Sea Buckthorn: A New Medicinal and Nutritional Botanical

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Foreword

Sea buckthorn is an ancient, relatively obscure medicinal plant that has recently been discovered to have previously unappreciated health benefits. Its berries are extraordinarily rich in vitamins and numerous antioxidant chemicals, and both the juice and extracts are useful for the preparation of a wide range of dietary supplements and fortified foods. Additional constituents in the fruit are exceptionally useful for treating skin conditions. Moreover, the presence of a variety of other medicinal components suggests that a wide range of illnesses may be alleviated by development of new medications based on extracts from the plant. Some attractive sea buckthorn health products have become available in the marketplace. Unfortunately, despite research indicating that there is considerable medicinal and market potential, the therapeutic properties of sea buckthorn are not yet well known to the scientific and technological communities, and this has retarded progress toward the development and popularization of manufactured health products. Accordingly, this comprehensive book devoted to chemical and therapeutic aspects of sea buckthorn is extremely timely.

There is a crisis in health care. Modern Western medicine has become astonishingly sophisticated, with an extraordinary arsenal of pharmaceuticals designed to alleviate most diseases. Regrettably, however, the cost of prescription drugs has skyrocketed, constituting the lion's share of health expenses and making some treatments prohibitively expensive. Worse, it appears that the continual gains in longevity achieved by modern medicine are ending, and our children may actually die at an earlier age. Why? Labor-saving devices have caused people to become much more sedentary than in the past, modern society is increasingly stressful and, most importantly, food consumption has become harmful. The major part of our diet is now made up of nutritionally unbalanced processed foods that are diabolically concocted with sugar, bad fats, salt, and artificial flavors to stimulate overeating and consequent obesity and health problems. It is critical that the processed foods currently making up the majority of our diet be reformulated to increase health. In this regard, sea buckthorn is like manna from heaven.

An ounce of prevention is worth more than a pound of cure. It is abundantly clear that small additions to diet will result in large gains in health, both for individuals and the population at large. Iodine was first added to sodium chloride (table salt) in the 1920s, to prevent the development of goiter. Vitamin D was first added to cow's milk in the early 1930s to aid in absorption of calcium and phosphorus, preventing rickets. From these early measures that increased the mineral and vitamin status of commercial food staples, food science has witnessed a huge growth in dietary supplementation. With the recent expanding market for health foods, marketing terms have developed, such as "medical foods" and "pharma foods". Two terms, "nutraceuticals" and "functional foods," have become widespread, although interpreted differently by different people. For simplicity, nutraceuticals may be thought of as dietary supplements that are consumed separately (as pills or capsules, for example) while, in functional foods, health-promoting constituents have been added to conventional food preparations. Because healthful nutraceuticals and fortified foods are much less expensive than drugs, they constitute a new, cost-effective health care system. It is apparent that plants are gold mines of the most desirable healthful dietary constituents and, among the plants that are now being used as sources of these beneficial chemicals, sea buckthorn is a rising star.

Most people do not realize that the skin (along with allied structures such as hair) is the largest of the body's organs. Moreover, despite the saying that beauty is only skin deep, the skin is the site of numerous health problems and skin health is essential to overall wellness. For many years, physicians have treated skin conditions with prescription ointments. However, today there are also many "over-the-counter" (i.e. non-prescription) preparations that consumers can use to promote skin health. The term "cosmeceutical" (cosmetic-nutraceutical) has now become commonplace to refer to non-prescription products that benefit the skin because of the surface (topical) absorption of biochemicals. Once again, constituents (notably essential fatty acids) in sea buckthorn have recently been shown to be extremely useful for skin problems, including burns, bedsores, eczema, and radiation injury. In addition to interest in developing prescription formulations with sea buckthorn constituents for the treatment by physicians of very serious skin damage, there is now a growing market for a wide range of personal body care products (such as soaps, shampoos, bubble baths, perfumes, creams, lotions, moisturizers, and lip balms) made with sea buckthorn to prevent damage by sun, wind, cold, dry air, abrasion, and exposure to household and industrial chemicals.

Ernest Small, Ph. D.

Preface

Worldwide, sea buckthorn is becoming recognized as a premium source of nutraceuticals. Products are appearing around the world such as Canada, China, Finland, India, Russia and the United States. A further brief exploration of the internet will demonstrate the wide acceptance of this fruit as an appealing medicinal plant. There are research and development projects under way in Canada aimed at processing and product development and production. Commercial operations are also appearing as the benefits and possibilities of this plant become known in the broader context and markets development. Much of this development is based upon the health promoting properties of the fruit and a fairly large, peer reviewed, literature has become available supporting many of these claims of health promotion.

The present book focuses on reviewing and documenting these claims and the supporting, publically available, literature. Composition is reviewed and brought up to date for such well known components as sugars, acids and the anti-oxidant, vitamin C, but especially details are provided for the lesser known antioxidants present in sea buckthorn. Antioxidants are increasingly recognized as important contributors to the medicinal properties of fruit, especially the tocopherols, carotenoids, and phenolic procyanidins present in significant concentrations in sea buckthorn. Omega fatty acids are also documented and discussed.

Dr. Sholberg demonstrates in Chapter 8 that several plant diseases are also susceptible to control by sea buckthorn products. Applied as sprays, application of sea buckthorn juices for plant disease control is revealed as a further, important, but lesser known application of this universal, all-in-one phyto-medicinal plant.

The authors hope this book will further promote the development of the growing Canadian Sea Buckthorn industry while providing value and information to an international audience.

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Chapter 1. Perspective of the crop potential

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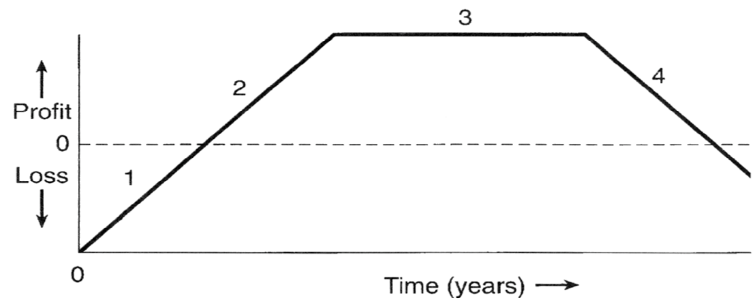
Sea buckthorn has proven itself to be extremely useful in land stabilization and reclamation in both the Old and New Worlds. However, its commercial value lies in its nutritional, cosmetic, and medicinal qualities. This large shrub has a longstanding reputation in China, where it has been harvested for at least 12 centuries, particularly as a medicinal plant. In Europe in the last half century, oil and extracts, mostly from the berries, have been incorporated into commercial medicinal and cosmetic products. Particularly in the last decade, interest has been generated in North America in the development of sea buckthorn as a commercial crop. However, it is but one of at least several dozens of promising crop possibilities under development for use in North America and by extension the rest of the world.

New crops are always a gamble and, before placing a bet, experienced gamblers consider as best they can the probability of success. In this brief analysis, some of the perils and promise of new crop ventures are discussed with particular reference to the possible advantages and disadvantages of sea buckthorn.

Plants are the basis of human nutrition and shelter, and the source of a large proportion of medicines. It has recently been estimated that there are about 400,000 species of higher or vascular plants (Govaerts 2001, Thorne 2002). While not all of these plants are equally useful, a staggering number of plant species are exploited by humans. At least 20,000 plant species have been used as human food among over 10,000 edible plants listed in encyclopedia (Tanaka, 1976). At least 5,000 of these are eaten regularly, and cultivated forms have been selected from about 3,000. However, only about 150 food plants are significant in world commerce. Prescott-Allen and Prescott-Allen (1990) listed what they considered to be the most important 103 species. The situation is rather comparable with medicinal species. Deans and Svoboda (1990) suggested that there are over 20,000 medicinal plants; Torkelson (1995) lists over 4,000 scientific names and more than 28,000 common names of medicinal plants; and Duke (2002) provides detailed information on over 800 of the most important medicinal plant species. Once again, relatively few species are cultivated commercially on a large scale. The above numbers suggest that for both food plants and medicinal plants, becoming commercially significant is not easy. The odds are about a thousand to one. A species needs to be very special in order to join the relatively elite club of important commercial plants.

Except in cases of monopoly, new offerings in the marketplace strongly tend to follow a characteristic life cycle of profitability, and it is extremely important that those investing in a relatively new crop like sea buckthorn understand this.

The figure provided below (p.8) illustrates the trend in a hypothetical fashion. Phase 1 is the period of investment in research and development required to bring the new product to the point of profitability; most new products do not survive this period. During phase 2, the market for the product expands to the point of saturation, becoming more profitable. Phase 3 is a stable period of profitability, at the end of which decline occurs. The most common cause of the decline in profitability is copy-cat competition, and the



more profitable and popular a given product becomes, the faster competition develops. A frequent consequence of such competition is over saturation of the market, the generation of surpluses, and business failure of those who remained dependent on the sale of the once profitable item. It really doesn't matter how good or important the product is — even the cereals upon which the world depends have become questionable investments because of surplus production.

Sea buckthorn is approximately in stage 2. Just how important it will become, and how long it will take to reach its maximum market penetration can not be predicted with much certainty, but at least some educated guesses can be made, based on performance to date, and on the nature of the marketplace in which sea buckthorn competes. The above profitability life-cycle diagram is pessimistic in suggesting that every crop will eventually become unprofitable, but there is an escape from this fate: research leading to new cultivars and new uses.

A disadvantage of sea buckthorn as a crop is that it is a shrub, which naturally takes 3-4 years before it becomes productive. Farmers often follow the maxim "find your market before you plant your seed," but this is much easier with an annual crop than with a perennial. Misjudging the marketplace with an annual crop could lead to wasting a season; the same exercise with a shrub (or a tree) can mean wasting a decade. The rapidity with which new cultivars can be developed and marketed is also necessarily slower than for annual crops, and it is much more difficult to scale production up rapidly to meet short-term demands. At the same time, there is a hidden advantage in the fact that sea buckthorn is a shrub. This means that growers are forced to plan ahead very carefully, and it discourages copy cats from quickly generating surpluses and depressing prices.

A great advantage of sea buckthorn is that its market value is generated primarily as value-added processing - and this is the case whether the products offered are for culinary, medicinal, or cosmetic purposes. The products have long shelf-life, and are therefore not limited to particular seasons or regions. The same certainly can not be said for the vast majority of fruits, which are generated seasonally, and are usually offered simply as a fresh dessert or snack, often with limited shelf life; further, most fruits are limited to their regions of production because of transportation costs.

China, Russia, and Mongolia are currently the largest producers of sea buckthorn. In China, fruit is harvested from over one million ha of wild sea buckthorn and almost 300,000 ha of cultivated plants. China has much cheaper labour and production costs than in most western countries, so new producers in North America, Europe, and elsewhere are faced with formidable, established competition. Such competition would be especially severe for extracts that are light enough to require minimal transportation costs.

Competing crops also need to be borne in mind, and in the case of sea buckthorn, this means considering crops that yield similar food products, and also similar medicinal products. The berries are too acidic to eat fresh for most palates, but make very tasty juice (used alone or in blends), jellies, marmalades, sauces, and liqueurs. Sea buckthorn fruits are among the most nutritious and vitamin-rich of all berries.

The content of vitamins C and E are very high. Sea buckthorn berries are also very rich in a variety of antioxidant chemicals (vitamins C and E; several carotenoids, including beta-carotene (pro-vitamin A); flavonoids; certain enzymes, and other substances). Provided that sea buckthorn juice can be supplied to the market at prices competitive with those of other fruits, there is at the very least the prospect of establishing sea buckthorn food products as commodities of particular interest to the natural and/or organic food sector. However, the number of species with edible fruits in the world is huge, particularly in tropical regions, and novelties can be expected to enter the marketplace from time to time, making it very difficult for new fruits to become commercial staples. Moreover, fruit products are now commonly supplemented, especially with vitamins, and so offering the consumer healthful, tasty products does not guarantee economic success. Sea buckthorn can produce very large harvests of berries, and it will need to be determined whether the cost of producing the crop for culinary purposes is competitive with similar currently available products.

In nature, an astonishing diversity of species survive in substantial part because most occupy distinctive ecological niches, thereby reducing direct competition. On the farm, however, most crops have been selected to grow in a rather common environment, marked by high agricultural inputs, particularly of fertilizer and water. Land that can supply such pampered conditions is at a premium, with the result that most crops are in direct competition with each other. This is not the case with sea buckthorn, giving it a very distinct advantage. The combination of adaptation to relatively dry, sterile soils, coupled with excellent winter hardiness and an inherent toughness in still other respects means that this species can thrive in marginal circumstances that would eliminate most other crops.

The source of most material for medicinal plants, culinary herbs, and spices is from the wild, rather than cultivated plants, and because of the relatively cheap availability of wild plants it is often not economically feasible to cultivate many species as crops. There are wild-growing sea buckthorn plants in Eurasia, but a more readily-available source of material is the huge areas that have been planted for land reclamation and erosion control. While these planting do not constitute "orchards," they nevertheless have been a principal source of fruit in Asia. Obviously such harvesting provides a unique advantage for the sea buckthorn industry in Asia, enjoyed by almost no other crop plant. Sea buckthorn has also been extensively planted for conservation purposes in North America, especially in Canada, but due to labour costs it is doubtful that the present, largely scattered planting can be economically harvested. Nevertheless, the possibility of establishing plants in North America for the dual purposes of both conservation and harvest is of interest. The prospect of growing sea buckthorn as a dual conservation/crop plant on marginal lands in North America, as is done in Asia, is particularly attractive.

The main sea buckthorn product in Asia is oil, which has been incorporated into commercial ointments for treating a wide variety of types of skin damage, including burns, bedsores, eczema and radiation injury. The presence of essential fatty acids, and their UV absorption properties, which are important for the maintenance of healthy skin, likely

accounts for some of the value of the oil as a skin conditioner. In western medicine and cosmetology, there are numerous products marketed for the same purposes. Sea buckthorn does have an attractive medicinal mystique, relating to its thousand-year history as an Asian medical plant, and an even older cosmetic reputation tracing to Greek and Roman times when it was thought to produce shiny, healthy hair on horses. Such a mystique often is important to the commercial success of medicinal plants (ginseng, for example), especially when combined with modern scientific demonstration of efficacy (in fact sea buckthorn has been demonstrated to be therapeutic for skin and hair conditions). Just how large a niche sea buckthorn can claim in western markets for natural medicinals and cosmetics remains to be demonstrated. These markets are extremely competitive, but the considerable success in Asia and the more recent occurrence of commercial products in Europe and North America are very promising indicators. A word of caution is in order: while the market for "nutraceuticals" (often defined as "natural food extracts with health-promoting properties"), as well as other natural medicinal and cosmetic products is growing rapidly in Western countries, popularity of given species can be short-lived.

While cultivars of sea buckthorn have been selected, compared to the established bush fruits such as raspberries and blueberries, sea buckthorn is at a relatively early stage of improvement — perhaps comparable to North American blueberries in the early 20th century. This is encouraging, suggesting that modern breeding techniques are likely to result in dramatic improvements. Certainly the considerable number of wild species that can be hybridized with common sea buckthorn provides a very substantial source of breeding germplasm.

For every crop, there is an ideal number of producers. Too little production means that insufficient material can be placed on the market to sustain interest. Too much production means that prices are depressed and profits decrease. For many new crops, limitations of processing and/or storage facilities are critical bottlenecks. Often, storage and processing capacity exists, but is remote from the sites of production, so that transportation is the problem. Ideally, there is balance among producers, processors, and marketers, as has generally developed for major, established crops and crop products. Almost inevitably, the development of new crops is accompanied by growing pains, and very often there is a period of several years during which some who were enthusiastic initially abandon the enterprise. Frequently a key to success is the backing of an influential manufacturer or retailer, which can result in sufficient market exposure for the merits of the product to become widely appreciated. Certainly supportive producer and marketing organizations are helpful, if not indeed indispensable. In North America, where the sea buckthorn industry is at a relatively embryonic stage, the initial developments in all of these regards are very promising.

Sea buckthorn, already well-established as a crop in Asia, appears to be a very promising new crop for the Western world, offering a wide variety of value-added products. While the crop and its products are faced with considerable competition, the prospects for success in the marketplace are attractive. The greatest need for the future development of sea buckthorn is research — to produce better cultivars, develop suitable agronomic management strategies, improve harvesting, storage, extraction, and processing technologies, and establish food and medicinal applications. Sea buckthorn is an excellent investment, deserving both governmental and private sector support.

Chapter 2. Next Generation of New Botanical

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Introduction

Sea buckthorn (*Hippophae rhamnoides* L.) is a hardy, deciduous shrub belonging to the family Elaeagnaceae (Rousi, 1971). It bears yellow through orange to red fruits which have been used for centuries in Europe and Asia for food, therapeutic, and pharmaceutical purposes (Bailey and Bailey, 1978). Sea buckthorn is a dioecious species with male and female flowers on separate shrubs. The gender of seedlings cannot be determined until the setting of flower buds in 3-4 years after seeding. Flowers are formed mostly on 2-year-old wood differentiated during the previous growing season (Bernath and Foldesi, 1992). Neither the male nor the female flowers produce nectar and does not attract insects; thus, pollination of female flowers depends entirely on the wind to spread pollen from male flowers. Sea buckthorn occurs as a native plant distributed widely throughout temperate zones between 27 and 69 EN latitude and 7 EW and 122 EE longitude (Rousi, 1971; Pan *et al.*, 1989) including China, Denmark, Finland, France, Germany, Mongolia, Netherlands, Norway, Poland, Russia, Sweden, and the United Kingdom (Pearson and Rogers, 1962).

Sea buckthorn fruits are among the most nutritious found in the plant kingdom. The importance of the plant is based on the nutritional value of the berries. The berries contain an essential oil and high concentration of vitamins A, B1, B2, B6, C, and E. Other important components such as carotene, fatty acid, palmitin, palmitolein acids, β-sitosterol have been used in therapeutic allications (Bernath and Foldesi. 1992). Vitamin concentration, especially C and E are as high as 360 mg/100 g and 160 mg/100 g of fruit weight, respectively. Sea buckthorn is also rich in flavonoid, carotenoid, and water and fat soluble vitamins. (Wahlberg and Jeppasson, 1992; Wolf and Wegert, 1993; Solonenko and Shishkina, 1989). The general population has increasing interest in the medicinal use of sea buckthorn and scientific research has shown that claimed therapeutic values of sea buckthorn have increasingly solid support from the scientific community. This trend is reflected in the number of scientific publications in the last 50 years. The number of publications increased from 11 published research papers from 1951 - 1960 to 37 from 1991 - 2000, and reached 100 from 2001 - mid of 2005 (Li, unpublished data). The quality and specific goals of the research has improved significantly in recent years.

Folklore and ancient tradition to scientifically proved therapeutic values

Historically, Chinese have used sea buckthorn medicinally for thousands of years starting in 618 A.D.(Zhou and Jiang, 1989). It is recorded in Tibetan medical classic 'rGyud Bzi' in 800's BC (Xu, 1994). In ancient Greece, leaves and young branches were added to horse fodder which resulted in weight gain and a healthy shine to the horse's coat. The generic Latin name, *Hippophae*, derives from this practice as it means shining horse (Lu, 1992). For its hemostatic and anti-inflammatory properties, sea buckthorn fruits are added to prescriptions in pulmonary, gastrointestinal, cardiac, blood and metabolic disorders in Indian and Tibetan medicine.

Sea buckthorn has long been used for relieving coughs, expelling phlegm, improving digestion, promoting blood circulation, relieving abdominal pain, and adjusting irregular

menstruation. It also has be used for aiding digestion and alleviating pain (Zheng, *et al.*, 1997). Clinical investigations on medicinal uses were initiated in Russia during the 1950's (Gurevich, 1956). In 1977, sea buckthorn was officially listed in the Chinese Pharmacopoeia by the Ministry of Public Health and the reputation of sea buckthorn as a medicinal plant was established. More than ten different drugs have been developed from sea buckthorn and are available in different forms, such as liquids, powders, plasters, films, pastes, pills, liniments, suppositories, and aerosols. The drugs can be used to treat oral, rectal, and vaginal mucositis, cervical erosion, radiation damage, burns, scalds, duodenal ulcers, gastric ulcers, chilblains, skin-ulcers and other skin damage caused by malnutrition (Abartene and Malakhovskis, 1975; Amosova *et al.*, 1998; Buhatel *et al.*, 1991; Chen, 1991; Cheng *et al.*, 1990; Dai *et al.*, 1987; Kukenov *et al.*,1982).

Sea buckthorn is regarded as a mild medicine with characteristic effects such as lowering fever, diminishing inflammation, counteracting toxicity and reducing abscesses, treating cough and colds, having mildly laxative effect, treating tumors, especially of the stomach and the esophagus, and treating different kinds of gynecological diseases in Tibetan medicine (Li and Guo, 1989). Different parts of sea buckthorn have been used for the treatment of disease in traditional medicine in various countries in the world. The uses of sea buckthorn medicinally are well documented in Asia and Europe.

The therapeutic wound-healing effect of sea buckthorn oil has been known for more than a decade (Kostrikova, 1989). Oil extracts obtained from fruits are used to treat liver diseases, inflammatory processes, gastrointestinal absorption disorders, and are applied externally for hemorrhaging. It has also been used successfully in the treatment of patients with melanosis and senile skin wrinkles (Zhong, *et al.*,1989). Oil extracts are used externally in dermatologic diseases such as eczema, psoriasis, lupus erythematosus, and chronic dermatitis. In ophthalmology, they are used in the treatment of trachoma, injuries or burns of the eye lid, and conjunctivitis (Guliyev *et al.*, 2004). Topical application of seed and pulp oils on burned, scalded, wounded, or radioactively damaged skin of both humans and experimental animals has shown healing and anti-inflammatory effects ((Vlasov, 1970; Mironov *et al.*, 1989, 1991; Li and Xu, 1993; Zhao, 1994; Xu, 1994; Yang, *et al.*, 2000; Cheng *et al.*, 2003). It reduces tissue inflammation, accelerates tissue regeneration and has been used to improve healing in 1st, 2nd and 3rd degree burns and skin grafts. In 79 cases of cervical erosion, the oil was applied daily for 5 days and relieved chronic cervicitis or cervical erosion in 50-74% of the cases observed (Xu and Qian, 1989). Also, it improved healing in 102 cases of radiation induced dermatitis, where burns were observed. The anti-inflammatory effect of the oil is usually considered to be brought about by the presence of ascorbic acid, tocopherol, plant sterols and especially, carotenoids, although evidence suggests that this effect can be enhanced by the unsaturated fatty acids (Vereshchagin and Tsydendambaev, 1995).

Sea buckthorn is regarded as the next generation of new botanical because of its considerable nutritional and therapeutic values for human beings. In a review article, Guliyev *et al.* (2004) indicated that the extracts of sea buckthorn branches and leaves are used to treat diarrhea, gastrointestinal and dermatologic disorders, colitis and enterocolitis in humans and animals in Mongolia and Russia. Leaves have also been applied as a compress in rheumatoid arthritis in Middle Asia. Flowers are used as a skin softener in Tajikistan.

There is evidence that sea buckthorn contains compounds that are anti-cancer in nature and these compounds are present in both the oil and juice fractions (Xu *et al.*, 2001). Sea buckthorn oil can inhibit tumor development of transplanted tumors and both the oil and

juice can kill S180 and P388 cancer cells and inhibit strains of human gastric carcinoma (Zhang, 1989). It can inhibit liver cancers induced by aflatoxin B1 and inhibit the formation of N-nitroso compounds preventing the induction of cancers.

Sea buckthorn fruit fractions, especially the oil, have been used in traditional medicine for treating cardiovascular disease both in animal experiments and clinical investigations (Xu and Chen, 1991; Jiang *et al.*, 1989; Li and Wang, 1998). Sea buckthorn seed oil has been administered to both humans and animals with hyperlipidemia, resulting in a reduction of total cholesterol and triacylglycerol in plasma and an increase in the high density lipoprotein (Jiang *et al.*, 1993). Supplementation with sea buckthorn oil has been reported to normalize the plasma lipid levels of hyperlipidemic subjects (Jiang *et al.*, 1993). Recently, Johansson *et al.* (2000) investigated the effects of CO_2 extracted sea buckthorn fruit oil and combined pulp and seed oil on risk factors of cardiovascular disease. Eleven healthy normolipidemic men consuming 5g of sea buckthorn oil per day for 4 weeks showed a clear decrease in the rate of adenosine-5-diphosphate-induced platelet aggregation along with reduced maximum platelet aggregation. This suggests a reduction of blood-clotting activity and by extension, a beneficial effect on cardiovascular risk factors.

Blended (seed and pulp) sea buckthorn oil is a rich source of C_{16} and C_{18} unsaturated fatty acids, as well as vitamin E (tocopherols), carotenoids and plant sterols (Beveridge *et al.*, 1999). Sitosterol has been reported to inhibit platelet aggregation (Zhao *et al.*, 1990) and since sitosterol is present in sea buckthorn oil in large amounts, provides a further mechanism for reducing cardiac risk because these compounds are capable of reducing the risk factors of cardiovascular disease as noted above. The concentration of phytosterols is much higher in the seed than in the pulp, although the relative quantities of pulp and seed in the fruit should be considered when calculating the total dietary contribution of sea buckthorn oils. In both fruit parts the major constituent is sitosterol which is capable of reducing platelet aggregation (Johansson *et al.*, 2000) and in the seed isofucosterol and/or obtusifoliol provide additional significant contributions.

Sea buckthorn oil possess anti-tumour activities, since the seed oil is known to retard tumour growth by 30-50% (Wang *et al.*, 1989). Seed oil (1.59 g/kg body weight) injected intraperitoneally significantly (30%) inhibited the growth rate of transplanted melanoma (B16) and sarcoma (S180) tumours in mice, without any influence on thymus or spleen weights (Zhang, *et al.*, 1989). The anti-tumour activity of oil from fruit residues (pulp) of sea buckthorn has been demonstrated by the significant increase in survival of mice bearing Ehrlich ascites carcinoma (Yang *et al.*, 1989). The survival time and life span increased in a dose-dependant manner when the oil at 125-500 mg/kg was administered intraperitoneally. Fractions extracted from the oil have no significant cytotoxic activity (50%) on human leukemia cell strain K562 at a concentration of 25 mg/ml (Yang *et al.*, 1989). The protective effect of sea buckthorn seed oil toward cervical cancer was thought to be due to the presence of vitamins A and E and β-carotene (Wu *et al.*, 1989).

The maximum dose for rats of sea buckthorn seed oil is 9.5 g/kg body weight. Adverse or toxic effects have not been observed with sea buckthorn oil even when stored at 18 °C for 2 years, and at doses 10-20 times greater than the maximum dose. In fact, the animals gained weight. No toxic effects were observed in acute (10 g/kg body wt) as well as chronic (0.5 g/kg for a month) consumption tests (Mironov *et al.* 1989). When administered to mice, rats, cats and dogs at 20-30 g/kg body weight by single or repeated injections over 2 months, sea buckthorn oil did not influence blood chemistry (number of erythrocytes,

haemoglobin, etc.) and the patho-morphology of animal organs (liver, stomach, kidney, spleen, lymphatic nodes, thyroid glands and adrenals) (Rachimov *et al.*, 1989).

Sea buckthorn oil is a good source of α-linolenic acid (18:3 n-3) and other unsaturated fatty acids. The potential of α- and γ-linolenic acids to assist in the treatment of such conditions as dermatitis, rheumatoid arthritis and platelet aggregation in clinical applications is discussed by Barre (2001) for evening primrose, borage, black currant and fungal oils, all high in α- and γ-linolenic acids. It was very difficult to present clear, convincing evidence for the efficacy of these oils in preventing human disease. In the light of repeated observations on the inhibition of platelet aggregation with sea buckthorn oil (Zhao *et al.*, 1990; Johansson *et al.*, 2000), it seems likely that this activity is not associated with these fatty acids. However, the oil has a unique composition of fatty acids, including a spectrum of mono-unsaturated acids and is a rich source of two essential fatty acids, linoleic and α-linolenic and both the seed and pulp oils are rich in oleic (18:1n-9) acid. There is increasing interest in the physiological role of mono-unsaturated acids relative to the modification of vascular endothelial surfaces and the role this may play in the reduction of thrombosis (Perez-Jimmenez *et al.*, 1999; Turpeinen *et al.*, 1998), psoriasis (Hodutu, 1999), and peptic ulcer (Abidov *et al.*, 2002).

Chapter 3. Products and Composition

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Introduction

Only six years ago the first comprehensive review of the production and composition of sea buckthorn introduced this remarkable plant to a Western audience (Beveridge *et al.*, 1999). This review highlighted the varied composition of this unique fruit and pointed out the potential industrial and medicinal uses for this plant. Since publication of that review, a fairly large literature has developed around this plant and knowledge of the composition and compositional variations across varieties and geography has expanded considerably. Also, the potential of this plant to provide an anti-oxidant profile to the diet contributing anti-aging, anti-cancer and health promoting activities is becoming recognized and this potential is being brought to the market through development of products in such diverse countries as India, the United States, Canada, and China.

In addition other countries are considering the possibilities, which information can be easily accessed through a web browser and the internet. The reputation of sea buckthorn as a "wonder plant" is taking on world wide significance and it is appropriate to review the recent literature concerning the composition of this fruit and its products.

Proximate Composition

Table 1 displays the protein, oil, moisture, and acid composition of the fluid (juice) expressed from sea buckthorn berries. The most important constituent of this fluid is its acid content because it is very high relative to most other fruits. The most dominant aspect of the flavour profile of sea buckthorn juice is its sourness (Beveridge *et al.*, 2004; Tang *et al.*, 2001; Tiitinen *et al.*, 2005) and this characteristic is certainly contributed by this high acid content. This acid content falls (TA, citric acid, approx. 3.5- 4.7 to 2.0-3.6) with increasing maturity of the berries at longer harvesting dates (Tang, 2002) depending on variety, but is still high relative to most other fruit well into September in Finland.

This newer data is in agreement with some previously reported values (Beveridge *et al.*, 1999), but is generally lower than previously reported by up to 3-4% malic acid. This is probably partly a variety effect as varieties are sought that have lower acidity to soften the sour notes inherent in the sea buckthorn flavour profile. It should also be remembered that sea buckthorn berries will remain on the trees well into the winter season, if not harvested, and will dehydrate, increasing acid content (and content of other components) with longer holding times. It is very important to record the time and conditions of harvest when reporting analytical data and such information was not available for previously reviewed data. These high acid contents contribute to pH values usually below 3.0 and ranging between 2.6 to 3.16, in agreement with previous reports (Beveridge *et al.*, 1999).

Moisture ranges from 73.4 to 86.8% , but these values should be considered carefully. Sea buckthorn juice has been observed to be very deliquescent and the measurement of moisture can be problematic in some samples since the base dry weight can be high in both moisture and oil determinations, especially if these dry weights are determined by freeze-drying.

Table 1. Chemical properties of Sea Buckthorn (Juice) from various sources.

Sample / variety	Protein	Oil (%)	Moisture	TA[1]	pH	Reference
Percolated, SCE, Sinensis, press juice	1.26 ± 0.09	0.060 ± 0.010	73.4 ± 0.093	5.05 ± 0.05	2.81 ± 0.05	Beveridge *et al.*, 2004
Percolated, Sinensis, press juice	1.40 ± 0.02	0.300 ± 0.076	73.7 ± 0.36	4.65 ± 0.00	2.81 ± 0.01	Beveridge *et al.*, 2004
Press Juice, Indian Summer				1.96 ± 0.30	3.16 ± 0.03	Beveridge *et al.*, 2002 b
Pulp, Two Vgbekistan wild samples	9.4, 9.1	7.77, 9.17	74.2, 65.4	2.7, 2.0	n/a	Chernenko *et al.*, 2004
Chinese sinensis (A)				4.23 ± 0.01	2.70 ± 0.00	Tang *et al.*, 2001
Danish rhamnoides (B)				3.69 ± 0.01	2.60 ± 0.00	Tang *et al.*, 2001
Finnish rhamnoides (C)				3.54 ± 0.01	2.63 ± 0.01	Tang *et al.*, 2001
A x C				4.46 ± 0.04	2.68 ± 0.01	Tang *et al.*, 2001
B x C				3.18 ± 0.0	2.72 ± 0.01	Tang *et al.*, 2001
Fin X Siberia mongonica				2.26 ± 0.01	2.84 ± 0.01	Tang *et al.*, 2001
Argustinka (2002) [2]		2.4 ± 0.3		2.8 ± 0.2	2.9 ± 0.1	Tiitinen *et al.*, 2005
Argustinka (2003)		2.1 ± 0.3		3.3 ± 0.1	2.9 ± 0.1	Tiitinen *et al.*, 2005
Botanicheskaya (2002)		3.2 ± 0.2		2.1 ± 0.2	2.9 ± 0.1	Tiitinen *et al.*, 2005
Botanicheskaya (2003)		2.9 ± 0.5		3.7 ± 0.2	2.6 ± 0.1	Tiitinen *et al.*, 2005
Oranzhevaya (2002)		0.7 ± 0.1		2.9 ± 0.1	2.8 ± 0.1	Tiitinen *et al.*, 2005
Oranzhevaya (2003)		0.9 ± 0.1		2.2 ± 0.1	2.8 ± 0.2	Tiitinen *et al.*, 2005
Prevoshodnaya (2002)		1.3 ± 0.2		2.1 ± 0.1	2.8 ± 0.1	Tiitinen *et al.*, 2005
Prevoshodnaya (2003)		2.1 ± 0.2		2.5 ± 0.1	2.8 ± 0.1	Tiitinen *et al.*, 2005
Raisa (2002)		3.3 ± 0.2		3.1 ± 0.2	2.9 ± 0.1	Tiitinen *et al.*, 2005
Raisa (2003)		2.9 ± 0.3		3.2 ± 0.1	2.7 ± 0.1	Tiitinen *et al.*, 2005
Trofimovskaya (2002)		3.6 ± 0.3		2.0 ± 0.1	2.8 ± 0.1	Tiitinen *et al.*, 2005
Trofimovskaya (2003)		3.0 ± 0.1		3.4 ± 0.2	2.7 ± 0.2	Tiitinen *et al.*, 2005
Chuiskaya (2002)		1.7 ± 0.3		2.0 ± 0.2	2.9 ± 0.1	Tiitinen *et al.*, 2005
Chuiskaya (2003)		2.9 ± 0.2		2.2 ± 0.1	2.8 ± 0.1	Tiitinen *et al.*, 2005
Den (1999)			82.4 – 84.8	3.0 – 4.1		Tang (2002)
Den (2000)			83.8 – 86.4			Tang (2002)
Chi (1999)			82.4 – 83.8	3.5 – 5.0		Tang (2002)
Chi (2000)			83.0 – 84.5			Tang (2002)
D × F1 (1999)			82.3 – 84.6	2.8 – 4.0		Tang (2002)
D × F1 (2000)			85.2 – 86.5			Tang (2002)
D × F2 (1999)			83.0 – 85.0	1.9 – 3.4		Tang (2002)
D × F2 (2000)			85.2 – 86.2			Tang (2002)
D × F3 (1999)			83.0 – 85.5	2.6 – 3.7		Tang (2002)
D × F3 (2000)			86.0 – 86.8			Tang (2002)
D × F4 (1999)			83.0 – 85.0	1.9 – 3.3		Tang (2002)
D × F4 (2000)			85.2 – 86.8			Tang (2002)
F × C (1999)			80.5 – 83.8	3.2 – 4.5		Tang (2002)
F × C (2000)			83.8 – 85.6			Tang (2002)

1. Values stated or converted to % Malic Acid ±. Values are standard deviations.
2. Year harvested.

Oil contents range from 0.3 to 9.17% but the higher values come from wild samples collected in Uzbekistan and may have been subjected to dehydration, or may be based upon dried berries. The literature is not clear. Otherwise the oil content varies quite widely around about 2% in the juice with the Hippophae rhamnoides L. subsp. senensis in Canada being particularly low in oil.

Contributors to oil content variation include harvest dates, geographic location and variety, as for other compositional components. However, another source of variation has not be previously considered. Oil is held in the fruit in cellular tissue masses which appear in the juice (Beveridge et. al., 2004; Beveridge and Harrison, 2005) and arise presumably from the parenchymal storage tissues in the fleshy portions of the fruit (Harrison and Beveridge, 2002). This oil bearing structure is derived from the fruit during the pressing procedure and the extent of extraction depends upon the condition of the parenchymal tissue, which condition depends in turn on the variety and maturity of the sea buckthorn fruit. The oil is held in the interior of the press- extruded cellular masses and the oil is partially extruded under pressure (Beveridge *et al.*, 2004) but must be extracted by the solvent extraction method for oil measurement purposes. Extraction with super critical carbon dioxide removes only about 80% of the juice oil (Beveridge *et al.*, 2004) but such measurements have not been made with other extractions solvents or systems. It is possible that the oil levels in sea buckthorn juices or fruit are being underestimated because of the retention of oil in storage tissues in the fruit or juice.

Protein is a juice component which is worthy of greater consideration. In other opalescent fruit juices it is the protein component of the cloud which confers the stable cloudy character to the juice (Beveridge, 2002). The protein content of Sinensis press juice ranged from 1.26 to 1.40% and was reported as 9.1 to 9.4 % in berries from Uzbekistan. The difficulties with the later analysis have been discussed above. Supercritical carbon dioxide extracted press juice provided a densely clouded juice of considerable cloud stability (Beveridge *et al.*, 2004) although the possible contribution of the protein content was not determined.

Sugars and Acids

Data relative to the content of specific sugars and specific acids are collected in Table 2. The only sugar components consistently reported in sea buckthorn are glucose and fructose although one report of an unknown component, presumably a sugar, was encountered (Beveridge *et al.*, 2002 a). In the vast majority of cases, the level of glucose exceeds that of fructose but there are a number of exceptions, five in the examined literature, so this cannot be considered an inevitable rule.

The exceptions are noted in table 2 as *Hippophae rhamnoides* L. subsp. *sinensis* from China, Danish and Finnish *H. rhamnoides* (Tang *et al.*, 2001), Oranzhevaya (2003) and Chuiskaya (2002) (Tiitinen *et al.*, 2005) in which fructose levels exceeds glucose levels. Since fructose is sweeter than glucose (van Nostrand, 2002), it is conceivable that this higher level of fructose could impact sensory properties and that these varieties are perceived as less sour and more desirable. This does not appear to be the case (Tang *et al.*, 2001) since the Chinese sinensis and Danish and Finnish *H. rhamnoides* were not determined to be either sweeter or more pleasant in sensory tests. However, other attributes such as acidity levels and the sugar/acid ratio impact the sensory perception and it might be prudent to control for these variables in future studies concerning the sensory desirability of sea buckthorn berries.

Glucose varies widely in absolute levels with variety and geographic location (Table 2). As the berry matures the level of glucose rises or remains approximately constant over the ripening period (Tang, 2002) but the cultivars Leikora and Hergo decreased over the ripening period (Raffo *et al.*, 2004). Fructose is generally lower in level than glucose, and is virtually

Table 2. Collection of values for specific sugars and acids in sea buckthorn juice and berries.

Sample	Glucose	Fructose	Organic Acids				ºBrix	Reference
			Ascorbic	Malic	Quinic	Citric		
Indian Summer	32.6 ± 2.56	10.8 ± 1.17	174.7 ± 30.7	13.8 ± 0.41	26.5 ± 0.09	2.21 ± 0.16	10.8 ± 0.46	Beveridge et al., 2002a
Press Juice	30.3 ± 2.15	11.1 ± 1.08	157.3 ± 22.6	15.1 ± 0.37	22.2 ± 0.79	2.12 ± 0.16	9.8 ± 0.0	Tang et al., 2001
Unspecified			154					Eccleston et al., 2002
Unspecified			122 ± 6.4					Rosch et al., 2003
Chinese Sinensis	23.2 ± 4.0	22.6 ± 7.0						Tang et al., 2001
Danish rhamnoides	2.7 ± 1.0	10.0 ± 1.0						Tang et al., 2001
Finish rhamnoides	3.3 ± 0.0	8.0 ± 0.0						Tang et al., 2001
Fin, Chinese Cross	16.2 ± 3.0	2.5 ± 2.0						Tang et al., 2001
Fin, Danish Cross	11.9 ± 2.0	1.1 ± 1.0						Tang et al., 2001
Fin, Mongolian Cross	20.0 ± 1.0	7.3 ± 3.0						Tang et al., 2001
Argustinka (2002)	24 ± 1	6 ± 1	68 ± 5	28 ± 1	10 ± 2		8.7 ± 0.1	Tiitinen et al., 2005
Argustinka (2003)	22 ± 1	7 ± 1	67 ± 5	36 ± 2	11 ± 2		8.5 ± 0.2	Tiitinen et al., 2005
Botanicheskaya (2002)	23 ± 1	2 ± 1	74 ± 5	18 ± 1	14 ± 2		7.4 ± 0.3	Tiitinen et al., 2005
Botanicheskaya (2003)	16 ± 3	3 ± 1	54 ± 1	35 ± 2	17 ± 2		7.5 ± 0.1	Tiitinen et al., 2005
Oranzhevaya (2002)	21 ± 1	15 1	17.6 ± 8	25 ± 1	12 ± 1		8.1 ± 0.2	Tiitinen et al., 2005
Oranzhevaya (2003)	29 ± 2	31 ± 4	128 ± 9	30 ± 3	18 ± 2		10.9 ± 0.3	Tiitinen et al., 2005
Prevoshodnaya (2002)	36 ± 1	16 ± 1	159 ± 5	16 ± 2	18 ± 1		10.3 ± 0.3	Tiitinen et al., 2005
Prevoshodnaya (2003)	39 ± 2	30 ± 2	87 ± 7	22 ± 2	18 ± 3		12.0 ± 0.2	Tiitinen et al., 2005
Raisa (2002)	39 ± 3	17 ± 2	46 ± 4	16 ± 1	16 ± 2		10.3 ± 0.4	Tiitinen et al., 2005
Raisa (2003)	19 ± 2	8 ± 1	29 ± 2	29 ± 2	26 ± 4		12.6 ± 0.1	Tiitinen et al., 2005
Trofimovskaya (2002)	43 ± 2	7 ± 1	107 ± 8	21 ± 2	11 ± 2		9.7 ± 0.2	Tiitinen et al., 2005
Trofimovskaya (2003)	24 ± 3	10 ± 2	120 ± 4	35 ± 3	12 ± 2		8.9 ± 0.3	Tiitinen et al., 2005
Chuiskaya (2002)	25 ± 1	21 ± 2	96 ± 3	17 ± 1	16 ± 2		9.1 ± 0.4	Tiitinen et al., 2005
Chuiskaya (2003)	29 ± 5	38 ± 4	68 ± 6	20 ± 1	18 ± 2		11.4 ± 0.1	Tiitinen et al., 2005
Sea Buckthorn (Non-specific)			80.3					Tiitinen et al., 2005
[1]DEN (1999)	2 - 2.5	1 - 1.5	180-160					Tiitinen et al., 2005
DEN (2000)	3 - 1.5	1 - 1.5						Tang, 2002
[1]CHI (1999)	2 - 34	2 - 3	650-900					Tang, 2002
CHI (2000)	4 - 31	1 - 1.5						Tang, 2002
DxF1 (1999)	8 - 17.5	2 - 13	90 - 175					Tang, 2002
DxF1 (2000)	6 - 14	1 - 1.5						Tang, 2002
DxF2 (1999)	6-11	1 - 1.5	180 - 230					Tang, 2002
DxF2 (2000)	5-9.5	1 - 1.5	180 - 220					Tang, 2002
DxF3 (1999)	3 - 9	1 - 1.5	180 - 220					Tang, 2002
DxF4 (1999)	2 - 4	1 - 1.5	110 - 180					Tang, 2002
DxF4 (2000)	7 - 9	1 - 1.5						Tang, 2002
[1]FxC (1999)	14 - 31	2.5 - 2.0	410 - 210					Tang, 2002
Finnish rhamnoides								Tang, 2002
Clones R3			170 - 120					Kallio *et al.*, 2002 a
Clones R4			135 - 65					Kallio *et al.*, 2002 a
Clones R1			200 - 135					Kallio *et al.*, 2002 a
Clones R2			190 - 110					Kallio *et al.*, 2002 a
H. sinensis S1			1150 - 1100 (var)					Kallio *et al.*, 2002 a
H. sinensis S6			900 - 850					Kallio *et al.*, 2002 a
cv. Leikora	3.5 - 4	1.2 - 2.1	350 - 265	29 - 23	10 - 8	1.1 - 0.85	7.6 - 6.9	Raffo *et al.*, 2004
cv. Askola	7.5 - 13	2.2 - 3.2	340 - 180	47 - 40	24 - 13	1.35 - 1.61	11.2 - 9.0	Raffo *et al.*, 2004
cv. Hergo	7.0 - 21	2.3 - 5.5	370 - 180	23 - 26	28 - 15	1.20 - 0.97	12.9 - 7.8	Raffo *et al.*, 2004

[1]DEN = Danish Variety; CHI = Chinese variety; DxF$^{1-4}$ = cross of Danish and Finish varieties; F x C = cross of Finnish and Chinese variety

constant over the ripening period for most varieties but fructose in the Chinese variety (CHI) rose markedly with ripening and fructose decreased in the Hergo variety (Tang, 2002; Raffo *et al.*, 2004). The level of sugars in conventional fruit is often determine by refractometer as °Brix and in sea buckthorn this measure ranges from 7.4 to 12.0 (table 2). This value should be interpreted with care since in sea buckthorn the acid levels are very high and contribute significantly to the °Brix reading. It is probably still a useful measure of soluble solids but should not be interpreted as sugar alone as is often done informally in other fruits.

The collection of data on ascorbic acid confirms the generally high levels characteristic of the sea buckthorn berry and products derived from them. Commonly these levels fall between 100 and 200 mg/100mL but exceptions up to the 1100 mg/100mL range exist, as do exceptions on the lower side (Raisa, 2003 for instance). At 100-200 mg/100mL, the level is higher that any other common unfortified fruit juice or product. Examination of the tabulated values would suggest that the highest values are present in the Chinese sinensis varieties with more intermediate values in the 300's mg/100mL more characteristic of the German varieties supporting the suggestion that genotype may be the most important influence determining ascorbic acid levels in different sea buckthorn populations (Kallio *et al.*, 2002b). For most varieties the ascorbic acid fell with increasing maturity or ripeness (Tang, 2002; Raffo *et al.*, 2004; Kallio *et al.*, 2002b) and the ranges of values shown in Table 2 provide some estimate of the range over which the decrease occurs. The exception to this general observation was the Chinese sinensis samples which were sampled from wild populations and showed fairly wide variations over the development season with no evidence of a decreasing trend.

Measurement of individual organic acids confirmed the generally high levels of total acid and that malic and quinic acids are the two major species. In the Canadian variety, Indian Summer, and two Russian varieties Prevoshodnaya and Raisa , quinic acid was the major species, but in the remaining Russian and German varieties, Leikora, Askola, and Hergo, malic was quantitatively the most important. Few reports of the presence of other organic acids were encountered but citric acid was reported in Indian Summer, Leikora, Askora and Hergo varieties at levels much lower than either malic or quinic acids. Beveridge *et al.*, (2002 a) reported the presence of both oxalic and tartaric acids at average levels of 0.29 ± 0.13 and 1.22 ± 0.95 mg/mL respectively. This latter report seems to be unique as no other reports of minor organic acids have been encountered beyond those noted in a previous review (Beveridge *et al.*, 1999).

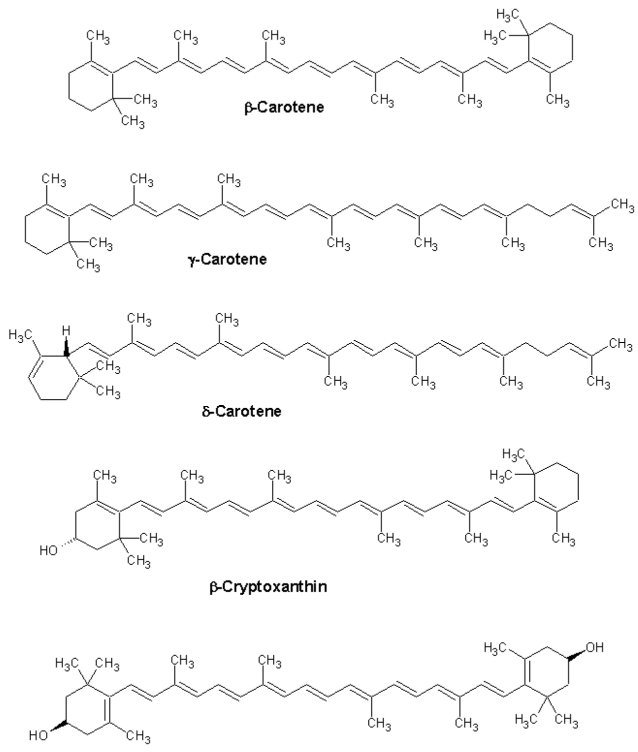
Fats and Lipids

Very little work has been done on carotenoid in sea buckthorn since the subject was reviewed earlier (Beveridge *et al.*, 1999) and little new data is available.

Beveridge et. al.(1999) reported sea buckthorn oil to contain 314 -2139 mg/100g carotenoid and Oomah (2003) reports pulp oil contains 5-10g/Kg and seed oil contains low levels at 20-85 mg/100g. The presence of β-carotene, γ-carotene, δ-carotene, β-cryptoxanthin and zeaxanthin in sea buckthorn has been confirmed by TLC, HPLC, and mass spectrometry (Oomah, 2003; Figure 1)

In plant systems, including sea buckthorn, carotenoids are held in the cells and membranes of the pulpy fruit where they contribute to the anti-oxidant properties and

Figure 1. Carotenoids reported to be in the sea buckthorn berry.



preservation of membrane lipids. The carotenoids are held as carotenolipoprotein complexes whose physiological function is unclear but which can be extracted from the pulpy fruit using buffers containing detergent (Pintea *et al.*, 2001).

Phytosterols

Phytosterols are well known for their cholesterol lowering activity (Beveridge *et al.*, 2002 a; Mellentin, 2005) and this property is exploited for the production of commercial products such as the margarine-type spread Benecol (Mellentin, 2005). However, phytosterols are also considered to have anti-inflammatory, anti-bacterial, anti-ulcerative and anti-tumour properties (Beveridge *et al.*, 2002 a). As such, these compounds contribute importantly to the nutritional and nutraceutical value of sea buckthorn oil containing products. Data on the content of individual phytosterols are scarce (Yang *et al.*, 2001) and the limited available data are summarized in Table 4 based on GC-MS data.

Quantitavely, the most important phytosterol present in both sea buckthorn seed and pulp oils is sitosterol (β-sitosterol) with a mixture of isofucosterol and obtusifoliol providing a second significant component in seed oils. Squalene is commonly reported in vegetable oils and can be quite high in ginseng oil (Beveridge *et al.*, 2002 a) but is not reported here. However, squalene is present in sea buckthorn products although at considerably lower levels than in ginseng oils (Drover, J. personal communication). The levels of total phytosterols in the seed oils are in the upper range of common vegetable oils and higher than in the pulp/skin lipids (Table 4). Ginseng seed oil contains 812 to 973 mg phytosterol per 100g oil for instance (Beveridge *et al.*, 2002 a), while the levels of phytosterols in *Hippophae rhamnoides* subsp.*sinensis* are higher than in *H. rhamnoides.*

Table 3. Fatty acid composition of individual polar lipids of the carotenolipoprotein complexes extracted from sea buckthorn (From Pintea *et al.*, 2001)

Percentage composition [a]							
Fatty acid	Phosphatidyl choline	Phosphatidyl choline + phosphatidylserine	Phosphatidic acid + diphosphatiddylglycerol	Phosphatidy lglycerol	Phosphatidyl-ethanolamine	Digalactosyldiacyl-glycerol	Monogalacto-syldiacyl glycerol
0.5833333	3	5.2	5.9	3.8	8.3	2.9	3.3
0.6666667	17.4	20.3	26.8	22.8	24.7	10.2	12.2
16:1 (9c)	2.8	2.8	9.2	11.9	4.8	1.9	10.1
0.75	12.4	14.1	9.9	8.3	14.3	14.8	5.9
18:1 (9c)	17.9	19.7	19.9	21.5	15.3	26.1	11.3
18:1 (11c)	4.3	2.2	3.5	5.8	9.1	-	6.4
18:2 (9c,12c)	7.1	9.3	8	9.2	5.3	12.2	14.4
18:3 (9c, 12c,15c)	14.3	13.6	4.6	4.7	6	27.3	17
0.8333333	3.1	2.3	2.6	2.4	8.6	2.9	-
20:1 (11c)	3.6	1.9	1.6	1.6	3.4	1.6	4.9
0.9166667	6.1	3.2	2.5	2.2	-	-	6
22:1 (13c)	7	5.3	5.3	5.6	-	-	8.3

[a] Mean of three determinations.

Table 4. Phytosterol content (mg/kg) in seeds and berry soft parts of two subspecies of sea buckthorn (adapted from Yang *et al.*, 2001).

Sterol	Seed Lipids		Pulp/Skin Lipids	
	sinensis	rhamnoides	sinensis	rhamnoides
Campesterol	33 4	40 8	4.8 1.3	3.8 0.5
Sitosterol	934 105	931 179	218 74	198 24
Stigmastanol	46 11	50 24	2.3 1.5	1.3 0.5
Isofucosterol + Obtusifoliol	244 56	201 55	nr	nr
Unidentified	33 19	5 2	nr	7.0 2.8
Stigmasta -5, 24(25) - dien - β - ol + unidentified sterol	22 4	17 7	nr	nr
Stigmasta - 7 - en - 3β - ol + cycloartenol	25 11	19 13	8.5 1.3	5.3 1.5
4», 14» - dimethyl -9β, 19 - cycloergost - 24(24) -en-3β-ol	12 4	17 9	10.3 6.1	6.5 3.1
Stigmasta - 7, 24(24) - diene - 3β - ol	26 7	12 3	7.6 2.1	5.5 1.7
4», 14», 24' - trimethylergosta - 8, 24(24) - dien-3β-ol	7 2	7 3	4.8 2.5	6.5 1.7
24-methyl-5»-cycloart-24(24)-en-3β-ol(24-methylenecycloartanol)	21 13	29 19	nr	3.3 1.2
Unidentified olerol	4 2	4 2	7.5 2.0	5.8 1.0
Citrostadienol	42 17	39 14	nr	nr
Stigmastadienol	nr	nr	5.5 1.3	3.3 0.5
Isofucosterol + aligmast-8-en-3β- ol + obtusifoliol	nr	nr	15.3 4.3	18.8 0.5
Stigmasta - 5, 24(25) - dien - β - ol + stigmasta - (8,24) - dien - 3β - ol + unidentified	nr	nr	3.8 1.7	5.0 2.8
Unidentified Sterol	nr	nr	5.0 1.2	4.8 3.1
Unidentified Sterol	nr	nr	6.5 1.7	4.5 1.7
TOTAL	1441 ± 52	1369 ± 281	309 ± 67.6	279 ± 35.4

nr - not reported or not detected.

Table 5. Fatty acid composition of sea buckthorn oils derived from sub species sinensis from China and subspecies rhamnoides from Finland.

	Seed Oil		Pulp/Peel Oil	
	sinensis	*rhamnoides*	*sinensis*	*rhamnoides*
0.66666666667	8.7 ± 0.7	7.4 ± 0.4	26.7 ± 4.5	27.8 ± 3.4
16:1 (n−7)	nr	nr	27.2 ± 6.4`	32.8 ± 3.2
0.75	2.5 ± 0.3	3.0 ± 0.6	1.3 ± 0.3	0.8 ± 0.2
18:1 (n−9)	19.4 ± 3.7	17.1 ± 2.0	17.1 ± 3.8	17.3 ± 2.3
18:1 (n−7)	2.2 ± 0.2	2.8 ± 0.7	8.1 ± 1.2	9.1 ± 0.8
18:2 (n−6)	40.9 ± 1.7	39.1 ± 2.1	12.7 ± 6.9	9.0 ± 3.1
18:3 (n−3)	26.6 ± 4.7	30.6 ± 3.0	7.1 ± 3.4	3.2 ± 1.6

Summary adapted from Yang and Kallio (2001)
nr = not reported or not present
Average values from 12 Chinese and 9 Finnish varieties
± values are standard deviation

Table 6. Volatile constituents of fruits of Hippophaë rhamnoides (adapted from Cakir, 2004)

Components	%
n − Heptanol	1.5
3 − Octenol	tr
Octanol	1.5
n − Undecane	3
Nonanol	3
Ethyl octenoate	0.7
Ethyl octanoate	9.9
Octyl acetate	2.3
Decanol	5.6
n − Tridecane	tr
n − Tetradecane	2.5
Ethyl decanoate	5.5
n − Pentadecane	1
Dodecanol	0.9
Ethyl dodecenoate[a]	39.4
Ethyl dodecanoate	3.7
n − Hexadecane	1.4
Tetradecanol	1.7
Ethyl myristate	1
Decanyl phenol	1
Tetramethylpentadecanone	0.6
n − Hexadecanol	0.7
n − Hexadecanoic acid	0.6
Ethyl palmitate	0.8
Tetramethylhexadecanol[a]	0.7
n − Eicosane	0.8
n − Heneicosane	0.9
n − Docosane	0.7
n − Tetracosane	0.7
n − Pentacosane	2.5
Aliphatic esters	63.3
Aliphatic alcohols	15.6
Aliphatic hydrocarbons	13.5
Others	2.2
Total	94.6

[a] The position of double bond could not be identified. tr = Trace (<0.6%)

Triglyceride Oils

The fatty acid composition of seed and pulp oils, as derived from 12 Chinese *H. sinensis* varieties and 9 Finnish *H. rhamnoides* varieties, are summarized in Table 5 (Yang and Kallio, 2001). This analysis is a slight over simplification since Cakir (2004) reports the presence of small amounts of caprylic (1.6%), capric (1.3%), and lauric (0.5%) acids along with traces of arachidonic, eicosenoic and arachidic acids in Turkish sea buckthorn oils, but Table 5 represents the major components. In terms of general patterns of fatty acid distributions, and as to quantitative levels of individual fatty acids these analysis conform reasonably well with limited data summarized earlier (Beveridge *et al.*, 1999). Oil extracted from seed is essentially based on C_{18} fatty acids with a small contribution from C_{16} saturated acids.

The seed oils from both *H. sinensis* and *H. rhamnoides* are highly unsaturated containing 89.1% and 89.6% unsaturated C_{18} fatty acids respectively. Of perhaps more importance is the fact that 67.5% and 69.7% respectively have double bonds in the 3 (n–3, omega 3) or 6 (n–6, omega 6) position from the methyl end of the carbon chain. Both are considered essential fatty acids for human metabolism since they are not produced by the body and must be obtained from the diet. The omega three fatty acids are increasingly seen as important nutrients because of their disease curbing activities.

Omega three fatty acids have been cited as reducing the risk of cardiac death, reducing the risk of death from cancer, especially breast, colorectal and perhaps prostate cancers (Talbot, 2005). Ground flax seed (45-60% omega 3, Gunstone *et al.*, 1994) has been shown to reduce prostate tumors (Donaldson, 2004) although cautious interpretation should be exercised since the ratio of omega 3 acids to omega 6 acids is low and this low ratio may moderate the apparent anti-cancer benefits of omega 3 acids (Donaldson, 2004). Nevertheless, while not as good a source as flax seed, sea buckthorn is a good source of omega 3 fatty acids, as good as fish oils, and provides a superior vegetable source of these important components. Common vegetable sources contain between 4% and 14% omega 3 acids (Gunstone *et al.*, 1994) while sea buckthorn contain 27 - 31% in seed oil and 3.2 - 7.1% in pulp oil.

Tsydendambaev and Vereshchagin (2003) have investigated the positional isomers of the triacylglycerol present in sea buckthorn seed and their distributional changes with increasing maturity or days after pollination. The positional isomers were made up of palmitic (P), oleic (O), linoleic (L) and linolenic (Le) acids primarily as would be expected from the fatty acid analysis. Examples include PLeLe, POO, LLeLe, and the variation over the growing season of individual triacylglycerides is documented.

Steam volatile constituents contributing to the odor and perhaps the flavour of sea buckthorn berries have been documented (Table 6, Cakir, 2004). `Quantitatively, the most important components were ethyl dodecenoate, ethyl octenoate, decanal and ethyl decanoate, however, the quantitative importance does not necessarily denote the importance of the component to the flavour or odor profile. To date no medicinal importance has been attached to these components.

Cutin is a polymer composed of a complex mixture of interesterified long chain ω-hydroxy acids usually with a C_{18} carbon skeleton. These ω-hydroxy acids may be released from cutin enzymatically or by saponification to release monomeric acids from the polymer. In sea buckthorn the major hydroxy acids released from cutin were 9,10-epoxy-18-hydroxyoctadecanoic (41%), 9,10-epoxy-18-hydroxyoctadec-12-enoic (30%), and dihydroxyhexadecanoic (15%) acids (Kallio *et al.*, 2006). To date no medicinal importance has been attached to any of the compounds mentioned in this paragraph.

Chapter 4. Sea Buckthorn Therapeutic Values

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Introduction

Sea buckthorn is regarded as the next generation of botanical because of its considerable therapeutic value. This miracle shrub has attracted considerable attention around the world from both researchers and commercial enterprises. There are many nutraceutical products on the market derived from sea buckthorn. It contains nutrients and bio-active substances such as vitamins, carotenoids, flavonoids, polyunsaturated fatty acids, free amino acids and elemental components, important for the health of human beings (Zeb, 2004).

Clinical trails and scientific studies undertake during the last few years confirm medicinal and nutritional value of sea buckthorn (Sabir *et al.*, 2005). Scientists continue to do research on its chemical components related to the nutraceutical and therapeutic claims. Clinical and preclinical trials have been conducted for some of the common human diseases, which demonstrated the therapeutic values of sea buckthorn scientifically.

Information from preclinical trials

1. Age-related macular degeneration

It has been reported that a high intake of lutein and zeaxanthin protects age-related macular degeneration and age-related cataract formation (Mares-Perlman *et al.*, 2002; Beatty *et al.*, 1999). This statement was further confirmed by the studies in which metabolites of zeaxanthin and lutein were isolated from human retina tissue (Bernstein *et al.*, 2001). With a liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, Weller and Breithaupt (2003) identified that sea buckthorn (*Hippophae rhamnoides* L.) is a valuable natural source of zeaxanthin esters for human consumption.

2. Anticarcinogenic

It was reported that the flavonoids (kaempferol, quercetin and myricetin), phenolic acids (p-coumaric, caffeic, ferulic, p-hydroxybenzoic, gallic and ellagic acids) and sterols have beneficial effects on human health as antioxidants and anticarcinogenes (Hakkinen *et al.*, 1999; Yang *et al.*, 2001). With an HPLC method, these compounds were identified at a high level in sea buckthorn berries (Hakkinen *et al.*, 1999; Yang *et al.*, 2001).

Anti-tumor effects of fruit juice and seed oil of *Hippophae rhamnoides* L. *in vivo* and its fruit juice in vitro as well as their influences on immune function were studied by Peizhen *et al.* (1989) in Research Institute, Lanzhou, China. The results of three experiments in mice showed that the inhibition rate of tumor growth (IRTG) of transplanted S180, B16 and P388 were over 30%, when fruit juice was injected intraperitoneally. When fruit juice was given, the IRTG of S180 was over 40%. When seed oil was injected intraperitoneally, the IRTG of transplanted B_{16} and S_{180} were both over 30%. The differences of these results compared with those of control groups were statistically significant ($p<0.01$ or 0.05). These results indicated the potential anti-tumor activities of the fruit juice and seed oil of *Hippophae rhamnoides* L. *in vivo*.

The results of this experiment showed no significant influence of fruit juice and seed oil on the weight of the thymus and spleen of the laboratory animals. The fruit juice

significantly increased the count of erythrocytes and leukocytes in the mice bearing B_{16} ($p<0.05$). The fruit juice and seed oil significantly increased the phagocytic rate and phagocytic index of macrophages ($p<0.01$). This implied that mal's immune function. It can be concluded that the inhibition rate for L1210 cells reached 100% when the concentration of fruit juice 10*u*g/ml was applied (Zhang, P. *et al.*,1989).

3. Antibacterial activities

In a study to investigate the *in vitro* antibacterial activities of sea buckthorn seed extracts in order to assess their potential as natural preservatives. Negi *et al.* (2005) found that the methanol extracts of sea buckthorn seeds exhibited high antibacterial activity, showing MIC of 200 ppm for *Bacillus cereus*, 300 ppm of *B. coagulan*s, *B. subtilis* and *L. monocytogenes*, and 350 ppm for *Yersinia enterocolitica*. It is suggested that selective extraction and concentration of phenols in methanolic extracts may be responsible for their higher biological activity.

4. Anti-inflammatory and analgesic

In an animal (mice and rats) experiments conducted by Yang and Kallio, 2001, the anti-inflammatory and analgesic effect of seed and pulp oils extracted from sea buckthorn berries were used to treat skin disorders. It was found that oils reduced acetic-acid-induced body writhing and topically applied oils reduced dimethylbenzene-induced ear swelling in mice. However, this treatment had no effect on egg-white-induced metatarsal inflammation in rats.

In a separate experiment, immunomodulatory activity of sea buckthorn leaf extract was evaluated in adjuvant induced arthritis (AIA) rate model. Sea buckthorn leaf extract was administered intraperitoneally to treat the inflammation which was induced by injecting complete Freund's Adjuvant (CFA) in the right hind paw of rats. Serial evaluation was carried out weekly after creation of inflammation. The results indicated that administration of sea buckthorn leaf extract on the same day or 5 days prior to inflammatory insult into the joints, significantly reduced the inflammation as compared to the untreated animals in a dose dependent manner (Ganju *et al.*, 2005). These observations suggest that the sea buckthorn leaf extract has a significant anti-inflammatory activity and has the potential for the treatment of arthritis.

In an experiment in canine, Varshney, *et al.* (2005 a) conducted a study for the evaluation of anti-inflammatory activities of *Hippophae rhamnoides* L. oil. The acute anti-inflammatory efficacy of sea buckthorn seed oil was investigated in 12 dogs of either sex. Localized acute inflammation was developed on the dorsal lumber area in all the animals. The development of inflammation and anti-inflammatory effects was monitored by skin thickness measurement by Vernier Calipers at different time intervals up to 7 days which revealed that the sea buckthorn seed oil has anti-inflammatory activity. The anti-inflammotory activity of sea buckthorn seed oil on sub acute inflammation was evaluated in 12 dogs till 21 days using cotton granloma inflammation model. However, the anti-inflammatory effect of sea buckthorn seed oil on sub acute inflammation in dogs was too mild to be detected.

The present study focuses on the effects of sea buckthorn leaf extract on nitric oxide (NO) production induced by lipopolysaccharide (LPS) in the marine macrophage cell line RAW 264.7. In addition, cell viability, free radical-scavenging activity and inducible nitric oxide synthase (iNOS) expression were also evaluated. Seabuckthorn leaf extract significantly inhibited the enhanced production of NO induced by LPS in a dose dependent manner.

Treatment with sea buckthorn did not reduce cell viability at any dose used. The extract showed significant scavenging of NO radicals released by the NO donor.

Treatment of macrophages with sea buckthorn leaf extract also caused a significant inhibition of iNOS activation. These observations suggest that the inhibition of net NO production by sea buckthorn leaf extract may be due to its scavenging activity and/or its inhibitory effects on iNOS activation. The study suggests that sea buckthorn leaf extract has significant anti-inflammatory activity and has potential for the treatment of inflammatory diseases (Padwad *et al.*, 2006)

5. Antimicrobial activity

Antimicrobial activity of Nordic berries including sea buckthorn and their phenols extracts on human pathogenic intestinal bacteria were investigated. Individual phenols responsible for antimicrobial activity were also identified (Puupponen-Pimia *et al.*, 2005). It was found that pathogenic bacterial strains, both Gram positive and Gram negative, were selectively inhibited by bio-active berry compounds. Phenols, especially ellagitannins, were strong inhibitors of Staphylococcus. Salmonella was only partly inhibited. Other compounds, such as organic acids, appeared to be responsible for the majority of the inhibitory effect. It was suggested based on the results that antimicrobial compounds present in berries may be of use in functional foods (Oksman-Caldentey, 2001).

6. Anti-viral

A new drug 'Hiporamin' was developed from purified tannin fraction of sea buckthorn leaves in respect of antimicrobial and antiviral activity. It has a wide spectrum of antiviral activity against Influenza viruses A and B, Herpes simplex type 1, adenoviruses type 2, HIP-1, and a mild antimicrobial activity in respect of gram+ and gram- microorganisms (Shipulina, 2001).

7. Apoptosis

The inhibitory and apoptosis-inducing effects of flavonoids from oil-removed seeds of sea buckthorn, *Hippophae rhamnoides*, (FSH) on liver cancer cell line BEL-7402 was investigated (Sun *et al.*, 2003). The results indicated that FSH has potent inhabitable effect on BEL-7402 cells by inducing apoptosis (Zhang, P. *et al.*, 2005).

8. Atopic dermatitis

Sea buckthorn berries are a rich source of vitamins A, C, and E, carotenes, flavonoids, and microelements such as sulfur, selenium, zinc, and copper (Beveridge, 2003). Which are edible and have been shown to protect from atopic dermatitis, hepatic injury, cardiac disease, ulcer, and atherosclerosis (Li, *et al.*, 2003; Yang *et al.*, 2000). Padmavathi *et al.*(2005) pointed out the mechanism of action in an animal (mouse) trial. They found that *Hippophae* inhibits benzo(a)pyrene-induced forestomach and DMBA-induced skin papillomagenesis. This decrease in carcinogenesis may be attritubted to the concomitant induction of phase II enzymes such as glutathione S-transferase and DT-diaphorase and antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in the mouse liver. It is concluded that *Hippophae* berry is able to decrease carcinogen-induced forestomach and skin tumorigenesis, which might involved up-regulation of phase II and antioxidant enzymes as well as DNA-binding activity of IRF-1, a known antioncogenic transcription factor causing growth suppression and apoptosis induction for its anticancer effect (Padmavathi *et al.*, 2005).

9. Boosting energy levels

It was reported that sea buckthorn, among others, can be used as dietary supplements to relieve fatigue and boost energy levels (Talbott, 2003).

10. Cancer cell proliferation

An investigation on the effect of berry and fruit extracts on the inhibition of cancer cell proliferation in vitro was conducted (Olsson *et al.*, 2004). Ten different extracts of fruits and berries including rosehips, blueberreis, black currant, black chokeberries, apple, sea buckthorn, plum, lingonberries, cherries, and raspberries were tested on cell proliferation of colon cancer cells HT29 and breast cancer cells MCF-7. The results indicated that fruit and berry extracts decreased the proliferation of both colon cancer cells HT29 and breast cancer cells MCF-7, and the effect was concentration dependent.

The inhibition effect for the highest concentration of the extracts varied 2-3-fold among the species, and it was in the ranges of 46-74% with an average of 62% for the HT29 cells and 24-68% with an average of 52% for the MCF-7 cells.

There were great differences in the content of the analyzed antioxidants in the extracts. The level of the vitamin C content varied almost 100-fold, and the content of total carotenoids varied almost 150-fold among the species. In addition, the composition and content of flavonolds, hydroxycinnamic acids, anthocyanins, and phenolics were found great differences among the 10 species.

In this investigation, the inhibition of cancer cell proliferation was found correlated with levels of carotenoids and vitamin C levels present at levels that can be found in human tissues. The same inhibition of cell proliferation could not be found by ascorbate standard alone. This correlation might indicate a synergistic effect of vitamin C and other substances. In MCF-7 cells, the anthocyanins may contribute to the inhibition of proliferation (Olsson *et al.*, 2004).

11. Carcinogenesis and Chemoprevention

It was reported that edible sea buckthorn (*Hippophae rhamnoides*) berries are a rich source of vitamins A, C, and E, carotenes, flavonoids, and micro elements such as sulfur, selenium, zinc, and copper. With unclear mechanism, the berries have been shown to protect from atopic dermatitis (Yang *et al.*, 2000), hepatic injury, cardiac disease, ulcer, and atherosclerosis. Padmavathi *et al.* (2005) found that the sea buckthorn berry inhibits benzo(a)pyrene-induced forestomach and DMBA-induced skin papillomagenesis in mouse. This decrease in carcinogenesis may be attributed to the concomitant induction of phase II enzymes such as glutathione S-transferase and DT-diaphorase and antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in the mouse liver. This was accompanied by a remarkable induction of the transcription factor interferon regulatory factor-1 in the sea buckthorn berry treated liver. The results indicated that sea buckthorn berry is able to decrease carcinogen-induced forestomach and skin tumorigenesis, which might involve up-regulation of phase II and antioxidant enzymes as well as DNA-binding activity of IRF-1, a known anti-oncogenic transcription factor causing growth suppression and apoptosis induction for its anti-cancer effect (Zhongrui and Shuzhen, 1993).

12. Cholesterol

In an animal trial, rats were divided into 5 groups and fed dietary fiber complex (DFC) at levels of 4, 16, and 64% for three month. Wheat fiber was used in the hypercholesterolemic control (HC) group and rats feeding on normal food were used as normal control (NC). Morphology of the small intesting, reticum and caecum were observed by light and electron microscope examination. Intestinal function was measured physically. The results showed that fecal weight and intestine wall were significantly raised in DFC group of higher level than NC and HC. It can be concluded that long term intake of DFC composed mainly of *Hippophae rhamnoides* L., bran, oat bran and guar gum at higher levels might induce some morphological changes of intestine and caecum. Therefore, DFC might be used at low level as an effective cholesterol-lowering agent. (Ma and Zhang, 2003)

13. Cytotoxicity

A study was conducted for the effect of the antioxidant activity of alcoholic extracts of sea buckthorn leaf and berry on nitric oxide induced cytotoxicity in J-774 macrophages (Geetha *et al.*, 2002 b). Sodium nitroprusside, which generates nitric oxide at the concentration of 500 mug.ml, induced cytotoxicity as revealed by decreased neutral red uptake by macrophages. The cytotoxicity of sodium nitroprousside was attributed to enhanced reactive oxygen species production, which in turn resulted in decrease in anti-oxidant levels.

Alcoholic leaf and fruit extracts of sea buckthorn at the concentration of 500 mug/ml were found to have a significant cytoprotective effect against sodium nitroprusside introduced oxidative stress. These extracts inhibited induced cytotoxicity, free radical production and maintained the anit-oxidant status identical to that of control cells. The alcoholic extracts of sea buckthorn was found to have significantly higher antioxidant activity than leaf extract against induced cytotoxicity in murine macrophages.

14. Gastric ulcer

It was reported that oils extracted from sea buckthorn seeds and pulp have traditionally been used in the treatment of disorders of skin and mucosa in China (Xing, *et al.*, 2002). A study was conducted on the experimental models of gastric ulcer in rats. Seed and pulp oil were orally administrated at a dosage of 7.0 ml/kg/day. The results indicated that the oil extracted from seed and pulp significantly reduced ulcer formation in water-immersion ($P<0.05$) and reserpine-induced ($P<0.01$) models in rats. In addition, administration at a dosage of 3.5 mg/kg/day significantly reduced the index of pylorus ligation-induced gastric ulcer ($P<0.05$) and sped up the healing process of acetic acid-induced gastric ulcer ($P<0.01$). These results suggested that the sea buckthorn seed and pulp oil have both preventive and curative effects against experimental gastric ulcers in rats.

15. Gastrointestinal tract and skin disorder

In Italy, it was reported, without explanation, that oil extracted from sea buckthorn berry can be treated for gastrointestinal tract and skin disorders (Paoletti and Raffo, 2004).

16. Genotoxic action

A study was conducted for the influence of sea buckthorn juice on the micronucleus frequency in bone marrow cells and sperm abnormality induced by cisplatin (cis-dichlordiammineplatinum-II) in male Swiss albino mice. Sea buckthorn juice prepared ex tempore was given to mice, at 0.3 ml, by gavage during 5 or 10 days. Three hours after the last

gavage, mice received cisplatin at doses either 1.2 mg/kg or 2.4 mg/kg i.p. Micronucleus frequency was studied in bone marrow polychromatic erythrocytes 24 h after the infection of the drug. The abnomality of sperm heads was studied by microscopy. the results indicated that the sea buckthorn juice decreased significantly the number of micronucleus in bone marrow cells induced by cisplatin at dose of 1.2 mg/kg by 36.5% and 47.9% (when it was given 5 and 10 days, consequently) and by 19.0% (P>0.05) at dose of 2.4 mg/kg. These results shown the sea buckthorn juice significantly decreased the damaging effect of cisplatin on sperm head at low dose, but not at the higher dose. Antigenotoxic effect of sea buckthorn juice was 45.0 % and 16.6 %, respectively. In conclusion, Nersesyan and Muradyan (2004) concluded that sea buckthorn juice decreased significantly the genotoxic effect of cisplain at dose of 1.2 mg/kg on somatic (Bone marrow) and germ (sperm) cells of mice.

17. Glyconeogenesis

An investigation was conducted for the effect of flavonoids from sea buckthorn seed oil (FSH) and pulp oil (FFH) on glycometabolism in mice. Male mice were randomly divided into three groups: control, FSH and FFH groups. Both FSH and FFH groups were given 3 dosages at 50, 100, and 150 mg/kg intragastorically. At the same time, the mice of control group were given physiological saline. The levels of serum glucose, serum cholesterol were determined when it lasted 7 and 14 days. After 16 days, glyconeogenesis test was made and liver glycogen was analyzed. The results indicated that the levels of serum glucose, serum cholesterol and serum triglyceride were significantly reduced by high dose of FSH and FFH, and glyconegensis was also obviously inhibited. In conclusion, FSH and FFH can decrease the levels of blood glucose and lipid in normal mice, and the effect of FSH and FFH on glycometabolism may be related to the control of glyconeogenesis (Cao *et al.*, 2003).

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19. Immunomodulatory effects and immunosuppression

A study was conducted for the immunomodulatory effects of sea buckthorn leaf extract on cellular and humoral immune. Oral feeding of chromium (30 mg/kg bw) significantly inhibited antibody production and S-RBC induced delayed-type hypersensitivity response. Administration of leaf extract at 100 mg/kg of body weight along with chromium significantly inhibited chromium-induced immunosuppression. To understand the immunomodulatory mechanism of leaf extract, in vitro studies were carried out suing rat lymphocytes. Addition of chromium resulted in a significant decrease in lymphocyte size and increased reactive oxygen species (ROS) generation. The leaf extract of sea buckthorn significantly inhibited chromium-induced ROS generation and maintained the cell size identical to that of control cells.

Chromium treatment markedly inhibited the mitochondrial transmembrane potential by larger lymphocytes in particular, while the sea buckthorn leaf extract restored the same significantly. It was concluded that the leaf extract of sea buckthorn has significant immunomodulatory activity and specifically activates the cell-mediated immnue response (Geetha *et al.*, 2005).

20. Ischemic cerebral infection

A study for the protection of sea buckthorn seed oil on ischemic cerebral infarction in rats and the mechanism of the action was conducted (Zhang, 1987). Focal cerebral ischemic model was made by middle cerebral artery occlusion in rats. Behavior obstacles of rats were observed. Cerebral infarction volume was determined by Magnetic Resonance Imaging. The results indicted that the administration of sea buckthorn seed oil at 0.7 and 0.35 g/kg could markedly reduce infarction volume after occlusion of middle cerebral artery in rats and also could ameliorate the behavior obstacles of rats. Cheng *et al.* (2003) concluded that the oil extracted from sea buckthorn seed had distinct protection to ischemic cerebral infarction in rats.

21. Memory impairment

An experiment was conducted for the effect of sea buckthorn juice on lead-induced memory impairment and neuronal damage in the brains of adult mice. Mice were exposed to lead acetate 10 mg/kg body weight for 20 days. In the water maze test, the swimming time was lengthened in mice treated with lead acetate, but this time period was decreased in mice received 20 and 40% sea buckthorn juice. The malondialdehyde levels were increased in lead-treated mice, which were reduced by 20 and 40% sea buckthorn juice in dose-dependant manner. the activities of acetylcholinesterase and monoamine oxidase A and B were significantly increased in the lead-treated group, which were decreased in mice by 40% sea buckthorn juice administration. The levels of norepinephrine, serotonin, and 5-hydroxyindole acetic acid were decreased significantly in the lead-treated mice, and the decreased were antagonized by 40% sea buckthorn juice, except for dopamine. These data suggested that the different doses of sea buckthorn juice protect against the lead acetate-induced deficits in learning and memory and changes in neurol-biochemical parameters (Xu *et al.*, 2005).

22. Lipase and lipoxygenase

The inhibitory effect of various extracts (lipophilic, hydrophilic-EtOH and hydrophilic-MeOH) from berries of six sea buckthorn cultivars, grown in North-Eastern Poland and

Byelorussia, on lipase and lipoxygenase activities was investigated. The total content of carotenoids in lipophilic and hydrophilic-MeOH extracts ranged from 293 to 816 mg/100 g and from 5.35 to 6.89 mg/100 g, respectively. β-carotene comprised between 84.3 and 95.6% of total carotenoids. The hydrophilic-MeOH extracts were rich sources of phenolics, but lower quantities of phenolics were also detected in both lipophilic and hydrophilic-EtOH extracts. The content of vitamin C in hydrophilic-EtOH extracts was 93-406 mg/100 g. Lipase activity was reduced by 40-70% and 13-50% by lipophilic and hydrophilic-EtOH extracts, respectively. The effect of hydrophilic-MeOH extract on lipase activity was negligible. The inhibitory effect of sea buckthorn extracts on lipoxygenase activity was reduced by sea buckthorn berry extract. Lipase activity was reduced by lipophilic and hydrophilic-ethanol extracts (Zadernowski *et al.*, 2002).

23. Human promyelotic leukemia cells

It was reported that the proliferations of human promyelotic leukemia HL-60 cells were inhibited as the concentrations of flavonols extracted from sea buckthorn were increased (Hibasami *et al.*, 2005). The order of the extent of growth inhibition by the flavonols at a concentrationof 20 microM is as follows: pentamethylquercetin >syringetin >isorhamnetin >quercetin >kaempherol >myricetin..

Apoptotic morphological changes of the nucleus, including chromatin condensation were induced in the HL-60 cells treated with quercetin, kaempherol and myricetin, respectively, but not in the cells treated with the other flavonols. The fragmentations of DNA by quercetin, kaempherol and myricetin, respectivly, to oligonucleosomal-sized fragments, a characteristic of apoptosis, were observed to be dose-dependent in the HL-60 cells. These findings suggest that growth inhibition by quercetin, kaempherol and myricetin, respectively, results from the induction of apoptosis by these flavonols. The other flavonols, pentamethylquercetin, syringetin, and isorhamentin, having methoxy (-OCh3) group inhibited more strongly than the above 3 flavonols without induction of apoptosis in the HL-60 cells. These finding suggest that mechanisms of growth inhibiton by pentamethylquercetin, syringetin and isorhamnetin are different from the apoptosis caused by quercetin, kaempherol and myricetin (Hibasami *et al*., 2005).

24. Mental and physical performance

In a review article, Li (2003) indicated that mental and physical performance is affect by health, ageing, stress, disease, nutrition and fluid intake and by the quality of the environment. Medicinal plants with beneficial effects on human health are listed. The effects of Asian and American ginseng (*Panax ginseng, Panax quinquefolium*), Siberian ginseng (*Eleutherococcus senticosus*), Indian ginseng (*Withania somnifera*), Echinacea (*Echinaces purpurea*), Sea buckthorn (*Hippophae rhamnoides*), Roseroot (*Rhodiola rosea*), Schizandra (*Schisandra chinensis*), Angelica (*Angelica sinensi*s), Garlic (*Allium sativum*), Reishi mushroom (*Ganoderma lucidum*), Goldenseal (*Hydrastic canadensis*) and Tea (*Camellia sinensis*) on mental and physical performance are evident.

25. Nicotine induced oxidative stress

A study was conducted to determine the effects of sea buckthorn seed extract and vitamin E as a positive control on nicotine-induced oxidative stress in rat blood, specifically alterations in erythrocyte malondialdehyde level, activities of some erythrocyte antioxidant enzymes, and plasma vitamin E and A levels (Suleyman *et al.*, 2002). There were 8 rats per each of the four groups studied, nicotine group (0.5 mg/kg/d, intra peritioneal,

i.p.), nicotine + vitamin E group (75 mg/kg/d, intragastric, i.g.), nicotine + sea buckthorn seed extract group (1 ml/kg/d, i.g.), and control group (receiving only vehicles) tested for a period of 3 weeks.

The results indicated that nicotine-induced increase in erythrocyte MDA level was prevented by both sea buckthorn seed extract and vitamin E. Nicotine-induced decrease in erythrocyte superoide dismutase activity was prevented by sea buckthorn seed extract, but not vitamin E. Sea buckthorn seed extract increased the erythrocyte glutathione peroxidase activity compared with nicotine and the vitamin E groups. Catalase activity was not affected. Vitamin E supplementation increased plasma vitamin E level. Plasma vitamin A level was higher in both vitamin E and sea buckthorn seed extract supplemented groups compared with nicotine and control groups. These results suggest that sea buckthorn seed extract can be used as a dietary supplement, especially by people who smoke, in order to prevent nicotine-induced oxidative stress.

26. Radiation-induced oxidative damage

It has been reported that the extract of the sea buckthorn berry provided protection to whole mice, various tissues, cells and cell organelles against irradiation. Goel *et al.* (2005) further investigated the effect of sea buckthorn berry extract for its effects on mitochondria isolated from mouse liver for superoxide anion, reduced (GSH) and oxidized glutathione (GSSG) levels. Pre-irradiation treatment of mice with sea buckthorn berry extract (30 mg/kg) significantly inhibited the radiation-induced increase in superoxide anions, GSSG, thiobarbituric acid reactive substances, NADH-ubiquinone oxidoreductase, NADH-cytochrome c oxidoreductase, succinate-cytochrome c oxidoreductase activity and mitochondrial membrance potential maximally at 4 h. This study suggests that pre-irradiation treatment of mice with sea buckthorn berry extract protects the functional integrity of mitochondria from radiation-induced oxidative stress.

27. Radiation protection

A study was conducted to understand the mode of action of alcoholic extract of whole sea buckthorn berries (RH-3) which has already been reported to render more than 80% protection against radiation induced mortality in mice (Kumar *et al.*, 2002), Direct and indicrect antixidant action (free radical scavenging and metal chelating potential) were assayed using 2-deoxy ribose degradation and 2,2′-bipiridyl assays. Effect of RH-3 on radiation and chemical oxidant mediated DNA damage was evaluated usign single cell gel electrophoresis (Comet assay) and alkaline halo assay. It is concluded that RH-3, evinced only a mild free radical scavenging activiy at concentrations used in the present study, therefore, its ability to protect DNA could mainly be attributed to direct modulation of chromatin organization (Kumar *et al.*, 2002; Agrawal and Goel, 2002).

Hippophae rhamnoides (RH-3) administered intraperitonially (i.p.) to mice 30 minutes before whole body irradiation and whole body survival, spleen colony forming units (CFU) and haematological parameters were studied. investigation of free radical scavenging and antioxidant potential, fenton reaction, radiation mediated OH radical scavenging and chemically generated superoxide anions scavenging were studied in vitro while inhibitin of lipid peroxidation was studied in liver homogenate of mice. The results indicated that a dose of 30 mg/kg body weight of RH-3 rendered 82% survival as comapred to no survival in irradiated control. RH-3 demonstrated radioprotective effect (Goel *et al.*, 2002).

Recently, *Hippophae rhamnoides* (RH-3, an alcoholic extract of whole berries) has been reported to render chromatin compaction and significantly inhibit radiation induced DNA strand breaks (Goel *et al.*, 2003 a). To investigate the mechanism of action of RH-3, a study was conducted. It indicated that RH-3 caused compaction of reversible (< 100 microg/ml) and irreversible (>100 microg/ml) nature which was related to the magnitude of DNA-protein cross-links formed. Maintenance of chromatin organization, induction of hypoxia, hydrogen atom donation, free radical scavenging and blocking of cell cycle at G2-M phase by interfering with topoisomerase I activity seem to contribute towards the radioprotective efficacy of RH-3 (Goel *et al.*, 2003 b).

In a separate study, Goel *et al.* (2003b) indicated that RH-3, an alcoholic extract of whole berries of Hippophae rhamnoides, has been demonstrated to provide radioprotective activity in terms of survival of mice against whole body lethal irradiation (10 Gy). It was concluded that reduction in the radiation induced loss of cellularity of crypts and villi and also decrease in frequency of apoptosis could have contributed towards rotection of mice treated with RH-3 before irradiation.

Hippophae rhamnoides (RH-3, an ethanol extract of whole berries) has been recently reported to elicit dose-dependent pro and antioxidant properties in vitro, induced apoptosis in murine thymocytes. In a concentration-dependent manner, RH-3 induced apoptosis in thymocytes in ex vivo conditions. The maximum effect was observed with 100 microg/mL of RH-3. Beyond this dose, the induction of apoptosis was inhibited, as seen on the ladder formation. However, apoptotic body formation, another indicator of apoptosis, was not manifested when various doses of RH-3 (20-200 microg/mL) were administered (Goel *et al.*, 2004).

During *in vivo* experiments in mice, the radioprotective dose of RH-3 (30 mg/kg. b.w.) Induced significantly DNA fragmentation in thymocytes studied spectrofluorimetrically. RH-3 treatment before irradiation *in vivo* enhanced radiation-induced apoptosis. These results were confirmed by hypodiploid population studied flow-cytometrically and also by ladder formation (Goel *et al.*, 2004).

28. Sulfur dioxide inhalation induced disorder

Ruan *et al.* (2003) conducted an *in vivo* experiment to study injury of sulfur dioxide inhalation on organs and germ plasm of mouse as well as protective effect of sea buckthorn seed oil against this injury. It showed that sulfur dioxide inhalation induced the change of the ratio between organ and body of mouse organs, such as liver, lung, kidney, and spleen, and a significant increase of number of micronuclei and polychromatic erythrocytes while sea buckthorn seed oil offered a protection against such injury.

29. Tendon and ligament injury

Sea buckthorn is one of Chinese herbal drugs that are traditionally used to promote tendon and ligament injuries. The total flavones from sea buckthorn, with major constituents including quercetin, isorhamnetin and kaempferol, have been demonstrated with most of thebioactive properties of sea buckthorn (Fu *et al.*, 2005). In a study to evaluate the potential effect of sea buckthorn flavones in the restoration of ultimate stress of healing patellar tendon in a well-established gap wound model in rats. 0.1 mg flavones was injected to wound 1 day after the injury. The results showed that the ultimate stress of the healing tendon was significantly promoted by injection of flavones, increasing from 30 to 50% as compared to control with saline. Excessive fibrotic response was not found in

flavone treated animal, but an enhanced collagen deposition and a better fibre alignment were observed. These results suggest that flavones from sea buckthorn may improve the ultimate stress of healing tendons at early stages, which implies possible earlier rehabilitation programme and better recovery (Fu *et al.*, 2005).

30. Thrombosis or thrombus

It was reported that a treatment of sea buckthorn berries and especially the ethanol extracts of the sea buckthorn twigs showed inhibition of thrombus formation and blood coagulation system in animals and humans (Xu and Chen, 1991). Sea buckthorn has antioxidant, anti-ulcerogenic and hepato-protective actions, and its berry oil is reported to suppress platelet aggregation. Although it is frequently used for patients with thrombosis, the likely mechanisms and effects of total flavones from sea buckthorn on thrombogenesis remain unclear.

It has been reported that aspirin prevents secondary events in cardiac diseases (Berger *et al.*, 2006). To compare flavones from sea buckthorn with aspirin, Cheng *et al.* (2003) conducted an investigation for the effect *in-vivo* of total flavones from sea buckthorn on thrombogenesis and *in vitro* on platelet aggregation, comparing them to those of aspirin. It was found that total flavones has an effect similar to that of asprin on *in-vivo* thrombogenesis. Based on these findings, a daily taking of total flavones is also expected to prevent such events. Furthermore, total flavones protects the oxidation of low-density cholesterol and inhibits inflammation by immunomodulation effects that might contribute to the prevention of the progression of atherosclerosis. In conclusion, total flavones prevented *in-vivo* thrombogenesis, probably due to the inhibition of platelet aggregation. The results of this study suggest a potential clinical approach for the prevention of cardiac and cerebral thrombosis.

31. Wound healing

A study was conducted to evaluate healing potential of sea buckthorn leaves in a pre-clinical study on rats using a cutaneous excision-punch wound model. Four full-thickness excision-type wounds of 8.0 mm diameter were created on the dorsal surface of rats under aseptic conditions. The aqueous lyophilized extract of sea buckthorn leaves, at doses of 0.5%, 1.0%, and 1.5% w/v prepared in proplyene glycol, were applied topically twice daily for 7 days. Control rats received the vehicle alone in an identical manner.

Wound granulation tissues were excised on eighth day after the wounding, and the hydroxyproline, hexosamine, total protein content, and antioxidant levels were determined. Wound surface area was also measured on the eighth day before wound excision to determine wound contraction. Topical application of 1.0% sea buckthorn leaf extract significantly augmented the healing process, as evidenced by increases in the content of hydroxyproline and protein as well as the reduction in woud area when compared with similar effects in response to treatment using providone-iodine ointment. The results suggest that aqueous leaf extract of sea buckthorn promotes wound healing, which may be due to increased antioxidant levels in the granulation tissue (Gupta *et al.*, 2005).

In a separate experiment, Varshney *et al.* (2005b) evaluated the therapeutic efficacy of sea buckthorn seed oil in the healing of aseptic full thickness cutaneous wounds in calves and compared with antiseptic ointment (5% povidone-iodine), a commercially available herbal ointment (Dermanol, Indian Herbs Research and Supply Company Ltd., Saharanpur, India) and a bland ointment base (liquid paraffin). The study was conducted on 12 male calves divided into 4 groups. In each animal, six full thickness equidimentional cuta-

neous wounds, 3 on either side of vertebral column at dorsal thoracolumbar region were created under local analgesia.

The wounds were dreassed with liquid paraffin, 5% povidone-iodine ointment, Dermanol, and sea buckthorn seed oil in group I, II, III and IV respectively for 28 days. The efficacy of different treatments was monitored on the basis of different clinical and haematological parameters at 0, 3, 7, 14, 21, and 28 days. the cardiac and respiration rates, rectal temperature and haematological parameters remained within normal physiological limit I all groups during the study. Clinically, the Dermanol and sea buckthorn seed oil treated wounds remained relatively drier and exhibited less pronounced inflammatory reactions during early stage of wound healing. The percent wound contraction was greater in Dermanol and sea buckthorn seed oil treated groups at different intervls till 28days. The wounds of these four groups also exhibited early formation of firm scab and its earlier shedding. Howeer, in between Dermanol and sea buckthorn seed oil treated groups, the overal wound healing was comparatively slightly better in Dermanol treated rounds (Varshney *et al*., 2003).

A study conducted by Tyagi *et al*., 2005, for the effect of sea buckthorn (*Hippophae rhamnoides* L.) seed oil was evaluated on the development of gastric erosions and ulcerations and its healing course in dogs. Twelve adult healthy dogs were randomly divided in to three groups. To induce gastric erosions and ulcers, a steroidal anti-inflammatory drug 'Dexamethasone' was administrated at 1 mg/kg intramuscularly once a day to all the animals for a period of 15 days. After 15 days, group I was kept as negative control and no treatment was given whereas group II animals were treated with sea buckthorn seed oil administered orally at 5 ml/animal twice a day till endoscopically identifiable gastric erosions healing. In group III, sea buckthorn seed oil was administered at the same dose starting from day 1 and continued till gastric lesions healing.

The clinical symptoms of gastric erosions/ulceration such as anorexia, vomiting, haematemesis, and melema etc. were found to be significantly milder in group III as compared to other groups. Endoscopic ulcer score was also significantly lesser in this group till 12th day and the disappearance of gastric lesions after 15th day was also faster in this group. The gastric lesions healing was slowest in group I. However, it was not much different than group II. The hemoglobin level also did not show as sharp decline in group III as in other two groups.

32. Subchronic arsenic toxicity

Gupta and Flora (2005) started a study to investigate the therapeutic efficacy of sea buckthorn (*Hippophae rhamnoides* L.) against the toxic effects of arsenic in mice. Twenty-five Swiss albino mice were exposed to arsenic (25 ppm) in drinking water for 3 months. Sea buckthorn fruit extracts (500 mg/kg bw for 10 days) were administered 3 months later. Mice were sacrificed, and blood and tissues were assayed for various biochemical indicators of oxidative stress and whether arsenic was removed from tissues. The results indicated that sea buckthorn fruit extracts significant protection from arsenic inhibition of blood delta-aminolevulinic acid dehydratase activity and restored blood reduced glutathione levels. Significant protection was also observed in altered hepatic, renal, and brain substances levels. The aqueous extract of sea buckthorn also provided protection aginst parameters indicative of liver injury auch as aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase activities.

A post-treatment with an aqueous extract of sea buckthorn fruits significantly protects against arsenic-induced oxidative stress but does not chelate arsenic, suggesting it may have a beneficial role as a supplementing agent during chelation of arsenic by other means (Gupta and Flora, 2005).

Information from clinical trials

1. Liver fibrosis

In a clinical study, Gao *et al.* (2003) appraised the effect of sea buckthorn on liver cirrhosis patients. Fifty cirrhosis patients were randomly divided into two groups. Group A of 30 patients were treated orally with 15 g sea buckthorn extract in fine granules (Sichuan Pharmaceutical Co., China), 3 times a day for 6 months. Group B of 18 patients, taking one vitamin B complex tablet, 2 tablets once, 3 times a day for 6 month. The results indicated that the serum levels of TNFα, IL-6, laminin and type IV collagen in group A were significantly higher than those in the group B. After a course of sea buckthorn extract treatment, the serum levels of laminin (LN), hyaluronic acid (HA), collagen types III and IV, total bile acid (TBA) decreased significantly as compared with those before and after treatment in the Group B. The sea buckthorn has notably shortened the duration for normalization of amino-tranferases. Based on these findings, it was concluded that sea buckthorn extract may be a promising drug for prevention and treatment of liver fibrosis but further well controlled clinical trials are required.

2. Hypertension and chronic cardiac insufficiency

The effect of total flavonoids of Hippophae (TFH) extracted from sea buckthorn was investigated for the improvement of myocardial hypertrophy (Xiao *et al.*, 2003). A immunohistochemical method was used to assess the inhibitory effect of TFH on the activation of NF-kappa B by stretching cultured cardiac myocytes. It was found that the NF- kappa B was activated by stretching cardiac myocytes in 10 hours and the TFH at the concentration of 1:400, 1:200, and 1: 100, respectively, partially and completely inhibited the activation. The results indicated that the blockade of activation of NF-kappa B might be a potential access to the improvement in myocardial function with the use of TFH for treatment of hypertension and chronic cardiac insufficiency.

3. Hypertension

Zhang, X. *et al.* (2001) conducted an investigation of the effect of total flavones of *Hyppophae* (TFH) extracted from sea buckthorn on sympathetic nerve activity of essential hypertensive patients and to determine whether TFH possesses inhibitory effect on sympathetic activity after supine isometric exercise. Eighty eight patients were randomly divided into three groups, TFH group (n=35), calcium antagonist nifedipine (CAN) group (n=33), and verapamil ER group (n=20) for heart rate, blood pressure and plasma catecholamines, measured before and after the treatment. It was shown after 8 weeks of treatment that TFH group did not alter the resting heart rate and plasma catecholamine concentration. In contrast, nifedipine produced an increase of noradrenaline and adrenaline level. Isometric exercise may significantly increase the heart rate, blood pressure and plasma catecholamine concentration in hypertensive patients. In TFH group, exercise did not increase the heart rate, blood pressure and plasma catecholamine concentration after treatment. In CAN group, however, the same isometric exercise significantly increased the heart rate, blood pressure and noradrenaline concentration after treatment. In ER group, the plasma catecholamine concentration did not change after treatment, but it was significantly increased after isometric exercise. These results indicated that TFH does not alter the sympathetic activity in treatment of hypertension and the inhibitory effect of TFH on sympathetic activity after supine isometric exercise may provide clinical benefits.

4. Cardiovascular homeostasis

A study was conducted for the beneficial role of sea buckthorn in the maintenance of cardiovascular homeostasis following cold stress in human. Ethanol extract of sea buckthorn berries at the daily oral dose of 500 mg was studied on various electro physiological and neurochemical parameters following cold stress among the positive cold stress responders continuously for 3 months. the results indicated that the average differences in cardiovascular responses such as systolic and diastolic blood pressure, and pulse rate which increased following cold presser test, decreased following three months of oral administration of sea buckthorn berry. The results indicated that sea buckthorn berry exhibited beneficial effects to reduce the cardiovascular reactivity following cold stress and thus it enhances the stress tolerance capacity as well as better adaptation towards stress (Dubey *et al.*, 2003).

5. Mucous membranes

A study on the protective and curative effects of sea buckthorn oils on diseases involving the mucosa of the stomach, urogenital tract and mouth have been conducted. The antioxidatvie properties of sea buckthorn oils, which is mainly due to its high content of tocopherols, tocotrienols and carotenoids. It is described in relation to the suppression of malondialdehyde formation, a product of lipid peroxidation. This study investigated the role of sea buckthorn oil in the treatment of 5 patients with chronic vaginal inflammation. The patients orally received capsules of sea buckthorn oil with omega 7, a mixture of oils from seed and berry soft parts, 3 g per day for 12 weeks. The results indicated that sea buckthorn oil provided significant improvement in 3 cases with severe vaginal inflammation. Oestrogen levels at the end of the treatment trial were equal to those obtained at baseline. None of the patients reported any adverse effects. It was concluded that oral administration of sea buckthorn oil is a promising alternative instead of prescription drug for treating chronic vaginal inflammation (Erkkola and Yang, 2003).

6. Platelet aggregation

A small-scale preliminary cross-over study was conducted by Johansson *et al.* (2000) to investigate the effects of supercritical CO_2 extracted sea buckthorn berry oil (SBO) on some risk factors of cardiovascular disease. Special features of the oil are high proportions of palmitic, oleic, palimitoleic, linoleic, and α-linolenic acids as well as vitamin E, carotenoids, and sterols. Twelve healthy normolipidemic men were recruited and each volunteer consumed SBO and fractionate coconut oil (CO) (control) 5g per day for a period of 4 weeks in a random order. Phospholipid fatty acids, plasma lipids, and glucose were unaffected by SBO supplementation. Instead, a clear decrease in the rate of adenosine-5′-diphosphate-induced platelet aggregation and maximum aggregation were found. This suggested the beneficial effects of SBO on blood clotting.

Johansson *et al.* (2000) concluded that the result of this study suggested SBO could be of value when treating persons with increased tendency to blood clotting. They further indicated that further studies on the dose-response effects are needed to assess the practical use of SBO as natural adjuctive form of therapy in the primary and secondary prevention of cardiovascular disease.

7. Coronary heart diseases

There is increasing evidence to support the hypothesis that free radical-mediated oxidative processes contribute to atherogenesis. More recently the ability of antioxidant nutrients to affect cell response and gene expression has been reported in vitro, providing a

novel mechanistic perspective for the biological activity of antioxidants.

Sea buckthorn is a rich source of antioxidants both aqueous and lipophilic, as well as polyunsaturated fatty acids (Eccleston *et al.*, 2002). A study was conducted for the effects of antioxidant-rich sea buckthorn juice on risk factors for coronary heart disease in humans. The objective was to characterize the antioxidant profile of sea buckthorn juice and to evaluate its effect on plasma lipids, LDL oxidation, platelet aggregation and plasm soluble cell adhesion protein concentration. Twenty healthy male volunteers were given either a placebo or sea buckthorn juice for 8 weeks. Additional daily intakes of vitamin C, α-tocopherol, β-carotene and flavonoids through sea buckthorn juice supplementation were 462, 3.2, 1.0 and 355 mg respectively. The results indicted that there were no significant changes in plasma total cholesterol, LDL-C, platelet aggregation or plasma intercellular cell adhesion moleculeI levels between treatment groups. Although a 20% and 17% increase in plasma HDL-C and triacylglycerol concentrations were observed. Sea buckthorn juice supplementation also resulted in a moderate decrease in the susceptibility of LDL to oxidation (Eccleston *et al.*, 2002).

8. Dermatological diseases

In a double-blind, placebo-controlled study of 49 atopic dermatitis patients who received 5g of sea buckthorn seed oil, pulp oil or paraffin oil daily for 4 months, Yang *et al.* (1999) found significant improvement of atopic dermatitis in groups receiving pulp oil and paraffin oil. Subjects consuming sea buckthorn seed oil showed no improvement in dermatitis (Yang *et al.*, 2000). Patients receiving pulp oil showed a small but significant increase in HDL cholesterol level (Yang *et al.*, 1999). In a following, randomized, placebo-controlled, double-blind study, 5g of sea buckthorn seed oil, pulp oil or paraffin oil was administered to sixteen patients with atopic dermatitis for 4 months (Yang *et al.*, 2000). Skin fatty acids were measured. The group supplemented with seed oil showed slightly increased proportions decosapentaenoic acid (22:5n-3) and decreased proportions of palmitic acid in skin glycerophospholipids. The high levels of linoleic and palmitoleic acids in seed and pulp oil, respectively, did not influence the fatty acid levels in skin glycerophospholipids (Yang *et al.*,2000) on short term dietary modification. Sea buckthorn oil can also be used as cleanser (Zou, 1997) and a cosmetic agent for the lips (Gercikovs and Zoludeva, 1998).

9. Gastric ulcerations

In a clinical trial, 468 ulcer patients treated by laser irradiation plus choline blocking agents with adjuvant α-tocopherol antioxidants from sea buckthorn oil provided evidence in favour of the antioxidant addition. Biochemical findings (vitamin E and malonic dialdehyde concentrations), electron microscopic, ++histo-enzyme and amino acids studies supported the clinical evidence. (Degtiareva et al., 1991)

Chapter 5. Preventive functions - Antioxidant

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Sea buckthorn is widely reputed to have considerable medicinal value (Beveridge *et al.*, 1999; Zeb, 2004; Eccleston *et al.*, 2002) including reduction of radiation damage, healing of burns, oral inflamation and gastric ulcers, and prevention of cancer and heart disease. The strong association between increased fruit and vegetable intake and disease prevention has been explained by the content of antioxidant phytochemicals (Halliwell, 1997; Eccleston *et al.*, 2002) and reduction of free radical damage associated with such disease states. Geetha *et al.* (2002c) reported strong anti-oxidant and immunodulatory properties in sea buckthorn which inhibited chromium induced free radicle production, apoptosis , and DNA fragmentation and stopped the chromium induced inhibition of lymphocyte proliferation. These model studies using alcoholic extracts suggest marked cytoprotective properties present in sea buckthorn.

The common antioxidants in plant sources are vitamin C (ascorbic acid), tocopherols, carotenoids and flavonoids all of which are present in sea buckthorn in exceptional quantities. Anti-oxidants in sea buckthorn fall into two categories, those that are held in the aqueous (juice) phase (eg. ascorbic acid, flavonoids), and those held in the lipid phase in the pulp or seed oil or incorporated into the membrane systems of the plant cells (eg. tocopherols, carotenoids).

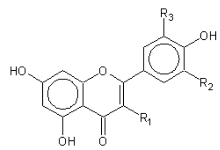
The exceptional levels of ascorbic acid present in sea buckthorn juices and products have already been documented in the section on organic acids. Vitamin C is one of the major contributors to the antioxidant activity present in the aqueous phase. The other major contributor(s) is a complex array of phenolic compounds which includes monomeric flavonols, monomeric flavanols such as catechin, various glycosides of these types of compounds and polymers of flavanol monomers of various degrees of polymerization, reported to degree of polymerization 65 (Le Bourvellec and Renard, 2005), termed collectively proanthocyanidins. The structural components making up a range of phenolic compounds in edible plants are shown in figure 1.

The range of phenolic acids and polymers are shown in figure 2. Proanthocyanidins (condensed tannins) are polymeric compounds of various sizes, composed of flavan-3-ol subunits and the example (Figure 2.) is based on catechin. The catechin sub units are normally linked through the C4→C8 carbons (Figure 2) or the C4→C6 carbons. In addition an ether bond C2→O7 is possible. The possible structures obtained by these various polymerizations and variations in the monomer subunits provide for complex, large molecules which are only now becoming understood, and methods developed for their analysis.

The structure, physical properties and measurement of proanthocyanidins, extracted from sea buckthorn fruit and bark (Xu *et al.*, 2006), has been reviewed by Santos-Buelga and Scalbert (2000). These compounds have been identified as dimers (ie. Procyanidin B_2), trimers, and procyanidin oligomers having a varied degree of polymerization extending to high numbers (Vanzani *et al.*, 2005; Vrhovsek *et al.*, 2004; Rosch *et al.*, 2004). This fraction has been identified (Vrhovsek *et al.*, 2004) with a broad peak in a defined HPLC normal phase system extending from just beyond 20 minutes to approximately 60 minutes elution

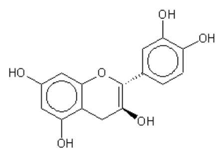
Figure 1. Some phenolic compounds found in sea buckthorn

Flavonols

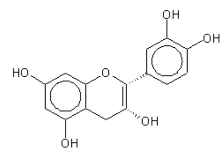


	R_1	R_2	R_3
Kaempferol	H	H	H
Quercertin	OH	H	OH
Myricetin	H	OH	OH
Rutin	rutinose	OH	H
Isorhamnetin	OH	H	OCH_3

Flavanols (Catechins)

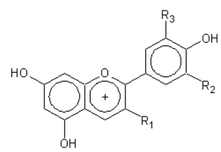


(+) - Catechin



(-) - Epicatechin

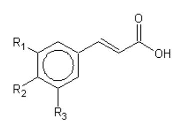
Anthocyanidins



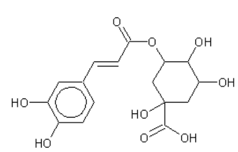
	R_1	R_2	R_3
Cyanidin	H	H	OH
Delphinidin	H	OH	OH
Pelargonidin	H	H	H
Peonidin	H	H	OCH_3
Kuromanin	glucose	H	OH

Figure 2. Phenolic acids and an example of procyanidin polymers found in sea buckthorn

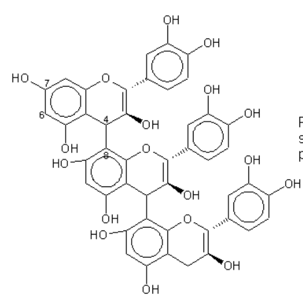
Phenolic Acids



	R_1	R_2	R_3
Cinnamic acid	H	H	H
Caffeic acid	H	OH	OH
Ferulic acid	H	OH	OCH_3
3-Coumaric acid	H	OH	H



Chlorogenic acid



Proanthocyanidin - representative structure of a catechin based trimeric procyanidin.

time. These proanthocyanidins are associated with very high antioxidant properties and are associated with antimicrobial, antiallergy, antihypertensive and anticancer activities (Santos-Buelga and Scalbert, 2000).

Specific phenolic compounds identified in sea buckthorn are listed in Table 1. There is general agreement that these phenolics are quercetin derived or derived from isomers of quercetin (isorhamnetin). Hakkinen *et al.*, (1999) found only quecertin in sea buckthorn and catechin, epicatechin gallic acid and other minor phenolics complete the composition (Table 3). In large measure the quercetin compounds exist as 3-0-glycosides with glucose, rutinose or rhamnose sugars making up the glycoside (Rosch *et al.*, 2004b).

Occasionally rhamnose is attached as a 7-O-rhamnoside. Of particular note there were no hydroxycinnamic acids detected (Table 1) and anthocyanins were either not present or were present in only small quantities. Maatta-Riihinen *et al.*, (2004) also detected no anthocyanins in sea buckthorn but reported trace quantities of the hydroxycinnamic acids ρ-coumaric, caffeic/ferulic acid. These findings are in contrast to the findings expected from common temperate fruit (eg. apples, pears, plums) where hydroxycinnamic acids are common and colors are derived from anthocyanin pigments. A small amount of myricetin glycoside and quercetin arabinoside were also reported (Maatta-Riihinen *et al.*, 2004).

Considering the anti-oxidant activity of sea buckthorn, it is notable that most of the activity associated with the juice or aqueous portion is derived from ascorbic acid or polymeric phenolics. In the data displayed in Table 1 (Rosch *et al.*, 2003), ascorbic acid represent 76.5% and 67.4% of the measured total anti-oxidant activity in non-enzymed and enzymed sea buckthorn mashes respectively, while polymeric proanthocyanidins represent respectively 20.1 and 29.0% respectively.

The next most active compound is (+)- catechin at 1.1 and 1.3%. Gao *et al.* (2000) also reported that the major anti-oxidant in seea buckthorn were vitamin C and phenols. Virtually all of the aqueous anti-oxidant activity in sea buckthorn can be ascribed to the three compounds cited. Some monomers, dimers and trimers of polymeric proanthocyanidins detected in sea buckthorn pomace by ESI-MS and NMR are shown in Table 2 as various polymers of catechin, epicatechin, gallocatechin and epigallocatechin.

It is known that ascorbic acid decreases with increased berry maturity (Tang, 2002; Raffo *et al.*, 2004) and it would be anticipated that the aqueous anti-oxidant capacity would fall with maturity also since ascorbic acid contributes much anti-oxidant activity to the berry. Crude aqueous extracts of sea buckthorn exhibited strong inhibitory activity toward lipid peroxidation induced by AMVN (2,2-azobis(2,4-dimethylvaleronitrile)) or ascorbate-iron (Gao *et al.*, 2000) and showed small but significant decreases in activity over the ripening period in the three Russian varieties tested.

Tocopherols (Vitamin E) are lipid soluble molecules exhibiting anti-oxidant properties through capture of free radicles by the OH function of the aromatic ring (Figure 3, 4). The structures of the various forms of tocopherols reported in sea buckthorn are shown in Figure 3 and 4 (Gunstone *et al.*, 1994).

There are several possible isomers of the tocopherols which differ in the substituents of the aromatic ring (tocopherols), the presence of double bonds in the phytyl side chain (tocotrienols), and the orientation of the methyl groups in the phytyl side chain (toco-

Table 1. Phenolic antioxidants in Sea Buckthorn.

Component	Concentration	Antioxidant Capacity	Reference
Flavanols			
Hippophoe rhamnoids -ssp. Sinensis	2900±61 mg/kg (berries)		Barl *et al.*, 2003
3 Chinese varieties	1487±12 (leaves)		Barl *et al.*, 2003
ssp. Mongolica	2910±60 (berries)		Barl *et al.*, 2003
Saskatchewan	1490±254 (leaves)		Barl *et al.*, 2003
"Flavanoids"	1182 mg/L		Eccleston *et al.*, 2002
Isorhamnetu rutinoside	355 mg/L		Eccleston *et al.*, 2002
Isorhamnetu glycoside	142 mg/L		Eccleston *et al.*, 2002
Quercetin rutinoside	35 mg/L		Eccleston *et al.*, 2002
Quecertin glycoside	35 mg/L		Eccleston *et al.*, 2002
Anthocyanins	0-0.4 mg/gm[1]		Olsson *et al.*, 2004
Quercetin	21± 3 mg/gm[1]		Olsson *et al.*, 2004
Quercetin glycosides	438±77 mg/gm[1]		Olsson *et al.*, 2004
Other flavonols	215±26 mg/gm[1]		Olsson *et al.*, 2004
Hydronycinnamic acid	non detected		Olsson *et al.*, 2004
Monomeric[2]			
(+) - catechin	19±1 mg/L	0.40 mm/L	Rosch *et al.*, 2003
(-) - epicatechin	2.8±0.1 mg/L	0.06 mm/L	Rosch *et al.*, 2003
Gallic acid	1.5±0.1 mg/L	0.07 mm/L	Rosch *et al.*, 2003
Protocatecheric acid	2.1±0.2 mg/L	0.08 mm/L	Rosch *et al.*, 2003
Quercetin 3-0- rutinoside	14.5±0.4 mg/L	0.15 mm/L	Rosch *et al.*, 2003
Quercetin 3-0-glucoside	9.0±0.4 mg/L	0.13 mm/L	Rosch *et al.*, 2003
Isorhamnetin 3-0- glucoside- 7-0 rhamnoside	75±4 mg/L	0.14 mm/L	Rosch *et al.*, 2003
Isorhamnetin 3-0- rutinoside	181±9 mg/L	0.13 mm/L	Rosch *et al.*, 2003
Isorhamnetin 3-0- glucoside	75±4 mg/L	0.07 mm/L	Rosch *et al.*, 2003
Isorhamnetin 7-0 rhamnoside	1.3±0.2 mg/L	0.01 mm/L	Rosch *et al.*, 2003
Isorhamnetin	1.4±0.3 mg/L	0.01 mm/L	Rosch *et al.*, 2003
Polymeric proanthocyanidins	351±16 mg/L	7.4 mm/L	Rosch *et al.*, 2003
Ascorbic acid	1220±64 mg/L	28.1 mm/L	Rosch *et al.*, 2003
Monomeric[3]			
(+) - catechin	26±2 mg/L	0.55 mm/L	Rosch *et al.*, 2003
(-) - epicatechin	5.2±0.3 mg/L	0.12 mm/L	Rosch *et al.*, 2003
Gallic acid	2.6±0.0 mg/L	0.12 mm/L	Rosch *et al.*, 2003
Protocatecheric acid	2.9±0.3 mg/L	0.10 mm/L	Rosch *et al.*, 2003
Quercetin 3-0- rutinoside	11.4±0.6 mg/L	0.12 mm/L	Rosch *et al.*, 2003
Quercetin 3-0- glucoside	7.7 ±0.1 mg/L	0.11 mm/L	Rosch *et al.*, 2003
Isorhamnetin 3-0- glucoside - 7-0- rhamnoside	66±3 mg/L	0.13 mm/L	Rosch *et al.*, 2003
Isorhamnetin 3-0- rutinoside	182±9 mg/L	0.13 mm/L	Rosch *et al.*, 2003
Isorhamnetin 3-0- glucoside	87±4 mg/L	0.08 mm/L	Rosch *et al.*, 2003
Isorhamnetin 7-0-rhamnoside	1.3±0.0 mg/L	0.01mm/L	Rosch *et al.*, 2003
Isorhamnetin	0.04±0.0 mg/L	<0.01 mm/L	Rosch *et al.*, 2003
Ascorbic acid	1220±85 mg/L	28.1 mm/L	Rosch *et al.*, 2003
Polymeric proanthocyanidins	573±6 mg/L	12.1 mm/L	Rosch *et al.*, 2003

[1]Values are per gram dry weight; [2]Values derived from raw, untreated berry mash; [3]Values obtained after treatment with carbohydrate hydrolying; enzymes.[4]Antioxidant capacity measure by ESR after reaction with Fremy's salt.

Table 2. Proanthocyanidin polymers identified in sea buckthorn (*Rosch et al.*, 2004).

Degree of Polymerization (DP)	Structure
1	EC, EGC
2	EC - EC
2	EC - EGC
2	EGC - EGC
2	GC - GC
2	C - C
2	C - EC
2	C - EGC
2	C - GC
2	GC - C
3	EC - EC - EC
3	EC - EC - EGC
3	EC - EGC - EGC
3	EGC - EGC - EGC
3	EGC - EGC - EC
3	EC - EGC - EC
3	EGC - EC - EC
4	detected, not identified

EC = epicatechin; EGC = epigallocatechin; GC = gallocatechin; C = catechin.

Figure 3. Tocopherols reported in sea buckthorn

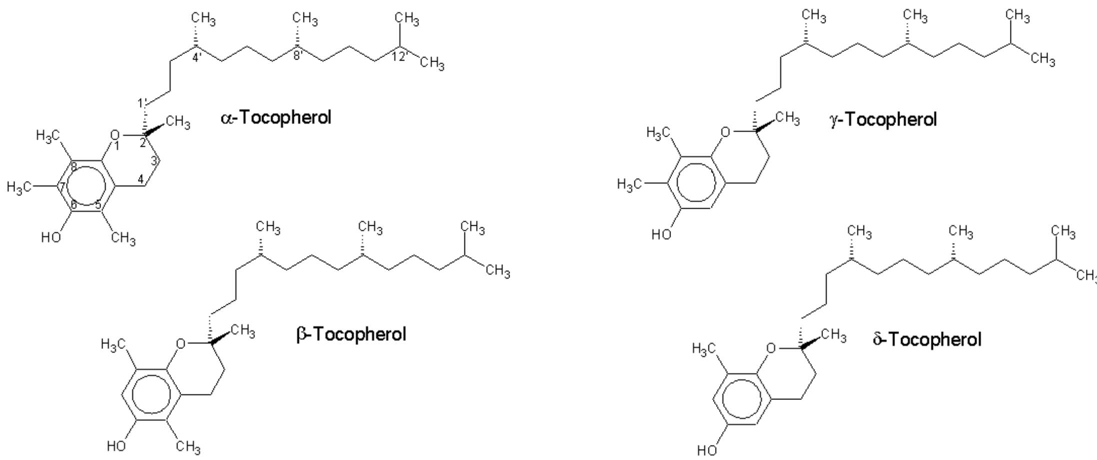
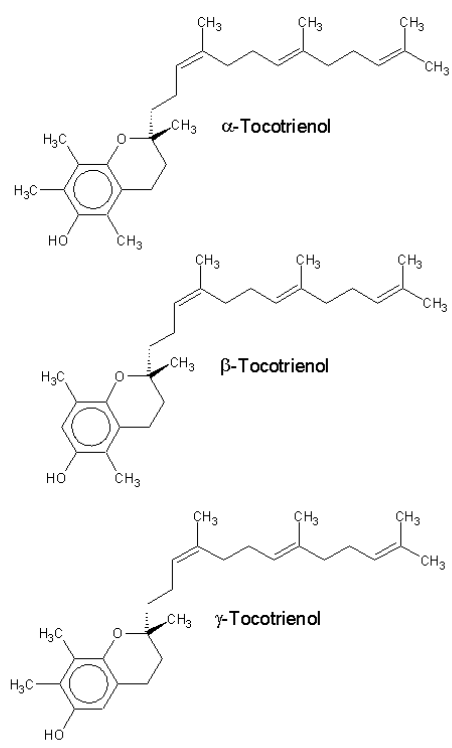


Figure 4. Tocotrienols reported in sea buckthorn



pherol stereoisomers, Figure 3) giving rise to a large number of possible isomers in both the tocopherol and tocotrienol forms. Not all of these possible isomers are present in sea buckthorn, and not all present the same Vitamin activity. For example, the calculation of biological vitamin E activity based on tocopherol content has been proposed by Eittenmiller *et al.* (1998) as $C_E = C_1 + 0.1C_2 + 0.03C_3$ where C_1 is α-tocopherol, C_2 is γ-tocopherol and C_3 is δ-tocopherol. In this representation γ-tocopherol and δ-tocopherol have only 10% and 3% respectively of the biological activity of α-tocopherol.

Vitamin E activity was earlier reported to range from 61-113 mg/100g in seed oil, from 162-255 mg/100g in pulp oil and to range from 390-540 mg/100g in the press residue (Beveridge *et al.*, 1999). Clearly, pulp oil is a better source of Vitamin E activity than seed oil, but the press residue is the best source. This would suggest that considerable Vitamin E active compounds are bound up with or held within the soft fleshy tissues, perhaps in cell walls or membranes. In more recent reports, α-, β-, γ- and δ- tocopherols and α-, β-, and γ- tocotrienols have been reported in sea buckthorn (Table 3).

In all samples, α- tocopherol is quantitatively the major form and β-, γ- and δ-tocopherol are minor constituents. Tocotrienols are quantitatively minor with two exceptions noted in the list. The Russian varieties (Zadernowski *et al.*, 2000) contain high levels of δ-tocopherol compared other varieties and this compound is also high in the seed of Chinese varieties (Kallio *et al.*, 2002b). Thus, in ripe berries, the major components contributing to the vitamin E activity of sea buckthorn is α- tocopherol with a major contribution from γ- tocopherol in seeds and from δ- tocopherol in some Russian varieties. Tocotrienols contribute only minor quantities in all reported cases. Zadernowski (2003) report high levels of γ- tocopherols early in the development of the Russian varieties (in addition to α- and δ- tocopherols) and, over development to maturity, the levels of γ-tocopherol fall to trace quantities while the levels of α-tocopherol and α-tocopherol increase by factors of ~2.5 and ~3.6 respectively. It is suggested that this results from early synthesis of γ-tocopherol and its transformation to the other two forms or to α-tocopherol with increasing maturity (Zadernowski *et al.*, 2003).

This statement indicates that Zadernowski *et al.* (2003) observed that α- and δ-tocpherols increased with increasing maturity increasing total tocopherol content. However, Kallio *et al.* (2002b) indicate a small rising trend for α-tocopherol, a decrease in β-tocotrienol (minor component) resulting in a slight increase, at best, in total tocopherol over an August 30 to November 30 time frame. This may explain some of the apparent differences in the two reports since Zadernowski *et al.*(2003) collected data from July 10 to December 29. The biggest part of the fall in γ-tocopherol and rise in α- and δ-tocopherol occurred in the July 10 to September 13 period with only small changes in tocopherol composition thereafter. This latter period of small change would approximately overlap the sampling period of Kallio *et al.* (2002b).

Very little work has been done on carotenoids in sea buckthorn since the subject was reviewed earlier (Beveridge *et al.*, 1999) and little new data is available. Beveridge et. al.(1999) reported sea buckthorn oil to contain 314 -2139 mg/100g carotenoid and Oomah (2003) reports pulp oil contains 5-10g/Kg and seed oil contains low levels at 20-85 mg/100g. The presence of β-carotene, γ-carotene, δ-carotene, β-cryptoxanthin and zeaxanthin in sea buckthorn has been confirmed by TLC, HPLC, and mass spectrometry (Oomah, 2003). In plant systems, including sea buckthorn, carotenoids are held in the cells and membranes of the pulpy fruit where they contribute to the anti-oxidant properties and preservation of membrane lipids. The carotenoids are held as carotenolipoprotein complexes whose physiological function is

Table 3. Tocopherols and Tocotrienol (mg/kg or mg/100gm oil)

Tocopherols and Tocotrienol (mg/kg or mg/100 gm oil)								
Sample	**Tocopherols**				**Tocotrienols**			**Reference**
	α	β	γ	δ	α	β	γ	
[1]**Whole berries**								
H. sinensis	70 ± 30	2.5	4.5 ± 2	~ 2	~ 2	~ 3	~ 2	Kallio *et al.* (2002 b)
H. mongolica	75 ± 25	5 ± 5	5 ± 5	~ 2	~ 2	~ 2	~ 2	mg / kg
[1]**Seeds**								
H. sinensis	45 ± 15	~ 10	55 ± 20	~10	nr	5 – 10	nr	
H. mongolica	165 ± 10	~ 13	95 ± 25	~ 12	nr	5 – 10	nr	
cv. Vadbaltycka	70 ± 7.6	nr	tr	36.6 ± 5.0	nr	nr	nr	Zadernowski
cv. Nevlejena	72.2 ± 5.2	nr	tr	40.5 ± 3.3	nr	nr	nr	*et al.* (2003)
cv. Otradnaja	83.3 ± 9.2	nr	tr	45.0 ± 6.9	nr	nr	nr	mg/100gm oil
cv. Podarok Sadu	72.0 ± 3.6	nr	0.25 ± 0.1	38.0 ± 3.9	nr	nr	nr	
cv.Trofimovskaja	63.4 ± 1.8	nr	tr	38.0 ± 1.0	nr	nr	nr	
cv. Hybrid 29-88	70.5 ± 3.3	nr	0.3 ± 3.0	nr	nr	nr	nr	
[1,2] **Berry seeds**								
H. sinensis	54 ± 30	~ 15	40 ± 25	~ 10	nr	~8	nr	Kallio *et al.* (2002 b)
H. rhamnoides	145 ± 20	~ 10	130 ± 7	~ 8	nr	~ 10	nr	mg/kg
H. mongolica	95 ± 5	~ 20	125 ± 10	20	nr	~ 6	nr	
[1,2] **Berry soft parts**								
Chinese sinensis	95 ± 35	10 ± 5	~ 5	nr	~ 3	10 ± 5	nr	
Chinese rhamnoids	35 ± 15	~ 3	~ 6	nr	~ 0	~ 0	tr	
Chinese mongolica	45 ± 20	~ 5	~ 6	nr	tr	~ 0	tr	
[1,2] **Berry Seeds, sinensis**								
Wenshui, China	25 – 82	7 – 21	25 – 75	5 - 7.5	nr	2 – 11	nr	
Xixian, China	225 – 140	9 – 13	65 – 95	5.5 – 10	nr	3 – 8.5	nr	
[1,2] **Berry Soft Parts, *H. sinensis***								
Wenshui, China	52 – 85	2 – 5.8	3.9 – 4.1	nr	nr	1.9 – 7.9	1 – 2	
Xixian, China	47 – 78	3 – 4.6	1 – 3.9	nr	nr	0 – 9.5	1 – 2	

[1] Estimated from a graphical presentation
[2] Values for Berry seeds and Berry soft parts given as maximum ranges over the mature period Aug. 30 to Nov. 30.
± Values are standard deviations
nr = not reported

unclear but which can be extracted from the pulpy fruit using detergent containing buffers (Pintea *et al.*, 2001).

Increasing information in the literature regarding the human disease preventive functions due to the antioxidant effects of sea buckthorn pulp and seed oil, juice, pomace, leaf, and twig have been reported recently.

1. Antioxidant and blood and cardiovascular system

A study was conducted by Shaov and Pshikova (2003) for the influence of the synchronized signals of action (SSA) which was formed in human organism by natural biological antioxidants from sea buckthorn on indices of the blood and cardiovascular system. The use of the natural biological antioxidants during 10 days normalized SaO2 fluctuation, pulse waves and blood distribution in tissues. SSA formed influence in the same way on recipients that were at the large distance from donor, influences were registered through

the short time - 6 min. A hypothesis was proposed about the distant control of physiological functions and organism adaptation processes through SSA, which was formed at the cell energetic and informational level by natural factors (biological antioxidants, adaptation to high mountains).

2. Antioxidant and its quality changes during fruit maturation

Different fractions of sea buckthorn fruits were investigated for antioxidant activity and its relationship to different phytonutrients (Gao et al., 2000). Antioxidant capacity of the lipophilic extract increased significantly and corresponded to the increase in total carotenoids. The phenolic fractions made a major contribution to the total antioxidant capacity. The lipophilic fractions were most effective if the comparison was based on the ratio between antioxidant capacity and content of antioxidant. The crude extract of sea buckthorn fruits showed that highest inhibitory effect in both 2,2-azobis (2,4-dimethylvaleronitrile) (AMVN) and ascorbate-iron induced lipid peroxidations. The aqueous and ascorbate-free extracts showed higher inhibition than the lipophilic extract in the AMVN assay, but lower inhibition in ascorbate-iron induced peroxidation. Gao *et al.* (2000) also pointed out that the capacity to scavenge radicals of the fruit crude extract, such as phenolic and ascorbate extracts, decreased significantly with increased maturation. These changes were strongly correlated with the content of total phenolics and ascorbic acid.

3. Antioxidant and immunodulatory properties

A study was conducted to determine the antioxidant and immunomodulatory properties of sea buckthorn leaves and fruits using lymphocytes as a model system. Chromium as potassium dichromate was used to induce oxidative damage. The production of free radicals by chromium and the ability of ethanol extracts of sea buckthorn leaf and fruit to inhibit the oxidative damage induced by chromium was investigated (Geetha et al., 2002 c). Addition of chromium (10 microg/ml) to the cells resulted in enhanced cytotoxicity, apoptosis, free radical production and decreased glutathion levels. Chromium also caused a significant inhibition of lymphocyte proliferation induced by both lipopolysaccharide and concanavalin A. Alcoholic extracts of leaves and fruits of sea buckthorn at a concentration of 500 microg/ml were found to inhibit chromium-induced free radical production, apoptosis, DNA fragmentation and restored the antioxidant status to that of control cells. In addition, these extracts were able to arrest the chromiun-induced inhibitionof lymphocyte proliferation. These observations suggest that the ethanol extracts of leaves and fruits of sea buckthorn have marked cytoprotective properties, which could be attributed to the antioxidant activity (Geetha et al., 2002 c).

4. Antioxidant and sea buckthorn pomace.

It was reported that residues such as peels and seeds that result from fruit juice production may contain substantial amounts of valuable natural antioxidants. In order to isolate the potential antioxidants gallocatechins and proanthocyanidins, sea buckthorn pomace was extracted by acetone-water and fractionated by Sephadex LH-20 gel chromatography and semipreparative HPLC. Five dimeric proanthocyanidins and nine trimeric proanthocyanidins were identified. The isolated flavan-3-ols and proanthocyanidins were potent in scavenging Fremy's salt, a synthetic free radical. They possessed antioxidant capacities that were higher or comparable to that of ascorbic acid or Trolox. On comparing the antioxidant capacities of monomeric flavan-3-ols and dimeric proanthocyanidins, no significant influence from the degree of polymerization was observed Rosch *et al.*(2004 a).

Rosch *et al.* (2004 b) reported that after flavonol glycosides, monomeric flavan-3-ols, and dimeric and trimeric proanthocyanidins were fractionated from an extract of sea buckthorn pomace by Sephadex LH-20 gel chromatography, oligomeric proanthocyanidins were eluted. The oligomeric fraction accounted for 84% of the total proanthocyanidins and 75% of the total antioxidant activity of the sea buckthorn pomace extract.

5. Antioxidant and coronary heart disease

There is increasing evidence to support the hypothesis that free radical-mediated oxidatve processes contribute to atherogenesis. Sea bucktorn is a rich source of antioxidants both aqueous and lipophilic, as well as polyunsaturated fatty acids. It was found that a 20% and 17% increase in plasma HDL-C and triacylglycerol (TAG) concentrations were observed with daily dose of sea buckthorn juice for 8 weeks. Sea buckthorn supplementation also resulted in a moderate decrease in the susceptibility of LDL to oxidation (Eccleston *et al.*, 2002).

6. Antioxidant activity of Sea buckthorn juice

The phenolic composition of sea buckthorn juice was investigated by HPLC with diode array and electrochemical detection (Rosch *et al.*, 2003). Flavonols were found to be the predominating polyphenols while phenolic acids and catechins represent minor components. Of the seven flavonols identified, isorhamnetin 3-O-glycoside were the most important representatives quantitatively. However, because of their structural properties, they were poor radical scavengers as shown by electron spin resonance spectroscopy. Phenolic compounds such as quercetin 3-O-glycosides, catechins, and hydroxybenzoic acids with a catechol structure exhibited good antioxidant capacities, but eheir concentration in sea bucktorn juice was small. These phenolic compounds, determined by HPLC, accounted for less than 5% of the total antioxidant activity of the filtered juice. Ascorbic acid was shown to be the major antioxidant in sea buckthorn juice. Because of its high concentration of 1.22g/L, it contributes approximately 75% to toal antioxidant activity. The remaining difference can be attributed to higher molecular weight flavan-3-ols (proanthocyanidins), which were determined photometrically after acid depolymerization to colored anthocyanidins (Rosch *et al.*, 2003).

7. Antioxidant and L-ascorbic acid

A study was conducted to identify the contents of L-ascorbic acid, phenolic substances and antixoidant properties of hydrophilic fraction extracted from various varieties of sea buckthorn berries (Shalkevich *et al.*, 2004). The contents of ascorbic acid was 27,4 - 80,5 mg/100g, phenolic substances 120,71 - 550,37 mg/100g. For the estimation of antioxidant properties 1,1-diphenyl-2-picrylhydrazyl, (DPPH) was used. Inhibition rate DPPH in all hydrophilic extractions was high and amounted 63,76-89,95%. Methanol fractions extracted from sea buckthorn berries with high contents of phenolic substances possessed the best antioxidant properties testified by maximum quantity of connected substance (DPPH - 13,04 mmol/g and 8,79 mmol/g accordingly).

8. Antioxidant and leaf extract

A study for the antioxidant activity of sea buckthorn leaves on chromium induced oxidative stress in male albino rats was conducted. Oxidative stress was induced in the rats by force-feeding of potassium dichromate equivalent to a dose of 30 mg/kg body weight of chromium for 30 days. Administration of chromium decreased the body weight

and increased organ to body weight ratio significantly. Chromium treatment significantly decreased reduced glutathione and increased malondialdehyde and creatine phosphokinase levels. In addition, it also enhanced glutamate oxaloacetate transforase and glutamate pyruvate transferase levels in the serium. Different doses of the alcoholic leaf extract of sea buckthorn were evaluated for the protection against the chromium induced oxidative stress. The results show that the leaf extract at a concentration of 100 and 250 mg/kg body weight protected the animals from the chromium induced oxidative injury significantly (Geetha *et al.*, 2003 a).

9. Antioxidant and bioactive phenols

An on-column capillary electrophoretic procedure for the determination of the antioxidative potential of bioactive phenols in sea buckthorn, Vaher *et al.* (2005) conducted an experiment by using hydrogen peroxide as a stressor, the kinetics of the oxidation of bioactive phenols including rutin, chlorogenic acid, quercetin, caffeic acid, gallic acid, and combinations of these compounds) and comapred with the oxidation rate of L-ascorbic acid as a reference. The concept was demonstrated for the determination of the antioxidative potential of various polyphenol mixtrues and of the methanol extract of the sea buckthorn. Vaher *et al.* (2005) claimed that the quercetin has the highest rate constant of oxidation among the tested phenolic compounds. However, they found that in the mixture of L-ascorbic acid and quercetin, the oxidation rate of L-ascorbic acid was enhanced and oxidation of quercetin was strongly inhibited compared with the other combinations of tested polyphenols.

10. Antioxidant and seed extracts

In an experiment, Negi *et al.*(2005) extracted sea buckthorn seeds with chloroform, ethyl acetate, acetone and methanol (MeOH) using a Soxhlet extractro for 8 h each of the solvent. The crude extracts were secreened for antioxidant and antibacterial activities. The reducing power and antioxidant activities evaluated in various in vitro models showed the highest activity for MeOH extract. The MeOH extract was also found to posses maximum antibacterial activity. The minimum inhibitory concentration (MIC) values, with respect to MeOH extrat for *Bacillus cereus*, *B. coagulans*, *B. subtilis*, *Listeria monocytogenes*, *Yersinia enterocolitic*a were found to be 200, 300, 300, 300, and 350 ppm, respectively. These results indicated the possibility of using sea buckthorn seeds extracts for medicinal uses and food preservation (Negi *et al.*, 2005).

11. Antioxidant and sodium nitroprusside induced cytotoxicity

A study for the antioxidant activity of ethanol extracts of sea buckthorn leaf and fruit on nitric oxide (NO) induced cytotoxicity in J-774 macrophages was conducted (Geetha *et al.*, 2002 b). It was found that the leaf and fruit ethanol extracts at concentration of 500 microg/ml were found to have a significant cytoprotective effect against sodium nitroprusside-induced oxidative stress. These sea buckthorn extracts inhibited sodium nitroprusside induced cytotoxicity, free radical production and maintained the antioxidant status identical to that of control cells. The fruit ethanol extract was found to have significantly higher antioxidant than leaf extract against sodium nitoprusside-induced cytotoxiity.

12. Sulfur dioxide exposure and sea buckthorn seed oil

In an animal trial, Wu and Meng (2003) investigated the effects of sulfur dioxide inhalation and protection by sea buckthorn seed oil from oxidative damage caused by sulfur

dioxide in male Kunming-strain mice. Sulfur dioxide at different concentrations (22+/- 2, 64 +/- 3, and 148 +/- 23 mg/m3) was administered to animals in treatment groups for 7 days, 6 h per day, while control groups were exposed to filtered air under the same condition. The activities of glutathione-S-transferase (GST) and glucose-6-phophate dehydrogenase (G6PD) and the contents of reduced glutathion (GSH) in brain, lung, heart, liver, and kidney of mice were measured. In the case of inhalation of a sulfur dioxide concentration of 148 +/- 23 mg/m3, the activities of GST and G6PD and contents relations were found between various sulfur dioxide exposed concentrations and the activities of GST and G6PD and the content of GSH. In groups given a high dosage (6 or 8 ml/kg) intraperitoneally, the level of thiobarbituric acid-reactive substances (TBARS) was decreased significantly ($p<0.05$) by the injection of sea buckthorn seed oil, and TBARS level exhibited a significant change in the glutathione redox system and indicted that sea buckthorn seed oil could contribute to the antixodiant effects in the case of sulfur dioxide exposure (Wu and Meng, 2003).

In a separate study, Ruan *et al.* (2003) conducted an experiment on the protective effects of sea buckthorn seed oil on mouse injury induced by sulfur dioxide inhalation. Micronuclei in the polychromatic erythrocytes of mouse bone marrow and the ratio between organ and body weight were determined and analyzed *in vivo* in order to study injury of sulfur dioxide inhalation on organs and germ plasm of mouse as well as protective effect of sea buckthorn seed oil against this injury. It showed that sulfur dioxide inhalation induced the change of the ratio between organ and body of mouse organs, such as liver, lung, kidney, and spleen, and a significant increase of number of micronuclei in the polychromatic erythrocytes, while sea buckthorn seed oil offered a protection against such injury (Ruan *et al.*, 2003).

Chapter 6. Chemical components contribute to the therapeutic claims

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Polyphenols, a group of substances with a broad spectrum of physiological activities, are abundant in medicinal plants used in traditional medicine. In addition to antioxidant properties, phenolic substances, primarily flavonoids, have numerous therapeutic effects (Kolesnikov and Gins, 2001). The clinical trials and scientific studies conducted recently in several countries confirm the values to medicinal use of sea buckthorn. The wide phytochemical composition of sea buckthorn contains vitamins, mineral elements, monosaccharides sugars, organic acids, free amino acids, large amount of carotenoids, volatile compounds and different flavonoids, phytosterols, and phenolic compounds (Zen, 2004). Flavonoids, such as leucocyanidin, catechin, flavonol (isorhamnetin, quercetin, quassin, and camellin), and a trace of flavanone are abundant in sea buckthorn leaves and fruits (Hakkinen *et al.*, 2000).

Phenols are effective against oxidation, tumorigenesis and radiation (Chen, 1988). A total flavonoid extract from sea buckthorn has anti-myocardial, anti-hyperlipemia and anti-fat-liver effects (Chai, 1989). Flavonoids have shown to remit angina and improve cardiac rhythm, improve functioning of the cardiovascular system, perhaps through a direct effect on the heart muscle, and treat coronary heart disease (Zhang, 1987). Sea buckthorn can also strengthen the immune system, increase human resistance against disease, and postpone senescence. It may also decrease peripheral vessel resistance to osmotic transfer and increase blood vessel elasticity (Xu *et al.*, 2001).

In a randomized, double blind, placebo controlled study, Wang *et al.* (2001) concluded that total flavonoids improved myocardial contractility and strengthened cardiac pump function in normal human subjects. China now produces acetylsalicylic flavonoid tablets as an prescription drug for heart function improvement. Sea buckthorn extracts can strengthen non-specific immunity functions, as demonstrated by anti-anaphylactic effects and increased phagocytic activity (Zhong, 1989).

Sea buckthorn oil is approved for clinical use in hospitals in Russia, and in China where it was formally listed in the "Pharmacopoeia" in 1977 (Xu, 1994). In early days, the most important pharmacological functions of sea buckthorn oil could be summarized as anti-inflammatory, antimicrobial, pain relief, and promotion of tissue regeneration (Li and Wang, 1998). The anti-inflammatory action of sea buckthorn oil is conferred by its high content of vitamin E, which can eliminate free radicals and decrease oxidation of unsaturated fatty materials in the membrane (Zhen et al., 1996). It also can be used for skin grafting, cosmetology, and treatment of corneal wounds. Russian researchers reported that 5-hydroxytryptamine (hippophan) isolated from sea buckthorn bark inhibited tumour growth (Sokoloff *et al.*, 1961). Others reported that the organic extract of sea buckthorn is safe and has adaptogenic immunomodulating properties and improves mental performance (Agrawal *et al.*, 2001 c).

Sea buckthorn extracts could improve the micro-circulation through scalp blood capillary vessels, and reduce hair loss. Sea buckthorn oil, which is safe and stable for a long period of time, is used as a major ingredient for the cosmetic cream, since it possesses therapeutic effects on skin (Singh, 2001). Agrawal *et al.* (2001a,b) reported that sea buckthorn is an effective remedial measure for the management of impaired mental functions, particularly to stop further momory loss among elderly people.

Sea buckthorn oil has been shown both in oral administration and topical application, to reduce inflammation, reduce pain and promote repair of tissue in animal models. Most health related data has been developed by using animal models such as induced gastric ulcerations, acute radio-dermatitis in rats and other radiation induced injuries (Zhang *et al.*,1989). Sea buckthorn oil, fed to guinea pigs, showed increased activity of some erythrocyte membrane enzyme systems and a reduced level of malondialdehyde (an oxidation product) in erythrocyte membranes (Rui *et al.*,1989). It was also reported that the oil can inhibit tumour development of transplanted tumours and both the oil and juice can kill S180 and P388 cancer cells and inhibit strains of human gastric carcinoma (Zhang *et al.*,1989). It can inhibit the liver cancers induced by aflatoxin B1 by inhibit the formation of N-nitroso compounds preventing the induction of cancers. Similar effects were found in limited clinical trials. Sea buckthorn seems to prevent lipid oxidation and these antioxidant activities are thought to contribute to the postponement of senility. There is evidence that sea buckthorn contains compounds that are anti-cancer in nature and these compounds are present in both the oil and juice fractions (Xu *et al.*, 2001).

Fruits of sea buckthorn have been used in Tibetan, Mongolian and Chinese traditional medicines for the treatment of different diseases for more than one thousand years (Zhou and Jiang, 1989). Sea buckthorn juice can be used as a disease preventive nutraceutical since it contains superoxides dismutase, which provides antioxidant activity, clearing free radicles from membrane systems (Jin, 1989). Sea buckthorn juice has a very high antioxidant activity, as high or higher than blueberries (Velioglu *et al.*, 1998). The therapeutic activity of the aqueous portion of sea buckthorn fruits may be due to this antioxidant capacity, which is attributed primarily to phenolic levels in the juice with which the activity correlates (Gao *et al.*, 2000). As well, some of the antioxidant activity would be expected to be contributed by the ascorbic acid known to be present in high amounts. Lipophilic antioxidant activity in sea buckthorn correlates, or is associated, with carotenoid levels in hexane extractives, although contributions from tocopherols (vitamin E) would be expected.

Sea buckthorn oil prevents lipid peroxidation in animals more effectively than vitamin E and the strong antioxidant activity of oil extracted from sea buckthorn fruit flesh protects erythrocyte membranes against peroxidation and cell aging in guinea pigs (Rui et al., 1989). Crude extracts of sea buckthorn fruit exhibit higher, metal ion catalysed, peroxidation inhibition than aqueous phenolic extracts. Hence, the anti-aging activity in the oil is postulated to be a component in the oil other than vitamin E, and carotenoid compounds seem probable candidates. However, fruit (pulp) oil of sea buckthorn contains a large number of bioactive substances (Schapiro, 1989) and more than 100 compounds have been identified from the unsaponifiable fraction of fruit oil, some of which may contribute to the biological activity of sea buckthorn oil (Wang *et al.*, 1989). Sea buckthorn oil triglycerides contain high concentrations of palmitoleic acid (>17%) that may have cholesterol and triglyceride lowering as well as stroke-suppressing effects, and the phytosterol, sitosterol, present in large amounts in the unsaponifiable fraction, is known to inhibit platelet aggregation. These effects will be noted in some detail in the following discussion.

In a study assessing the reparative effect on skin wounds in rabbits, sea buckthorn oil stimulated the regeneration of skin and complete healing was achieved in 13-14 days compared to 19-21 days for the control treated with sunflower oil. Gastric ulcers in rats induced by acetic acid shows healing, estimated as ulcer area reductions to 6.0 + 0.7 mm^2 for sea buckthorn oil administered by esophagus compared to reduction to 21 + 1.3 mm^2 for the control sunflower oil (Mironov *et al.* 1989). Animal studies showed that sea buckthorn oil had anti-inflammatory activity by inhibiting the development of the inflammatory reaction in the hypodermic skin tissue of mice (Lebedeva *et al.*, 1989), and appears to suppress the secretion of gastric juice in the gastrointestinal tract of cats and dogs (Khaidarov *et al.*, 1989). It quickly alleviates and cures esophagal and duodenal inflammations in rats. The anti-ulcer constituent, purported to be β-sitosterol-β-D-glucoside, is present at 10 times higher levels in seed oil than in pulp oil (Jiang et al., 1989). Sea buckthorn oil extends by 30 %, the survival period of radiation-treated mice by slowing the increase in polychromatic erythrocytes.

Treatment of operative wounds in ear, nose and throat with sea buckthorn oil has been documented. Sea buckthorn oil has also been used successfully in the clinical treatment of 56 cases of traumatic perforation of the tympanic membrane (Xu *et al.*, 2001). Sea buckthorn oil eases pain , decreases allergic reactions and facilitates exfoliation of the post-tonsillectomy nick membrane and has therefore been used in the treatment of the postoperative wounds of tonsillitis (Fayman,1991). Sea buckthorn cream made essentially from sea buckthorn oil has been reported to have therapeutic effects on chloasma, melanosis, keratoderma, xeroderma, recurrent dermatitis and other skin conditions when tested in 350 people in China (He *et al.*, 1989). It is also used in cosmetics as a natural plasticizer and emulsifier, in skin-regenerating compositions, and as natural UV filters.

Sea buckthorn oil posseses anti-tumor activities, since the seed oil is known to retard tumour growth by 30-50% (Wang *et al.*, 1989). Seed oil (1.59 g/kg body weight) injected intraperitoneally significantly (30%) inhibited the growth rate of transplanted melanoma (B_{16}) and sarcoma (S_{180}) tumors in mice, without any influence on thymus or spleen weights (Zhang *et al.*, 1989). The anti-tumor activity of oil from fruit residues (pulp) of sea buckthorn has been demonstrated by the significant increase in survival of mice bearing Ehrlich ascites carcinoma (Yang *et al.*, 1989). The survival time and life span increased in a dose-dependant manner when the oil was administered intraperitoneally at 125-500 mg/kg. Fractions extracted from the oil have no significant cytotoxic activity (50%) on human leukemia cell strain K562 at a concentration of 25 mg/mL (Yang *et al.*, 1989). The protective effect of sea buckthorn seed oil toward cervical cancer was thought due to the presence of vitamins A and E and β-carotene (Wu *et al.*, 1989).

Sea buckthorn oil is a good source of α-linolenic acid (18:3n-3) and other unsaturated fatty acids. The potential of α- and γ-linolenic acids to assist in the treatment of such conditions as dermatitis, rheumatoid arthritis and platelet aggregation in clinical applications is discussed by Barre (2001) for evening primrose, borage, black currant and fungal oils, all high in α- and γ-linolenic acids. It was very difficult to present clear, convincing evidence for the efficacy of these oils in preventing human disease. In the light of repeated observations of the inhibition of platelet aggregation with sea buckthorn oil (Zhao *et al.*, 1990; Johansson *et al.*, 2000), it seems likely that this activity is not associated with these fatty acids. However, the oil has a unique composition of fatty acids, including a spectrum of mono-unsaturated acids and is a rich source of two essential fatty acids, linoleic and α-linolenic and both the seed and pulp oils are rich in oleic (18:1n-9) acid.

There is increasing interest in the physiological role of mono-unsaturated acids relative to the modification of vascular endothelial surfaces and the role this may play in the reduction of thrombosis (Perez-Jimenez *et al.*, 1999; Turpeinen *et al.*, 1998), treat psoriasis (Hodutu, 1999), and peptic ulcer (Abidov *et al.*, 2002). Endothelial cells produce a large number of substances involved in adhesion and transendothelial migration of circulating leucocytes into the vascular cell wall, in addition to coagulation and fibrinolysis. All of these factors are involved in atherosclerotic development.

Leaves and bark also possess curative and preventative properties. Leaves contain vitamin C, carotenoids, and phenolic compounds. The bark of skeletal branches contains the alkaloid serotonin, this with its associated form, hippophaein, play an important role in stimulating the activity of the central nervous system. It is reported to be protective against radioactivity and swelling (unpublished data).

Chapter 7. Documentation of sea buckthorn publications supporting human health and wellness

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Introduction

Sea buckthorn (*Hippophae rhamnoides* L.) is considered as the next generation of new botanical because of its considerable medicinal value. In Asia, the use of sea buckthorn for health reasons started thousands of years ago and its use is still part of medical practice in China and many developing countries. Recently, developing and understanding of chemical composition and potential medicinal and cosmetic functions has attracted increasing attention from the scientific community around the world generating numerous research publications documenting the actual or potential health benefit of sea buckthorn.

This chapter is directed toward documenting individual literature citations in six major areas of research describing health benefits and describing briefly the benefits claimed or demonstrated.

Health of the circulatory system

1. Cardiovascular disorder

(A) Effect on cardiac function. Sea buckthorn (*Hippophae rhamnoids* L.) could raise the blood pressure of rats, widen the pulse pressure gap, enhance the contractility of myocardium, improve the diastolic function, and strengthen the whole heart pump function (Xu and Qian,1989).

(B) Effect on antagonization to myocardial ischemia. Total flavonoids of sea buckthorn could antagonize myocardial ischemic of rats induced by pituitrin, lower the whole blood viscosity of myocardial ischemia under the low shearing speed caused by ligation of coronary artery I rats; and enhance the tolerance of anoxic of mice under normal pressure and low pressure significantly. These results showed that total flavonoids from sea buckthorn has the effect of antiischemic and anoxic of myocardium (Xu and Qian,1989).

(C) Effect on anti-arrhythmia. Total flavonoids from sea buckthorn could lower the incidence of auricular fibrillation of mice induced by calcium chloride. acetylcholine, and recover the sinus rhythm. It could antagonize ventricular arrhythmia induced by aconitine, adrenalin and barium chlopide significantly (Xu and Qian,1989).

(D) Effect on antilipemic. Total flavonoids from sea buckthorn could lower serum cholesterol of hyperlipidemic rats and prevent elevation of lopid of rats, rabbits and chichens caused by hyperlipidemic diet (Xu and Qian,1989).

(E) Clinical application. The therapeutic effect of angina pectoris of coronary heart disease was 70% with marked significance, 20% with improvement and 8% without response. It has no aide effect observed (Xu and Qian,1989).

2. Contractile function

A study was conducted for the role of total flavonoids of sea buckthorn (*Hippophae rhamnoides* L.) seed and pulp oil in improving contractile function of stretched cardiac myocyte. The results indicated that flavonoids of the traditional Chinese drug *Hippophae rhamnoides* is effective in improving the contractile function of stretched cardiac myocyte in low dosage (Wang *et al.*, 2000). The influence of sea buckthorn oil on cyclophosphamide, farmorubicin and dioxadet mutagenicity was studied. The oil decreased significantly the cytogenetic action of cyclophosphamide and farmorubicin, but not of dioxadet action. (Nersesian *et al.*, 1990).

3. Chronic cardiac insufficiency

The effect of total flavonoids of sea buckthorn fruit and seed oil on the improvement in myocardial hypertrophy. The results supported that the blockade of activation of NF-kappa B might be a potential access to the improvement in myocardial function with the use of total flavonoids for treatment of hypertension and chronic cardiac insufficiency. (Xiao *et al.*, 2003).

4. Cytoprotective property

(A) A study was conducted for the cytoprotective properties of ethanol extraction of sea buckthorn leaves. Exposure of cells to hypoxia for 12 h resulted in a significant increase in cytotoxicity and decrease in mitochondrial transmembrance potential compared to the controls. Pretreatment of cells with ethanol leaf extract of sea buckthorn at 200 mug/ml significantly inhibited cytotoxicity, reactive oxygen species (ROS) production and maintained antioxidant levels similar to that of control cells (Narayanan *et al.*, 2005).

(B) Long-term administration of the common sea buckthorn bark and sprout extract improves the hormonal-metabolic organism status in rats disturbed by a stress factor (immobilization). The drug administration led to normalization of the altered functional activity indices of the neuro-endocrine system (disturbed adrenocorticotropin, 11-deoxy-cortisol, insulin, urea, and glucose levels) by affecting the production of glucocorticoids and increasing the hypothalamus sensitivity with respect of regulatory signals. (Krylova *et al.*, 2000).

5. Antioxidant

(A) The anti-oxidant activity of alcoholic extracts of leaf and fruit of seabuckthom (SBT) on nitric oxide (NO) induced cytotoxicity in J-774 macrophages. Sodium nitroprusside (SNP) generated NO induced cytotoxicity as detected by decreased macrophages uptake of neutral red at 500 mug/ml, induced cytotoxicity as revealed by decreased neutral red uptake by macrophages. The cytotoxicity of SNP was attributed to enhanced reactive oxygen species (ROS) production, which in turn resulted in decreased in anti-oxidant levels. Ethanol extracts of sea buckthorn leaf and fruit at the concentration of 500 ug/ml were found to have a significant cytoprotective effect against SNP-induced oxidative stress. These extracts inhibited SNP-induced cytotoxicity, free radical production and maintained the anti-oxidant status identical to that of control cells. The alcoholic fruit extract of SBT was found to have significantly higher anti-oxidant activity than leaf extract against SNP-induced cytotoxicity in murine macrophages. (Geetha, *et al.*, 2002 b).

(B) In a clinical study, sea buckthorn juice was used to characterize the antioxidant profile and to evaluate its effect on plasma lipids, LDL oxidation, platelet aggregation and

plasma soluble cell adhesion protein concentration. Twenty healthy male volunteers were given either a placebo or sea buckthorn juice for 8 weeks with daily intakes of vitamin C, alpha-tocopherol, beta-carotene and flavonoids through sea buckthorn juice supplementation were 462, 3.2, 1.0 and 355 mg respectively. There were no significant changes in plasma total cholesterol, LDL-C, platelet aggregation or plasma intercellular cell adhesion molecule 1 (ICAM-1) levels between treatment groups. Although it is not statistically significant, a 20% and 17% increase in plasma HDL-C and triacylglycerol (TAG) concentrations were observed. Sea buckthorn juice supplementation also resulted in a moderate decrease in the susceptibility of LDL to oxidation. (Eccleston *et al.*, 2002).

(C) A study to determine the effects of sea buckthorn (*Hippophae rhamnoides* L.) extract (HRe-1) and also vitamin E as a positive control on nicotine-induced oxidative stress in rat blood. Malondialdehyde level, activities of some erythrocyte antioxidant enzymes, and plasma vitamin E and A levels were determined. The results indicated that HRe-1 extract can be used as a dietary supplement, especially by people who smoke, in order to prevent nicotine-induced oxidative stress. (Suleyman *et al.*, 2002). Tannins from sea buckthorn has the effect on mitochondrial respiration. It inhibits the respiratory chain at the third point of conjugation in the transfer of electrons from cytochrome c to oxygen (Spiridonov *et al.*, 1997).

(D) An experiment was conducted to determine the anti-oxidative potential of various polyphenol mixtures and of the methanol extract of the sea buckthorn seed and pulp oil. In most cases quercetin has the highest oxidation rate constant among the tested phenolic compounds. Synergistic effect was evident in the mixture of L-ascorbic acid and quercetin, the oxidation rate of L-ascorbic acid was enhanced and oxidation of quercetin was strongly inhibited compared with the other combinations of tested polyphenols.(Vaher *et al.*, 2005).

(E) This study investigated the effects of sulfur dioxide (SO_2) inhalation and protection by sea buckthorn seed oil from oxidative damage caused by SO_2 in male Kunming-strain mice. One approach was set up to study the effects of SO_2 inhalation on changes of the mice antioxidant defense system.SO_2 at different concentrations (22 ± 2, 64 ± 3, and 148 ± 23 mg/m^3) was administered to animals in treatment groups for 7 days, 6 h per day, while control groups were exposed to filtered air under the same condition. The activities of glutathione-S-transferase (GST) and glucose-6-phosphate dehydrogenase (G6PD) and the contents of reduced glutathione (GSH) in brain, lung, heart, liver, and kidney of mice were measured. In the case of inhalation of a SO_2 concentration of 148 ± 23 mg/m^3, the activities of GST and G6PD and contents of GSH in the brain, lung, heart, liver, and kidney were significantly decreased. Another approach was taken to determine whether sea buckthorn seed oil could maintain the glutathione redox system and prevent oxidative damage to lung induced by SO_2. In groups given a high dosage (6 or 8 ml/kg) intraperitoneally, the level of thiobarbituric acid-reactive substances was decreased significantly ($p < 0.05$), and the activity of GST was increased significantly ($p < 0.05$). Overall GST activity and TBARS level exhibited a significant negative correlation ($r = 0.891$, $p < 0.05$). The observations showed that SO_2 inhalation resulted in a significant change in the glutathione redox system and indicated that sea buckthorn seed oil could contribute to protector agaist SO_2 exposure (Wu and Meng, 2003).

(F) A study was conducted to determine quality and quantity of the main components of sea buckthorn oils produced by different technological procedures, and to evaluate their

antioxidant activity. An influence of thermal shock to antioxidant activity of sea buckthorn oils (pulp oil and extracted seed soil) was also evaluated. The results have demonstrated that extracted oil has 2.4 times more carotenoids and more potent antioxidant. It was also found that after storing oil samples at 60 ± 2 °C, antioxidant activity decreased (Kasparaviciene *et al.*, 2004).

(G) The antioxidant, immune-modulators and anti-stress properties of sea buckthorn (*Hippophae rhamnoides* L.) oil were studied in-vitro using rat spleenocytes and macrophages and in-vivo using male albino rats. Addition of chromium to the cells resulted in enhance cytotoxicity, free radical generation, and decreased glutahione levels. Chromium also caused a significant inhibition of Concanavalin a stimulated inter-leukin production in lymphocytes.

The leaf extract of sea buckthorn inhibited chromium induced free radical production, apoptosis and restored the antioxidant status to that of control cells. The leaf extract at a concentration of 100 and 250 mg/kg body weight protected the animals from chromium induced oxidative injury significantly. The extract was also found to have a significant anti-stress and adaptogenic activity when tested in cold-hypoxia-restrain (C-H-R) stress animal model. These observations suggest that the leaf extract of sea buckthorn has significant cyto-protective, immunomodulatory and anti-stress activities (Geetha *et al.*, 2003 b).

(H) Different fractions of sea buckthorn fruits were investigated for antioxidant activity and its relationship to different phytonutrients. Capacity of the crude extract decreased significantly with increased maturation. The changes were strongly correlated with the content of total phenolics and ascorbic acid. Antioxidant capacity of the lipophilic extract increased significantly and corresponded to the increase in total carotenoid. The crude extract of fruits showed the highest inhibitory effect in both 2,2-azobis(2,4-dimethylvaleronitrile) (AMVN) and ascorbate-iron induced lipid per oxidations. The aqueous and ascorbate-free extracts showed higher inhibition in the AMVN assay, but lower inhibition in ascorbate-iron induced peroxidation, than the lipophilic extract (Gao, X. *et al.*, 2000).

(I) A study was conducted for the extraction of carotenoid-lipoprotein complexes from sea buckthorn fruit pulp. These complexes contain most of the valuable health promoting components (carotenoids, lipids, proteins, vitamins, minerals) of sea buckthorn fruits in a stable and bio-available form (Socaciu and Noke, 2003).

(J) In a study to reveal hepato-protective properties of the sea buckthorn seed and sea buckthorn pulp oils in chinchilla rats exposed to tetrachloromethane by using the organ-specific enzyme sorbitoldegydrogenase (SDG). The results indicated that the sea buckthorn seed and sea buckthorn pulp oils promoted normalization of SDG level 5 and 8 days respectively earlier than in the control group, which can be interpreted that sea buckthorn oil has apparaent hepatoprotective properties. (Lipkan and Oliinyk, 2000).

(K) A study of total flavonoids of *Hippophae rhamnoides* (TFHR) effect on the immune system (Xu and Qian, 1989). Enhancement of non-specific immunity effect. This effect can be shown by the significant increase of body weight, thymus index and spleen index on the laboratory mice, the enhancement of the phagocy tie function of macrophage as well as its phagocytic index. The elevation of the contents of serum lysozyme in mice and serum total complement of guinea pig was evident.

Enhancement of special immunity effect. The effect can be manifested by humoral immunity, such as the increase of contents of immunoglobulin, hemolysis, hemagglutinin, and by cell-mediated immunity, such as the increase of T lymphocyte, the modulation of the lymphoboast transformation an specific rosette formation. It also had the antagonistic effect of immune inhibition caused by immune inhibitor (Xu and Qian, 1989).

(L) In a study to determine the antioxidant and immunomodulatory properties of seabuckthorn using lymphocytes as a model system. Chromium (VI) as potassium dichromate was used to induce oxidative damage. Addition of chromium (10 microg/ml) to the cells resulted in enhanced cytotoxicity, apoptosis, free radical production and decreased glutathione levels. Chromium also caused a significant inhibition of lymphocyte proliferation induced by both lipopolysaccaride and concanavalin A. Alcoholic extracts of leaves and fruits of seabuckthorn at a concentration of 500 microg/ml were found to inhibit chromiun-induced free radical production, apoptosis, DNA fragmentation and restored the antioxidant status to that of control cells. These observations suggest that the alcohollic extracts of leaves and fruits of seabuckthorn have marked cytoprotective properties, which could be attributed to the antioxidant activity. (Geetha *et al.*, 2002 a).

(M) Hiporamin, a new drug with antiviral properties from polyphenols of sea buckthorn leaves, is a purified tannin fraction from sea buckthorn (*Hippophae rhamnoides*), possesing a wide spectrum of antiviral activity. The experimental data have shown that the new domestic vegetative preparation Hiporamin has unique biological properties, combining antiviral activity in respect of a wide spectrum of pathogenic RNA- and DNA-containing viruses (influernza viruses strains A and B, herpes simplex type 1, Adenoviruses type 2, HIV-1), a mild anti-microbial activity in respect of Gram + and Gram - microorganisms and having interferon induction properties (Tolkachev and Shipulina, 2003).

(N) Flesh seed oil from *Hippophae rhamnoide*s L. inhibited malondialdehyde formation of liver induced by carbon tetrachloride, acetaminophen, and ethyl alcohol, decreased significantly the activity of serum glutamic pyruvic transminase and significantly checks the depletion of glutathione damaged liver induced by acetaminophen. The microscopic and electron-microscopic examination has shown that the seed oil can lighten live injury. (Cheng *et al.*, 1990; Cheng *et al.*, 1994).

6. Anti-aging

Some researchers used sea buckthorn seed oil to study the effect of guinea pigs' red cell membrance on antilipid perooxidization. The results showed that the effect of sea buckthorn oil on retarding aging of red cells with antilipid peroxidization is greater than vitamin E of the same quantity. It was concluded that the effective ingredient of HR may be developed as a health drug for anti-aging (Xu and Qian, 1989).

It has been suggested that lutein and zeaxanthin may decrease the risk for age-related macular degeneration. Surprisingly, oleoresins rich in zeaxanthin are not yet available on the market. Several authors have reported enhanced stability of esterified xanthophylls, so plants containing zeaxanthin esters were investigated to establish valuable sources for the production of durable oleoresins. Liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (LC-(APCI)MS) was used to unequivocally identify zeaxanthin esters of a standard mixture and in several plant extracts (Weller and Breithaupt, 2003).

Zeaxanthin esters were quantified on the basis of their respective molecular masses using zeaxanthin for calibration; total zeaxanthin was determined after saponification of aliquots of the extracts. Thus, dried wolfberries (Lycium barbarum), Chinese lanterns (Physalis alkekengi), orange popper (Capsicum annuum), and sea buckthorn (*Hippophae rhamnoides*) proved to be valuable zeaxanthin ester sources. The present LC-MS method allows for an even more detailed analysis of zeaxanthin esters than reported previously (Weller and Breithaupt, 2003).

7. Dietary supplement

(A) A study to determine the effects of sea buckthorn (*Hippophae rhamnoides* L.) fruit extract and also vitamin E as a positive control on nicotine-induced oxidative stress in rat blood, malondialdehyde level, activities of some erythrocyte antioxidant enzymes, and plasma vitamin E and A levels. The results suggest that the extract can be used as a dietary supplement, especially by people who smoke, in order to prevent nicotine-induced oxidative stress. (Suleyman *et al.*, 2002).

(B) Twenty healthy male volunteers were given either a placebo or sea buckthorn juice for 8 weeks. Additional daily intakes of vitamin C, α-tocopherol, β-carotene and flavonoids throguh sea buckthorn juice supplementation were 462, 3.2, 1.0 and 355 mg respectively. There were no significant changes in plasma total cholesterol, LDL-C, platelet aggregation or plasma intercellular cell adhesion molecule 1 levels between treatment groups. Although not significant, a 20% and 17% increase in plasma HDL-C and triacylglycerol (TAG) concentrations were observed (Eccleston *et al.*, 2002).

Control of radiation injury

1. Anti-lipid peroidation

Sea buckthorn seed oil markedly inhibited malondialdehyde formation of liver induced by carbon tetrachloride, acetaminophen and ethyl alcohol on mice. Seed oil 4.75 g/kg body weight could lower serum glutamic pyruvic transminase levels induced by carbon tetrachloride and acetaminophen. It blocked also depletion of glutathione damaged liver induced by acetaminophen. The mechanism of heptoprotective actions of sea buckthorn seed soil might be related to anti-lipid peroxidation. (Cheng and Yu, 1992).

2. Radio-protective function

(A) An alcoholic extract of whole berries of *Hippophae rhamnoides*, has been demonstrated to provide radio protective activity in terms of survival of mice against whole body lethal irradiation (10 Gy). Administration of RH-3 (a preparation of *Hippophae rhamnoides*) before irradiation (-30 min.) increased the number of surviving crypts in the jejunum bya factor of 2.02 ($p<0.05$) and villi cellularity by 2.5 fold)$p<0.05$) in comparison to the irradiated control. (Goel *et al.*, 2003).

(B) The whole extract of the fresh berries of *Hippophae rhamnoides* L (RH-3) was investigated for its effects on mitochondria isolated from mouse liver to determine for RH-3 mediated radioprotective manifestation . The pre-irradiation treatment of mice with RH-3 significantly inhibited the radiation-induced increase in superoxide anion, GSSG, thiobarbituric acid reactive substances (TBARS). This study suggests that pre-irradiation treatment of mice with RH-3 protects the functional integrity of mitochondria from radiation-induced oxidative stress. (Goel *et al.*, 2005).

(C) A dose of 30 mg/kg body weight of RH-3 (*Hippophae rhamnoides* L. preparation) rendered 82% survival as compared to no survival in irradiated control. The endogenous CFU counts in mouse spleen on 10th post-irradiation day with and without RH-3 demonstrated radioprotective effect. It was concluded that free radical scavenging, acceleration of stem cell proliferation and immunostimulation are the radioprotective attributes (Goel *et al.*, 2005).

(D) Effect of RH-3 (a preparation of *Hippophae rhamnoides*) on radiation and chemical oxidant mediated DNA damage was evaluated using single cell gel electrophoresis (Comet assay) and alkaline halo assay. RH-3 inhibited 2-deoxy ribose degradation in a dose dependent manner (IC 50 approximately 500 mincrog/ml), 2.2'-bipiridyl assay revealed the inability of RH-3 to chelate Fe2+ ions. RH-3 inhibited radiation and tertiary butyl hydroperoxide induced DNA strand breaks in a dose dependent manner and at concentrations of 100 and 120 microg/ml the length of comet trail was considerably reduced and became almost similar to that of untreated control. RH-3, evinced only a mild free radical scavenging activity at concentrations used in the present study, therefore its ability to protect DNA could mainly be attributed to direct modulation of chromatin organization. (Kumar *et al.*, 2002).

(E) Immunostimulatory activity of alcohol extracted *Hippophae rhamnoides* berry, which may play an important role in the manifestation of its radioprotective efficacy (Prakash *et al.*, 2005).

Cancer control or inhibition

(A) The berry extracts of sea buckthorn decreased the proliferation of both colon cancer cells HT29 and breast cancer cells MCF-7, and the effect was concentration dependent. The inhibition of cancer cell proliferation seen in these experiments correlated with levels of some carotenoids and with vitamin C levels. The same inhibition of cell proliferation could not be found with ascorbic acid standards alone. This correlation might indicate a synergistic effect of vitamin C and other substances. In MCF-7 cells, the anthocyanins may contribute to the inhibition of proliferation (Olsson *et al.*, 2004.).

(B) It was found that sea buckthorn leaves could inhibit the growth of pre-cancer disorder of liver carcinoma which was induced by aflatoxin B1 in rats.The extract of sea buckthorn oil which has the direct cytotoxin effect of antagonizing human erythroleukemia cell line (K562) in vitro, could kill 50% of tumor cells in low concentration. It also prolonged the life of mice bearing Ehrlich ascites carcinoma. The HR juice has anti-neoplastic activity of the above tumor cell and gastric cancer cell (Xu and Qian,1989).

(C) Investigation of the inhibitory and apoptosis-inducing effects of flavonoids from oil-removed sea buckthorn seed on liver cancer cell line BEL-7402 was conducted. The resutls indicated that flavonoids has potent inhibitive effect on BEL-7402 cell line in a concentration-dependent manner. Flavonoid exerts its inhibitive effect on Bel-7402 cells by inducing apoptosis. (Sun *et al.*, 2003).

(D) Keeping in view the beneficial effects of sea buckthorn oil in the management of a variety of wounds through its diverse mechanism of action, the present research was undertaken to three phases: A. Toxicity studies of sea buckthorn oil upon topical application. B. Studies for the evaluation of anti-inflammatory activities of sea buckthorn. C. Studies for the evaluation of burn wound healing activity of sea buckthorn. The results

indicated that 1. sea buckthorn oil is non-irritating and does not have any kind of toxic effects upon its topical application. 2. Sea buckthorn oil has anti-inflammatory properties upon oral feeding in dogs (Varshney, 2003).

(E) Recent epidemiologic studies continue to show an association between high dietary intake of β-carotene and other carotenoids, particularly α-carotene and lutein, and reduced risk of lung cander (Cooper *et al.*, 1999a). However, the science community has not found conclusive evidence for a specific role of carotenoids in disease prevention (Cooper *et al.*, 1999b).Promising areas of investigation include characterizing biologic activities of carotenoids and gaining further insight into whether they may serve primarily as markers for a healthy lifestyle of diet (Cooper *et al.*, 1999a).

Key epidemiologic studies show associations between high dietary intakes of certain carotenoid containing fruits and vegetables and reduce risk of prostate, breast, head and neck cancers (Cooper *et al.*, 1999b). There is evidence that dietary fat, soy proteins, vitamin E, and selenium affect risk for prostate cancer (Fair *et al.*, 1997). On the other hand, β-carotene has not been consistently assoicated with reduced risk of prostate cancer in observational epidemiologic studies (Giovannucci *et al.*, 1995).

A number of dietary factors have been investigated for their role in breast cancer etiology, including caloric intake, dietary fat, protein, fiber, vitamins A, C, and E, carotenoids, and fruit and vegetables (Cooper *et al.*, 1999b). A comprehensive review of the epidemiologic literature on diet and breast cancer was published. it indicated that higher β-carotene consumption was associated with lower risk of breast cancer. However, the results were only significant in four out of eleven studies. (Clavel-Chapelon *et al.*, 1997).

It was reported that significant inverse associations between fruit and vegetable intake and cancers of the head and neck in 30 of 33 studies. Fruits in particular were reported to be protective in cancers of the esophagus, oral cavity, and larynx and were significantly associated with reduced risk in 28 of 29 studies (Block *et al.*, 1992). Epidemiologic and experimental data have suggested that retinoids and carotenoids may have chemopreventive activity causeing regression of oral leukoplakia, a premalignant lesion, thus preventing its progression to oral cancer (Ramaswamy *et al.*, 1996).

(F) Administration of sea buckthorn oil and anti-tumor preparations is possible and desirable in the complex treatment of neoplasma. Toxic effects of cytostatics on metabolism drugs were used together with sea buckthorn oil. (Abartene and Malakhovakis, 1975).

(G) An investigation was conducted to study the possibility of the effect of sea buckthorn seed oil on the hematopoitic reconstitution after high does chemotherapy. The mice were fed with sea buckthorn seed oil and counts of various blood cells. The results indicated that the counts of erythrocytes of were significantly elevated. The blood cell counts in myelosuppression mice fed with sea buckthorn seed oil exceeded those in control group, and the mortality was decreased. It can concluded that sea buckthorn oil can improve the hematopoiesis of erythroid linage. Like G-CSF, OHR can stimulate the recovery of hematopoiesis after chemotherapy (Chen *et al.*, 2003).

Wound healing

1. Anti-gastro-ulcerative activity

(A) β-sitosteriol-β-D-glucoside and its aglycone from sea buckthorn seed oil were investigated for their anti-gastro-ulcerative activity in rats with two models, chronic acetic acid-induced ulcers and cold stress-induced ulcers. Both the glucoside and its aglycone showed antiulcerative activity in chronic acetic acid-induced gastric ulcer models. The effect of aglycone appears better than the glucoside's. glucoside also showed visibly antiulcerative effects on cold stress-induced ulcers. (Xiao *et al.*, 1992).

(B) A screening test was conducted on mice with the use of a model of neurogenic damage to the stomach revealed anti-ulcerative activity in extracts of sea buckthorn. The results indicated that an extract of sea buckthorn produces has anti-ulcerative effect on being administered to animals with 'acute' ulcers and in treatment of chronic peptic ulcer. (Amosova *et al.*, 1998).

2. Anti-inflammatory

(A) Anti-inflammatory property of thick extract from sea buckthorn (*Hippophae rhamnoides* L.) fruit pulp of suppressed the development of carrageenin-induced oedema, UV-induced erythema and exudative peritonities. In most cases, the extract from sea buckthorn was superior to rutin (Sabynich *et al.*, 1994).

(B) Many researchers study the anti-inflammatory effect of sea buckthorn (*Hippophae rhamnoids*) oil from different points of view. Some used sea buckthorn seed oil to treat rat gastric ulcer caused by chronic reserpine method and acetic acid method with accelerated healing effect. Some used sea buckthorn oil to treat liver injury induced by carbon tetrachloride, alcohol, acetaminophen and others with liver-protective effect method. Some researchers produced acute radiation disease of mice by ^{60}Co radiation and radioactive esophagitis and radiative dermatitis by X-ray radiation.

All these experiments received good therapeutic effect with sea buckthorn oil. Some applied it clinically. In the treatment of 102 cases of radioactive dermatitis, mucositis, ulcer and burn, therapeutic effects were observed on all these cases by the application of HR oil compounds. These results indicated that sea buckthorn oil could reduce inflammation and diminish swelling, and accelerate the regeneration of tissue. It was concluded that a new drug can be developed for reducing inflammation, analgesic and accelerating the healing of ulcer (Xu and Qian,1989).

3. Anti-ulcerative effect

(A) Ethanol and aqueous extracts of 30 herbal medicines, including *Hippophae rhamnoides*, used traditionally for the treatments of gastric ulcers were screened in vitro for the anti-*Helicobacter pylari* actions. Significant anti- *Helicobacter pylori* actions with MICs around 60.0 ug/ml were exhibited by the ethanol extracts of *Hippophae rhamnoides* (Yang, *et al.*, 2005).

(B) The antiulcerogenic effect of a hexane extract (Hre-1) from sea buckthorn (*Hippophae rhamnoides* L.) was tested on indomethacin- and stress-induced ulcer models. As a result Hre-1 was found to be active in preventing gastric injury (Suleyman *et al.*, 2001.).

(C) Oils from sea buckthorn seeds and berries have traditionally been used in the treatment of disorders of skin and mucosa in China. Compared with the negative control, oral administration of CO_2 extracted seed and pulp oils, 7.0 ml/kg body weight/day significantly reduced ulcer formation in water-immersion and reserpine-induced models in rats. The results suggested that the CO_2 extracted sea buckthorn seed and pulp oils have both preventive and curative effects against experimental gastric ulcers in rats. (Xing *et al.*, 2002).

4. Dermatological damages and wounds

(A) Preparations of sea buckthorn are recommended for external use in cases of burns, bed sores, and other skin-complications induced by confinement to bed or treatment with X-ray and other radiations. (Pentegova, 1983).

(B) Sea buckthorn seed oil dressing was applied on the patients with burn wounds as an inner dressing and covered by disinfecting dressing. The oil dressing was changed every other day until wound healing. The results indicated that sea buckthorn seed oil dressing alleviated the swelling and effusion of the wounds and relieved the pains (Wang *et al.*, 2006).

(C) During in-vivo experiments in mice, the radioprotective dose of sea buckthorn (*Hippophae rhamnoides* L.) seed oil (30 mg/kg body weight) induced significant DNA fragmentation in thymocytes studied spectrofluorimetrically. This treatment before irradiation in-vivo enhanced radiation-induced apoptosis. These results were confirmed by hypodiploid population studied flow-cytometrically and also by ladder formation. Sea buckthorn treatment was pro-oxidative in nature because it depleted tholes and enhanced lipids per oxidation after 8 hours of treatment. (Goel *et al.*, 2004).

(D) An ethanol extract of sea buckthorn whole berries has been demonstrated to provide radio protective activity in terms of survival of mice against whole body lethal irradiation (10 Gy). Administration sea buckthorn berry extract before irradiation (-30 min.) increased the number of surviving crypts in the jejunum bya factor of 2.02 ($p<0.05$) and villi cellularity by 2.5 fold ($p<0.05$) in comparison to the irradiated control. (Goel *et al.*, 2003).

(E) In an animal trial, the effect exerted by sea buckthorn seed extract on the healing of experimental wounds in rats is studied dynamics of histomorphologically. The markedly stimulating effect on the healing process is explained by the rich content of vitamins (A, C, E, etc.) and microelements (sulfur, selenium, zinc, copper etc.) in the extract used. (Ianev *et al.*, 1995).

(F) Observing wound surface area and its reduction over time suggested that sea buckthorn oil healed open sores as well as an artificially prepared sea buckthorn oil imitation 'Aekol' and better than traditional wound healing remedies (Kostrikova *et al.*, 1990).

(G) Sea buckthorn seed oil speeds healing of chemical burns inflicted on rabbits eye. The effect was attained in the phases of atrophic disturbances and epithelialization (Agrawal and Goel, 2002).

It was observed that sea buckthorn fruit oil inhibits benzo(a)pyrene-induced forestomach and DMBA-induced skin papillomagenesis in mouse. The results indicated that sea buckthorn fruit is able to decrease carcinogen-induced forestomach and skin tumorigenesis. This might be accomplished by up-regulation of phase II and antioxidant enzymes as well as DNA-binding activity of IRF-1, a known antioncogenic transcription

factor causing growth suppression and apoptosis induction for its anticancer effect. (Padmavathi *et al.*, 2005).

Research group in Finland has published at least indicative results showing that sea buckthorn oils inhibit platelet aggregation and increase the HDL-cholesterol level but not the LDL-cholesterol level in man. The auricular and mesenteric micro circulation was improved in mice and rats, respectively (Kallio and Yang 2003). The oils also relieved symptoms and improved indicators of atopic dermatitis. Topical treatments of scalds and burns in rats by sea buckthorn oils reduced swelling, redness and secretion. In addtion, the oral administration of berry oil seemed to improve mucosal conditions in women with severe vaginal problems. In experimental animal models, the protection of gastric acylglyceros of special fatty acids, sterols, tocopherols, tocotrienols and carotenoids has benericial effects on health (Kallio and Yang, 2003).

Anti-microbial properties

(A) Infection by *Helicobacter pylori* has been ascertained to be an important etiologic impetus leading usually to chronic active gastritis and gastric ulcer with growing incidences worldwide. A standard strain (ATCC 43504) and five clinic isolates of *Helicobacter pylori* were used as the test pathogens, the antibacterial action was assessed in vitro with ethanol extracts of 30 Chinese herbal medicines which have been frequently prescribed since ancient times for treating gastritis-like disorders. Among these 30 herbal medicines, the ethanol extracts of *Abrus cantoniensis* (Fabaceae), *Saussurea lappa* (Asteraceae) and *Eugenia caryophyllata* (Myrtaceae) were strongly inhibitory to all test strains at approximately 40 ug/ml (MIC, minimum inhibitory concentration), and *Hippophae rhamnoides* (Elaeagnaceae), *Fritillaria thunbergii* (Liliaceae), *Magnolia officinalis* and *Schisandra chinensis* (Magnoliaceae), *Corydalis yanhusuo* (Papaveraceae), *Citrus reticulata* (Rutaceae), *Bupleurum chinense* and *Ligusticum chuanxiong* (Apiaceae) substantially active with MICs approximately up to 60.0 ug/ml (Yang, *et al.*, 2005).

The antibacterial actions of the aqueous extracts of the same herb medicines, those derived from *Cassia obtusifolia* (Fabaceae), *Fritillaria thunbergii* and *Eugenia caryophyllata* were remarkably inhibitory against all the six *Helicobacter pylori* strains. The work compared almost quantitatively the magnitude of the anti-*Helicobacter pylori* actions of the 30 most prescribed gastritis-treating Chinese herbal drugs, and located as well some source plants where potent anti-*Helicobacter pylori* phytochemicals could be characterized. (Yang, *et al.*, 2005)

(B) Sea buckthorn (*Hippophae rhamnoides* L.) seeds were successively extracted with chloroform, ethyl acetate, acetone and methanol (MeOH) using a Soxhlet extractor for 8 h each solvent. The crude extracts were screened for antioxidant and antibacterial activities. The reducing power and antioxidant activities were evaluated in various in vitro models (1,1-diphenyl-2-picrylhydrazine and liposome model system) showed the highest activity for MeOH extract. The MeOH extract was also found to posses maximum antibacterial activity. These results indicated the possibility of using seabuckthorn seeds for medicinal uses and food preservation (Negi *et al.*, 2005).

(C) A study was conducted for the antimicrobial properties of phenolic compounds present in sea buckthorn berries from Finland against probiotic bacteria and other intestinal bacteria, including pathogenic species. Antimicrobial activity of pure phenolic com-

pounds representing flavonoids and phenolic acids, and eight extracts from common Finnish sea buckthorn berries, was measured against selected Gram-positive and Gram-negative bacterial species, including probiotic bacteria and the intestinal pathogen Salmonella. Antimicrobial activity was screened by an agar diffusion method and bacterial growth was measured in liquid culture as a more accurate assay. Myricetin inhibited the growth of all lactic acid bacteria derived from the human gastrointestinal tract flora but it did not affect the Salmonella strain. In general, berry extracts inhibited the growth of Gram-negative but not Gram-positive bacteria. These variations may reflect differences in cell surface structures between Gram-negative and Gram-positive bacteria. Cloudberry, raspberry and strawberry extracts were strong inhibitors of Salmonella. Sea buckthorn berry and blackcurrant showed the least activity against Gram-negative bacteria. In conclusion, different bacterial species exhibit different sensitivities towards phenolics. The authors indicated that the significance and impact of this study is these properties can be utilized in functional food development and in food preservative purposes (Puupponen-Pimia *et al.*, 2001).

(D) An investigation was conducted for the effects of phenolics extracts from Nordic berries, including sea buckthorn, on pathogenic intestinal bacteria and to identify single phenolic compounds being responsible for antimicrobial activity. Eight human pathogens, pathogenic bacterial strains, both gram-positive and gram-negative were selectively inhibited by bio-active berry compounds. Phenolic compounds, especially ellagi-tannins, were strong inhibitory compounds against Staphylococcus bacteria. Salmonella bacteria were only partly inhibited by the berry phenolic, and most of the inhibitions seemed to originate from organic acids. It was concluded that antimicrobial properties of berries could be utilized in functional foods and pharmaceutical industry (Puupponen-Pimia *et al.*, 2005).

It was reported that sea buckthorn seed oil can treat some otorhinolaryngologic diseases (Paskar, 1973), and the oil may also control bacterial flora in acute rhinopharyngitis. (Paskar and Iuzefovich, 1974) and foot rot in sheep (Pivnicov *et al.*, 1989).

Chapter 8. Sea buckthorn juice for plant disease control

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Introduction

Plant diseases caused by bacterial and fungal pathogens reduce the world's food supply and often produce toxic substances that can cause disease in humans. Products that are considered organic are urgently needed for many plant diseases not adequately controlled by presently available methods. Sea buckthorn juice extracts or tea made from Sea buckthorn leaves would be ideal substances for the control of plant disease because they are organic, and are sustainable products that are not harmful to the environment. This chapter lists attempts by our laboratory to use Sea buckthorn juice extracts to control plant diseases in apple, grape, and stone fruits. Information on general Sea buckthorn horticulture and chemical composition of the products used in these trials can be found in, "Sea Buckthorn (*Hippophae rhamnoides* L.): Production and Utilization", by Li *et al.* (2003).

Diseases caused by Fungi

Apple powdery mildew

The powdery mildews are important diseases of a wide range of plants. Many products have been used to control these diseases. Organic products that includes milk, plant and mineral oils, plant extracts from *Reynoutria sachalinensis* (giant knotweed), neem, and compost tea have been found to be effective to varying degrees (Bélanger and Labbé 2002). However, as far as we are aware there are no reports on the use of Sea buckthorn extracts for the control of powdery mildew.

Apple powdery mildew is caused by the obligate fungus *Podosphaera leucotricha* and is an important disease especially in areas where irrigation is used to grow fruit (Jones and Sutton 1996; Turchek 2004). Conventional growers have several fungicides that will adequately control powdery mildew on apple preventing russeting of fruit. Organic growers rely almost completely on sulphur to control this disease. There is a need for an alternative and effective organic product other than sulphur for control of apple powdery mildew led to our testing Sea buckthorn juice extracts and tea made from its leaves.

The trials were conducted in the greenhouse on 'McIntosh' seedlings at the two-leaf stage. The seedlings were grown in trays and then transplanted into pots with a single plant per pot. Each treatment was applied to a single apple seedling replicated five times. Details on the procedure used for this trial can be found in the 2004 Pest Management Research Report (Sholberg and Boulé 2003). The treatments were applied with a hand atomizer approximately 20 mL per plant applied after 7 to 10 day intervals for a total of three applications per trial. Powdery mildew was rated as follows: over 50% of leaf covered = 3; less than 50% of leaf covered = 1; and no leaf powdery mildew symptoms = 0. Sea buckthorn treatments, rates, and powdery mildew incidence and severity are found in Table 1.

Table 1. Greenhouse trials using sea buckthorn (SBT) to conrol powdery mildew.

Trial	Treatment And Rate	Average Powdery mildew index[a]
1	Distilled water	14.8 a
1	SBT juice residue 1.5% v/v	9.8 ab
1	SBT centrifuged pulp oil 1.5% v/v	9.2 abc
1	SBT juice cv. *H. sinensis*	3.8 bc
1	SBT juice cv. Indian Summer	2.5 bcd
1	SBT 'Indian Summer' diluted 50% v/v	1.5 def
1	SBT seed oil 1.5% v/v	1.3 ef
1	SBT juice and seed oil 1.5% v/v	1.0 ef
1	Standard organic (Sulphur 2.0 g/L)	0.8 ef
1	SBT pulp oil 1.5% v/v	0.2 f
1	Standard fungicide (myclobutanil 0.1 g/L)	0.2 f
2	Distilled water	11.4 a
2	SBT 'Indian Summer' Tea 50% v/v	10.2 ab
2	SBT 'Indian Summer' leaf extract 2% v/v	8.2 abc
2	SBT 'Indian Summer' supernatant	2.5 de
2	Standard fungicide (myclobutanil 0.1 g/L)	4.2 bcde
3	Distilled water	33.0 a
3	Autoclaved SBT supernatant *H. sinensis*	11.6 b
3	SBT *H. sinensis* supernatant	7.6 bc
3	SBT *H sinensis* supernatant 50%	12.0 b
3	Standard fungicide (myclobutanil 0.1 g/L)	4.4 d

[a]Index values for each planted were counted and the average for the five plants was statistically analyzed using the analysis of variance and means were separated with the Waller-Duncan *k*-ratio t-test (k= 100). Values in the column with the same letter are not significantly different.

Extracts of Sea buckthorn were effective against apple powdery mildew in all three trials conducted in the greenhouse on 'McIntosh' apple seedlings. The oils from Sea buckthorn seed and pulp appeared to be the most effective extracts followed by juice and the supernatant from the centrifuged product. In the first trial the pulp oil was as effective as the standard myclobutanil (Nova™) fungicide treatment (Table 1). It was also noted that diluted *Hippophae rhamnoides* L. subsp. *sinensis* Rousi (Sinensis) fruit juice was more effective than pure juice. In the second trial only *Hippophae rhamnoides* L. cv. Indian Summer fruit extracts were tested (Table 1). The supernatant was as effective as the standard fungicide treatment reducing powdery mildew to very low levels when compared to the water as a control. Leaf extracts tested and tea made from leaves were not significantly different from the water control indicating that these products were not active enough to control powdery mildew. In the third trial the incidence of powdery mildew was extremely high in the control (Table 1). All three supernatant extracts produced after centrifuging Sinensis were effective and significantly reduced powdery mildew. Autoclaved supernatant remained effective indicating that the active ingredient of sea buckthorn was relatively stable and did not breakdown after heating.

One field trial was conducted with 'Indian Summer' Sea buckthorn juice. The results of this trial were disappointing. The juice caused russetting of 'Jonagold' apple fruit and likely made the apples unsaleable. Sea buckthorn seed or pulp oil would likely have been better choices for this study because they were more effective in the greenhouse trial although they came from sources outside our laboratory. Research is needed on the use of Sea buckthorn extracts to determine how to avoid russetting. Earlier timing and lower application

rates, or alternation with sulphur treatments could be the answer. However, where russetting is not a concern, such as on nursery trees, Sea buckthorn juice is a viable alternative to conventional fungicides.

Grape powdery mildew

Grape powdery mildew also known as Oidium is caused by the obligate fungus, *Erysiphe necator* (=*Uncinula necator*). It is the most important disease of grapes and is managed with cultural practices and by the application of fungicides (Thind *et al.* 2004; Jarvis *et al.* 2002). Several organic alternatives are known with petroleum and plant oils being comparatively effective (Sholberg 2004). Sulphur remains the most important organic material used on grapes for powdery mildew. Sea buckthorn juice could be an alternative to sulphur if they were effective because of their desirable organic properties.

A field trial was conducted at PARC on 16 year-old 'Pinot noir' grape vines (Sholberg and Walker 2004). The experimental design was a randomized complete block with five replicates per treatment. Each replicate consisted of five vines with the three middle vines being evaluated for powdery mildew. The treatments were applied approximately every two weeks starting on 29 May at prebloom, and ending on 16 September one week before harvest on 23 September, 2003. Percent incidence and severity of leaf and cluster powdery mildew were evaluated on 15 July and 3 September and again at harvest.

Sea buckthorn juice or the standard myclobutanil (Nova™) fungicide treatment applied six times over the growing season at a rate of 13.3 g/100L of water or 133 g/ha reduced disease incidence in this trial (Table 2). Both treatments reduced disease severity although Sea buckthorn juice was not as effective as the fungicide. Sea buckthorn juice did reduce disease severity almost in half after the first reading on 15 July and continued to reduce disease severity after the second powdery mildew reading on 3 September.

Table 2. Grape field trial on incidence and severity of powdery mildew on 'Pinot noir' grapes treated with sea buckthorn (SBT) juice in 2003

Date evaluated	Treatment and rate	% leaf powdery mildew		% cluster powdery mildew	
		Incidence	Severity	Incidence	Severity
15 July	Control	99.6 a *	51.8 a	100.0 a	46.3 a
15 July	50% SBT juice	98.8 a	24.2 b	098.0 a	23.5 b
15 July	Fungicide program	64.0 b	08.0 c	078.0 a	08.4 c
3 September	Control	099.2 a	74.7 a	100.0 a	90.8 a
3 September	50% SBT juice	100.0 a	49.8 b	100.0 a	41.8 b
3 September	Fungicide program	087.2 a	20.3 c	068.0 b	13.4 c

* Numbers followed by the same letter are not significantly different according to the Waller Duncan *k*-ratio t test ($k = 100$).

This trial indicates that Sea buckthorn juice could be an alternative control for grape powdery mildew if it does not impact grape quality. More research is needed on its use to find the most effective concentration and timing of application. Sea buckthorn products with activity against grape powdery mildew should also be considered as materials for alternating with sulphur to reduce the overall amount of sulphur used on grapes.

Stone fruit brown rot

Brown rot is considered the most important disease of stone fruit (Ram and Bhardwaj 2004) which is caused by three closely related fungi—*Monilinia fructicola*, M. laxa, and *M. fructigena*. The distribution of the pathogens overlap to some extent, but M. fructigena was eliminated from North America, and *M. fructicola* has not been detected in Europe (Ogawa *et al*. 1995). The disease has two phases, the blossom blight phase and the fruit rot phase. Fruit rot is the most destructive phase of the disease and is more common on mature fruit before and after harvest. Because Sea buckthorn juice was effective against apple and grape powdery mildew to some extent it was thought that it could potentially control fruit brown rot.

A dip trial with sea buckthorn undiluted juice, 1% Sea buckthorn pulp oil, and 1% Sea buckthorn seed oil was conducted at PARC on ripe apricot and peach fruit (Sholberg and Boulé 2003). Three replicates of 10 fruit per replicate were dipped for 1 min in 2 L of each Sea buckthorn treatment. Following treatment fruit were inoculated with spores of *Monilinia* spp. and incubated in the dark at 13°C for a week. Incidence of brown rot was recorded by counting the number of fruit with brown soft areas with gray to tan sporulation.

Sea buckthorn juice was not effective against brown rot on apricot (Table 3) but Sea buckthorn seed oil diluted in water to 1% significantly reduced peach brown rot. Further studies on the use of Sea buckthorn seed oil to control brown rot are warranted based on this trial. More information is needed on rates and the effects it has on fruit quality and appearance.

Table 3. Percent fruit brown rot on 'Blenhiem' apricot and 'Red Haven' Peach after dipping in Sea buckthorn (SBT) juice or 1% seed or pulp oil

Treatment and rate	Percent brown rot incidence	
	Apricot	Peach
Water control	83 bc*	93 a
SBT juice cv. Indian Summer	100 a	Not treated
SBT pulp oil 1%	Not treated	69 b
SBT seed oil 1%	77 bc	73 b
Iprodione 0.5g/L (Rovral)	30 d	4 c

*Numbers followed by the same letter are not significantly different according to the Waller Duncan *k*-ratio t test (*k* = 100).

Diseases caused by Bacteria

Fire blight of apple

Fire blight is a devastating disease caused by the bacterium, *Erwinia amylovora* that attacks many plants in the family *Rosaceae* including most notably apples, pears and Quince (Turechek 2004). Fire blight is endemic to North America and has spread to Western Europe, including Italy where recent large epidemics have occurred. The disease is not know to occur in South America, or Australia, but occurs in New Zealand and possibly Japan (Bonn and van der Zwet 2000). Fire blight symptoms can be divided into five different stages as follows: blossom blight, shoot blight, canker blight, trauma blight, and rootstock blight. The disease is difficult to control effectively as evidenced by the wide range of chemical products that have been tested (Psallidas and Tsiantos 2000). These

chemical products can be divided into three categories, copper compounds, antibiotics, and a few miscellaneous compounds. Biologically at least three bacterial antagonists have been developed for the control of fire blight, *Pseudomonas flurorescens* (strain A506), *Erwinia herbiocola* (strain C9-1) and *Bacillus subtilis* (Serenade) (Johnson and Stockwell 2000). Several other bacterial antagonists have been tested around the world but as yet none of them are available from commercial sources. None of these biological treatments are as effective as the antibiotic streptomycin which is the treatment of choice. However there are concerns with the wide spread use of streptomycin on plants because of concerns with the transfer of antibiotic resistance to human pathogens. Thus, there is an urgent need for a safe and effective material that is environmentally safe for control of fire blight.

Research at Pacific Agri-Food Research Centre evaluated several juice components of Sea buckthorn fruits for control of *E. amylovora* in the laboratory (Table 4). Nutrient agar plates were covered with actively growing *E. amylovora* cells and shortly after disks that contained 10 to 20 µL of Sea buckthorn juice or juice component were placed on the inoculated plates. Inhibition zone including disk (6 mm in diameter) was measured 24 to 48 h after plating and incubation at room temperature.

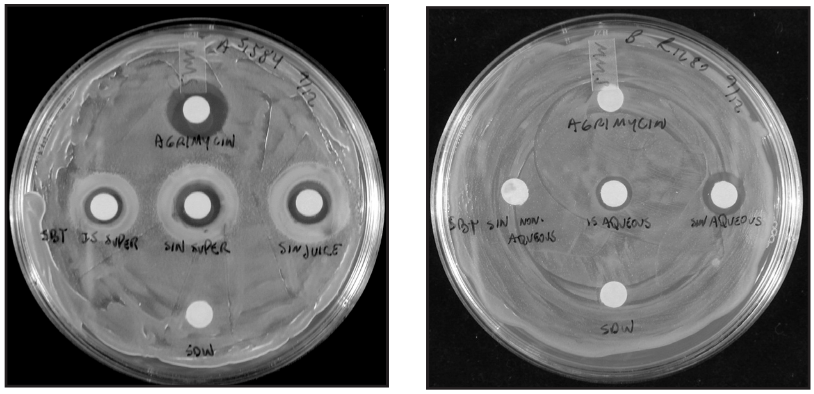
Table 4. Diameter (mm) of inhibition zone produced by sea buckthorn components against *Erwinia amylovora* isolates on nutrient agar

Treatment	*E. amylovora* isolate			
	1280*	1477	1584	1604
Streptomycin 100 ppm	0.0 d**	9.7 a	8.5 a	7.6 a
SBT 'Sinensis' juice	4.3 b	5.0 b	4.3 bcd	4.7 b
SBT 'Sinensis' supernatant	4.0 b	4.3 bc	5.3 b	4.3 bc
SBT 'Indian Summer' juice	3.0 c	3.0 d	3.3 de	3.0 d
SBT 'Sinensis' aqueous phase	5.0 a	4.3 bc	5.0 bc	4.7 b
SBT 'Indian Summer' aqueous phase	3.0 c	2.0 e	2.7 ef	2.3 de
SBT 'Sinensis' non-aqueous phase	0.0 d	0.0 f	0.0 g	0.0 f
SBT 'Indian Summer' non-aqueous	0.0 d	0.0 f	0.0g	0.0 f
SBT 'Sinensis' juice autoclaved	4.3 b	4.0 bc	4.7 bc	4.3 bc
SBT juice supernatant filtered	4.3 b	3.7 bc	4.0 cd	3.3 cd
Sterile Distilled Water Control	0.0 d	0.0 f	0.0 g	0.0 f

*This isolate is resistant to streptomycin.
** Numbers followed by the same letter are not significantly different according to the Waller Duncan *k*-ratio t test ($k = 100$).

Streptomycin was the most effective treatment against three isolates of *E. amylovora* known to be sensitive to streptomycin. In the case of streptomycin sensitive isolate 1584, streptomycin produced the largest zone of inhibtion as expected (Table 4; Fig. 1). On the otherhand, isolate 1280 was not inhibited by streptomycin and the Sea buckthorn 'Sinensis' aqueous phase was the most effective material. Thus in this case the Sea buckthorn extract was more effective than streptomycin. The trial also showed that the nonaqueous phase of Sea buckthorn did not inhibit *E. amylovora* and juice from the 'Sinensis' cultivar was more inhibitory than the 'Indian Summer' cultivar. The compound(s) in the aqueous phase that inhibited all four isolates from Sea buckthorn 'Sinensis' juice were not denatured by autoclaving. This indicates that the inhibitory compounds are not sensitive to heat and likely are relatively simple compounds.

Figure 1. Zones of inhibition for *E. amylovora* when challenged with streptomycin or sea buckthorn extracts.



(A) *E. amylovora* isolate # 1584 susceptible to streptomycin. Note zones around Agrimycin (=streptomycin), Sea buckthorn supernatant extract 'Indian Summer', Sea buckthorn supernatant extract 'Sinenesis', and Sea buckthorn juice 'Sinensis'.

(B) *E. amylovora* isolate 1280 resistant to streptomycin. Note zones only around Sea buckthorn aqueous extracts. Sterile distilled water = SDW was used as the control.

Table 5. Percent 'Gala' apple flowers blighted and shoots wilted by *E. amylovora*

Treatment and Rate	% Fire blight incidence*	
	Flowers	Shoots
Control	69 a**	31 a
Oxytetracycline 130 ppm	63 ab	30 a
Sea buckthorn juice	46 bc	17 ab
Streptomycin 135 ppm	40 c	12 b

*These values are means of six replication of 'Gala' potted apple trees.
**Numbers followed by the same letter are not significantly different at $p \leq 0.05$ according to the Waller-Duncan k-ration (k=100) test. Raw data were arcsin transformed before analysis.

A field trial with Sea buckthorn juice as a treatment to control blossom fire blight on 'Gala' apples was conducted at PARC, Summerland, BC in a screenhouse (Unpublished data). Treatments were replicated six times on single tree replicates. Sea buckthorn juice and streptomycin treatments were applied with a spray bottle on 3 May, 2002 at 25% bloom and again at full bloom 10 days later. Twenty-four hours later the blossoms were inoculated with a cell suspension of *E. amylovora* and 24 h after that the blossoms were wetted for 3 h with water applied by overhead misters. Clusters displaying symptoms of fire blight indicated by blackening of flowers were recorded 10 days later and wilted shoots were recorded 1 month after inoculation. Sea buckthorn juice was as effective as streptomycin in reducing the number of blighted blossoms (Table 5). It also appeared to reduce the number of infected shoots. Sea buckthorn could be a new alternative treatment for fire blight where an organic treatment is urgently needed and showed promise by inhibiting the *E. amylovora* bacterium in a plate test in the laboratory and reducing blossom fire blight incidence in the field. Subsequent trials in 2004 and 2005 have not been as clear as this trial was because of various problems such as insufficient blossoms on the trees or high temperatures in the screen house and the trial needs to be repeated with a more defined Sea buckthorn product. Sea buckthorn juice will not be a dependable treatment for control

of fire blight until it can be predicted before it is applied whether it will have sufficient activity to control the disease. This means that an assay for the active component(s) will be necessary for this promising control to be used commercially.

Conclusion

Extracts of Sea buckthorn juice show promise for the control of various plant diseases on tree fruit crops and grapes. It appears that the oil based Sea buckthorn extracts are most effective against powdery mildew and brown rot and the Sea buckthorn aqueous extracts and/or juice are most effective against bacterial plant pathogens such as *E. amylovora*. More research is needed on the use of Sea buckthorn for control of plant diseases especially to discover the components that prevent plant disease. In this chapter we have only touched the surface on the use of Sea buckthorn extracts for disease control. There are several other diseases not mentioned here that likely could be suppressed and possibly controlled by these extracts. A focused research program is recommended to test these products against a wide range of plant diseases using both laboratory and field studies.

References

Abartene, D. Y. and A. I. Malakhovskis. 1975. Combined action of a cytostatic preparation and sea buckthorn oil on biochemical indices. Lietuvos Tsr. Mokslu Akademijos darbai Serija c Biologijos modslai 1, 167-171.

Abidov, M. T., V. Kh. Zhilov, A. A. Ovchinnikov, and A. P. Khokhlov. 2002. Peptic ulcer treatment: Galavit combined with tocopherol and kaolin or almagel in rose or sea buckthorn oil. Russian Patent (RU 2160104).

Agrawal, A., B. B. Khuntia, and G. P. Dubey. 2001a. Sea buckthorn modulates the mental health status and its effect on immunosce-nescence among the elderly - a double blind placebo control study. In Proceedings International Workshop on Sea Buckthorn. A resource for Health and Environment in Twenty First Century. Feb. 18-21, 2001. New Delhi, India. p. 213-20.

Agrawal, A., B. S. Tewari, and G. P. Dubey. 2001 b. Role of sea buckthorn (Hippophae rhamnoides) in the management of age related memory disorder. In: Proceeding International Workshop on Sea Buckthorn. A Resource for Health and Environment in Twenty First Centry, Feb. 18-21, 2001. New Delhi, India. p. 225-30.

Agrawal, A., S. P. Dixit, A. Mishra, and G. P. Dubey. 2001 c. Determination of safety and efficacy profile of sea buckthorn (Hippophae rhamnoides L.). In: Proceeding International Workshop on Sea Buckthorn. A Resource for Health and Environment in Twenty First Centry, Feb. 18-21, 2001. New Delhi, India. p. 235-38.

Agrawal, P.K. and H.C. Goel. 2002. Protective effect of Rh-3 with special reference to radiation induced micronuclei in mouse bone marrow. Indian J. Exp. Biol. 40, 525-30.

Amosova, E.N., E.P. Zueva, T.G. Razina, V.F. Turetskova, O.V. Azarova, S.G. Krylova, and E.D. Gol'dberg. 1998. The search for new anti-ulcer agents from plants in Siberia and the Far East. Eksp Klin Farmakol 61, 31-5.

Bailey, L.H. and E.Z. Bailey, 1978. Hortus Third. A concise dictionary of plants cultivated in the United States and Canada. MacMillan Pub. Co. Inc. 1290 p.

Barl, B., L. Akhov, D. dunlop, S. Jana, and W. R. Schroeder. 2003. Flavonoid content and composition in leaves and berries of sea buckthorn (*Hippophae* spp.) of different origin. Acta Hort. 626, 405-13.

Barre, D. E. 2001. Potential of evening primrose, borage, black currant, and fungal oils in human health. Ann. Nutr. Metab. 45, 47-57.

Beatty, S., M. Boulton, D. Henson, H. Koh, I. J. Murray. 1999. Macular pigment and age-related macular degeneration. Br. J. Ophthalmol. 83, 867-77.

Bélanger, R. R. and C. Labbe. 2002. Control of powdery mildew without chemicals: prophylactic anc biological alternatives for horticultural crops. In: The powdery mildew, a comprehensive treatise. R. R. Belanger, W. R. Bushnell, A. J. Dik, and T. L. W. Carver (eds.). APS Press. St. Paul, Minn. p. 256-67.

Berger, J. S., M. C. Roncaglioni, F. Avanzini, G. Tognoni, and D. KL. Brown. 2006. Aspirin for the primary prevention of cardiovascular events in women and men: a sex-specific meta-analysis of randomized controlled trials. JAMA 295, 306-13.

Bernath, J. and D. Foldesi. 1992. Sea buckthorn (*Hipppophae rhamnoides* L.): a promising new medicinal and food crop. J. Herbs, Spices, Meidicnal Plants. 1, 1-2, 27-35.

Bernstein, P. S., F. Khachik, L. S. Carvalho, G. J. Muir, D. Y. Zhao, and N. B. Katz. 2001. Identification and quantitation of carotenoids and their metabolites in the tissues of the human eye. Exp. Eye Res. 72, 215-223.

Beveridge, T., T. S. C. Li, B.D. Oomah and A. Smith. 1999. Sea Buckthorn Products: Manufacture and Composition. J. Agric. Food Chem. 47:3480-3488.

Beveridge, T. H. J. 2002. Opalescent and cloudy fruit juices: formation and particle stability. Crit. Rev. Food Sci. Nutr. 42, 317-37.

Beveridge, T.H.J., T.S.C. Li and J.C.G. Drover. 2002 a. Phytosterol content in american ginseng seed oil. J. Agric. Food Chem. 50:744-750.

Beveridge, T. H. J., J. E. Harrison, and J. Drover. 2002 b. Processing effects on the composition of sea buckthorn juice from *Hippophae rhamnoides* L. cv. Indian Summer. J. Agric. Food Chem. 50, 113-6.

Beveridge, T. H. J. 2003. Chemical composition and some physical properties. In: Li, T.S.C. and T.H.J. Beveridge (eds.) Sea buckthorn (*Hippophae rhamnoides* L.): Production and utilization. Nat. Res. Council Canada, Ottawa, Canada. p.79-88.

Beveridge, T. H. J., M. Cliff, and P. Sigmind. 2004. Supercritical carbon dioxide percolation of sea buckthorn press juice. J. Food Qual. 27, 41-54

Beveridge, T. H. J. and J. E. Harrison 2005. Centrifugal yield and further microscopic characterization of sea buckthorn juice products. J. Food Qual. 28, 257-66.

Block, G., B. Patterson, and A. Subar. 1992. Fruit, vegetables, and cancer prevention: a review of the epideminological evidence. Nutr. Cancer. 18, 1-29.

Bonn, W. G., and van der Zwet, T. 2000. Distribution and economic importance of fire blight. *In* Fire blight: the diseases and its causative agent, *Erwinia amylovora*. *Edited by* J. L. Vanneste, CABI Publishing, Wallingford, UK. Pp. 37-53.

Buhatel, T., S. Vesa, and R. Morar. 1991. Data on the action of sea buckthorn oil extract in the cicatrization of wounds in animals. Bul. Inst. Agron. Cluj0Napoca Ser. Zootech. Med. Vet. 45, 129-133.

Cakir, A. 2004. Essential oil and fatty acid composition of the fruits of *Hippophae rhamnoides* L. (sea buckthorn) and *Myrtus communis* L. from Turkey. Biochem. System. and Ecology 32:809-816.

Cao, Q., W. Qu, Y. Deng, Z. Zhang, W. Niu, and Y. Pan. 2003. Effect of flavonoids from the seed and fruit residue of *Hippophae rhamnoides* L. on glycometabolism in mice. Zhong Yao Cai 26, 735-7.

Chai, Q. 1989. The experimental studies on the cardiovascular pharmacology of sea buckthorn extract from *Hippophae*

rhamnoides L. In: Proceedings of the first International Symposium on Sea Buckthorn. Oct. 19-23, 1989. Xi-an, China p. 392-397.
Chen, J. H. 1991. Effect of the immunomodulating agents (BCG) and the juice of HRL on the activity of splenic NK cells and LAK cells from tumor bearing mice. Chin. J. Microbiol. Immunol. 11, 105-108.
Chen, T. 1988. Preliminary research onthe biochemical components of sea buckthorn oil from Gansu, China. Seabuckthorn 1, 35-38.
Chen, Y., X. Zhong, T. Liu, and Z. Ge. 2003. The study on the effects of the oil from *Hippophae rhamnoides* in hematopoiesis. Zhong Yao Cai. 26, 572-5.
Cheng, J. Y., K. Kondo, Y. Suzuki, Y. Ikeda, X. Meng, and K. Umemura. 2003. Inhibitory effects of total flavones of *Hippophae rhamnoides* L. on thrombosis in mouse femoral artery and in vitro platelet aggregation. Life Sci. 72, 2263-71.
Cheng, T., T. Li, Z. Duan, Z. Cao, Z. Ma, and P. Zhang. 1990. Acute toxicity of flesh oil of *Hippophae rhamnoides* L. and its protection against experimental hepatic injury. Chung Kuo Chung Yao Tsa Chih 15, 45-47.
Cheng, T. J. and Z.Yu. 1992. Protective action of seed oil of *Hippophae rhamnoides* L. against experimental liver injury in mice. Zhonghua Yu Fang Yi Xue Za Zhi 26, 227-9.
Cheng, T. J., J. K. Pu, L.W. Wu, Z.R.Ma, Z. Cao, and T. J. Li 1994. An preliminary study on hepato-protective action fo seed oil of *Hipppophae rhamnoides* L. and mechanism of the action. Zhongguo Zhong Yao Za Zhi 19, 367-70.
Cheng, T. J., Y. B. Wang, L. P. Gao, Y. F. Sun, and J. Zhang. 2003. The protection of seed oil of *Hippophae rharmnoides* on ischemic cerebral infarction in rats. Zhongguo Zhong Yao Za Zhi. 28, 548-50.
Chernenko, T. V., N. T. Ul'chenko and A. I. Glushenkova. 2004. Fruits of two sea buckthorn varieties. Chem. Natural Compounds 40, 529-31.
Clavel-Chapelon, F., M. Niravong, and R. R. Joseph. 1997. Diet and breast cancer review of the epideminological literature. Cancer Detect. Prev. 21, 426-40.
Cooper, D. A., A. L. Eldridge, and J. C. Peters. 1999 a. Dietary carotenoids and lung cancer: a review of recent research. Nutrition Reviews. 57, 133-145.
Cooper, D. A., A. L. Eldridge, and J. C. Peters. 1999 b. Dietary carotenoids and certain cancers, heart disease, and age-related macular degeneration: a review of recent research. Nutrition Reviews 57, 201-274.
Dai, Y. R., C. M. Gao, Q. L. Tain, and Y. Yin. 1987. Effect of extracts of some medicinal plants on superoxide dismutase activity in mice. Planta Med. 53, 309-310.
Deans, S. G. and K. P. Svoboda. 1990. Biotechnology and bioactivity of culinary and medicinal plants. AgBiotech News Info. 2, 211-6.
Degtiareva, I. I., Ets, Toteva, E. V. Litinskaia, A. V. Matvienko, N. N. Iurzhenko, L. N. Leonov, E. V. Khomenko, and V. P. Nevstruev. 1991. Degree of lipid peroxidation and vitamin E level during the treatment of peptic ulcer. Klin Med. (Mosk) 69, 38-42.
Donaldson, M.S. 2004. Nutrition and cancer: A review of the evidence for anti-cancer diet. Nutr. Journal 3:19.
Dubey, G. P., A. Agrawal, and S. P. Dixit. 2003. Role of sea buckthorn (*Hippophae rhamnoides*) in the maintenance of cardiovascular homeostasis following cold stress. J. Natural Remedies 3, 36-40.
Duke, J. A. 2002. Hanbook of medicinal herbs. 2nd edition. CRC Press, Boca Raton, FL, USA. 936 p.
Eccleston, C., B. Yang, R. Tahvonen, H. Kallio, G. H. Rimbach, and A. M. Minihane. 2002. Effects of an antioxidant-rich juice (sea buckthorn) on risk factors for coronary heart disease in humans. J. Nutritional Biochem. 13, 346-54.
Erkkola, R., B. Yang. 2003. Sea buckthorn oils: Towards healthy mucous membrances. AGROFood industry hi-teck. May/June 2003. 5 p.
Eittenmiller, R.R., W.O. Landen and J. Augustin. 1998. Vitamin analysis in Food Analysis, edited by S.S. Nielsen, Aspen Publishers, Gaithersburg MD, USA. p281-291.
Fair, W. R., N. E. Fieshner, W. Heston. 1997. Cancer of the prostate: a nutritional diesease? Urology 50, 840-8.
Fayman, B. A. 1991. Treatment of operative wounds in ear, nose, and throat with sea buckthorn oil. Seabuckthorn 4, 7.
Fu, S. C., C. W. Hui, L. C. Li, Y. C. Cheuk, L. Oin, J. Gao, and K. M. Chan. 2005. Total flavones of Hippophae rhamnoides promotes early restoration of ultimate stress of healing patellar tendon in a rat model. Med. Eng. Phys. 27, 313-21.
Ganju, L., Y. Padwad, R. Singh, D. Karan, S. Chanda, M. K. Chopra, P. Bhatuagar, R. Kashyap, R. C. Sawhney. 2005. Anti-inflammatory activity of Seabuckthorn (*Hippophae rhamnoides*) leaves. Int. Immunopharmacol. 5, 1675-84.
Gao, X., M. Ohlander, N. Jeppsson, L. Bjork, and V. Trajkovski, 2000. Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. J Agri. Food Chemistry. 48, 1485-1490.
Gao, Z. L., X. H. Gu, F. T. Cheng, and F. H. Jiang. 2003. Effect of sea buckthorn on liver fibrosis: a clinical study. World J. Gastroenterol 9, 1615-7.
Geetha, S., M. S. Ram, V. Singh, G.Ilavazhagan, and R. C. Sawhney. 2002 a. Antioxidant and immunomodulatory properties of seabuckthorn (*Hippophae rhamnoides*) - an in vitro study. J. Ethnopharmacology 79, 373-8.
Geetha, S., M. S. Ram, V. Singh, G. Ilavazhagan, and R. C. Sawhney. 2002 b. Effect of seabuckthorn on sodium nitroprusside-induced cytotoxicity in murine macrophages. Biomed Pharmacother, 56, 463-7.
Geetha, S., M. Sai Ram, V. Singh, G. Ilavazhagan and R.C. Sawhney. 2002 c. Anti-oxidant and immunomodulatory properties of sea buckthorn (*Hippophae rhamnoides*) – an in vitro study. J. Ethnopharm. 79:373-378.
Geetha, S., M. S. Ram, V. Singh, G. Havazhagan, and R. C. Sawhney. 2003 a. Evaluation of antioxidant activity of leaf extract of seabuckthorn (*Hippophae rhamnoides* L.) On chromium (VI) induced oxidative stress in albino rats. J. Ethnopharmacol 87, 247-51.
Geetha, S., M. Sairam, S. K. Grover, H. M. Divekar, V. Singh, G. Ilavazhagen, and R. C. Sawhney. 2003 b. Cytoprotective, immunomodulatory and antistree potential of sea buckthorn. In: Proc. 1st Congress of International Sea buckthorn

Association. Sept. 14-18, 2003. Berlin, Germany. p.108-124.
Geetha, S., V. Singh, M. S. Ram, G. Ilavazhagan, P. K. Banerjee, and R. C. Sawhney. 2005. Immunomodulatory effects of sea buckthorn (*Hippophae rhamnoides* L.) Against chromium (VI) induced immunosuppression. Mol. Cell Biochem. 278, 101-9.
Gercikovs, L. and I. Zoludeva. 1998. Cosmetic agents for the lips. Patent in Latvia (LV12065).
Giovannucci, E., A. Ascherio, E. B. Rimm. 1995. Intake of carotenoids and retinol in relation to risk of prostate cancer. J. Natl. Cancer Inst. 67, 1767-76.
Goel, H. C., J. Prasad, S. Singh, R. K. Sagar, I. P. Kumar, and A. K. Sinha. 2002. Radioprotection by a herbal preparation of *Hippophae rhamnoides*, RH-3, against whole body lethal irradiation in mice. Phytomedicine 9, 15-25.
Goel, H. C., I. P. Kumar, N. Samanta, and S. V. Rana. 2003 a. Induction of DNA-protein cross-likes by *Hippophae rhamnoides*: implications in radioprotection and cytotoxicity. Mol. Cell Biochem. 245, 57-67.
Goel, H. C., C. A. Salin, and H. Prakash. 2003 b. Protection of jejunal crypts by RH-3 (a preparation of *Hippophae rhamnoides*) against lethal whole body gamma irradiation. Phytother. Res. 17, 222-6.
Goel, H. C., P. Indraghanti, N. Samanta, and S. V. Ranaz. 2004. Induction of apoptosis in thymocytes by *Hippophae rhamnoides*: implications in radioprotection. J. Environ. Pathol. Toxical Oncol. 23, 123-37.
Goel, H. C., D. Gupta, S. Gupta, A. P. Garg, and M. Bala. 2005. Protection of mitrochondrial system by *Hippophae rhamnoides* L. against radiation-induced oxidative damage in mice. Phar. Pharmacol. 57, 135-43.
Govaerts, R. 2001. How many species of see plants are there? Taxon 50, 1085-90.
Guliyev, V. B., M. Gui, and A. Yildirim. 2004. *Hippophae rhamnoides* L.: chromatographic methods to determine chemical composition, use in traditional medicine and pharmacological effects. J. Chromatogr B. Analyt. Technol. Biomed Life Sci. 812, 291-307.
Gunstone, F.D., J.L. Harwood and F.B. Padley. 1994. The Lipid Handbook 2nd edition. Chapman and Hall, One Penn Plaza, New York, New York. 101119 USA. p393-394.
Gupta, R. and S. J. Flora. 2005. Therapeutic value of *Hippophae rhamnoides* L. against subchronic arsenic toxicity in mice. J. Med. Food 8, 353-61.
Gupta A, R. Kumar, K. Pal, P. K. Banerjee, R. C. Sawhney. 2005. A Preclinical Study of the Effects of Seabuckthorn (*Hippophae rhamnoides* L.) Leaf Extract on Cutaneous Wound Healing in Albino Rats. Int J Low Extrem Wounds. 4, 88-92.
Gurevich, S. K. 1956. The application of sea buckthorn oil on ophthamology. Vestn. Ottamol. 2, 30-33.
Hakkinen, S. H., S. O. Karenlampi, I. Marina Heinonen, H. M. Mykkanen, and A. Ritta Torronen. 1999. Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. J. Agric. Food Chem. 47, 2274-9.
Hakkinen, S. H., S. O. Karenlampi, H. M. Mukkanen, and A. R. Torronen. 2000. Influence of domestic processing and storage on flavonol contents in berries. J. Agric. Food Chem.48, 2960-5.
Halliwell, B. 1997. Antioxidants and human disease: a general introduction. Nutr. Rev. 55:44-52.
Harrison, J. E. and T. Beveridge. 2002. Fruit structure of *Hippophae rhamnoides* cv. Indian Summer (sea buckthorn). Can. J. bot. 80, 399-409.
He, X., Z. Fang, Z. Liang, and L. Jiang. 1989. Devlopment and utilization of sea buckthorn fruit cosmetic. In: Proceedings First International Symposium on Sea Buckthorn. Oct. 19-23, 1989. Xian, China. p. 320-1.
Hibasami H, A. Mitani, H. Katsuzaki, K. Imai, K. Yoshioka, and T. Komiya. 2005. Links Isolation of five types of flavonol from seabuckthorn (*Hippophae rhamnoides*) and induction of apoptosis by some of the flavonols in human promyelotic leukemia HL-60 cells. Int J Mol Med. May;15, 805-809.
Hodutu, M. 1999. Medicinal preparations containing catina oil and vitamins for treatment of psoriasis. Patent in Canada (CA 2209625).
Ianev, E., S. Radev, M. Balutsov, K. Klouchek, and A. Popov. 1995. The effect of an extract of sea bucktorn (*Hippophae rhamnoides* L.) On the healing of experimental skin wounds in rats. Khirugiia (Sofiia) 48, 30-3.
Jarvis, W. R., Gubler, W. D., and Grove, G. G. 2002. Epidemiology of powdery mildew in agricultural pathosystems. *In* The powdery mildew, a comprehensive treatise. *Edited by* R. R. Bélanger, W. R. Bushnell, A. J. Dik, and T. L. W. Carver, APS Press, St. Paul, Minn. Pp. 169-199.
Jiang, Z., D. Qian, and S. Chou. 1989. An experimental study of *Hippophae rhamnoides* seed oil against gastric ulcer. In: Proceedings First International Symposium on Sea Buckthorn. Oct. 19-23, 1989. Xian, China. p. 401-2..
Jiang, Y. D., Y. C. Zhou, C. F. Bi, J. M. Li, I. X. Yang, and S. X. Zhao. 1993. Clinical investigation of effects of sea buckthorn seed oil on hyperlipidemia. Hipppophae 6, 23-24.
Jin, Y. 1989. Super-oxide dismutase firm fruit foliage of common sea buckthorn (*Hippophae rhamnoides*) L. In: Proceedings of the First International Symposium on Sea Buckthorn. Oct. 19-23, 1989. Xi'an, China. p. 350-7.
Johansson, A. K., H. Korte, B. Yang, J. C. Stanley, and H. P. Kallio. 2000. Sea buckthorn berry oil inhibits platelet aggregation. J.. Nutritional Biochem. 11, 491-5.
Johnson, K. B., and Stockwell, V. O. 2000. Biological control of fire blight. *In* Fire blight: the diseases and its causative agent, *Erwinia amylovora*. *Edited by* J. L. Vanneste, CABI Publishing, Wallingford, UK. p. 319-337.
Jones, A. L., and Sutton, T. B. 1996. Powdery mildew. *In* Diseases of tree fruits in the East. *Edited by* A. L. Jones and T. B. Sutton, Michigan State University, East Lasing, Mich. p. 11-12.
Kallio, H., B. Yang, P. Peippo, R. Tahvonen, and R. Pan. 2002a. Triacylglycerols, glycerophospholipids, tocopherols, and tocotrienols in berries and seeds of two subspecies (ssp. sinensis and mongolica) of Sea Buckthorn (*Hippophae rhamnoides*). Agric. Food Cehm. 50, 3004-9.
Kallio, H., B. Yang and P. Peippo. 2002b. Effects of different origins and harvesting time on vitamin C, tocopherols and tocotrienols in sea buckthorn (*Hippophae rhamnoides*) berries. J. Agric. Food Chem. 50:6136-6142.

Kallio, H. and B. Yang. 2003. Health effects of sea buckthorn (*Hippophae rhamnoides* L.) Oils: Chinese and russian knowledge and claims supported by Finnish research. In: Proc. 1st Congress of International Sea buckthorn Association. Sept. 14-18, 2003. Berlin, Germany. p.82-89.

Kallio, H., R. Niemnen, S. Tuomasjukka, and M. Hakala. 2006. Cutin composition of five Finnish berries. J. Agric. Food Sci. 54, 457-62.

Kasparaviciene, G., V. Briedis, and L. Ivanauskas. 2004. Influence of sea buckthorn oil production technology on its antioxidant activity. Medicina (Kaunas) 40, 753-7.

Khaidarov, K., L. Rachimov, and L. D. Lebedeva. 1989. The influence of sea bucktorn oil on the organs of vital importance, motor and secretory functions of the gastrointestinal tract. In: Proceedings First International Symposium on Sea Buckthorn. Oct. 19-23, 1989. Xian, China. p. 399-400.

Kolesnikov, M. P. and V. K. Gins. 2001. Phenolic substances in medicinal plants. Applied Biochem. And Microbiology. 37, 437-465.

Kostrikova, E. V. 1989. Experimental study of wound-healing effect of the preparation 'Aekol' (artificial sea buckthorn oil). Ortop. Travmatol. Prot. 1, 32-36.

Kostrikova, E. V., L. D. Goridova, and I. V. Gusak. 1990. Application of 'Aekol' preparation for combined treatment of open injuries. Ortop Travmatol Protez. 1990 Dec; (12) 42-7.

Krylova, S. G., O. N. Konovalova, and E. P. Zueva. 2000. Correction by common sea buckthorn bark and sprout extracts of hormonal and metabolic distrubances during stress in rats. Eksp. Klin. Farmakol. 2000 July-Aug. 63(4), 70-3.

Kukenov, M. K., F. D. Dzhumagalieve, N. G. Tatimova, and S. B. Bespaev. 1982. Study of medicinal plant reserves and distribution atlas compilation in the Kazakh-SSR USSR and prospects of their use in public health service. Izvest. Akad. Nauk. Kaz. SSR Ser. Biol. 1, 3-6.

Kumar, I. P., S. Namita, and H. C. Goel. 2002. Modulation of chromatin organization by RH-3, a preparation of *Hippophae rhamnoides*, a possible role in radioprotection. Mol. Cell Biochem. 238, 1-9.

Lebedeva, L., I. Rachmov, and K. Kchaidarov. 1989. Screening investigation of the anti-inflammation activity of sea buckthorn oil. In: Proceedings First International Symposium on Sea Buckthorn. Oct. 19-23, 1989. Xian, China. p. 398-9.

Li, F. and T. Guo 1989. Application of *Hippophae rhamnoides* L. in Tibetan Medicine. In: Proceedings of the International symposium on Sea buckthorn. Xi'an, China. Oct.19-23, 1989. The Secretariat of International Symposium on Sea buckthorn. p. 409-412.

Li, T.S.C. and Wang, L. C. H. 1998. Physiological components and health effects of ginseng, Echinaces, and sea buckthorn. In: Functional Foods, G. Mazza (ed.) Technomic Publ. Co. Inc. Lancaster, PA. p. 329-56.

Li, T.S.C. 2003. The range of medicinal plants influencing mental and physical performance. In: David H. Watson (ed.) Performance functional foods. Woodhead Publishing Ltd. Cambridge, England. p. 38-60.

Li, T.S.C., T. H. J. Beveridge, and B. D. Oomah. 2003. Nutritional and medicinal values. In: Li, T.S.C. and T.H.J. Beveridge (eds.) Sea buckthorn (*Hippophae rhamnoides* L.): Production and utilization. Nat. Res. Council Canada, Ottawa, Canada. p.101-108.

Li, Y. and M. Xu. 1993. Preliminary report on the anti-bacterial effect of sea buckthorn oil. Hippophae 6, 28-29.

Lipkan, G. M. and O. A. Oliinyk. 2000. Hepatoprotective effect of the sea buckthorn-and-pink oil. Lik. Sprava. 2000 Sept. (6), 96-9.

Lu, R. 1992. Sea buckthorn: A multipurpose plant species for fragile mountains. Int. Centre for Integrated Mountain Development. Katmandu, Nepal. 62 p.

Ma, Z. and X. Zhang. 2003. Effect of chronic intake of dietary fiber complex on the intestinal structure and function in hyperchlesterolemic rats. Yei Sheng Yan Jiu 2003 July, 32, 354-9.

Maatta-Riihinen, K.R. A. Kamal-Eldin, P.H. Mattila, A.M. Gonzalez-Paramas and A.R. Torronen. 2004. Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. J. Agric. Food Chem. 52:4477-4486.

Mares-Periman, J. A., A. E. Millen, T. L. Ficek, and S. E. Hankinson. 2002. The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. Overview. J. Nutr. 132, 518S-524S.

Mellentin, J. 2005. The future of functional foods. Nutraceutical World, Nov. p.54,56,58,60,62.

Mironov, V. A., T. N. Guseva-Denskaya, Y, Dubrovina, G. A. Osipov, E. A. Shabanova, A. A. Nikulin, N. Sh. Amirov, and I. G. Trubitsina. 1989. Chemical composition and biological activity of extracts from sea buckthorn fruit components. Khim-Farm. Zh. 23, 1357-64. (in Russian)

Mironov, V. A., T. N. Guseva-Donskaya, Y. Dubrovina, G. A. Osipova, E. A. Shabanova, A. A. Nikulin, N. S. Amirov, and I. G. Trubitsina. 1991. Composition and biological activity of lipid extracts from Armenian sea buckthorn. In Nov. Biol. Khim. Farm. Oblep. AN SSSR. Novosibrirsk, Russia. p. 114-121. (in Russian).

Narayanan, S., D. Ruma, B. Gitika, S. K. Sharma, T. Pauline, M. S. Ram, G. Ilavazhagan, R. C. Sawhney, D. Kumar, and P. K. Banerjee. 2005. Antioxidant activities of sea buckthorn (*Hippophae rhamnoides*) during hypoxia induced oxidative stress in glial cells. Mol. Cell Biochem. 278, 9-14.

Negi, P. S., A. S. Chauhan, G. A. Sadia, Y. S. Rohinishree, and R. S. Ramteke. 2005. Antioxidant and antibacterial activities of various seabuckthorn (*Hippophae rhamnoides* L.) Seed extracts.

Nersesyan, A. and R. Muradyan. 2004. Sea buckthorn juice protects mice against genotoxic action of cisplatin. Exp. Oncol. 26, 153-5.

Nersesian, A.K., V. N. Zil'flan, V. A. Kumkumadzhian, and N. V. Proshian. 1990. Antimutagenic properties of sea buckthorn oil. Genetika 26, 378-80.

Ogawa, J. M., Zehr, E. I., and Biggs, A. R. 1995. Brown rot. *In* Compendium of stone fruit diseases. J. M. Ogawa, E. I. Zehr, G. W. Bird, D. F. Ritchie, K. Uriu, and J. K. Uyemoto (eds.), APS Press, St. Paul, Minn. Pp. 7-10.

Oomah, D.B. 2003. Sea Buckthorn Lipids in Sea Buckthorn (*Hippophae rhamnoides* L.): Production and Utilization. T.S.C.

Li and T.H.J. Beveridge editors. NRC-CNRC. NRC Research Press. Ottawa, Ontario, Canada. p51-68.
Olsson, M. E., K. Gustavsson, S. Andersson, A. Nilsson, and R. Duan. 2004. Inhibition of cancer cell proliferation in vitro by fruit and berry extracts and correlations with antioxidant levels. J. Agric. Food Chem. 52, 7264-71.
Padmavathi, B., M. Upreti, V. Singh, A. R. Rao, R. P. Singh, and P. C. Rath. 2005. Chemoprevention by *Hippophae rhamnoides*: Effects on tumorigenesis, Phase II and antioxidant enzymes, and IRF-1 transcription factor. Nutr. Cancer 51, 59-67.
Padwad, Y., L. Ganju, M. Jain, S. Chanda, D. Karan, P. Kumar Banerjee, and R. Chand Sawhney. 2006. Effect of leaf extract of sea buckthorn on lipopolysaccharide induced inflammatory response in murine macrophages. Int. Immunopharmacol. 2006 Jan;6(1), 46-52.
Pan, R.Z., Z. Zhang, Y. Ma, Z. San, and B. Deng. 1989. The distribution characters of sea buckthorn (H. rhamnoides L.) and its research progress in China. In Proc. the First International Symposium on Sea Buckthorn, Oct. 19-23, 1989. Xi'an, China. p. 1-16.
Paoletti, F. and A. Raffo. 2004. Chemical composition and use of sea buckthorn (*Hippophae rhamnoides* L.) berries. Ingredienti Alimentari. 3(5), 26-32.
Paskar, N. P. 1973. Treatment of some otorhinolaryngologic diseases with sea buckthorn oil. Vestn. Otorinolaringol. 35, 40-2.
Paskar, N. P. and F. S. Iuzefovich 1974. Action of sea buckthorn oil on the bacterial flora in acute rhinopharyngitis. Zh Ushn Nos Gorl Bolezn. 1974 Mar-Apr. 0(2), 98-9.
Pearson, M.C. and J. A. Rogers. 1962. Biological flora of the British Isles. No. 85. *Hippophae rhamnoidues* L. J. Ecol. 50, 501-9.
Peizhen, Z., X. Ding, M. Lina, L. Dexing, and L. Dexing, L. Lanping. 1989. Anti-tumor effects of fruit juice and seed oil of *Hippophae rhamnoides* and their influences on immune function. In: Proceedings of the International Symposium on Sea buckthorn (*H. rhamnoides* L.). The Secretariat of International Symposium of Sea Buckthorn. Xi-an, China. 393-381.
Pentegova, V.A. 1983. Biology, chemistry and pharmacology of sea buckthorn, 'Nauka' Adad, Nauk, SSSR. Novosibirsk. p. 124.
Perz-Jimmenez, F., P. Castro, J. Lopex-Miranda, J. Paz-Rojas, E. Blanco, A. Lopex-Segura, F. Velasco, C. Marin, F. fuentes, and J. M. Ordovas. 1999. Circulating levels of endothelial function are modulated by dietary mono-unsaturated fats. Atherosclerosis 145, 351-8.
Pintea, A., A. Marpeau, M. Faye, C. Socaciu and M. Gleizes. 2001. Polar lipid and fatty acid distribution in carotenolipoprotein complexes extracted from sea buckthorn fruits. Phytochem. Anal. 12:293-298.
Pivnicov, I., G.I. Brad, and D. Moisiu. 1989. Controlling foot rot in sheep by a treatment incorporating the oil and a paste of sea buckthorn (*Hippophae rhamnoides*). Zootehnie-si-medicina-veterinara 39, 12, 42-49.
Prakash, H., M. Bala, A. Ali, and H. C. Goel. 2005. Modification of gamma radiation induced response of peritoneal macrophages and splenocytes by *Hippophae rhamnoides* (RH-3) in mice. J. Pharm. Pharmacol. 57, 1065-72.
Prescott-Allen, C. and R. Prescott-Allen. 1990. How many plants feed the world? Conservation Biology 4, 365-74.
Psallidas, P. G., and Tsiantos, J. 2000. Chemical control of fire blight. *In* Fire blight: the diseases and its causative agent, *Erwinia amylovora*. *Edited by* J. L. Vanneste, CABI Publishing, Wallingford, UK. Pp. 199-234.
Puupponen-Pimia, R., L. Nohynek, C. Meier, M. Kahkonen, M. Heinonen, A. Hopia, and K. M. Oksman-Caldentey. 2001. Antimicrobial properties of phenolic compounds from berries. J. Appl. Microbiol. 90, 494-507.
Puupponen-Pimia, R., L. Nohynek, S. Hartmann-Schmidlin, M. Kahkonen, M. Heinunen, K. Maatta-Riihinen, and K. M. Oksman-Caldentey. 2005. Berry phenolics selectively inhibit the growth of intestinal pathogens. J. Appl. Microbiol. 98, 991-1000.
Rachimov, I. P., L. d. Lebegdeva, and K. K. Khaidarov. 1989. the experimental toxicology of the sea buckthorn oil. In: Proceedings First International Symposium on Sea Buckthorn. Oct. 19-23, 1989. Xian, China. p. 371-2.
Raffo, A., F. Paoletti and M. Antonelli. 2004. Changes in sugar, organic acid, flavonol and carotenoid composition during ripeneing of berries of three sea buckthorn (*Hippophae rhamnoides* L.) cultivars. Eur. Food Res. Technol. 219:360-368.
Ram, V., and Bhardwaj, L. N. 2004. Stone fruit diseases and their management. *In* Diseases of fruits and vegetables, Volume II. *Edited by* S. A. M. H. Naqvi, Kluwer Academic Publishers, the Netherlands. p. 485-510.
Ramaswamy, G., V. R. Rao, S. V. Kumaraswamy, and N. Anantha. 1996. Serum vitamins' status in oral leucoplakias - a preliminary study. Eur. J. Cancer B Oral Oncol. 32B, 120-2.
Rosch, D., M. Bergmann, D. Knorr, and W. Kroh-Lothar. 2003. Structure antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice. J. Agri. Food Chemistry 51, 4233-9.
Rosch, D., A. Krumbein and L. Kroh. 2004. Antioxidant gallocatechins, dimeric and trimeric proanthocyanidins from sea buckthorn (*Hippophae rhamnoides*) pomace. Eur. Food Res. Technol. 219:605-613.
Rosch, D., A. Krumbein, C. Mugge, and L. W. Kroh. 2004a. Structural investigations of flavonol glycosides from sea buckthorn (Hipppophae rhamnoides) pomace by NMR spectroscopy and HPLC-ESI-MS(n), J. Agric. Food Chem. 52, 4039-46.
Rosch, D., C. Mugge, V. Fogliano, and L. W. Kroh. 2004b. Antioxidant oligomeric proanthocyanidins from sea buckthorn (*Hippophae rhamnoides* L.) Pomace. J. Agric. Food Chem. 52, 6712-8.
Rousi, A. 1971. The genus Hippophae L. A taxonomic study. Ann. Bot. Fenn. 8, 177-227.
Ruan, A., H. Min, Z. Meng, and Z. Lu. 2003. Protective effects of seabuckthorn seed oil on mouse injury induced by sulfur dioxide inhalation. Inhal. Toxicol. 15, 1053-8.

Rui, L., Y. Gao and R. Su. 1989. Effects of sea buckthorn oil on lipids peroxidation of guinea pigs erythrocyte membranes. In Proceedings the First International Symposium on Sea Buckthorn. Oct. 19-23, 1989. Xi-an, China. p. 358-64.

Sabir, S. M., H. Maqsood, S. D. Ahmed, A. H. Shah, and M. Q. Khan. 2005. Chemical and nutritional constituents of sea buckthorn (*Hippophae rhamnoides* ssp. turkestanica) berries from Pakistan. Ital. J. Food Sci. n.4, vol. 17, 455-462

Sabynich, L. V., L. A. Sibileva, L. S. Belova, V. S. Chuchalin, I.M. Sedykh, A. A. rychkov, A.S. Saratikov, and T.P. Prishchep. 1994. Antiinflammatory properties of thick extract from fruit pulp of *Hippophae rhamnoides* L. Rastital nye-resursy. 30 (3), 70-74.

Santos-Buelga, c., and A. Scalbert. 2000. Proanthocyanidins and tannin-like compounds - nature occurrence, dietary intake and effects on nutrition and health. J. Sci. Food Agric. 80:1094-1117.

Schapiro, D. C. 1989. Biochemical studies on some hopeful forms and species of sea buckthorn in USSR. In: Proceedings first International Symposium on Sea Buckthorn, Oct. 19-23, 1989. Xi'an, china. p. 64-66.

Shalkevich, M., S. Czaplicki, and R. Zadernowski. 2004. Contents of L-ascorbic acid, phenolic substances and antioxidant properties of hydrophilic tractions of sea buckthorn (*Hippophae Rhamnoides* L). Samokhvalovichi (Belarus), IFG. 2004, p. 331-5.

Shaov, M. T. and O. V. Pshikova. 2003. To the problem of remote control of physiological function of organism. Fiziol Zh. 49, 169-73.

Shipulina, L. D. 2001. A study on the anti-viral activity and other biological properties of Hiporamin - a new anti-viral drug from sea buckthorn leaves. In: Proceedings of International Workshop on Sea buckthorn. New Delhi, India. p.212-213.

Sholberg, P. L. 2004. Management of grape diseases in arid climates. *In* Diseases of fruits and vegetables, Volume II. *Edited by* S. A. M. H. Naqvi, Kluwer Academic Publishers, the Netherlands. p. 53-80.

Sholberg, P. L., and Boulé, J. 2003. Use of organic treatments for control of brown rot of apricots in 2002. 2003 Pest Management Research Report 42: 165-166. Online http://www.carc-crac.ca/english/ECIPM/ecipm.htm

Sholberg, P. L., and Walker, M. 2004. Efficacy of oils and IBR liquid against powdery mildew of grape, 2003. 2004 Pest Management Research Report 43: 180-184. Online http://www.carc-crac.ca/english/ECIPM/ecipm.htm.

Singh, V. 2001. Sea buckthorn (*Hippophae rhamnoides* L.)- A wonder plant of dry temperate Himalayas. In: Proceedings of an International Workshop on sea buckthorn. A resource for health and environment in twenty first century. February 18-21, 2001. New Delhi, India. p.39-42.

Socaciu, C. and A. Noke. 2003. Devlopment of new cosmetic products derived from carotenoid-lipoprotein complexes of sea buckthorn fruits. In: Proc. 1st Congress of International Sea buckthorn Association. Sept. 14-18, 2003. Berlin, Germany. p.125-129.

Sokoloff, B. K., M. Gunaoka, C. C. Fujisawa, E. Saelhof, D. B. Raniguchi, and C. Miller. 1961. An oncostatic facter present in the bark of *Hippophae rhamnoides*. Growth 25, 401-9.

Solonenko, L. P. and E. E. Shishkina. 1989. Electrophoretic and amino acid analysis of proteins in the sea buckthorn fruit. Proc. Int. Symp. Sea buckthorn (*H. rhamnoides* L.). Xian, China. p.91-95.

Spiridonov, N. A., V. V. Arkhipov, A. G. foigel, O. N. Tolkachev, S. A. Sasov, A. B. Syrkin, and V. N. Tolkachev. 1997. The cytotoxicity of *Chamaenerium angustifolium* (L.) Scop. and *Hippophae rhamnoides* L. tannins and their effect on mitochondrial repiration. Eksp. Klin. Farmakol. 60, 60-3.

Suleyman, H., L. O. Demirezer, M. E. Buyukokuroglu, M. F. Akcay, A. Gepdiremen, Z. N. Banoglu, and F. Gocer. 2001. Antiulcerogenic effect of *Hippophae rhamnoides* L. Phytother. Res. 15, 625-7.

Suleyman, H., K. Gumustekin, S. Taysi, S. Keles, N. Oztasan, O. Aktas, K. Altinkaynak, H. Timur, F. Akcay, S. Akar, S. Dane, and M. Gul. 2002. Beneficial effects of Hippophae rhamnoides L. on nicotine induced oxidative stress in rat blood compared with vitamin E. Biol. Pharm. Bull. 25, 1133-6.

Sun, B., P. Zhang, W. Qu, X. Zhang, X. Shuang, and H. Yang. 2003. Study on effect of flavonoids from oil-removed seeds of *Hippophae rhamnoides* on inducing apoptosis of human hepatoma cell. Zhong Yao Cai 26, 875-7.

Talbott, S. M. 2003. Supplements for boosting energy levels. from Talbott (ed.), A guide to understanding dietary supplements. Haworth Press, NY. p. 181-216.

Talbot, G. 2005. Omega-3 fatty acids; what they are and why we need them. Food Sci & Technol. 19:46-48.

Tanaka, T. 1976. Tanaka's cyclopedia of edible plants of the world. Keigaku Publ. Co., Tokyo, Japan. 924 p.

Tang, X., N. Kaviainen and H. Tuorila. 2001. Sensory and hedonic characteristics of juice of sea buckthorn (*Hippophae rhamnoides* L.) origins and hybrids. Lebensm.-Wiss. u.-Technol. 34:102-110.

Tang, X. 2002. Intrinsic change of physical properties of sea buckthorn (0) and implications for berry maturity and quality. J. Hort. Sci. Biotech. 77, 177-85.

Thind, T. S., Arora, J. K., Mohan, C., and Raj, P. 2004. Epidemiology of powdery mildew of downy mildew and anthracnose diseases of grapevine. *In* Diseases of fruits and vegetables, Volume I. *Edited by* S. A. M. H. Naqvi, Kluwer Academic Publishers, the Netherlands. Pp. 621-638.

Thorne, R. F. 2002. How many species of seed plants are there. Taxon 51, 511-2.

Tiitinen, K. M., M. A. Hakala, and H. P. Kallio. 2005. Quality components of sea buckthorn (*Hippophae rhamnoides*) varieties. J. Agric. Food Chem. 53, 1692-9.

Tolkachev, O. N. and L. D. Shipulina. 2003. Antiviral polyphenols from sea buckthorn leaves as the source of drug Hiporamin. In: Proc. 1st Congress of International Sea buckthorn Association. Sept. 14-18, 2003. Berlin, Germany. p.90-103

Torkelson, A. R. 1995. The cross name index to medicinal plants. CRC Press, Inc. Boca Raton, FL, USA. 3 vols.

Tsydendambaev, V.D. and A.G. Vereshchagin. 2003. Changes in triacylglycerol composition during ripening of sea buckthorn (*Hippophae rhamnoides* L.) seeds. J. Agric. Food Chem. 51:1278-83.

Turechek, W. W. 2004. Apple diseases and their management. *In* Diseases of fruits and vegetables, Volume I. *Edited by* S. A. M. H. Naqvi, Kluwer Academic Publishers, the Netherlands. Pp. 1-108.

Turpeinen, A. M., A. M. Pajari, B. Freese, R. Sauer, and M. Mutanen. 1998. Replacement of dietary saturated fatty acids by insaturated fatty acids: effects on plateler protein kinase C activity, urinary content of 2.3-dinor-TXB2 and in vitro platelet aggregation in healthy man. Thromb. Haemostasis 80, 649-55.

Tyagi, S. P., A. C. Varshney, A. Kumar, V. Singh. 2005. Therapeutic and prophylactic efficacies of sea buckthorn in gastric erosions and ulcerations in dogs. Abstracts of 2nd International Sea Buckthorn Association Conference. August 26-29, 2005. Beijing, China. p. 52.

Vaher, S. Ehala, and M. katjurand. 2005. On-column capillary electrophoretic monitoring of rapid reaction kinetics for determination of the antioxidative potential of various bioactive phenols. Electrophoresis 26, 990-1000.

Van Nostrand 2002. Van nostrand's Scientific Encyclopedia, 9th Edition. Vol. 2. John Wiley and Sons Inc. New York. 3399 p.

Vanzani, P., M. Rossetto, A. Rigo, U. Vrhovsek, F. Mattivi, E. D'Amato and M. Scarpa. 2005. Major phytochemicals in apple cultivars: Contributing to peroxyl radical trapping efficiency. J. Agric. Food chem. 53:3377-3382.

Varshney, A. C. 2003. Therapeutic evaluation of sea buckthorn oil in cutaneous burn wound healing in bovine: A clinical-haematological stody. In: Proc. 1st Congress of International Sea buckthorn Association. Sept. 14-18, 2003. Berlin, Germany. p.104-7.

Varshney, A. C., S. P. Tyagl, A. Kumar, and V. Singh. 2005 a. Studies for the evaluation of anti-inflammatory activies of *Hippophae rhamnoides* oil in canine. The Global Seabuckthorn Research and Development No. 2, Vol. 3, 22-25, 41.

Varshney, A. C., S. P. Tyagi, R. Rana. 2005 b. Therapeutic efficacy of sea buckthorn and dermanol in the healing of cutaneous wounds in bovine. Abstracts of 2nd International Sea Buckthorn Association Conference. August 26-29, 2005. Beijing, China. p. 51.

Velioglu, Y. S., G. Mazza, L. Gao, and B. D. Oomah. 1998. Antioxidant activity and total phenolics in selected fruits, and vegetables and grain products. J. Agric. Food Chem. 46, 4113-7.

Vlasov, V. V. 1970. *Hippophae* oil in the treatmetn of superficial skin burns. Vestn. Dermatol. Vnenraol. 44, 69-72.

Vrhovsek, U., A. Rigo, D. Tonon and F. Mattivi. 2004. Quantitation of polyphenols in different apple varieties. J. Agric. Food chem. 52:6532-6538.

Wahlberg, K. and N. Jeppsson. 1990. Development of cultivars and growing techniques for sea buckthorn. Sver. Lantbruksuniv. Verksamhetsb. Balsgaard (Sweden) 1990; 1988-1989: 80-93.

Wang, H., H. Ge, and J. Zhi. 1989. The components of unsaponifiable matters in sea buckthorn fruit and seed oil. In: Proceedings first International Symposium on Sea Buckthorn, Oct. 19-23, 1989. Xi'an, china. p. 81-90.

Wang, Z. R., L. Wang, H. H. Yin, F. J. Yang, Y. O. Gao, and Z. J. Zhang. 2000. Effect of total flavonoids of *Hippophae rhamnoides* on contractile mechanics and calcium transfer in stretched myocyte. Space Med. Med. Eng (Beijing). 13, 6-9.

Wang, B., Y. Feng, Y. Yu, H. Zhang, and R. Zhu. 2001. Effects of total flavones of *Hippophae rhamnoids* L. (Sea buckthorn) on cardiac function and hemodynamics in healthy human subjects. Rich Nature Nutroceutical Laboratories Inc. www.richnature.com. 4 p.

Wang, Z. Y., X. L. Luo, and C. P. He. 2006. Management of burn wounds with Hippophae rhamnoides oil. Nan Fang Yi Ke Da Xue Xue Bao. 26(1), 124-5.

Weller, P. and D. E. Breithaupt. 2003. Identification and quantification of zeaxanthin esters in plants using liquid chromatography-mass spectrometry. J. Agric. Food Chem. 51, 7044-9.

Wolf, D. and R. Wegert. 1993. Development of cultivars and growing techniques for sea buckthorn. In: Cultivation and utilization of wild fruit crops. Bernhard Thalacker Verlag GmbH & Co. p. 23-29. (in German).

Wu, A., Y. Su, J. Li, Q. Liu, J. Lu, X. Wei, C. Cian, Y. Lai, and G. Wang. 1989. The treatment of chronic cervicitis with *Hippophae* oil and its suppository (129 cases analysis). In: Proceedings First International Symposium on Sea Buckthorn. Oct. 19-23, 1989. Xian, China. p. 404-6.

Wu, D. and Z. Meng. 2003. Effect of sulfur dioxide inhalation on the glutathione redox system in mice and protective role of sea buckthor seed oil. Arch. environ. Contam. Toxicol. 45, 423-8.

Xiao, M., Z. Yang, M. Jiu, J. You, and R. Xiao. 1992. The antigastroulcerative activity of beta-sitosterol-beta-D-glucoside and its aglycone in rats. Hua Xi Yi Ke Das Xue Xue Bao 23, 98-101.

Xiao, Z., W. Peng, B. Zhu, and Z. Wang. 2003. The inhibitory effect of total flavonoids of *Hippophae* on the activation of NF-kappa B by stretching cultured cardiac myocytes. Sichuan Da Xue Xue Bao Yi Xue Ban. 34, 283-5.

Xing, J., B. Yang, Y. Dong, B. Wang, J. Wang, and H. P. Kallio. 2002. Effects of sea buckthorn (*Hippophae rhamnoides* L.) seed and pulp oils on experimental models of gastric ulcer in rats. Fitoterapia 73, 644-59.

Xu, M. and Z. H. Qian. 1989. A survey of medical research of Hippophae rhamnoides L. in China. Proc. Internat. Symp. Sea Buckthorn. Xian, China. Oct. 19-23, 1989. p.329-332.

Xu, M.1994. The medical research and exploitation of sea buckthorn. Hippophae 7, 32-84. (in Chinese).

Xu, M., X. Sun, and J. Cui. 2001. The medicinal research on sea buckthorn. In: Proc. International Workshop on Sea Buckthorn. A resource for health and environment in twenty first century. Feb. 18-21, 2001, New Delhi, India. p.12-23.

Xu, Q. and C. Chen. 1991. Effects of oil of *Hippophae rhamnoides* on the experimental thrombus formation and blood coagulation system. Research and Development of Natural products33, 70-73.

Xu, X., B. Xie, S. Pan, E. Yang, K. Wang, S. Cenkowski, A. W. Hydamake, and S. Rao. 2006. A new technology for extraction and purification of proanthocyanidins derived from sea buckthorn bark. J. Sci. Food Agriculture. 86, 486-92.

Xu, Y., G. Li, C. Han, L. Sun, R. Zhao, and S. Cui. 2005. Protective effects of *Hippophae rhamnoides* L. Juice on lead-induced neurotoxicity in mice. Biol. Pharm. Bull. 28, 490-4.

Yang, J., X. Wang, Y. Liu, G. Li, L. Ren, J. Jing, H. Zhang, C. Zhong, and R. Su. 1989. Preliminary studies on the effects of oil from fruit residues of sea buckthorn upon anti-tumors. In: Proceedings First International Symposium on Sea Buckthorn. Oct. 19-23, 1989. Xian, China. p. 382-4..

Yang, B., K. O. Kalimo, L. M. Mattila, S. E. Kallio, J. K. Katajisto, O. J. Peltola, and H. P. Kallio. 1999. Effects of dietary

supplementation with sea buckthorn (*Hippophae rhamnoides*) seed and pulp oils on atopic dermatitis. J. Nutr. Biochem. 10, 622-30.

Yang, B., K.O. Kalimo, R. L. Tahvonen, L. M. Mattila, J. K. Katajisto, and H. P. Kallio. 2000. Effect of dietary supplementation with sea buckthorn (*Hippophae rhamnoides*) seed and pulp oils on the fatty acid composition of skin glycerophospholipids of patients witha atopic dermatitis. J. Nutr. Biochem. 11, 338-40.

Yang, B. and H.P. Kallio. 2001. Fatty acid composition of lipids in sea buckthorn (*Hippophae rhamnoides* L.) berries of different origins. J. Agric. Food Chem. 49:1939-1947.

Yang, B., R.M. Karlsson, P.H. Oksman and H.P. Kallio. 2001. Phytosterols in sea buckthorn (*Hippophae rhamnoides* L.) berries: Identification and effects of different origins and harvesting times. J. Agric. Food Chem. 49:5620-5629.

Yang, L., C. Xu, Q. Zhang, J. Y. Liu, and R. X. Tan. 2005. In vitro anti-*Helicobacter pylori* action of 30 Chinese herbal medicines used to treat ulcer diseases. J. Ethnopharmacology 98, 329-33.

Zadernowski, r., M. Naczk, H. Nowak-Polakowska, and J. Nesterowicz. 2002. Effect of sea buckthorn (*Hippophae rhamnoides* L.) Berry extracts on the activity of lipase and lipoxygenase. J. Food Lipids 9, 249-258.

Zadernowski, R., M. Naczk and R. Amarowicz. 2003. Tocopherols in sea buckthorn (*Hippophae rhamnoides* L.) berry oil. J. Am. Oil Chem. Soc. 80:55-58.

Zen, A. 2004. Important therapeutic uses of sea buckthorn (*Hippophae*): A review. J. Biological Sci. 4, 687-693.

Zhang, F., J. Gao, and Y. Guo. 1989. Predication of medical application prospects of sea buckthorn oil on the basis of its recent advances. In: Proceedings First International Symposium on Sea Buckthorn. Oct. 19-23, 1989. Xian, China. p. 339-47.

Zhang, M. 1987. Random control tests of treating the ischemic cardiopathy with TFH. J. China Cardiovasc. Dis. 15, 97-99.

Zhang, P., Y. C. Mao, B. Sun, M. Qian, and W. J. Qu. 2005. Links Changes in Apoptosis-related Genes Expression Profile in Human Breast Carcinoma Cell Line Bcap-37 Induced by Flavonoids from Seed Residues of *Hippophae Rhamnoides* L. Ai Zheng 24, 454 - 60. in Chinese.

Zhang, P., X. Ding, L. Mao, D, Li, and L. Li. 1989. Anti-tumor effects of fruit juice and seed oil of *Hippophae rhamnoides* and their influences on immune function. In: Proc. International Symposium Sea Buckthorn (*H. rhamnoides* L.). Xian, China. The Secretariat International Symposium Sea Buckthorn. p. 373-381.

Zhang, M. 1987. Random control tests of treating the ischemic cardiopathy with TFH. J. China Cardiovasc. Dis. 15, 97-99.

Zhang, P. 1989. Anti-cancer activities of sea buckthorn seed oil and its effects on the weight of immune organs. Seabuckthorn 2 (3), 31-34.

Zhang, X., M. Zhang, Z. Gao, J. Wang, and Z. Wang. 2001. Effect of total flavones of *Hippophae rhamnoides* L. on sympathetic activity in hypertension. Hua Zi Yi Ke Da Xue Xue Bao 32, 547-50.

Zhao, J., C. Y. Zhang, Y. Xu, G. Q. Huang, Y. L. Xu, Z. Y. Wang, S. D. Fang, Y. Chen, and Y. L. Gu. 1990. The antiatherogenic effects of components isolated from pollen typhae. Thromb. Res. 38, 957-66.

Zhao, Y. 1994. Preliminary report on the effects of sea buckthorn oil on thirty-two cases of burn or scald. Hipppophae 7(3), 36-37.

Zhen, H. J., X. Y. Chen, Q. Z. Yang, and F. C. He. 1996. Effects of sea bucktorn oil on immune function of mice. J. Lanzhou Univ. (Nat. Sci.) 26, 95-98. (in Chinese)

Zheng, Z. H., Z. H. Dong, and J. Yu. 1997. Modern study of traditional Chinese Medicine. Beijing University of Traditional Chinese Medicine. Xue Yuan Press, Beijing, China.

Zhong, F., Y. Jiang, and X. Ge. 1989. Study on the immunopharmacology of the components extracted from *Hippophae rhamnoides* L. In: Proceedings of the first international symposium on sea buckthorn Oct. 19-23, 1989. Xi'an, China. p. 368-70.

Zhongrui, L. and T. Shuzhen. 1993. Clinical observation on curative effect of oral sea buckthorn seed oil on cancers under chemotherapy. Hippophae 6(4), 39-41.

Zhou, Y. and Jiang, J. 1989. Medical and health-care functions and applications of sea buckthorn. Hippophae 2(2), 35-42. (in Chinese)

Zou, X. 1997. Multifunctional medicinal super cleansers. Chinese Patent 1141-266.