

# EFFECTS OF ORAL SUPPLEMENTATION AND TOPICAL APPLICATION OF SUPERCRITICAL CO<sub>2</sub> EXTRACTED SEA BUCKTHORN OIL ON SKIN AGEING OF FEMALE SUBJECTS

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## Summary

Sea buckthorn (*Hippophaë rhamnoides*) seed and pulp oil is known to promote skin regeneration, speed up wound healing and ease skin inflammation. The present study was carried out to investigate the anti-ageing effects of oral supplementation of a standardized supercritical CO<sub>2</sub>-extracted sea buckthorn oil and topical skin application of supercritical CO<sub>2</sub>-extracted sea buckthorn seed oil. Sixty female subjects of age 50-70 years were randomly divided into two groups, thirty subjects in each group. In one group, the subjects took sea buckthorn oil capsules, four capsules (4 x 0.5 g oil) per day, for three months. In the other group, the subjects applied topically sea buckthorn seed oil rejuvenating night cream on the face, twice per day, for three months. Skin hydration status, elasticity, surface roughness, luminosity and cutaneous thickness were determined using non-invasive instrumental measurements before, after one month and at the end of treatments. Both treatments significantly improved the skin hydration status and the overall skin elasticity of the subjects ( $P < 0.001$ ). Oral supplementation with the sea buckthorn oil capsules resulted in decreases in the mean roughness (Ra) and maximum roughness (Rz) of the skin surface, indicating anti-wrinkle efficacy of the product. Topical application of the sea buckthorn oil cream increased cutaneous thickness, suggesting

positive structural changes and improvement in collagen synthesis in the skin. This is the first study showing beneficial effects of orally used sea buckthorn oil in reducing the signs of skin ageing. This is also an important breakthrough demonstrating the great potential of sea buckthorn oil in supporting the health and well being of healthy skin through internal use.

## Riassunto

E' noto come l'olio ricavato dai semi e dalla polpa dell'olivello spinoso promuova la rigenerazione della pelle, accelera la sua cicatrizzazione riducendo i processi infiammatori.

Il presente studio è stato condotto per verificare gli effetti anti-età della supplementazione orale dell'olio di olivello spinoso estratto dai suoi semi con il metodo di CO<sub>2</sub> ad alta pressione.

Sessanta donne di età compresa tra i 50 e i 70 anni sono state suddivise, in modo casuale, in due gruppi da 30 individui. In un gruppo i soggetti assumevano 4 capsule al giorno dell'olio (4x0,5 g) per tre mesi. Nell'altro gruppo i soggetti applicavano topicamente due volte al giorno, sempre per tre mesi, una crema da notte contenente lo stesso olio.

Dopo il primo mese ed alla fine del trattamento sono state controllate con metodologia non invasiva l'idratazione, l'elasticità, la rugosità, la luminosità e lo spessore della cute.

Entrambi i trattamenti hanno migliorato significativamente lo stato di idratazione e l'elasticità ( $p < 0,001$ ).

La supplementazione orale con le capsule dell'olio di olivello spinoso poneva in risalto sia una riduzione della rugosità media (Ra) che di quella massima (Rz) riscontrata a livello della superficie cutanea, ponendo in risalto l'efficacia anti-rughe del prodotto.

L'applicazione topica dello stesso olio incrementava lo spessore medio della pelle raggiungendo un positivo incremento della produzione di collagene.

Questo è il primo studio che, ponendo in evidenza l'effetto benefico che l'olio di olivello spinoso svolge nel ridurre i segni dell'età, dimostra la grande potenzialità insita nel suo uso per migliorare il benessere generale e l'aspetto della cute.

## INTRODUCTION

Lipids are important components of the diet for maintaining the health and the functions of the skin. Triacylglycerols, free fatty acids, sterols, squalene, ceramides and some polar lipids are essential components of the stratum corneum, forming the barrier structure of the skin (1). Phospholipids and sterols are components of cell membranes responsible for both the transduction of signals and the transportation of substances. Polyunsaturated fatty acids are precursors of local hormones regulating the inflammation and proliferation of skin cells. Insufficient or unbalanced dietary intake of lipids results in dry, scaly and inflammatory skin (2).

Polyunsaturated fatty acids have been shown to have potential in inhibiting melanin synthesis and reducing UV-induced hyperpigmentation (3). In topical applications, CO<sub>2</sub>-extracted lingonberry seed oil rich in linoleic (18:2n-6) and  $\alpha$ -linolenic (18:3n-3) acids increased skin moisture and lightened the skin tone of normal skin and age spots (4).

Oxidation damages of cellular components are often the primary cause of aging and onset of diseases. Environmental factors such as UV-radiation, pollution and temperature change pose constant oxidative stress to the skin. A proper supply of antioxidants through diet and dietary supplementation forms an indispensable element of modern skin care.

Sea buckthorn (*Hippophaë rhamnoides*) is a Eurasian plant that has been used in traditional health care since ancient times. The berry of sea buckthorn is a rich source of a wide range of lipophilic and hydrophilic bioactive compounds. Sea buckthorn seed oil is rich in the two essential fatty acids, linoleic and  $\alpha$ -linolenic acids. Oil isolated from the pulp/peel fraction of sea buckthorn berries contains high levels of monounsaturated fatty acids, palmitoleic acid (16:1n-7) and oleic acid (18:1n-9). Both seed oil and pulp

oil are rich sources of tocopherols, tocotrienols, plant sterols and natural carotenoids. The carotenoid content can be as high as 0.3-0.5% in the pulp oil (5). Sea buckthorn seed oil and pulp oil have been shown to inhibit lipid oxidation, protect cell membranes from oxidation damage, reduce inflammation and promote tissue regeneration (6; 7; 8; 9; 10; 11).

Sea buckthorn pulp oil and seed oil from different sources have been shown to be effective in treating skin wounds, burns and scalds (12; 13; 14) as well as irradiation dermatitis (15; 16). Topical application of test creams containing 3-5% CO<sub>2</sub> extracted sea buckthorn seed oil or pulp oil effectively scavenged free radicals formed by UVA radiation (by 40-50%) and protected skin lipids from UVA-induced photo-oxidation by 60-80%. The test creams also reduced skin inflammation and speeded up the restoration of skin barrier function after repeated tape-stripping (17). Oral supplementation of capsules of supercritical CO<sub>2</sub>-extracted sea buckthorn seed oil and pulp oil improved the conditions of atopic skin. The symptom improvement in the seed oil group was positively correlated with the incorporation of sea buckthorn fatty acids into the plasma lipids (18). So far, no scientific reports have been published on the effects of sea buckthorn oil on healthy skin.

## MATERIALS AND METHODS

The aim of the study is to investigate the effects of oral supplementation and topical application of supercritical CO<sub>2</sub>-extracted sea buckthorn oil on healthy mature skin using non-invasive skin measurement methods (19; 20). Changes in skin hydration, elasticity, colorimetry, skin roughness and cutaneous thickness were investigated during and after the treatments.

## SUBJECTS

Sixty Caucasian females of age 50-70 (mean age 61 years) were recruited. The subjects did not have skin diseases or other pathological events during the period immediately before or during the study period. Other exclusion criteria included topical application and systematic use of drugs, pregnancy, breast-feeding, and history of intolerance of drugs and cosmetic products. All the subjects were informed of the purpose and the procedures of the study and signed written consents before starting the trial. The subjects were allowed to interrupt the treatments based on their own will or medical reasons related or not related to the treatments. Details of any cases of dropping out were reported.

## TEST PRODUCTS

The sea buckthorn oil capsule (Aromtech Ltd, Kiviranta/Tornio, Finland) was a vegetable based product, with filling material consisting of 100% SBA24® Sea Buckthorn Oil, a standardised composition containing CO<sub>2</sub> extracted sea buckthorn seed and pulp oil. The sea buckthorn

oil cream (LUMENE Group, Espoo, Finland) was a rejuvenating night cream containing 1% sea buckthorn seed oil manufactured by Aromtech using supercritical CO<sub>2</sub> extraction technology. The cream was an oil-in-water-emulsion with an oil/aqueous-phase ratio of approx. 27/73. The active ingredients of the cream include sea buckthorn seed oil, acetyl hexapeptide-8, palmitoyl pentapeptide-4, glycerin, biosaccharide gum-1, saccharide isomerate, sodium hyaluronate. The composition of SBA24® Sea Buckthorn Oil and supercritical CO<sub>2</sub>-extracted sea buckthorn seed oil is presented in Table I.

## STUDY DESIGN

The study was carried out at the Institute of Skin and Product Evaluation (Milan, Italy) in compliance with the principles of Good Laboratory Practice and Good Clinical Practice as well as the principles established by the World Medical Association in the Declaration of Helsinki. The study was approved by the Ethical Committee of ISPE srl.

**TABLE I**

*Composition of SBA 24 sea buckthorn oil and sea buckthorn seed oil*

	Fatty acids (weight %)						
	16:0	16:1n-7	18:0	18:1n-9	18:1n-7	18:2n-6	18:3n-3
<b>Seed oil</b>	7	1	2	18	3	37	31
<b>SBA 24</b>	24	24	1	14	5	18	13
	Carotenoids (mg/100 g)		Plant sterols (weight% of oil)		Tocopherols and tocotrienols (mg/100 g)		
<b>Seed oil</b>	30		0.5		100		
<b>SBA 24</b>	180		1.2		400		

The subjects were randomly divided into two groups, thirty subjects in each group. In group I, the subjects took 4 capsules per day for three months. The subjects were advised not to apply any skin care products on the forehead. In group II, the subjects applied the sea buckthorn test cream on the face. All the subjects were advised to avoid UV radiation on the skin of the face and forehead throughout the duration of the study. At the beginning, after 1 month and 3 months of treatment, non invasive instrumental measurements of the selected parameters were performed on the forehead of the subjects of group I and on the periocular area of the subjects of group II. Instrumental measurements included skin hydration, skin elasticity, skin surface roughness, skin luminosity, and cutaneous thickness. The instrumental measurements were carried out in a bioclimatic room (temperature:  $24 \pm 2^\circ\text{C}$ ; relative humidity:  $50 \pm 10\%$ ).

## Instrumental measurements

### Hydration status

The skin hydration status was measured using a Corneometer CM 825 (Courage + Khazaka Electronic GmbH, Köln, Germany). The physical principle the instrument is based on the measurement of capacitance (21). The device consists of a square-shaped sensor (area:  $49\text{ mm}^2$ ) on a mobile axis connected to the base unit by a spiral cable. When the front surface of the sensor is pressed against the skin, a number appears on the liquid crystal display. This reading, directly proportional to the amount of water contained in stratum corneum and the hydration level of cutaneous surface, is expressed in corneometric unit (c.u.), an arbitrary unit of the instrument. The measurement range of the corneometer was 0-150 c.u.

### Elasticity

The elasticity of the skin was measured using a Cutometer SEM 575 (Courage + Khazaka Electronic GmbH, Köln, Germany). The instrument measured the vertical deformation of the skin sucked into the opening of a measuring probe by a constant negative pressure of 350 mbars lasting for one second. The negative pressure was then annulled, and the skin was allowed to relax for two second. An optical system measured the variations in electrical capacitance, which were proportional to the rise (in millimeters) of the skin surface being measured. Three measurement cycles (1 cycle: suction/release) were performed for each measurement. The three suction/release cycles represented three successive curves providing the deformation parameters relating to the elastic features of the skin (22; 23). Figure 1 presents the demonstration of the deformation curve of one measurement cycle. The skin rises are shown on Cartesian axes, where the deformation of the skin (expressed in mm) is a function of time (expressed in seconds). The effects of the supplementation on skin elasticity were investigated by monitoring the changes in maximal deformation ( $R0 = U_F$ ), overall elasticity ( $R2 = U_A / U_F$ ), and viscoelastic ratio ( $R6 = U_V / U_E$ ) during and after the treatments.  $U_A$ ,  $U_V$ , and  $U_E$  represent total deformation recovery at the end of the stress-off period, visco-elastic creep occurring after the elastic deformation, and elastic deformation of the skin due to the application of stress delivered by the instrument, respectively.

### Luminosity

Skin luminosity ( $L^*$  value) was measured using a Chroma Meter CR-300 (Minolta Camera Co. Ltd, Osaka, Japan).  $L^*$  values ranged from 0 to 100, where 0 corresponded to black colour and 100 to white (24).

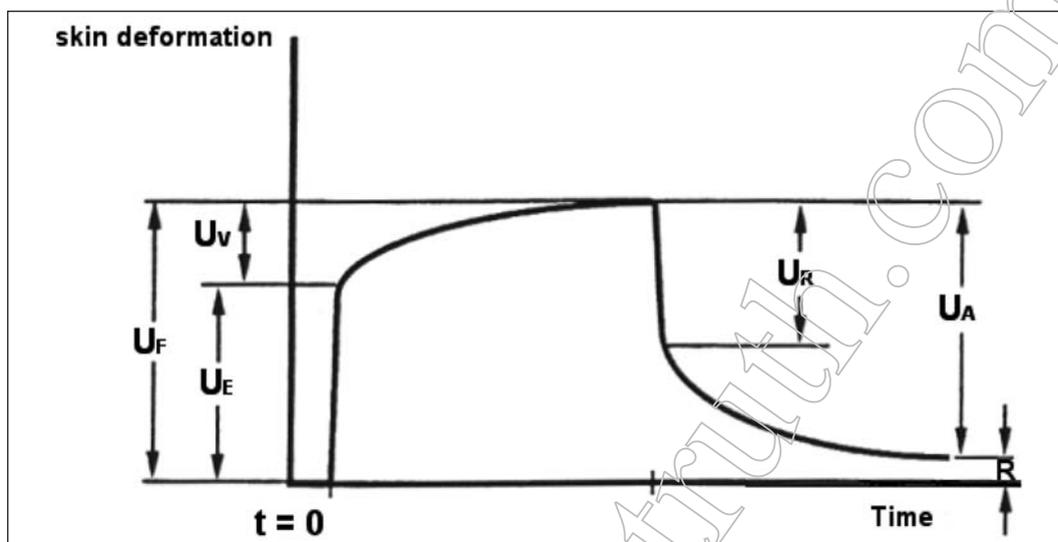


Fig. 1 Skin deformation curve of one measurement cycle using a cutometer.  $U_E$ , elastic deformation of the skin due to the application of stress by the instrument;  $U_V$ , viscoelastic creep occurring after the elastic deformation;  $U_R$ , elastic deformation recovery due to stress removal;  $U_A$ , total deformation recovery at the end of the stress-off period;  $U_F$ , maximal deformation of the skin.

## Surface roughness

To obtain negative imprints of the skin surface, skin replicas were prepared using adhesive discs with an internal diameter of 24 mm and an external diameter of 40 mm (3M, St. Paul, Minnesota) and fast hardening synthetic polymer SILFLO (Flexico Ltd, Potters Bar, Herts, United Kingdom). The adhesive disc was put onto the subject's skin in order to delimit the investigated area and to avoid skin stretching during the polymer application. A small amount of polymer was then spread onto the internal area of the disc and left *in situ* for a few minutes until it hardened. The disc was then removed, and a replica with negative imprints of surface structure of the skin obtained.

The skin replicas were then analysed by an image analysis system Quantilines (Monaderm, Monaco) allowing analysis of a range of relief parameters (25; 26). The parameters  $R_a$  representing mean roughness and  $R_z$  representing

maximum roughness were calculated and used for evaluation of the effects of sea buckthorn oil treatment on skin surface roughness (27). An image covering an area of 12 x 9 mm<sup>2</sup> of the surface of each skin replica was acquired through a High Performance CCD video-camera (Cohu, Inc., San Diego, CA, USA).

## Cutaneous thickness

The thickness of the skin was measured using a Dermascan C<sup>®</sup>, high resolution ultrasound scanner (Cortex Technology, Denmark) operated in A-scan (amplitude-scan) mode (28; 29; 30).

## Data analysis

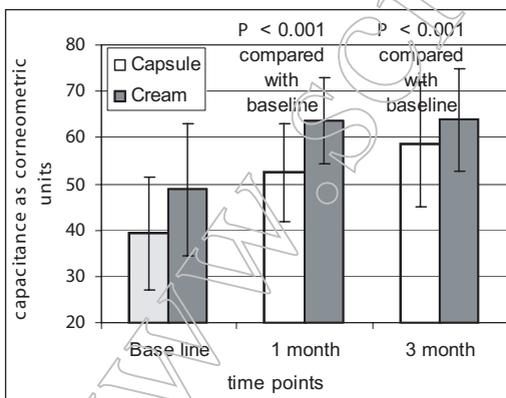
For each instrumental measurement, mean values and standard deviations were calculated for results at each of the three time points, base-

line, after one and three months of supplementation. The values obtained at the three check points were compared by Analysis of Variance and Tukey's test. Differences reaching a confidence level of 95% ( $P < 0.05$ ) were considered statistically significant.

## RESULTS

### Skin hydration status

Skin capacitance measured as corneometric units significantly increased in both groups during and at the end of the study (Figure 2). After one month of supplementation with SBA24 sea buckthorn oil, the capacitance was increased by 33.6%, after three months of treatment by 48.6%, from the baseline value ( $P < 0.001$ ). Correspondingly, one-month application of sea buckthorn oil cream increased the capacitance of the skin by 30.5% ( $P < 0.001$ ). This increase was maintained during the rest of the study period. No difference in skin hydration status was found between the group receiving the sea buckthorn oil capsules and the group using the sea buckthorn oil cream.

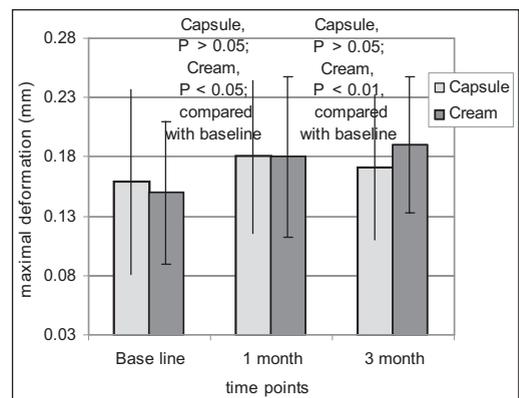


**Fig. 2** Capacitance expressed as corneometric units before, during and after supplementation of sea buckthorn oil capsule and topical application of sea buckthorn oil cream.

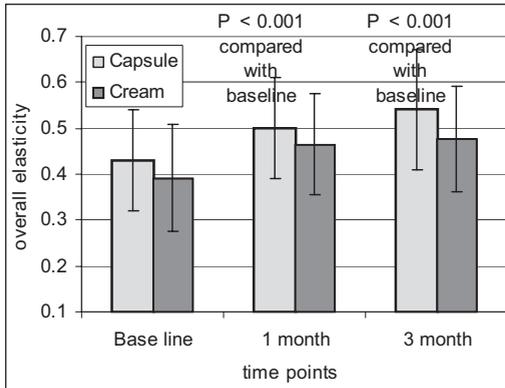
### Skin elasticity

Topical treatment with sea buckthorn cream slightly increased the maximal deformation of the skin, whereas no clear effect was observed from the supplementation of SBA24 sea buckthorn oil (Figure 3). Both treatments significantly increased the overall elasticity of the skin (Figure 4). After one and three months of treatment, overall elasticity was increased from the baseline by 16.3% and 25.8%, respectively, in the capsule group, 18.6% and 21.9%, respectively, in the cream group. At the same time some decrease in skin visco-elastic ratio was detected in both groups during and after the treatments, although the changes did not reach a statistically significant level (Figure 5).

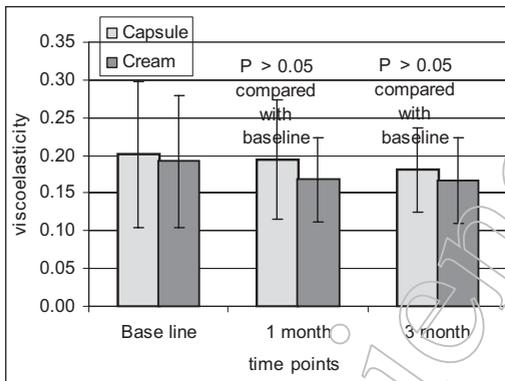
The clear increase in the overall elasticity and the decreasing trend in the visco-elastic ratio demonstrated clear positive effects of the test products in improving the elasticity of the skin.



**Fig. 3** Maximum deformation of the skin before, during and after supplementation of sea buckthorn oil capsule and topical application of sea buckthorn oil cream.



**Fig. 4** Overall elasticity of the skin before, during and after supplementation of sea buckthorn oil capsule and topical application of sea buckthorn oil cream.

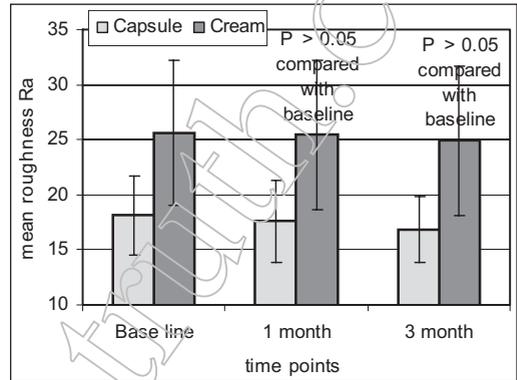


**Fig. 5** Viscoelasticity of the skin before, during and after supplementation of sea buckthorn oil capsule and topical application of sea buckthorn oil cream.

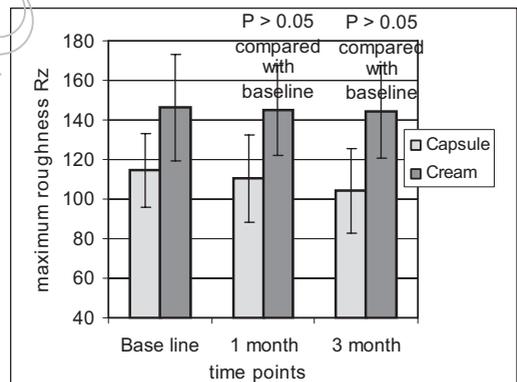
## Surface roughness

The mean roughness (Ra) of the skin surface was decreased by 3.0% after one month of supplementation and by 7.4% after three months of supplementation of SBA24 from the baseline value (Figure 6). The maximum roughness parameter Rz was decreased from the baseline value by 3.6% and 9.2%, respectively, after one and three months of the supplementation (Figure 7). The results indicate potential of oral supplementation SBA24 sea buckthorn oil capsules in

decreasing big wrinkles and fine lines of the skin. The cream treatment did not show clear effects on the skin surface roughness in the study period.



**Fig. 6** Mean roughness (Ra) of the skin before, during and after supplementation of sea buckthorn oil capsule and topical application of sea buckthorn oil cream.



**Fig. 7** Maximum roughness (Rz) of the skin before, during and after supplementation of sea buckthorn oil capsule and topical application of sea buckthorn oil cream.

## Luminosity and thickness

Throughout the study skin luminosity remained rather constant in both groups (Figure 8), indicating no clear effects of the two treatments on the skin tone of the subjects. The cream treatment

significantly increased the cutaneous thickness ( $P < 0.05$  after one month of treatment,  $P < 0.001$  after three months of treatment) (Figure 9). Increase in skin thickness may have resulted from improvement of hydration level and increase of collagen synthesis and are often associated with increased elasticity of the skin. Despite the deviation, the average cutaneous thickness remained rather constant in the capsule group during the study (Figure 9).

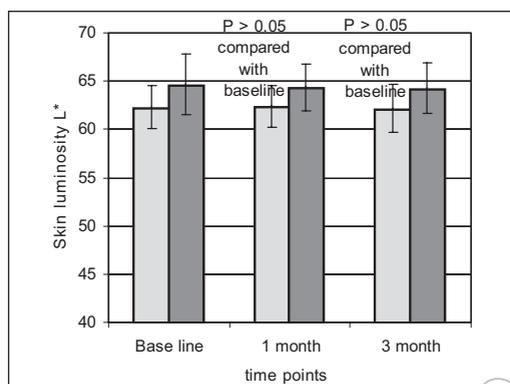


Fig. 8 Luminosity of the skin before, during and after supplementation of sea buckthorn oil capsule and topical application of sea buckthorn oil cream.

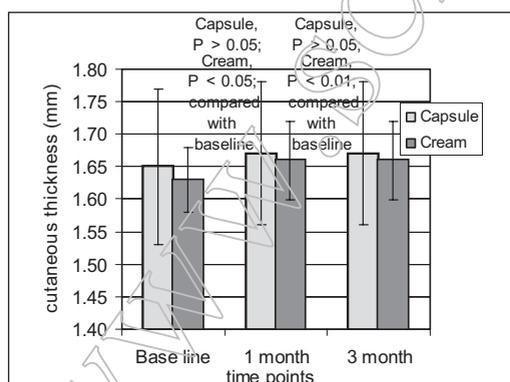


Fig. 9 Cutaneous thickness of the skin before, during and after supplementation of sea buckthorn oil capsule and topical application of sea buckthorn oil cream.

## DISCUSSION

With an increasing awareness of the importance of diet and nutrition in health management, “beauty from within” is becoming an important part of the strategy for comprehensive skin care. Dietary supplementations and functional foods have great potential in promoting the health and well-being of the skin through different biological activities such as enhancing collagen synthesis, scavenging free radicals and reducing the tendency of inflammation.

Beneficial effects of sea buckthorn oil on skin have been widely reported. However, most of the previous studies have investigated the efficacy of sea buckthorn oils in treating skin problems such as wounds, burns, skalds, and different types of dermatitis. To our knowledge, this paper is the first report on the positive effects of dietary supplementation of sea buckthorn oil in improving hydration status and reducing the ageing of healthy and mature skin. SBA24 sea buckthorn oil and the sea buckthorn seed oil used in the present study are standardised natural products manufactured by environmental friendly supercritical CO<sub>2</sub> extraction technology. By avoiding thermal and oxidative damage during the manufacturing process, the bioactive lipids were kept in the natural form in these products.

Essential fatty acids, long chain alcohols, and sterols in the products are essential nutrients supporting the regeneration of skin cells and restoration of skin barrier structure. Both SBA24 sea buckthorn oil and the sea buckthorn seed oil are rich in natural  $\alpha$ - and  $\gamma$ -tocopherols. It is known that natural  $\alpha$ -tocopherol has a better bioavailability and is more effective as an antioxidant and vitamin E in internal applications compared with the synthetic forms (31). Recent studies suggest that  $\gamma$ -tocopherol is not only an effective antioxidant but also a potent anti-inflammatory compound (32). Tocopherols,

tocotrienols, and carotenoids are natural antioxidants working synergistically, protecting skin from the oxidative damage caused by stress, ageing, and the unfavourable factors in the environment (33; 34).

It is also important to notice that oral supplementation with sea buckthorn oil resulted in improvement in the skin conditions of the subjects comparable to the effects shown by the topical treatment with the sea buckthorn oil cream. While effects of topical treatments tend to be restricted to the skin, dietary supplementation of sea buckthorn oil positively influences the well being of the whole body. The beneficial effects of ingested SBA24 sea buckthorn oil on the skin, mucous membranes, the heart and the vascular system have been demonstrated by earlier studies (18; 35; 36).

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