***p*-value[a]**
Pre-treatment	63.96±13.44	60.74±9.44	0.168
After 8 weeks	67.46±10.82	65.26±11.24	
$\overline{D}$±SD	3.50±10.14	4.52±7.38	0.344
p-value[b]	0.052	0.003**	

Notes: [a]By independent t-test. [b]By paired t-test.
$\overline{x}$, mean. $\overline{D}$, mean difference.

Table 4. Median, median difference and standard deviation (SD) of transepidermal water loss (TEWL), at weeks 0 and 8 of treatment with astaxanthin in the groups administered every other day or daily.

TEWL	**Astaxanthin EOD (n=24) (median±SD)**	**Astaxanthin OD (n=25) (median±SD)**	***p*-value[c]**
Pre-treatment	18.57±5.81	17.4±3.63	0.2
After 8 weeks	18.87±9.12	18.63±5.35	
Median difference	0.42±5.92	1.13±4.11	0.39
p-value[d]	0.079	0.071	

Notes: [c]By Mann-Whitney U test. [d]By Wilcoxon test.

Table 5. Median, median difference and standard deviation (SD) of melanin index at weeks 0 and 8 of treatment with astaxanthin in the groups administered every other day or daily.

Melanin index	Astaxanthin EOD (n=24) (median±SD)	Astaxanthin OD (n=25) (median±SD)	*p*-value[c]
Pre-treatment	312.17±61.13	297±56.8	0.43
After 8 weeks	302.67±60.72	307±59.64	
Median difference	-7.84±25.53	2.67±20.27	0.08
p-value[d]	0.34	0.2	

Notes: [c]By Mann-Whitney U test. [d]By Wilcoxon test.

4. Discussion

Medicinal plants have been used in Thailand from the past to the present, and nowadays, they are popular to be developed into products in various forms such as medicines, dietary supplements, cosmeceuticals, and cosmetics. Consumer behavior is increasingly turning to natural products to avoid the side effects of synthetic chemicals. Many plants in nature are rich in compounds that can fight free radicals and inhibit the formation of melanin pigment effectively. *D. regia* plant contains polyphenolic compounds such as flavonols, anthocyanins, and phenolic acids as bioactive secondary metabolites that are responsible for their antioxidant activity.[1] This is the first study to compare the effectiveness of taking a dietary supplement of astaxanthin 4 mg daily versus every other day on skin elasticity, skin moisture, TEWL and melanin index. It was found that the skin elasticity of both groups improved with statistical significance, but there were no statistically significant differences between the two groups. The moisture content of the skin was statistically and significantly improved in the 4 mg daily astaxanthin group, and the 4 mg every-other-day astaxanthin group showed a strong tendency for improvement in the moisture content of facial skin (p-value = 0.052). TEWL and melanin index showed no statistically significant improvements in either group.

Skin ageing is the result of both intrinsic and extrinsic processes, which contribute simultaneously to a progressive loss of skin integrity.[14-15] The mechanism of elasticity improvement by astaxanthin could be explained as a result of collagen fibre recovery. Astaxanthin promotes collagen fibre recovery by protecting the dermal layer from singlet oxygen damage.[11,16] In addition, astaxanthin inhibits the expression of matrix metalloproteinases in different cells, including macrophages and chondrocytes. Thus, astaxanthin can suppress the destruction of collagen, elastin, and glucosaminoglycans. Moreover, it can also increase the expression of wound healing biological markers such as collagen type Iα1 and basic fibroblast growth factor (bFGF) .[6] We evaluated the effects of astaxanthin 4 mg daily compared with 4 mg every other day for eight weeks. The findings of this study confirm the idea of not having to take astaxanthin every day for people seeking improved skin elasticity. According to the findings in both groups, skin elasticity was significantly improved after 8 weeks of treatment and there were no differences in the increase in skin elasticity for either group. Hence, administering astaxanthin at only 4 mg every other day is enough to help improve skin elasticity, possibly because astaxanthin has a long half-life of 24-30 hours.[13]

Both UV radiation and dryness cause the progression of wrinkle formation. UV irradiation contributes to wrinkle formation by inducing MMP secretion from dermal fibroblasts via cytokines, such as IL-1α, IL-6, and TNF-α, released by UVB-exposed keratinocytes. [12] IL-1α may also induce other proinflammatory cytokines such as IL-6 and IL-8. [12,17] Therefore, the mechanism by which astaxanthin improves skin moisture levels may be due to astaxanthin inhibiting both inflammatory cytokine secretion from epidermal keratinocytes and MMP-1 secretion by dermal fibroblasts in response to UVB irradiation. [12] The findings of this study indicate that taking 4 mg of astaxanthin daily improved skin hydration after the end of 8 weeks, which was significantly different from astaxanthin every other day. The group who took 4 mg of astaxanthin every other day showed a strong tendency for improved moisture content of the skin (p-value = 0.052) . Therefore, increasing the study period for the every other day group might lead to more skin moisturizing effects. After evaluating the results of the study, it can be concluded that astaxanthin can improve both skin elasticity and the moisture content of the skin, but a more extended period of administration might be required to improve the moisture content of the skin.

TEWL is a marker for the barrier functions in the corneocyte layer. Taking astaxanthin might normalize the corneocyte condition, protecting against keratinocyte differentiation and cornification from oxidative damage-induced inflammation in the epidermis. Atopic skin patients who have high TEWL may be treated by taking astaxanthin.[11] In this study, it was found that taking astaxanthin at 4 mg daily or every other day does not reduce TEWL, possibly because the supplement dosage or study period were insufficient.

This study found that neither group of astaxanthin administration had an improved melanin index, possibly because this study was conducted during the summer of Thailand and the fruit harvesting period. Although most of the research participants were civil servants who did not have direct exposure to the sun, the situation of the COVID-19 outbreak made the borders close and foreign workers difficult to find. Consequently, most of the volunteers had to go and help harvest the fruit, whether in their own orchards or those of acquaintances. Therefore, the participants were exposed to more sunlight than usual. As a result, the interpretation of the results in this study might be incomplete. If the study had been conducted during a period without this limitation or with an increased dosage of astaxanthin or an extension of the study period, the results might have been better. If the melanin index is improved, the effect on melanin levels should be studied further later.

5. Conclusion

The results showed that taking astaxanthin at 4 mg every other day significantly improved skin elasticity with no difference compared to taking 4 mg astaxanthin daily. Furthermore, taking 4 mg astaxanthin every other day tended to improve skin moisture content. This is only a preliminary study to determine the effect of astaxanthin. Further studies should be performed to see results at 2, 4, 6, 8 weeks or more, to see the direction of the results when the maximum effect is achieved. A long-term follow-up could show when the effect would decrease after stopping astaxanthin supplementation.

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Conflicts of Interest

The authors declare no conflict of interest.

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