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Effects of SEA BUCKTHORN OIL (Hippophaë rhamnoides) on skin:

EASTERN TRADITION AND MODERN RESEARCH

INTRODUCTION

Sea buckthorn (*Hippophaë*), a genus in the family *Elaeagnaceae* is a berry-bearing hardy plant naturally distributed in Asia and Europe and cultivated in these regions and North America. *Hippophaë rhamnoides* is the major species of sea buckthorn. It is further classified into nine different subspecies, of which *H. rhamnoides subsp. rhamnoides* is native to Northern and Western Europe, the rest being found mainly in China and Eastern Europe (Rousi, 1971).

Since ancient times sea buckthorn berry has been used as a valuable source of nutrient by local people, and its application in Asian traditional medicines dates back to more than one thousand years ago. In more than 300 ancient prescriptions sea buckthorn is used to improve blood circulation, reduce inflammation, regenerate skin and mucous membranes and treat gynaecology disorders. Examples are found in "Four Books of Pharmacopoeia" (773 – 783 A.D.) and "Jing Zhu Ben Cao"(Introduction to Pharmacology, 1835 A.D.).

- Obstruction by sputum: *H. rham.* L. extract and *Ladimiria souliei ling.* In serious cases, use *H. rham.* L. powder, *Ladimiria souliei ling*, Na2B4O5(OH)4. 8H2O, NH4Cl, and Na2O5.2H2O. ...
- Clotted menstruation can be cured by taking *H. rham.* L. powder with borax and Mauritis arabica, ground into powder and taken orally.

Composition of sea buckthorn berry and oils

The nutritional and pharmaceutical properties of sea buckthorn are due to its special composition. The pearl-shaped berry is known as "nutrient bomb" and is claimed to contain more than two hundred bioactive compounds, among which natural vitamins, antioxidants, essential fatty acids, and plant sterols have been well investigated (Yang, 2001; Yang and Kallio, 2002). For example, the level of vitamin C can be as high as 2% in fresh sea buckthorn berry juice, about 30 times higher than the common level found in strawberry (Kallio *et al.*, 2002).

One special feature of sea buckthorn is that the berry contains oil both in seed (~10%) and in the soft parts (pulp oil from flesh and peel) (1%-5% f.w.). The seed oil is rich in essential fatty acids, linoleic (18:2n-6, 30%-40%) and α-linolenic (18:3n-3, 30-40%) acids, and the pulp oil is the richest source of palmitoleic acid (16:1n-7, up to 50% of total fatty acids), rarely found in the plant kingdom. Both seed oil and pulp oil contain high levels of natural vitamin E (tocopherols and tocotrienols) and plant sterols. In addition, the soft part oil is especially rich in natural carotenoids. Table 1 summarises the composition of sea buckthorn seed oil and pulp oil. The origin (subspecies) and harvesting time of the berries as well as oil isolation technology influence the oil composition (Yang 2001; Yang and Kallio, 2001; Yang *et al* 2001).

Sea buckthorn oil and skin

Oral administration of sea buckthorn oils improves atopic dermatitis.

Atopic dermatitis (AD) is an inflammatory skin disease characterised by dry, itching and lichenous skin. Impaired epidermal barrier function and abnormal synthesis of eicosanoids, the mediators of epidermal inflammation and hyperproliferation, are involved in these skin changes. Acylceramides rich in linoleic acid (18:2n-6) are essential components of the epidermal barrier system. Polyunsaturated fatty acids in phospholipids are essential components for the maintenance of the proper fluidity of cell membranes, which in turn is important for signal transduction

TABLE 1. Composition of seed and soft parts of sea buckthorn berry (YANG, 2001).

	Fatty acids (weight %)						
	16:0	16:1n-7	18:0	18:1n-9	18:1n-7	18:2n-6	18:3n-3
Seed oil	6-10	< 0.5	2-4	15-20	2-4	35-40	20-35
Pulp oil	15-40	15-50	1-2	10-20	5-10	5-15	5-10

	Carotenoids (mg/100 g)	Plant sterols	Tocopherols and tocotrienols (mg/100 g)
Seed oil	10-50	1-2%	100-200
Pulp oil	100-400	2-3%	100-400

and substance transportation. Polyunsturated fatty acids are also precursors of eicosanoids. Abnormality in the level of polyunsaturated fatty acids has been found in plasma, skin, umbilical cord and breast milk of mothers of children at high risk of atopic diseases. This ubiquitous abnormality in AD patients is thought to be due to deficiencies in intake, incorporation and metabolism of essential fatty acids (Manku *et al*, 1984).

A placebo-controlled, double-blind study was carried out to investigate the effects of seed and pulp / peel oils of sea buckthorn on atopic dermatitis (Yang *et al*., 1999). Forty-nine patients took 5 g of seed oil, pulp / peel oil or paraffin oil daily for four months. During follow up, dermatitis improved in the pulp/peel oil and seed oil groups. Supplementation with seed oil increased the proportion of linoleic, α-linolenic, and eicosapentaenoic (20:5n-3) acids in plasma phospholipids and α-linolenic acid in plasma neutral lipids. Positive correlations were found between symptom improvement and the increase in the proportions of α-linolenic acid in plasma lipids, suggesting positive effects of α-linolenic acid (Figure 1). The effects of α-linolenic acid were probably related to its metabolism to eicosapentaenoic acid, which in turn resulted in competitive inhibition of the synthesis of the 4-series leukotrienes from arachidonic acid by the increased synthesis of the 5-series leukotrienes from eicosapentaenoic acid. Pulp/peel oil treatment increased the proportion of palmitoleic acid in plasma lipids (Yang *et al* 1999).

In addition to fatty acids, the high level of carotenoids in the pulp oil and vitamin E and phytosterols in both seed and pulp oils may also have contributed to the improvement of AD (Yang *et al* 1999).

Sea buckthorn oils have tissue-regenerative effect and promote healing of skin burns and wounds

Sea buckthorn oil has been used in the treatment of burns and scalding of skin. Vlasov (1970) used sea buckthorn oil for topical treatment of 122 patients with 1st, 2nd, and 3rd degree skin burns for 8 – 12 days. The results were compared with treatment using streptomycin, sulfanilamide emulsion, cod liver oil petrolatum, potassium permanganate, and topically used antibiotics, respectively. Sea buckthorn oil was reported to be equally effective as other treatments. Topically-applied sea buckthorn oil was used to treat 32 patients with 1st – 3rd degree burns on skin, combined with light-heating. The treatment period lasted for five – seven days. All the patients were cured after seven days' treatment (Zhao *et al* 1994). Mekhtiev *et al* (1991) used, topically, sea buckthorn oil extracted from oil press cake of berries with petroleum ether in the treatment of experimental scalds (by hot water) on the skin of rabbits.

Patients with traumatic tympanic rupture/ perforation were treated with topically applied sea buckthorn oil (Fan & Xu, 1989). The injuries were healed after 2 – 28 days' treatment. In a clinical experiment involving tympanic perforation caused by mechanical injuries (Fan *et al* 1991), 56 patients were treated with topically applied sea buckthorn oil (an oil saturated cotton sheet was placed over the perforation area, and oil was added to the cotton sheet 2-3 times a week). Forty-seven cases healed, the average healing time being 13 days Fan *et al* 1991).

Sea buckthorn oil treatment accelerates the regeneration, especially epithelisation and granulisation process of injured skin. In addition, the oil has clear anti-inflammatory and analgesic effects (Quirin and Gerard, 1993; Kallio *et al* 1999).

Sea buckthorn oils have anti-inflammatory effect and improve irradiation dermatitis

Irradiation dermatitis is common skin problems resulting from exposure to UV and other irradiations. Erythema, inflammation and liquid secretion are typical symptoms of irradiation dermatitis. Sea buckthorn oil is effective in protecting skin from UV-damage and in relieving the dermatitis symptom caused by irradiation (Quirin and Gerard, 1993).

Dermatitis (2nd degree with inflammation, wet peeling, secretion and partial ulcer) caused by X-ray irradiation on legs of NIH female mice were treated with topically applied sea buckthorn seed oil, pulp oil and combined seed/pulp oil (Zhang *et al* 1988). After five to seven days of treatment, inflammation and secretion were reduced. After two weeks of treatment, ulcer areas were healed, and skin colour became normal.

Zhang and his colleagues (1988) also treated 13 cases of vulvitis and perineal inflammation caused by irradiation therapy for cervix cancer, vulva cancer, and anorectic cancer with topically applied (three to four times a day) sea buckthorn oil. Eleven (84.6 %) patients improved after treatment. Eight patients with irradiation dermatitis in the axilla (armpit) and clavicle area were also treated with topically applied sea buckthorn oil. Eighty-seven percent of the patients showed improvement after the treatment (Zhang *et al* 1988).

Sea buckthorn oils protect lipids and cell membranes from oxidation under normal conditions and cold exposure

Oxidation is a key process in the development of ageing, tissue damage

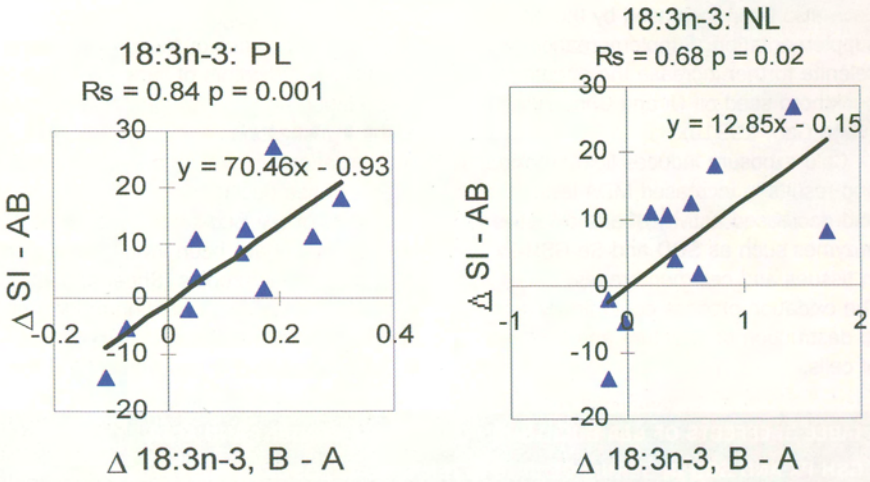

Figure 1. *The skin symptom improvement (Δ IS – AB) is positively correlated with incorporation of α-linolenic acid (Δ 18:3n-3, B – A) into plasma phospholipids (PL) and neutral lipids (NL) after one month of seed oil supplementation (Yang et al 1999).*

TABLE 2. EFFECTS OF SEA BUCKTHORN SEED OIL ON MDA LEVEL AND SE-GSH-PX ACTIVITIES IN ERYTHROCYTES (MEAN ± SE) (JI AND GAO, 1991A).

	Basic diet (A)	Basic diet + sea buckthorn seed oil (B)	Basic diet + sea bucktorn seed oil + Se (C)
MDA (μmol/g.Hb)	0.69 ± 0.20	0.23 ± 0.04*	0.18 ± 0.03*
Se-GSH-Px (Eu/g.Hb)	291.17 ± 28.51	337.28 ± 27.08**	525.04 ± 38.03**

** p < 0.05; ** p < 0.01*

and many diseases. Oxidation of membrane components leads to damage of membrane structure, resulting in malfunction or even total loss of function of cell membranes.

Human skin is constantly exposed to many different kinds of oxidative stress e.g. UV-irradiation, active oxygen, and oxygen and nitrogen free radicals. Skin has an extraordinary need of anti-oxidants to cope with the environmental stress and to maintain normal functions.

Sea buckthorn seed and pulp oils have been shown to slow down the oxidation process and to stabilise the membrane structure (Ji and Gao, 1991a; Ji and Gao, 1991b). Malonaldehyde (MDA) levels in tissue and cell membranes have been measured as an indicator of lipid oxidation. Activities of many enzymes involved in reaction to oxidation have also been determined.

Diet containing 10% sea buckthorn seed oil decreased MDA level in cell membrane of erythrocyte ghosts by 68% and in liver homogenate by 35% compared with the 100% basic diet (Ji and Gao, 1991a; Ji and Gao, 1991b). Sea buckthorn seed oil supplementation has also increased glutathione peroxidase (Se-GSH-Px) activities in erythrocytes by 16% (Ji and Gao, 1991a; Ji and Gao, 1991b). Levels of sialic acid (by 46%) and sulfhydryl group (-SH, by 73%) (Ji and Gao, 1991b) as well as Na,K-ATPase activity (by 44%) (Ji and Gao, 1991a) in the erythrocyte cell membrane have also been increased by the oil supplementation. Supplementation with selenite further increase the effects of sea buckthorn seed oil (Ji and Gao, 1991a; Ji and Gao, 1991b).

Cold exposure induces lipid peroxidation and results in increased MDA level and decreased activity of anti-oxidative enzymes such as SOD and Se-GSH-Px in tissues and cell membranes. The oxidation process could finally lead to destruction of structure and function of cells.

Feeding rats and guinea pigs with sea buckthorn pulp oil suppress the MDA production in erythrocytes under cold exposure (Table 3) (Song and Gao, 1995). In addition, the oil treatment increased the activity of anti-oxidative enzymes Se-GSH-Px and superoxide dismutase (SOD) in serum (by 20%) and liver (by 35%). The level of sialic acid on erythrocyte membranes has also been increased by the oil supplementation. Electron microscopic examination showed that sea buckthorn oil protected mitochondria of hepatic cells from cold exposure-caused damages. Pure vitamin E showed less protective effects compared with sea buckthorn oil.

Lipid peroxidation induces MDA production in cell membranes. Interactions between MDA and membrane structural components such as protein and phospholipids lead to changes in structure and function of the membranes. The level of sulfhydryl group (-SH) in membrane proteins not only reflects the gluthathione (GSH) level and reductive capacity of cells, but also indicates the oxidation status of cell membranes. Se-GSH-Px metabolises MDA and protects cell-membranes. Sialic acid is an important component of glycoproteins on the surface of cell membranes. As an component of receptors, it participates in many activities such as recognition, adhesion and signal transduction. The level of sialic acid on erythrocyte membranes has been found to decrease with ageing of the cells. Decreased acitivities of Na, K-ATPase and G-6-PD are indicators of increased lipid peroxidation and –SH oxidation. Results of the investigations above suggested anti-oxidation and membrane-stabilising effects of sea buckthorn oils.

Effects of sea buckthorn seed oil on LDL oxidation have been investigated with *in vitro* oxidation models (Shi *et al* 1994). In the Cu^{2+}-catalysed oxidation model, LDL from normal subjects was incubated in $CuCl_2$ solution (50 µmol/l) at 37°C for 24 hours in the presence of sea buckthorn seed oil at different concentrations (0, 0.3, 0.6, 1.2, 2.4, 4.8%, v/v). In cell-catalysed oxidation models, the LDL samples (0.03 µmol/l, 0.5 ml/incubation cell) were incubated together with mouse abdominal macrophages or bovine endodthelial cells at 37°C for 24 hours in the presence of sea buckhton seed oil (0, 0.04, 0.08, 0.16, 0.32%, v/v). Sea buckthorn seed oil significantly decreased MDA concentration in the Cu^{2+}-catalysed oxidation system, already at a concentration of 0.3%, compared with negative control (by 24%, $P < 0.05$). The speed of conjugated diene formation was significantly decreased by addition of sea buckthorn oil at 0.02% in Cu^{2+}-catalysed oxidation system. Dose-responsive effects were observed in both the MDA levels and conjugated diene formation. MDA levels in the cell catalysed oxidation system were also significantly decreased (by 18%-75%) by sea buckthorn seed oil addition with clear dose-responsive effects.

TABLE 3. EFFECTS OF SEA BUCKTHORN OIL ON MDA CONTENT AND ACTIVITY OF GSH-PX AND SOD IN SERUM OF RATS AFTER COLD EXPOSURE (SONG AND GAO, 1995).

Group	MDA (nmol/ml)		GSH-Px (U/ml)		SOD (U/g. Hb)	
	1st week	2nd week	1st week	2nd week	1st week	2nd week
A, 100% Basic diet (room temperature)	4.5 ± 0.1b	4.1 ± 0.2b	41.3 ± 2.2	49.1 ± 2.9b	1270.8 ± 43.8b	1314.5 ± 44.2b
B, 100% Basic diet (cold exposure)	5.3 ± 0.3	7.3 ± 0.3	50.5 ± 2.3	42.2 ± 0.5	1478.3 ± 62.4	1056.8 ± 49.5
C, 80% basic diet + 20% sea buckthorn oil (cold exposure)	3.8 ± 0.1b	4.3 ± 0.1b	44.1 ± 1.9	52.6 ± 2.5b	1253.0 ± 58.4b	1360.5 ± 30.5b

b, $p < 0.05$ or $p < 0.01$, compared with group B.

Supercritical CO_2 extraction of sea buckthorn oil

The conventional methods for oil isolation from seeds and dried fruit soft parts are solvent extraction with hexane, although mechanical methods such as centrifugation have been used for obtaining oil from the berry juice and pulp. The solvent extraction methods have clear disadvantages of leaving solvent residue in the oils and causing environmental pollution.

Supercritical CO_2 extraction (CO_2-SFE) is a modern and environment-friendly technology for organic solvent-free extract. It is especially suitable for isolating lipids, aromas and valuable oils. Physical properties of supercritical CO_2 i.e. high density and low viscosity and low surface tension ensure the production of aseptic oils (Quirin and Gerard, 1993). The mild temperature condition applied in the process maintains the natural structures of the bioactive components. Sea buckthorn oils isolated by CO_2-SFE are valuable standardised oils of superior quality compared to those isolated with conventional methods. These oils are premium natural active ingredients for cosmetics and skin care products.

Conclusions

Sea buckthorn berry has been used as a valuable source of nutrients and medicinal ingredients since ancient times. Oils from seed and soft parts of sea buckthorn berry are rich in special fatty acids and natural vitamins, anti-oxidants and plant sterols. Due to the unique combination of multiple nutrients, sea buckthorn oils have a wide range of

beneficial effects on skin and the general well being of human cells.

Supercritical CO_2-extraction (CO_2-SFE) is a new, gentle, and environment-friendly technology suitable for isolating valuable oils from natural sources. CO_2-SFE sea buckthorn oils are natural, free of organic solvent, and are thus optimal ingredients for skin care products. PC

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