Seabuckthorn (Hippophae L.): *A Multipurpose Wonder Plant,* Vol. 2 (V. Singh, *Editor-in-Chief, Daya Publishing House, New Delhi, India, 2005), p. 3-69*

Free Radicals, Diseases, Anti-oxidants and Anti-oxidant Properties of Seabuckthorn *(Hippophae rhamnoides* L.)

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SUMMARY

The present review paper discusses the mechanism of free radical generation, their role in diseases, antioxidant properties and anti-oxidants of seabuckthorn and some other bioactive substances. While living being is dependent on oxygen for survival, a small part of it is degraded into reactive free radicals (0_2^-, H_2O_2 OH^-, $N0^-$ etc.). Various factors causes the release of free radicals, which damage the organic molecules like protein and DNA etc., causing various pathological disorders like Parkinson, Alzheimer type dementia, cancer and atherosclerosis etc. A number of enzymes and non-enzymes like vitamin C, E, carotenoids and flavonoids are known to neutralize the free radicals.

Seabuckthorn *(Hippophae* L.), which grows widely in cold regions of Asia and Europe, is one of the plants, being viewed a strong antioxidant. Seabuckthorn flavonoids significantly reduced the active oxygen radicals level of PMA-stimulated PMN. Addition of the seed oil declined the formation of MDA and conjugated diene in the Cu^{2+}-catalysed oxidation system. MDA levels in the cell-catalysed oxidation system were also significantly declined by addition of the oil. Alcoholic extracts of leaves and fruits of seabuckthorn at higher doses of 500 pg / ml were found to inhibit chromate induced cytotoxicity, apoptosis and free radical generation in rat spleenocytes prevented chromate induced inhibition of lymphocyte proliferation. Seabuckthorn fruit oils have shown positive effects on the lipid peroxidation, immune function, mucosa and skin. The seabuckthorn oil showed promising effect on the cardiovascular diseases. Animal and *in vitro* studies have also indicated anticancer and anticarcinogenic effects of its oil.

Fruit of seabuckthorn is very rich in anti-oxidants like vitamin C, which varies from 30 mg to 2740 mg/100 g. Fruits of *H. salicifolia* and *H. rhamnoides* ssp. *sinensis* and ssp. *yunnanensis* are rich source of vitamin C, whereas *H. rhamnoides* subsp. *turkestanica* and ssp. *mongolica* are richer in oil than other species. Total carotenoids content in seabuckthorn fresh fruit varies generally from 1 mg to 120 mg/100 g, whereas content of β-carotene varies from 0.2 to 17 mg/100 g. Lowest seed oil vitamin E content was estimated in *H. salicifolia* (46.9 mg/100 g oil) and the highest in *H. rhamnoides* subsp. *turkestanica* (159 mg/100 g oil). The highest vitamin E content in pulp oil was found in *H. rhamnoides* subsp. *sinensis* (248 mg/100 g oil). Pulp oil part has generally been richer in α-tocopherol than the seeds. α-Tocopherol constitutes up to 96 per cent of the total tocopherols in fruit pulp oil, being clearly the major isomer. In seed, y-tocopherol (up to 40 per cent of total tocopherols) is another dominating isomer, in addition to α-tocopherol. The main flavonoids identified in seabuckthorn are leucocyanidin, catechin, flavonol and trace flavanone. Metallothionein acts as detoxying agent for heavy metals and as free radical scavenger for most toxic radical, hydroxyl radical (HO^-). Other useful compounds are sterols and fatty acids like linoleic and α-linolenic acids etc.

Key words: free radicals. diseases, seabuckthorn (Hippophae rhamnoides L.), anti-oxidant properties, vitamins, carotenoids, flavonoids, metallothionein, sterols, tannins, oil, fatty acids and amino acids etc,

INTRODUCTION

Although for a living being oxygen is necessary for life, it has been accepted that oxygen is also toxic. Explanation is in the "Superoxide theory of oxygen toxicity" which was postulated by Irwin Fridowich and Joe McCord in 1969 and states that oxygen is toxic because some of it is metabolized to make superoxide radical. It has been found that either under the low level of endogenous anti-oxidant defense or increasing level of ROH, oxidative damage to the cells occurs, finally leading to several disease conditions (Halliwell, 1997). Several disorders have been related to ROS mediated, *e.g.* cardiovascular diseases, neurodegenerative diseases, rheumatoid arthritis, cystic fibrosis, gastrointestinal ulcerogenesis, metabolic disorders and AIDS (Halliwell and Gutteridge, 1990; Das *et al,* 1997; Olanow *et al,* 1996; Halliwell, 1998); Well-studied ROS mediated diseases are Alzheimer's disease (Butterfield, 1996), Parkinson's diseases (Wiseman and Halliwell, 1996; Winyard *et al.,* 1997), cancer (Li and Trush, 1995; Yanbo *et al.,* 1997), Down's syndrome (Buscigilo and Yankner, 1995), oxidative modification of low-density lipoprotein in atherosclerosis (Steinberg *et al.,* 1989) and ischemic reperfusion injury in different tissues heart, brain, kidney, liver and gastro-intestinal tract (McCord, 1987). In atherosclerosis (Maffick *et al,* 1997) and ischemic injury in heart (Ashraf, 1997) and brain (Phillies, 1997), role of ROH has also been well studied. Recently, it has been found that ROS plays a major role in stress-induced gastric ulcer and inflammatory bowel diseases (Das and Banerjee, 1993; Das *et al.,* 1997; Murthy *et al,* 1997; Bandyopadhyay *et al,* 1999).

Some drugs may also cause oxidative stress as a side effect, *e.g.* penicillamine is a drug used for the treatment of rheumatoid arthritis. An unfortunate side effect of this drug is the creation of penicillamine-derived radicals that bind to proteins and create new antigens, perhaps explaining the autoimmune syndrome, which is developed in some patients treated with penicillamine (Aruoma, 1996; Halliwell, 1997). Injuries to body tissues are also known to release free radicals, which cause further damage. Tissue injury, by almost any mechanism, will activate phagocytes, which produce O/ and H_20_2. Cell breakdown releases catalytic metal ions. Mitochondria can be damaged so that they become "leakier," releasing more electrons to 0_2 to form 0_2^-. For these and many other reasons, tissue injury can lead to oxidative stress as well (Ames *et al,* 1993; Diplock 1994; Frei, 1994). Detail mechanisms of oxygen radical mediated diseases (Thomas and Kalyanaraman, 1997) and formation of various oxygen derived free radicals and role of anti-oxidant defense system in controlling the ROS mediated pathological conditions have been reviewed by Halliwall (1998) and Bandyopadhyay *et al.* (1999).

Seabuckthorn, which grows widely in temperate Himalayas, China, central Asia and -Europe, has been found to be a strong anti-oxidant (Geetha *et al.,* 2003). Since ancient times, seabuckthorn has been traditionally used in Indian System of Medicine as early as 500-5000 BC in Ayurveda (Uniyal and Uniyal, 2001), Amchi System of Indian Himalayan (Brijlal *et al,* 2001), Tibetan System of Medicne in China (Li and Guo, 1989). Indian scientists discovered as early as 1962, the inhibitory effect of seabuckthorn bark on tumors (Ambaye *et al.,* 1962), however, real credit to start the work on the modem medicines of seabuckthorn goes to Russian scientists during 1950s (Gurevich, 1956; Akulinin, 1958). The oil and juice, commercially produced from its fruits and extracts from leaves, are used in Russian traditional and official medicine for the wound healing, antibacterial, as anti-ulcer and antiinflammatory (antiphlogistic) agent and as the multivitamin product (Eidel'nant 1998; Pomerantseva *et al.,* 1986; Maksyutina 1985; Zadorozhnyi *et al.,* 1989). The oil products from the seabuckthorn fruits and vegetative parts of plant possess hepatoprotective activity (Fedorov *et al,* 1996). In the official medicine of Russia, the plant extracts are commonly used as the components of various medicinal, compositions in dermatology, stomatology, ophthalmology, veterinary and cosmetology etc. A number of medicinal and cosmetic products, as well as food additives have been recently produced from the seabuckthorn extracts (Mauz *et al,* 1997; Miyazaki 1997; Wu *et al,* 1997; Verbuta *et al,* 1996; Petrescu *et al.,* 1996). Liquid extracts of shoots and bark increase the selectivity of cytostatics with a mild inhibiting action on tumors, and possess immunomodulating activity (Zueva *et al,* 1995). The alcoholic extracts of seabuckthorn shoots and barks were reported to be active anti-neoplastic agents on experimental tumors (Goldberg *et al,* 1996). Extracts of fruits and vegetative parts of seabuckthorn exhibited hepato-protective activity (Fedorov *et al,* 1996). A chemical composition and biological action of active principles from different organs of seabuckthorn have been reviewed in a number of monographs (Rastit. Resursy, 1988; Eidel'nant, 1998; Matafonov, 1983). In China, positive effects of seabuckthorn oil on heart diseases have also been reported (Xu and Chen, 1991; Jiang, *et al.,* 1993; Li and Wang 1994).

Studies carried out on biochemical characteristics revealed that fruit, leaves and other parts of seabuckthorn are very rich sources of variety of anti-oxidants and other bioactive substances (Yang and Kallio, 2002; Lu Rongsen, 2003) with medicinal properties, which make this plant a very promising raw material for the industrial utilization, particularly for the preparation of products for health protection and treatment of various diseases. Present paper highlights the mechanism of free radical release, their role in diseases, anti-oxidant properties, anti-oxidants and other bioactive compounds of seabuckthorn and their role in health protection.

FREE RADICALS

Oxygen is necessary for various life processes, therefore, the survival of living beings. Generally, 0_2does not exhibit extreme reactivity due to quantum-mechanical restrictions and on reduction results into water (In Bandyopadhyay *et al.,* 1999):

$$O_2 + 4\,H^+ + 4\,e^- \longrightarrow 2\,H_2O$$

However, about 1-3 per cent or even more of the O_2 taken in is converted to several reactive oxygen species (ROS) like O_2^-, H_2O_2 (hydrogen peroxide) and ^+OH (Reactive hydroxyl radical), as a result of sequential univalent reduction of 0_2:

$$O_2 \longrightarrow O_2^- \longrightarrow H_2O_2 \longrightarrow {}^+OH \longrightarrow H_2O$$

As mentioned above, $4e^-$ reduces O_2 into H_2O, as restricted by cytochrome oxidase of the mitochondria electron transport chain in respiring cells, without releasing O_2^- or H_2O_2. However, there is a probable leakage of single electron at the specific site of this transport chain, consequently inappropriate single electron reduction of O_2 to O_2^- (Loschen *et al.,* 1974; Chance *et al,* 1979; Fridovich, 1983). When the electron transport chain is highly reduced and the respiratory rate is dependent on ADP availability, there is an enhancement of leakage of electrons at the ubisemiquinone and ubiquinone sites, resulting in the production of O_2^- and H_2O_2 (Blake *et al.,* 1987; Bast *et al.,* 1991). Peroxisomal oxidases, flavoproteins, A-amino acid oxidase, L-hydroxy acid oxidase and fatty acyl oxidase participate in the production of H_2O_2 (Chance *et al.,* 1979; Bast *et al.,* 1991).

Under certain conditions, cytochrome P-450, P-450 reductase and cytochrome β-5 reductase in the endoplasmic reticulum, produces O_2^-, and H_2O_2 during their catalytic cycles (Turrens and Boveris, 1980). Xanthine oxidase's catalytic cycle has also been found as an important source of O_2^- and H_2O_2 during tissue damages. Xanthine oxidase, generated by proteolytic cleavage of xanthine dehydrogenase during ischemia, upon reperfusion in the presence of 0_2, acts on xanthine or hypoxanthine to produce O_2^- and H_2O_2 (McCord, 1987; Halliwell and Gutteridge, 1990). The phagocytic cells, like neutrophils, when activated during phayocytosis, produce O_2^- and H_2O_2 through activation of NADPH oxidase (Morel *et al.,* 1991). In inflammated tissue, neutrophil accumulation is one of the major reasons of oxidative damage due to ROS production. Further, spontaneous dismutation of O_2^- at neutral pH or dismutation by superoxide dismutase, results in H_2O_2 generation (Fridovich, 1983). Ramasarma (1982) has reviewed various biological sources for the H_2O_2 generation.

Generation of ^+OH *in vivo* requires the occurrence of trace amount of transition metals like Fe or Cu, except during ionizing radiation. ^+OH is produced by a simple mixture of Fe^{2+} salt and H_2O_2

$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^+OH + OH^-$ (Fenton reaction)

Traces of Fe^{3+} can further react with H_2O_2 and produce O_2^- as follows:

$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2++} + O_2^- + H^+$

Fortunately redox-active free Fe or Cu is not found in biological system, as these transitional metal ions remain bound to protein, membranes and nucleic acids or low molecular weight chelating agents like citrate, histidine or ATP (Halliwell and Gutteridge, 1984). However, these metal may be released from same metaloprotein during ischemic condition and cellular acidosis (Chevion *et al.,* 1993) and causing the generation of *OH, as mentioned above. High level of reactive Fe in *Substantia nigra* and increased oxidative damage in nigral neuronal cells (Schapira *et al.,* 1993) have been associated with Parkinson disease. Similar observations have been found in Huntington's and Alzheimer's diseases. Fairbum *et al.* (1993) have also suggested a role of catalytic Fe in ^+OH mediated damages of inflammatory joint decease. Low pH in the hypoxic tissue (acidosis), ascorbate or O_2^- can mobilize the ferritin Fe from the protein and even from microenvironment of phagocytes and the released Fe can thereby generate ^+OH (Biemond *et al.,* 1984). H_2O_2has also been found to liberate redox active Fe from hemoglobin and myoglobin (Halliwell and Gutteridge, 1990).

O_2^- is reported to be toxic to a cell growing under aerobic conditions and super oxide dismutage (SOD) provides defense against it (Fridovich, 1986). Under hydrophobic conditions, it shows high reactivity unlike aqueous solutions, where it shows a poor reactivity. However, it can not cross a biological membrane due to its charged state, with the exception of erythocyte membrane, which has an "anion channel". An indirect deleterious action of 0_2^- is mediated by its dismutation to H_2O_2, which is sensitive to catalase. While H_2O_2 shows a limited toxicity in a transition metal free system, however, H_2O_2 is long lived and membrane permeable and may diffuse to a considerable distance (Turrens and Boveris, 1980). ^+OH on the other hand, is extreme reactive, having a very short half-life With a very limited diffusion capacity. But it can attack and degrade almost every molecule in its vicinity (Halliwdl, 1991). Therefore, the magnitude of damage to the molecules or cells by H_2O_2 and 0_2^- increases in the presence of the transition metal ions due to the generation of more harmful ^+OH by the Haber-Weise-catalyzed reaction (Halliwell and Gutteridge, 1984).

ROLE OF FREE RADICALS IN DISEASES

In the case of disturbed balance between formation of free radicals and antioxidant defense, in the cell we have oxidative stress and the free radicals can play a role in the development of various diseases. Over productions of ROS have been implicated in the etiology of host degenerative diseases including cardiovascular diseases, diabetes, cancer, Alzheimer's disease, retinal degeneration, ischemic dementia, other neurovegetative disorders and aging. In addition they also play a role not only in acute conditions, such as trauma, stroke, and infection, but also in physical exercise and stress. ROS can attack the vital molecules (lipids, proteins, nucleic acids etc.) and structures of the cell, which may cause genetic modification or even cell death.

Lipid Peroxidation

Cell membranes, made of large amount of polyunsaturated fatty acid (PUFA), are highly prone to oxidative attack, which affects membrane permeability, fluidity and metabolic processes. Although lipid peroxidation is not initiated by O_2^- and H_2O_2, however ^+OH, alkoxy radicals (RO) and peroxy radicals (ROO) play a role in initiating the oxidative modification of lipids (Turrens and Boveris, 1980; Gardener, 1989). The occurrence of double bond, near to methylene group, makes the methylene C-H bond of PUFA weaker and therefore H becomes more susceptible to abstraction. Peroxy radicals act as reaction initiators as well as the products of lipid peroxidation, therefore leading to a self-perpetuating process. Lipid peroxy radicals react with other lipids, proteins and nucleic acids, propagating thereby the transfer of electrons and causing the oxidation of the substrates.

Oxidative Damage to Proteins

Free radicals, released from mitochondrial electron transport chain, can stimulate protein degradation. Oxidative protein damage results by metabolic processes, which degrade a damaged protein to promote the synthesis of a new protein. Damage to DNA polymerases could alter their fidelity. It has been suggested that a modification in the conformation of DNA polymerase could explain the frequency of close proximity double mutations that occur secondarily to a wide range of genetic stresses (Feig *et al,* 1994; Madzak and Sarasin, 1991). In cataractogenesis, oxidative modification plays an important role in cross-linking of crystalline lens protein, resulting in high molecular weight aggregates, loss of solubility and lens opacity (Gupta *et al.,* 1992). Lipofus-cin-an aggregate of peroxidized lipid and protein, has been found accumated in lysosome of aged cells, Alzheimer's disease brain cells and Fe-overloaded hepatocytes (Wolf *et al.,* 1986). α-1-protease inhibitor has been found in an oxidatively modified form in the rheumatoid joint (Zhang *et al.,* 1990). It has been found that catalytically inactive or less active and more thermolabile forms of enzyme and a big increase in die level of protein carbonyl content, an index of metal-catalysed oxidation of proteins (Oliver et *al.,* 1984; Stadtman and Oliver, 1991). Content of glyceraldehydes-3-P-dehydrogenase, aspartate aminotransferase and phosphoglycerate kinase in human erythrocytes, decrease with age alongwith an increase in protein carbonyl level (Oliver *et al,* 1987). An age related oxidative modification of human ceruloplasmin, a Cu containing protein in human plasma, has also been reported (Musci *et al.,* 1993). An oxidative inactivation of glutamine synthetase occurs during ischemic-reperfusion injury of gerbil brain (Oliver *et al,* 1990). Oxidative protein damage can also affect the activity of DNA repair enzymes. The mechanism of oxidative damage of proteins by ROS has been studied in vitro by Fisher and Stadtman (1992).

Damages to DNA

Free radicals can cause oxidative damages to DNA, nuclear as well as mitochondrial. ROS may cause base modification, deoxyribose oxidation, breakage of strand and damage of DNA-protein cross-links. ^+OH may generate various complexes or products from the DNA bases, *e.g.* C-8 hydroxylation of guanine to produce 8-oxo-7,8 dehydro-2'-deoxyguanosine, the action of which as a promutagen as well as in altering the binding of methylase to the oligomer so as to inhibit methylation of adjacent cytosine have been reported to cancer development (Weitzman *et al.,* 1994; Counts and Goodman, 1994). ROS has also been related to cause mutation in the p^{53} tumor suppressor gene (Hussain *et al.,* 1994). ROS has also been found to induce various early response or stress response genes *e.g.* c-fos, c-jun and heme oxygenase-1 etc., leading to cell proliferation and transformation (Watts *et al.,* 1995). ROS may also interfere with normal cell signaling, causing modifications of gene expression and resulting in cancer by redox regulation of transcriptional factors/activator and or by oxidatively modulating the protein kinase cascades.

Beside nuclear damage, ROS mediated oxidative damage of mitochondrial DNA involves strand breaks and base modification, leading to formation of abnormal components of electron transport system, which results in generation of more ROS through increased leakage of electrons and therefore more cell damage, consequently causing aging and even cancer (Richter, 1988).

Disorders Related to DNA Damage *Schistosomiasis*

Rosin *et al.* (1994) have used the schistosomiasis model to study the interrelationship between inflammation oxidative DNA damage, chromosomal instability and disregulated cell proliferation. It was found that infection with *schistosoma haematobium* produces chronic bladder inflammation and is associated with increased cancer at this stage. High level of genetic damage in the bladder cells was observed in the infected individuals (Rosin *et al.,* 1994; Rosin, 1992)

Cancerogenesis

Numerous investigators have proposed participation of free radicals in carcinogenesis, mutation and transformation, particularly in the past 10 years. Although there is no definitive evidence that free radicals involvement is obligatory in these processes, it is clear that their presence in biosystem could lead to mutation, transformation and ultimately cancer (Simic, 1988). Induction of mutagenesis, the best known of the biological effect of radiation, occurs mainly through damage of DNA by the ^{+}OH radical and other species produced by radiolysis of water, and also by direct radiation effect on DNA. The reaction of ^{+}OH radicals are mainly addition to double bond of pyrimidine bases and abstraction of hydrogen from the sugar moiety resulting in chain scission of DNA. These effects can cause cell mutagenesis and carcinogenesis. Lipid peroxides are also suspected of being responsible for the activation of benzo (a) pyrene and other carcinogens, as well as for the production of some types of promoter.

Lung cancer

Cigarette smoke is rich in carcinogens such as nitrosamines, acrolein and carcinogenic hydrocarbons, but ROS may also contribute to cancer development, as smoke is rich in ROS and oxides of nitrogen (Eiserichet el., 1994; Pryor and Stone, 1993). Higher levels of oxidative DNA base damage have been found in lung cancer tissue (Olinski *et al.*, 1992) and in cells exposed to cigarette smoke (Kiyosawa *et al,* 1990). Farther, a 4-10 fold elevation of urinary 8-OhdG excretion has been reported in the smokers (Loft ef *al.,* 1992,1993).

Liver Cancer

Hepatocellular carcinoma is a major reason of death in many countries of Asia and Africa. Primary hepatoma has often been associated with chronic infection with hepatitis viruses B or C (Blumberg *et al.,* 1975) or infection of aflatoxin (Ross *et al.,* 1992). Chronic hepatitis (Stein, 1991) has been associated with the presence of inflammatory cells, presumably producing ROS and RNS. Increased levels of 8-OHdG have been observed in DNA from livers with chronic hepatitis (Hagen *et al.,* 1994; Shimoda *et al.,* 1994). Aflatoxin frequently produce G to T transversions, which is a predominant substitution at Codon 249 in p53 found in aflatoxin-associated tumors. This transversion can also be produced by oxidative damage (Hussain *et al.,* 1994).

Breast Cancer

Higher level of DNA base damage with a characteristic attack of ^{+}OH has been found in invasive ductal carcinoma (Malms and Haimanot, 1991; Malins *et al.,* 1993), *e.g.* 9 fold increase in 8-OHG, 8-hydroxyadenine and 2,6-diamino-4 hydroxy-5 formamidopyrimidine in DNA from invasive ductal carcinoma (Malins and Haimanot, 1991; Malins *et al.,* 1993). DNA damage by ROS is also implicated in inflammatory breast disease (Jaiyesimi *et al.,* 1992), where malignant progression can occur (In Wiseman and Halliwell, 1996).

Inflammatory Bowel Disease (IBD)

IBD, a term has been given to a series of chronic inflammatory diseases of the gastrointestinal tract, including ulcerative colitis and Crohn's diseases. ROS has been found in excess in IBD and plays an important role both in its pathogenesis of IBD (Simmonds and Rampton, 1993; Grisham, 1994) and increased risk of cancer in certain IBDs. The main sources of ROS in the gut are phagocytes, which accumulate in the mucosa of patients with IBD and generate ROS upon activation.

DNA from colon biopsies from patients with ulcerative colitis had increased level of 8-OHG, 2-hydroxy-adenine 8-hydroxyadenine and 2,6-diamino-5-forma mido-pyrimidine (H. Wiseman *et al.* in Wiseman and Halliwell, 1996). These lesions, indicating ^{+}OH attack, could signify increased DNA damage and or decreased repair. The constitutive and ROS induced activity of PARP has been shown to be declined in patients with IBD and colon cancer (Markowitz *et al.,* 1988).

Oxidative Stress and Apoptosis

ROS may cause pathological disorders as well as cell death by two mechanisms, *i.e.* necrosis and apoptosis. In case of necrosis, cell ruptures, releasing its materials, that also include Cu and Fe ions, which act as pro-oxidants to generate ROS, especially ^{+}OH, causing oxidative damage to the adjacent cells. In apoptosis, a programmed cell death occur as a result of activation of suicidal mechanism of cell and cell does not normally rupture to affect the surrounding cells, unlike necrosis. Several studies have established that oxidative stress induces apoptosis in different cellular systems (Korsmeyer *et al.,* 1995; Hasnain *et al.,* 1999)). ROS mediated oxidative stress has been related to apoptic cell death that results in neurodegenerative diseases (Olanow *et al.,* 1996), progressive loss of T lymphocytes in human immuno-deficiency virus infection (AIDS) (Ameisen, 1992; Flores, 1997) and in myocardial ischemia-reperfusion injury (Ashraf, 1997; Khalid and Ashraf, 1993). The mechanism of ROS mediated apoptosis has not been understood, except for the involvement of H_20_2, which has been believed to play a role in the signaling pathway that controls the activity of some transcription factors (Sen and Packer, 1996; Begum *et al.,* 1999). Recent studies on a *in vitro* cultured *Spodoptera frugiperda* insect cells, have identified p35 gene product, which acts directly as an anti-oxidant, scavenging the free radicals therefore preventing the ROS mediated apoptosis (Sah *et al.,* 1999).

Aging

The human body is in constant battle to keep from aging. Strong experimental evidence supports the free radical theory of aging. In 1956, first time it was Harman, who proposed free radical theory of aging. An increasing number of diseases and disorders, as well as aging process itself, demonstrate link either directly or indirectly to these reactive and potentially destructive molecules. However, not much is known about the mechanism of aging and what determines the lifespan of a person. Leading theories attribute these to programmes written in DNA and/or to the accumulation of cellular and functional damage. Reduction of free radicals or decreasing their rate of production may delay aging and the onset of degenerative conditions associated with aging.

The contents of ROS indicated oxidatively damaged lipids; proteins and DNA have been found to increase with age (Harman, 1981; Sohal and Orr, 1995). Mutation in mitochondrial DNA also leads to the formation of defective respiratory enzymes (Wei *et al.,* 1998), resulting in lesser ATP synthesis and generating more ROS to cause further oxidative damage, therefore starting a vicious cycle, which is responsible for aging and age related disorders. Recently, Melatonin, a pineal hormone, having antioxidant activity (Reiter *et al.,* 1998; Srinivasan, 1999) has been found to decrease with age (Reiter *et al.,* 1998). Low intake of calories have been found to delay aging through decreased production of mitchondrial O_2^- and H_2O_2 and increased production of anti-oxidant defenses, consequently decreased production of oxidatively damaged proteins, lipids and DNA (Sohal and Weindruch, 1996; Weindruch and Sohal, 1997).

DEFENCE AGAINST FREE RADICALS

Cells possess multiple anti-oxidant defenses for protection from ROS. Since these defenses are not in excess, cells go through continuous oxidative damage. However, antioxidant defenses are generally inducible in response to oxidative damage. Antioxidant defense system against oxidative stress is composed of several lines, and the antioxidants are classified into four categories based on function (Noguchi *et al.,* 2000):

1. First line of defense is the preventive antioxidants, which suppress the formation of free radical (enzymes: glutathione peroxidase, catalase; selenoprotein, transferrin, ferritin, lactoferrin, carotenoids etc.)

2. Second line of defense is the radical scavenging antioxidants suppressing chain initiation and/or breaking chain propagation reactions: radical scavenging antioxidants

3. Third category: repair and de novo antioxidant (some proteolitic enzymes, repair enzymes of DNA etc.)

4. A fourth line is an adaptation where the signal for the production and reactions of free radicals induces formation and transport of the appropriate antioxidant to the right site.

Antioxidants act as radical scavenger, hydrogen dpnors, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents. Both enzymatic (primary defence) and non-enzymatic antioxidants (secondary defence) exist in the intracellular and extracellular environmental to detoxify ROS. To provide maximum intracellular protection these scavengers are strategically compartmentalized throughout the cell.

PRIMARY DEFENCE AGAINST FREE RADICALS

The effect of ROS is neutralized by primary defense systems of human body, which consists of different enzymes, *i.e.,* superoxide dismutase, glutathione peroxidase, Heme peroxidase and catalase (Table 1.1). The human body does not synthesize an overwhelming excess of its antioxidant defense systems. Instead, there is a balance in the human body between production of ROS and level of antioxidant defense. An imbalance between ROS and antioxidant defense systems may lead to chemical modifications of biologically important macromolecules like DNA, proteins or lipids, which are possible pathobiochemical mechanisms in the initiation or development of several diseases in human body. Oxidative damage to important biomolecules is not only a deleterious pathway, but also influences of ROS on gene regulation or the immune system might impair bodily functions (Ames *et al,* 1993; Esterbauer *et al,* 1992; Diplock, 1994; Sies, 1993).

Table 1.1 Enzymatic Antioxidants

Enzymatic Antioxidants	*Location*	*Properties*
Superoxide dismutase (SOD)	Mitochondria, cytosol	Dismutase superoxide radicals
Glutathione peroxidase (GSH)	Mitochondria and cytosol	Removes hydrogen peroxide and organic hydroperoxide
Catalase (CAT)	Mitochondria and cytosol	Removes hydrogen peroxide
Heme peroxidase		Scavenges the endogenous H_2O_2

Source. Neda Mimica-Dukic, 1997.

Superoxide Dismutose (SOD)

This is the first enzyme involved in the anti-oxidant defence. This metaloprotein is found in the cytosol and mitochondrial matrix (Fridovich, 1983). Its biosynthesis has been related to the intracellular fluxes of $0\sim_2$ (Crapo and McCord, 1976; Rister and Balchner, 1976). McCord and Friovich (1969) discovered superoxide dismutage (SOD) in bovine erythrocyte. In various forms, it has been widely found in other animals. It neutralizes the harmful effect of superoxide radical (0_2^-) and converts it into hydrogen peroxide and oxygen:

$$2O_2^- + 2H^+ \xrightarrow{(SOD)} H_2O_2 + O_2$$

SOD has been found to have therapeutic effect on the cancer, bone marrow injuries, radiation damage, pneumonia and loss of immunity (Tean, 1978; Qingsheng, 1984). Presently, it is isolated from the animals for medical purposes. Superoxide Dismutage has also been found in some plants like seabuckthorn.

Glutathione Proxidase

It is a seleno-enzyme, two-third of which is present in the cytosol and rest in the mitochondria (Freeman and Crapo, 1982). It catalyses the reaction of hydroperoxides with reduced glutathione (GSH) to form glutathione disulphide (Meister and Anderson, 1983) and the reduction product of the hydroperoxide (Chance *et al.,* 1979).

Heme Peroxidase

Heme peroxidase, like horseradish peroxidase, lactoperoxidase etc. catalyses the oxidation of a wide variety of electron donors with the help of H_20_2 and thereby scavenges the endogenous H_2O_2 (Das *et al.,* 1995; Mazumdar *et al,* 1997).

Catalase

It is a hemoprotein and catalyses the decomposition of H_2O_2 to water and oxygen and therefore protects the cell from H_2O_2 and ^+OH (Deisseroth and Dounce, 1970). The enzyme is present in the peroxisomes of almost all die mammalian cells (Chance *et al.,* 1979).

SECONDARY DEFENCE AGAINST FREE RADICALS

Secondary defense against ROH involves small molecules, which react with radicals to produce another radical compound, known as "Scavengers". These scavengers produce a lesser harmful radical species, called "anti-oxidant", *e.g.* a-tocopherol (vitamin E), ascorbate (vitamin C) and reduced glutathione (GSH) may act in combination to act as cellular anti-oxidants.

Recent studies have also found strong association between prevention of diseases like cancer and cardiovascular and intake of fruit and vegetables, rich in anti-oxidants like vitamin C, E and carotenoids (Halliwell, 1997). Flavonoids have also been found to be preventive against cancer and cardiovascular diseases and acts as a strong anti-oxidant (Rice-Evans *et al,* 1997). Besides antioxidant enzymes of human body, which protect us from ill effect of free radicals, we get other antioxidants from the diet. Dietary antioxidants include vitamin C, vitamin E and carotenoids, flavonoids, polyphenols and terpenes (Table 1.2). Oxidative stress is one of major causes of aging and diseases like cancer, cardiovascular, Parkinson diseases, Alzheimer type dementia and atherosclerosis, which can be curtailed by anti-oxidants like vitamin C, E, carotenoids, flavonoids and dismutage present in seabuckthorn. Epidemiological studies have certainly found a correlation between the increased consumption of food rich in antioxidants and a decreased risk of several diseases like cardiovascular and cancer. Therefore, an increased intake of fruits and vegetables can be recommended. However, further studies are required to establish whether antioxidant supplementation beyond dietary intake levels is of benefit for health improvement (Frei, 1994; van Poppel and Goldbohm, 1995; Verhagen *et al.,* 1995).

VITAMIN C

Consumption of fresh fruits and vegetables has been related to be inversely correlated with cancer of stomach, pancreas, oral cavity and oesophagus and to a lesser extant of the breast, cervix, rectum and lung (Block, 1992; Block *et al,* 1992), where protective role of vitamin C has been emphasized. It was found that vitamin C inhibits the formation of carcinogenic N-nitroso compounds like N-nitroamines (Mirvish, 1994; Licht *et al,* 1998).

Ascorbic acid present in foods is readily available and easily absorbed by active transport in the intestine (Sauberlich, 1985). Ascorbic acid is sensitive to air, light, heat and easily destroyed by prolonged storage and over processing of food. Ascorbic acid being a water-soluble compound is easily absorbed, but it is not stored in the body. Hence ascorbic acid has to be regularly supplemented through diet or tablets to maintain ascorbic acid pool in the body. Ascorbic acid is generally non-toxic but at high doses (2-6 g/day) it can cause gastrointestinal disturbances or diarrhoea (Anderson *et al,* 1997; Johnson, 1999). A deficiency of ascorbic acid leads to scurvy. It is characterized by spongy swollen bleeding gums, dry skin, open sores on the skin, fatigue, impaired wound healing and depression (Olson, 1999). The most Ascorbic add is known to enhance the availability and absorption of iron from non-heme iron sources (Hallberg, 1981). Ascorbic acid supplementation is found to facilitate the dietary absorption of iron. The new average daily intake level that is sufficient to meet the nutritional requirement of ascorbic add or recommended dietary allowances (RDA) for adults (>19 yaers) are 90 mg/day for men and 75 mg/day for women (Fiei and Traber, 2001). Consumption of 100 mg/ day of ascorbic acid is found to be sufficient to saturate the body pools (neutrophils, leukocytes and other tissues) in healthy individuals. Based on the clinical and epidemiological studies, it has been suggested that a dietary intake of 100 mg/day of ascorbic add is associated with reduced incidence of mortality from heart diseases, stroke and cancer (Carr and Frei, 1999).

Table 1.2 Non Enzymatic Antioxidants

Enzymatic Antioxidants	***Location***	***Properties***
Vitamin C	Aques phase of cell	Acts as free radical scavenger and recycles vitamin E
Vitamin E	Ceil membrane	Major chain-breaking antioxidant in cell membrane
Uric acid	Product of purine metabolism	Scavenger of OH radicals
Glutathione	Nonprotein thiol in cell	Serves multiple roles in the cellular antioxidant defense
a-lipoic acid	Endogenous thiol	Effective in recycling vitamin C, may also be an effective glutathione substitute
Carotenoids	Lipid soluble antioxidants, located in membrane tissue	Scavengers of reactive oxygen species, singlet oxygen quencher
Bilirubin	Product of heme metabolism in b100d	Extracellular antioxidant
Ubiquinones Metals ions sequestration: transferrin, ferritin, lactoferrin Nitric oxide	Mitochondria	Reduced form are efficient antioxidants Chelating of metals ions, responsible for Fenton reactions Free radical scavenger, inhibitor of LP

Source: Neda Mimica-Dukic, 1997.

The widely known health beneficial effect of ascorbic add is for the prevention or relief of common cold. Pauling (1970) suggested that ingestion of 1-2 g of ascorbic acid effectively prevents/ ameliorate common cold. Ascorbic acid plays a critical role in wound repair and healing/regeneration process (Shukla, 1969).

As a scavenger of ROS, ascorbate has been found to be effective against the superoxide radical anion, the hydroxyl radical and singlet oxygen. In aqueous solutions, vitamin C also scavenges reactive nitrogen oxide species effidently, preventing the nitrosation of target molecules. Studies carried out in vitro have proven that vitamin C is capable of regenerating tocopherol from the tocopheroxyl radical, which is formed on inhibition of lipid peroxidation by vitamin E. This process transports the radical load from a lipophilic compartment to an aqueous compartment, where it is taken care of by efficient enzymic defense systems (Bendich *et al,* 1986; Niki *et al,* 1985; Levine *et al,* 1996).

Lipids peroxidation and oxidative modification of low-density lipoproteins (LDL) are implicated in development of atherosclerosis (Steinbrecher *et al.,* 1990). Vitamin C protects against oxidation of isolated LDL by different types of oxidative stress, including metal ion dependent and independent processes (Frei, 1997). Addition of iron to plasma devoid of ascorbic acid resulted in lipid peroxidation, whereas endogenous and exogenous ascorbic acid was found to inhibit the lipid oxidation in iron-over loaded human plasma (Berger *et al.,* 1992). Similarly, when ascorbic acid was added to human serum supplemented with Cu^{2}*, antioxidant activity rather than pro-oxidant effects were observed.

A number of studies have been carried out in humans to determine the protective effect of ascorbic acid supplementation (500-100 mg/day) on *in vivo* and *ex vivo* lipid peroxidation in healthy individuals and smoker. The findings are inconclusive as ascorbic acid supplementation showed a reduction or no change in lipid peroxidation products (Anderson *et al.,* 1997; Fuller *et al.,* 1996; Nyyssonen *et al.,* 1997; Samman *et al.,* 1997; Wen *et al.,* 1997). Considerable biochemical and physiological evidence suggests that ascorbic acid functions as a free radical scavenger and inhibit the formation of potentially carcinogenic N-nitroso compounds from nitrates, nitrite in stomach and thus offer protection against stomach cancer (Schorah *et al.,* 1991; Sobala *et al.,* 1991; Drake *et al.,* 1996).

Supplementation of vitamin C has been observed to decrease the mucosal DNA damage (Dyke *et al.,* 1994), which indicates a protective role of vitamin C against the gastric cancer. However, supplementation of vitamin C could not affect the chemically induced tumors in the bladder and colon cancers rodents (Byers and Perry, 1992). Therefore, vitamin C role in controlling the tumors is believed to be uncertain.

Consumption of vegetables thrice a day and fruits twice a day have been suggested for sufficient intake of antioxidants. Besides these intakes of vegetables and fruits, consumption of moderate vitamin C and a-tocopherol has been desirable for smokers (Diplock, 1994; Halliwell, 1994; Block,1992; Block *et al,* 1992; Mirvish, 1994). As large intake of vitamin C or E were not protective in case of breast cancer in women, it is yet to be investigated to what extent dietary changes can decrease the oxidative damage of DNA or optimal intake of fruits, vegetable or antioxidant suitable for the human body (Wiseman and Halliwell, 1996).

CAROTENOIDS

Carotenoids, a fat-soluble group of naturally occurring plant pigments, are a sub-classification of the terpenes. There are approximately 600 known carotenoids, 50 of which are present in our diets, mostly from fruits and vegetables. Twenty have been identified in the human body. Chemically, carotenoids are classified in 2 main groups: carotenes and xanophylls. Carotenes refer to the carotenoids that contain only carbon and hydrogen (beta-carotene and lycopene). Xanthophylls refer to compounds that contain in addition a hydroxyl group (lutein, zeaxanthin, and beta-cryptoxanthin), a keto group (canthaxanthin) or both (astaxanthin). Perhaps the most familiar of phytonutrients, carotenoids function as powerful antioxidants and immuno-potentiaters. Diets rich in carotenoids are linked with a decreased risk of heart disease, cancer, and degenerative eye diseases like muscular degeneration and cataracts (Functional Foods and Nutraceuticals, 2003). Alpha-carotene, beta-carotene, and cryptoxanthin are the main vitamin A precursors.

Carotenoids are considered potent membrane antioxidants due to their reactivity with singlet oxygen. Ranked by antioxidant power we can list astaxanthin, canthaxanthin, beta-carotene, zeaxanthin, and lutein as all stronger than vitamin E.

Leafy green vegetables contain mostly lutein and zeaxanthin. Carrots, oranges, sweet potatoes and squash contain mainly carotenes. Interestingly, the yellow yolk of eggs is rich in lutein, and the astaxanthin group from red algae is what makes salmon pink.

Lutein and zeaxanthin are the only carotenoids identified in the macula. There they filter blue light from the retina and inhibit oxidative damage. Such damage leads to macular degeneration, the leading cause of blindness in these over 65.

Researchers at the University of Utah Medical School found that lutein intake is inversely associated with colon cancer (Slattery, 2000). A study on serum carotenoids levels in women in India with breast cancer showed lutein and zeaxanthin to be significantly lower than in healthy controls, at least in postmenopausal women (Ito, 1999).

Lycopene is most abundant in tomatoes with smaller amounts in pink grapefruit, watermelon, guava, and rose hips. Lycopene makes up approximately 50 per cent of the total carotenoids in b100d plasma of those persons consuming western diets. It protects against prostate, cervical, breast, digestive tract and lung cancers, and perhaps atherosclerosis. (Agarwal and Rao, 2000).

Astaxanthin, found mostly in red yeasts and red algae, is now fed to salmon, trout, crabs, krill and shrimp in "fish farms" to provide the red and pink color of their natural red algae eating wild brethren. This most powerful of the carotenoid antioxidants has been shown to enhance secondary immune response in humans, and help reduce symptoms of H. pylori, CTS and RA (Naguib, 2003). Beta-carotene intake is associated with reduced risk of breast, stomach, esophageal, and pancreatic cancers (Nishino *et al.,* 2000). Researchers at John Hopkins reported in 1994 that smokers with the lowest b100d levels of beta-carotene had approximately a 350 per cent greater risk of heart attack as compared to non-smokers with high beta-carotene levels.

Beta-cryptoxanthin, found mostly in fruits like oranges, tangerine and papayas, is second only to beta-carotene as a source of vitamin A. Cryptoxanthin, again demonstrating the uniqueness of each phytonutrient, is the only carotenoid that appears to be related inversely to bladder cancer risk (Zeegers, 2001).

Some carotenoids rich foods like carrots and tomatoes yield more beta-carotene and lycopene, respectively, when cooked. Lutein and lycopene require fat for optimal uptake of carotenoids whereas dietary fiber inhibits its absorption of lutein, lycopene, and beta-carotene by 40 per cent to 75 per cent (Reidl *et al,* 1999; Roodberg *et al,* 2000).

VITAMIN E (TOCOPHEROLS)

Vitamin E is an important dietary antioxidant and includes all tocols and tocotrienol derivatives. Their most important antioxidant function appears to be the inhibition of lipid peroxidation, scavenging lipid peroxyl radicals to yield lipid hydroperoxides and a tocopheroxyl radical. The tocopheroxyl radical might be either reduced by ascorbate and GSH or further oxidized to the respective quinone. As only small amounts of tocopheryl quinone are detectable in human b100d and tissues, the regenerative pathway appears to be favoured.

Vitamin E deficiency in man causes defects in development of nervous system in children and hemolysis (Sokol, 1996). Low intakes of vitamin E and other anti-oxidants results into certain types of cancer and atherosclerosis (Gey *et al,* 1991; Knekt, 1993; Rimm *et al,* 1993). It has been suggested that supplementation with these anti-oxidants may decrease the risk of these and other degenerative processes (Blot *et al.,* 1993). Among the tocopherols, a-tocopherol is the most bioactive (30 per cent), whereas the P-tocopherol has 25-50 per cent bioactivity and y-tocopherol has 10-35 per cent (Dillard *et al,* 1983).

Several studies have discussed the role of antioxidants like vitamin E, in curing the cardiovascular diseases. The primary role of these antioxidants in reducing the risk of cardiovascular disease has been found through the inhibition of peroxidation in LDL. The epidemiological evidence for a protective role of the antioxidants in cardiovascular disease has been found strongest for vitamin E. Several epidemiological studies established a relationship between vitamin E intake and cardiovascular disease. These studies have found significant reduction in cardiovascular diseases in persons, who had consumed vitamin E supplements. However, in one study, it has been observed that use of both vitamin E and C supplements together was significantly more protective and effective against cardiovascular diseases than use of vitamin E supplements alone, or no use of supplements (van Poppel *et al.,* 1994; Losonczy *et al,* 1996; Kushi *et al,* 1996).

PHENOLICS AND FLAVONOIDS

Flavonoids are phenolic compounds that belong to the recently popular phytochemicals, *i.e.;* compounds derived from plant material with potential beneficial effects in human health. Flavonoids are a large group of polyphenolic antioxidants that occur in several fruits, vegetables, and beverages such as tea, wine, beer etc. They are efficient antioxidants, capable of scavenging free radicals. Quercetin, kaempferol, myricetin and their glycosides are the most common flavonoids in the beverages. They can be found in onion and tea, together with fruit juice, they make up the main intake of the flavonoids. It should be mentioned, however, that the bioavailability of these compounds is rather poor. They are rapidly conjugated in phase II detoxification reactions and levels of free flavonoids in human plasma are very low.

Although secondary plant metabolites, flavonoids are an important part of the human diet and they represent an active principle of some medicinal plants. There are over 5,000 individual flavonoids known, probably a consequence of their structural diversity (Bors *et al,* 1996; Rice-Evans *et al,* 1996). The basic structure of the major flavonoid groups (flavanone, dihydroflavonol, flavone, anthocyanidin and flavanol) is same, however their differences within each of these groups are usually by the degree of aromaticity, the arrangement and number of hydroxyl substituents, and the extent and type of glycosylation of these groups. The preferred glycosylation site is the 3 position and glucose is the most usual sugar residue (other residues being galactose, xylose, etc.).

Flavonoids have been shown to inhibit the lipid peroxidation processes (Ursini *et al,* 1994; Laughton, 1989), inherent in the auto-oxidation of linoleic acid, oxidation of low-density lipoproteins (LDL), peroxidation of phospholipid, microsomal, and mitochondrial membranes, lysis and peroxidation in human erythrocytes and erythrocyte ghosts, antioxidation of brain homogenates and deterioration of the lysosomal membrane. It is likely that several functions of flavonoids account for these effects on lipid peroxidation; flavonoid acting as metal chelators, chain breaking antioxidants, and/or freeradical scavengers.

The biological, pharmacological and medicinal properties of the flavonoids have been extensively reviewed (Cody *et al,* 1998; Das, 1990). The wide range of effects elicited by flavonoids is an expression of their functional group chemistry, underlying their antioxidant and prooxidant properties, mutagenic, anticarcinogenic, and biocidal effects, interaction with signal transduction processes and beneficial effects in inflammatory and immunomodulatory systems.

ANTI-OXIDANT PROPERTIES OF SEABUCKTHORN

Several studies have revealed that plants produce potent anti-oxidants to control the oxidative stress caused by oxygen and sun beams etc. As oxidative stress is known to be one of the major causes of aging (Harman, 1957), diseases like Parkinson, Alzheimer type dementia and atherosclerosis (Ames *et al,* 1993), therefore, use of plant as a source of anti-oxidants can significantly reduce the severity and progression of these diseases. Seabuckthorn is a plant, widely grows in cold regions of Himalayas and Europe (Singh, 2003), which has been used as a medicinal plant in traditional medicines like Ayurveda (Uniyan and Uniyan, 2001) and Tibetan System of Chinese Medicines (Fuying and Tlanming, G. 1989). Since 1950s, many medicinal preparations of seabuckthorn have been clinically used to treat various disorders and diseases in former Soviet Union and China, however, it is only recently that some authentic scientific studies have been conducted particularly on anti-oxidative efficacy of seabuckthorn fruit and leaf extracts (Rui *el al.,* 1989; Ji and Gao, 1991a; Song and Gao, 1995; Ji and Gao, 1991a, b; Song and Gao, 1995; Rui and Gao, 1989; Shi *et al.,* 1994; Liu *et al.,* 2002; Geetha *et al.,* 2002, 2003; Khanum *et al.,* 2004). Seabuckthorn pulp oil contains high levels of palmitic and palmitoleic acids, whereas seed oil is rich in linoleic and a-linolenic acids, Both oils are rich in tocopherols and phytosterols. These bioactive substances and the high levels of carotenoids are believed to play an important role in the anti-oxidative properties found in both oils of seabuckthorn.

Ju *et al.* (1989) studied the scavenging effect of total flavonoid of seabuckthorn on oxygen radicals. 1 pg/ml of seabuckthorn flavonoids effectively inhibited the CL response of PMA-stimulated human polymorphonuclear leukocytes (PMN). 1.7 pg/ml of seabuckthorn flavonoid significantly reduced the active oxygen radicals level of PMA-stimulated PMN; 0.03-3 pg/ml of seabuckthorn flavonoids could remarkable scavenged superoxide in xanthine/xanthint oxidase (Xan/Xo) system and the effect was concentration dependent.

Antioxidative activities of seabuckthorn pulp oil and vitamin E (at the same dose as in seabuckthorn pulp oil) were analyzed by Song and Gao (1995) in rats under cold exposure and by Rui and Gao (1989) in guinea pigs. At the end of the study, MDA content in erythrocytes in the negative control group and the vitamin E supplemented group had significantly enhanced by 75 per cent ($p<0.01$), as compared to the positive control group, whereas the values in the seabuckthorn oil group were near to those of the positive control group. Electron microscopic analysis found that seabuckthorn oil protected mitochondria of liver cells from cold exposure-caused damages. In an 8-week study conducted on the guinea pigs, addition of seabuckthorn pulp oil (40 g/kg feed, vitamin E content 50 mg/kg feed) to a vitamin E free diet declined MDA-induced erythrocyte hemolysis and the MDA level of erythrocyte cell membranes. The oil supplementation enhanced the level of sulfydryl groups and the activities of Na, K-ATPase and glucose-6-phosphate dehydrase (G-6-PD) of erythrocyte cell membrane.

Ji and Gao (1991a, b) studied the effects of seabuckthorn seed oil on the cell membranes in rats. Wistar rats, 33 in numbers (12-week-old), were randomly divided into 3 groups, lived for 8 weeks on (a) 100 per cent basic diet (vitamin E, 5.2 mg/100 g; Se, 0.2 ppm), (b) 90 per cent basic diet plus 10 per cent seabuckthorn seed oil (vitamin E, 15.7 mg/100 g; Se, 0.2 ppm), or (c) 90 per cent basic diet plus 10 per cent seabuckthorn seed oil, supplemented with sodium selenite (vitamin E, 15.7 mg/100 g; Se, 2.0 ppm). The seabuckthorn oil-supplemented diets enhanced the MDA level in the cell membrane of erythrocyte ghosts by 68 per cent and in liver homogenate by 35 per cent, as compared to the 100 per cent basic diet. Seabuckthorn seed oil supplementation increased the glutathione peroxidase (GSH-Px) activities in erythrocytes by 16 per cent. Contents of sialic acid (by 46 per cent) and the sulfydryl group (-SH, by 73 per cent), as well as Na, K-ATPase activity (by 44 per cent) in the erythrocyte cell membrane, were elevated by the oil supplementation.

Shi *et al.* (1994) studied the effects of seabuckthorn seed oil on the oxidation of LDL with *in vitro* oxidation models. In the Cu^{2+}-catalyzed oxidation model ($CuCl_2$,50 mmmol/l), LDL (0.1 mmmol/l) from normal subjects was incubated in the presence of seabuckthorn seed oil at different levels (0, 0.3, 0.6, 1.2, 2.4, 4.8 per cent, v/v) at 37°C for 24 hours. In cell-catalyzed oxidation models, the LDL samples (0.03 mmmol/l) were incubated together with mouse abdominal macrophages or bovine endothelial cells in solutions containing the seed oil at different levels (0, 0.04, 0.08, 0.16, 0.32 per cent, v/v) at 37°C for 24 hours. Addition of the seed oil significantly declined the formation of MDA and conjugated diene in the Cu^{2+}-catalyzed oxidation system. MDA levels in the cell-catalyzed oxidation system were also significantly declined (by 18-75 per cent) by addition of the oil. The effects of seabuckthorn seed oil were dose-responsive in both types of oxidation models.

Liu *et al.* (2002) also studied the antioxidative effect and protective effect of seabuckthorn oils on the myocardium in mice. 70 ICR mice were divided into different groups, given oral seabuckthorn oil or negative control under different exercise programmes. The serum Aspartate aminotransferase (GOT), SOD and MDA contents in heart and ultrastructure of myocardial cells were analyzed at sedentary state, immediately after exercise and 24 hours after exercise). Seabuckthorn oil enhanced the SOD levels after exercise and decreased the MDA levels at sedentary state and 24 hours after exercise. In addition, the oil treatment maintained the integrity of mitochondria, endoplasmic reticulum and myofibre in the myocardium.

Geetha *et al.* (2002) studied the effect of anti-oxidant and immunomodulatory properties of seabuckthorn, using lymphocytes as a model system. Addition of chromium (10 µg/ml) to the cells resulted in enhanced cytotoxicity, apoptosis, free radical production and decreased glutathione level. Water and alcoholic extracts of leaves and fruits of seabuckthorn were studied for anti-oxidant activity. Alcoholic extracts of leaves and fruits of seabuckthorn at higher doses of 500 µg/ml were found to inhibit chromate induced cytotoxicity, apoptosis and free radical generation in rat spleenocytes prevented chromate induced inhibition of lymphocyte proliferation. Further leaf and fruit extracts of seabuckthorn were found to stimulate lymphocyte proliferation even in the absence of mitogens (ConA / PLS), indicating the presence of intrinsic immunomodulatory substances in seabuckthorn. Therefore, seabuckthorn has a cytoprotective effects against chromium induced cytotoxicity, improves the antioxidant defence system of cell by increasing the intracellular GSH levels and inhibiting free radical production and also have a immunomodulatory activity. However leaf extract showed better results in all parameters than the fruit extract. Patro *et al.* (2001), while using rat brain homogenate, also found anti-oxygenic property in non-polar fractions, which are rich in ^-carotene and xanthophylls. Polar fractions, rich in flavonoids and phenolics also showed anti-oxygenic activity. Fractions containing fatty acids did not show any anti-oxygenic activity. Geetha *et al.* (2002), in another experiment, studied the anti-oxidant activity of leaf extract of seabuckthorn. Oxidative stress was created in the rats by potassium dichromate equivalent to a dose of 30 mg/kg body weight of chromium (VI) for 30 days. Chromium treatment significantly decreased reduced glutathione (GSH) and increased malondialdehyde (MDA) and creatine phosphokinase (CPK) levels. Alcoholic leaf extract of seabuckthorn protected the animals from chromium induced oxidative damage significantly at a concentration of 100 and 250 mg/kg BW.

Different extracts from seabuckthorn were investigated by Gao (2005) for the antioxidant activity. The crude extracts of 50 per cent EtOH from all 3 tested cultivars of seabuckthorn showed high activity to scavenge 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation radicals.

Trolox Equivalent Antioxidant Capacity (TEAC) ranged 19.8 to 25.2 µmol/g. The antioxidant activity was highly correlated with the contents of total phenolics and ascorbate (r = 0.78 to 0.98).

Khanum *et al.* (2004) developed an herbal mixture of 90 per cent seabuckthorn leaves and 10 per cent other medicinal herbs and spices. The efficiency *in vitro* was compared with that of tea available in market. The study found the seabuckthorn mixture a rich source of flavonoids (550 µg/100g). *In vivo* studies on rats, where in oxidative stress was induced chemically, using HCH, were conducted by providing the mixture at levels of 1 per cent and 2 per cent. HCH induced oxidative stress by increasing the content *of* malnodialdehyde (197 per cent) and conjugated dienes (116 per cent) apart from reducing the GSH (68 per cent) the activity of catalase (70.4). Seabuckthorn herbal mixture decreased the malondialdehyde from 197 per cent to 114 per cent and 10 per cent at 1 per cent and 2 per cent levels, respectively, whereas the level of GSH increased from 68 per cent (in HCH treated rats) to 91 per cent at 1 per cent and 142 per cent at 2 per cent mix levels respectively. Both *in vitro* and *in vivo* studies showed a very strong anti-oxidant activity of seabuckthorn herbal mixture.

ANTI-OXIDANTS OF SEABUCKTHORN

While Several studies have proven strong anti-oxidant properties of seabuckthorn (Rui *et al.,* 1989; Song and Gao, 1995; Liu *et al.,* 2002; Geetha *et al.* 2002), seabuckthorn fruit and leaves have been found rich in anti-oxidants like superoxide dimutase enzyme, vitamin C, E, carotenoids, flavonoids etc. The biological properties of the seabuckthorn oil are determined by its non-saponificated part, which includes anti-oxidants like tocopherols, carotenoids and other compounds like vitamin K and sterols (Yang and Kallio, 2002). It was estimated that content of unsaponified matters in fruit oil ranged from 1.1 per cent-2.8 per cent in seabuckthorn growing in western Pamirs (Glazunova *et al,* 1991) to 3.7 per cent in Transbaikal (Zham'jatsan, 1973; cit. of Glazunova *et al,* 1983). The Siberian and the Caucasian forms had similar values (3.2-3.7 per cent and 3.0-3.45 per cent, respectively) (Shugam, 1969; cit. of Trofimov, 1976; Abutalybov *et al,* 1978; Muraviev *et al,* 1985) and almost same content was in middle Asian populations (Glazunova *et al.,* 1983,1991).

SUPEROXIDE DISMUTASE (SOD)

Superoxide dismutase (SOD) is considered as an important soluble anti-oxidant. Yuehua *et al.* (1989) isolated SOD from seabuckthorn fruit and foliage, which explores the utility of seabuckthorn as an important source of anti-oxidant. Activity of SOD (unit/gfw) extracted from seabuckthorn fruit and leaves was much higher than other plants (Jin and Tao, 2003). They studied the chemical composition of this enzyme and found absence of tryptophan amino acid, a characteristic of this enzyme. Antioxidant activity of SOD drug was studied by Jin and Tao (2005) in rats. The preparation reduced the free redicals and lipid peroxidation.

Some studies have shown that the berries of seabuckthorn contain high content of SOD. *H. salicifolia* contains the highest content of SOD (130.2 U/g). *H. goniocarpa* ssp. *litangensis* contains the lowest content of SOD *e.g.* 39.4 U/g. There is an obvious trend that the SOD content of the berries is correlated with the vitamin C content. That means the species and subspecies that contain more vitamin C. such as *H. rhamnoides* ssp. *sinensis, H. rhamnoides* ssp. *yunnanensis* and *H. salicifolia,* also contain more SOD (Lu Rongsen, 2003).

VITAMIN C

Studies have proven that the vitamin C content of seabuckthorn fruit is 4-100 fold higher than many other fruits or vegetables. Vitamin C is a major anti-oxidant present in the seabuckthorn fruit juice, varying in content from 30 to 2740 mg/100 g of berries (Lu Rongsen, 2003; Li and Schroeder, 1996;

Tang *et ah,* 2001; Singh and Sawhney, 2005). Studies conducted in various countries have found considerable variation in vitamin C content both among and within natural populations, attributed to genetic factors, degree of ripeness at harvest and environmental conditions.

In Chinese forms, Lu Rongsen (2003) found vitamin C in a high range of 1680.5-1889.0 mg/100 g in the fruit of *H. salcifolia,* followed by ssp. *sinensis* (1351 mg) and ssp. *yunnanensis* (887.7-1261.4). Kallio *et al.* (2002a) reported that berries of subsp. *sinensis* were more rich in vitamin C (420-1320 mg/ 100 mL of berry juice, with mean value 860 mg/100 ml) than berries of subsp. *rharnnoides* (70-290 mg/ 100 mL) and subsp. *mongolica* (90-120 mg/100 mL). A low content of 48-200 mg/100 has been reported in subsp. *mongolica* in Mangolia (Jamyansan and Badgaa, 2005). Lower content of vitamin C has been reported in berries of European subsp. *rharnnoides* (30-290 mg/100 g berry weight) (Rousi and Aulin, 1977; Yao, 1993). Low values of vitamin C, *i.e.* 26-150 mg/100 g has also been found in Romanian seabuckthorn (Mladin *et al,* 2001), 49-101 mg in Estonia (Jalakas *et al,* 2003), some higher values of 150 to 340 mg/100 g were estimated for 4 cultivars in Germany (Albrecht, 1990) and 100-373 mg in Hungry (Bemath and Foldesi, 1992) (Table 1.3).

Indian Himalayas is characterized with occurrence of seabuckthorn with higher values of vitamin C. High contents of vitamin C of 950 mg/100g (Singh *et al,* 1995) and 2740 mg/100 g (Singh and Sawhney, 20Q5) have been recorded for *H. salicifolia* growing in Lahaul, Elimachal Pradesh Himalayas, India, Vitamin C in the range of 225-642 mg/100g has been found in *H. rharnnoides* ssp. *turkestanica* populations growing also in Lahaul Himalayas. *H. rharnnoides* ssp. *turkestanica* in Ladakh Himalayas, India have been found very rich in Vitamin C upto 1400 mg/100 g (Dwivedi *et al,* 2005). Chauhan *et al.* (2001) reported a medium value of 516 mg/100g in H. *rharnnoides* ssp. *turkestanica* growing in Indian Himalayas (Table 1.3). Over all both Indian species are quite rich in vitamin C.

The east European climatype in Russia is characterized by a range of 4 to 418 mg, the lowest range, varying from 1 to 151 mg, on the average, with the maximum of 319 mg. In the Siberian climatype, high vitamin C forms are found in the Altay Mountain up to 792 mg (Demina, 1964). Aksenova and Dolgacheva (1998) estimated vitamin C in the range of 85-309 mg/100g in high yielding cultivars at Moscow Botanical Garden at Moscow State University. A range of 100-200 mg vitamin C was estimated in biotypes from Buryatia, Russia (Myahanova, 1998). A high value of 1294 mg in the central Asian forms have been found in the Kyzyl-Ungur river valley in the Tien-Shan ecotype (Eliseev, 1976). The vitamin C in the fruits of the western Pamirs ecotype is rather high, ranging from 53 to 594 mg. Other studies (Gachechiladze *et al,* 1981; Glazunova *et al,* 1983,1984; Korzinnikov *et al,* 1983) have found that the maximum vitamin content of 468-594 mg was estimated in the seabuckthorn fruits at 2530-3500 m above the sea level in the Shakh-Dara, Pyanj, Gunt, Pamir rivers valleys. The minimal vitamin C content in the seabuckthorn fruits was found in the east Khazakhstan ecotype (43 to 195 mg). There, seabuckthorn grows in highly unfavourable conditions of the saline semi-desert (Besschetnov, 1980; Korovina *et al,* 1993). In the Caucasian, the highest characteristic variation was observed in the North Caucasian, Azerbaijan and Black Sea area ecotypes, varying from 1-18 mg to 150-319 mg, which was considerably lesser than that of the above mentioned climatypes. The minimal changeability range has been found in closed areas of the Central Caucasus (19.5-57.5 mg) and Sevan (12.0-35.5 mg) (In Korovina, 2005).

While climatic conditions during growth and maturation may affect to some extent vitamin C accumulation, studies (Tang, 2002) suggested decreasing trends in vitamin C concentration during fruit ripening. In a study conducted on 6 clones from native stands in southwestern Finland, the content of vitamin C acid decreased in all clones by 25-60 per cent over a time span of 34 days from August 17 to September 21 (Rousi and Aulin, 1977). In a recent Finnish study (Kallio *et al,* 2002a), a quite linear decline from the beginning Of September to the end of November was observed in 4 different ssp. *rhamnoides* clones cultivated in Helsinki, Finland (Table 1.3).

Table 1.3: Vitamin C Variations in the Fruits of Seabuckthorn Species

Species/Subspecies	***Place***	***Vitamin C (mg/100g)***	***Reference***
H. rhamnoides ssp. *rhamnoides*	German forms	150-310	Darmer, 1952
H. rhamnoides	German cultivars	160-340	Albrecht, 1990
H. rhamnoides	German forms	190-200	Prokkola *et al.,* 2003
H. rhamnoides ssp. *rhamnoides*	Rihtniemi, Finland	165-293	Rousi & Aulin, 1977
H. rhamnoides	Moscow, Russia	85-309	Aksenova & Dolgacheva, 1998
H. rhamnoides	Kyzyl-Ungur river valley, Central Asia	1294	Eliseev, 1976
H. rhamnoides	Altay, Siberia	88-792	Demina, 1964
H. rhamnoides	Western Pamirs,	53.0 to 594	Gachechiladze *et al.,* 1981; Glazunova *et al.,* 1983

H. rhamnoides	East Khazakhstan,	43 to 195.5	BeSschetnov, 1980; Korovina *etal.,* 1993
H. rhamnoides	Buryatia, Russia	100-200	Myahanova, 1998
H. rhamnoides	Central Caucasian	19.5-57.5	Igoshina *et al.,* 1987
H. rhamnoides	Black Sea area, Caucasian	15.6-319.0	Igoshina *et al.,* 1987; Eliseev., 1976
H. rhamnoides	*Azerbaijan*	110-304	Novruzov *et al.,* 2001
H. rhamnoides ssp. *sinensis*	Maoxian Sichuan, China	867.5-1351.5 (1109.5)	Lu Rongsen, 2003
H. rhamnoides ssp. *yunnanensis*	Zhongdian Yunnan, China	887.7-1261.4 (1074.6)	Lu Rongsen, 2003
H. rhamnoides ssp. *turkestanica*	Yining, Xinjiang, China	128.5-130.8 (129.7)	Lu Rongsen, 2003
H. rhamnoides ssp. *fluviatilis*	Munich Germany	383.5-515.5 (449.5)	Lu Rongsen, 2003
H. salicifolia	Maoxian Sichuan, China	1680.5-1889.0 (1784.7)	Lu Rongsen, 2003.
H. rhamnoides ssp. mongolica	Habahe Xinjiang, China	67.5-150.2 (108.9)	Lu Rongsen, 2003
H. goniocarpa ssp. *litangensis*	Litang, Sichuan, China	205.6-224.6 (215.1)	Lu Rongsen, 2003
H. goniocarpa ssp. *goniocarpa*	Hongyuan Sichuan, China	43.4-165.4 (104.4)	Lu Rongsen, 2003
H. gyantsensis	Lhasa, Tibet, China	9.34-51.5 (30.4)	Lu Rongsen, 2003
H. tibetana	Hongyuan Sichuan, China	122.6-130.5 (126.6)	Lu Rongsen, 2003
H. rhamnoides ssp. *turkestanica*	Lahaul, India	219-642	Singh *etal.,* 1995
H. salicifolia	Lahaul, India	947	Singh *etal.,* 1995
H. salicifolia	Lahaul, India	2740	Singh & Sawhney, 2005
H. rhamnoides ssp. *turkestanica*	Himalayas, India	516	Chauhan *etal.,* 2001
H. rhamnoides ssp. *turkestanica*	Ladakh, India	138-1400 (769)	Dwivedi *etal.,* 2005
H. rhamnoides ssp. *mongolica*	Indian Summer, Canada	100-300	Li *et al.,* 1999 in Schroeder *etal.,* 2001.
H. rhamnoides ssp. mongolica	Mangolia	48.0-200.0	Jamyansan & Badgaa, 2005
H. rhamnoides	Italian forms	210-270	Antonelli *etal.,* 2003
H. rhamnoides	Estonia	49-101	Jalakas *et al.,* 2003
H. rhamnoides	Romania	26-150	Mladin *et al.,* 2001
H. rhamnoides	Hungry	100-373	Bernath & Foldesi, 1992

Noter. Value in parenthesis is mean of the range.

CAROTENOIDS

Like vitamin E, carotenoids belong to the group of lipophilic antioxidants present in lipoproteins. Carotenoids are well known for their wide distribution in plant kingdom. Various colours of ripe berries of seabuckthorn, ranging from yellow to bright red are related to occurrence of carotenoids. Fruits of seabuckthorn differ from other plants by occurrence of considerably high content of lipid-soluble carotenoids. The main constituents of all carotenoids are tetraterpene, *i.e.* compounds consisting of 8 isoprene fragments or their derivatives. Composition of carotenoids of seabuckthorn fruit has been widely studied, *i.e.* Ignatev (1948), Protsenco (1953) and Savinov (1954,1956).

Out of 600 carotenoids known in the nature, 39 have been identified in seabuckthorn fruits. Total carotenoid content in seabuckthorn fresh fruit varies generally from 1 mg to 120 mg/IOCg, whereas content of β-carotene varies from 0.2 to 17 mg/100g (Korzinnikov *et al.,* 1981; Wen *et al,* 1991; Chenef *al.,* 1990; Ma and Cui, 1988;

Yang and Liu, 1989; Shapiro *et al.,* 1978; Ali-Zade *et al.,* 1978; Mekhtiev *et al.,* 1979; Eliseev *et al.,* 1979; Kabulova *et al,* 1985; Maisuradze and Malyona, 1988; Shirko and Radyuk, 1989; Shapiro *et al.,* 1978,1979; Kondrashov, 1979).

The composition of carotenoids affects the colour of seabuckthorn fruits (Dalgatov *et al,* 1985, Lian *et al,* 2000). Red and orange-red fruits are richer in carotenoids as compared with the less intensely coloured fruits like yellow and orange-yellow (Lian *et al,* 2000). Different growth conditions influence the carotenoid content in the soft parts of the berries. Wei and Guo (1996) observed the oik from berries growing on hill slope contain higher content of carotenoids as compared with berries from seabuckthorn growing on the riverbank areas. Carotenoid content has been found increasing , with the maturation of fruit (Zhang *et al,* 1989). Soft part (pulp) oil of ssp. *sinensis* growing in Shanxii province of China had a maximum carotenoid level of 2140 mg/100g (Wei and Guo, 1996) and a *S* minimum value of 2.1 mg/ 100g in north Caucasus (Shaftan *et al,* 1986). Generally carotenoid level is maximum in peel oil followed by fruit pulp oil and seed oil.

It is also established that carotenoids of seabuckthorn fruits consist of a-carotene, p-carotene, lycopene and zeaxanthin (Jmyrko *et al,* 1978; Protsenko, 1953). Many studies have found B-carotene to be a major carotenoid, making 15-55 per cent of total carotenoids, depending on the place (Gachechiladze *et al,* 1981; Lian *et al,* 2000). a-carotene, y-carotene, dihydroxy-P-carotene, lycopene, zeaxanthin and canthaxanthin have been viewed to be the other carotenoids in seabuckthorn berries: (Lagazidze *et al,* 1984; Lian *et al,* 2000; Kallio *et al,* 1989), more studies are to be done on the identification -of carotenoids in seabuckthorn.

Shnaydman and Shugam (1971) have found the occurrence of 12 constituents in the seabuckthorn fruits. The following substances were isolated from fruit, which are α-, β-, γ-carotene, lycopene, polcislycopene A, C, B, ruboxanthin and tarxanthin (Shugam, 1969). The phytophulin and xanthin were isolated by Shnaydman (1973). Neamt *et al* (1976) have isolated flavoxanthin, luteine, violxanthin, neoxanthin and neo-cryptoxanthin. Novruzov (1981) and Mamedov (1984), Bagirov and Nasudari: (1998,1999) and Bagirov *et al.* (1993) found 9 carotenoids in the composition of carotenoids of 26 various seabuckthorn forms in Azerbaijan. Among them, these were identified α-, β-carotene, lycopene, polycislycopene and zeaxanthin. In all studied forms, β-carotene, criptoxanthin, cintaxanin, auroxanthin, in 3 kinds of β-zeaxanthin, zeaxanthin, lutein-epoxide and flavoxanthin have been found. Side by side with those carotenoids, identified in just one form was also found out γ-carotene, neurosporin, mutachrome etc. as well. Seabuckthorn carotenoids are dominated by β-carotene, α-carotene, lycopene, polyzlycopene-3 and zeaxanthin (Schapiyo, 1989).

One of the important parameters determining the quality of seabuckthorn oil is the content of carotenoids present in it. Savinoy (1948) estimated that the oil of seabuckthorn fruit contains 49.8 per cent of β-carotene and 50.2 per cent of xanthophylls. Nearly, half sum of carotenoids in the pulp oil is carotene. Carotenoids are accumulated in the pulp oil more than in the seeds or whole fruits (Novruzov, 2005). The oil of red fruits has the highest content of carotenoids (872-1240 mg). Oil obtained from yellow fruits contains 360-571 mg, from orange ones, 425-1250 mg, orange-red, 482-1270 mg (Novruzov, 2005). Shishkina (1976) showed that seabuckthorn oil contains a-, β-carotene, lycopene, zeaxanthin, taraxanthin and phytidfaene. Besides, other studies found lutein, flavoxanthin, cryptoxanthin, vkdaxanthin (Neamt *et al.,* 1976). According to Jmyrko *et al.* (1978), qualitative composition of carotenoid of pulp and seed oil are equally consisted of β-carotene, zeaxanthin, cantoxanthin, 3,3-dihydroxi-retrocarotene, 4,4-diceto-P-carotene, dihydroxy- β-carotene. However it should be noted, that composition may change. It was found that pulp and seed oils considerably differ in their qualitative composition (Novruzov, 1981).

Comparison of carotenoids with A-vitamin activity is of great interest. Table 1.4 below indicates the biological activity of carotenoids found in the seabuckthorn fruits as compared with P-carotene, vitamin-A the activity of which is equal to 100 per cent. As this table indicates α-, β-carotene, neo-β-carotene and β-zeaxanthin, α-Apo-10-carotene, cintexanthin have a certain vitamin-A activity (In Novruzov, 2005).

Table 1.4: Vitamin-A Activity of the Carotenoids of Seabuckthorn

Carotenoids	***Configuration of Stereoisomer***	***Activity of Trance-carotene* (%)**	***References***
β-carotene	Full trance	100	Berezovski, 1973
neo-β-carotene	9 mono-cis	38	
α-carotene	Full trance	53	
γ-carotene	Full trance	27	
cryptoxanthin	Full trance	57	
pro-γ-carotene	Penta-cis	44	

α-Apo-10-carogynal	Full	70	

Source. In Novruzov. 2005.

β-carotene is most important and dominant among the carotenoids of seabuckthorn. Its content varies depending upon species, place of origin and the component analyzed, *i.e.,* the seed oil from Shaanxi, China, contains 160 mg/100g P-carotene, but that from Hetain, Xinjiang Province, China, contains 1160 mg/100g. In the pulp ой, the carotenoid content is about are 900-1000 mg/100g from the Pamirs region (Mironov, 1989).

Biological Properties

No individual of carotenoids shows high antioxidant activity, however, as a whole group, the carotenoids play an important role in the antioxidant defense (Erdman *et al.,* 1993; Mangels *et al.,* 1993; van Poppel and Goldbohm, 1995). The biological properties of seabuckthorn oil has been explained mainly by presence of carotenoid, particularly P-carotene, one of the most active bioactive substances and a precursor of vitamin A, therefore it promotes the health of the body (Lapic *et al.,* 1983; Shvenik *et al,* 1983). Further, it was found that these properties of oil are characterized by the presence of other ingredients as well (Loginov *et al.,* 1983; Rahimv *et al,* 1983).

Wound Healing

Carotene form of V_A, improves the metabolic normalization and regenerates the injured tissues. Deficiency of carotenoids in human food leads to poor growth, weak eyesight, decreases in tolerance to diseases and affects the mucous membrane of gastrointestinal tract. Carotenoids are effective remedies against bum, frostbites, ulcers and various gynaecological problems. Carotenoids and vitamin E present in seed oil accelerate the epithelization process in chronic cervisitis. β-carotene has been used in China for preparing the drug capsule for curing bed shores (Qingping *et al,* 1999).

Stress

Carotenoids are the major source of vitamin A. Nutrient deficiency of vitamin A in humans causes the growth disturbances, poor eye sight, reduced disease tolerance, besides affecting the mucus membrane of gastrointestinal tract (Berezovski, 1973, Gotke and Wolf, 1990; Protsenko, 1953, Savinov, 1948). Carotenoids raise physical and physiological capacity of organism to work in stress conditions (Bogdanov *et al,* 1986; Brudacher and Weisler, 1985).

Cancers

During the last few years, the interest in vitamin A, particularly carotenoids as an anti-cancer substance has increased. Carotenoids, particularly β-carotene and cantaxanthine act as anti-carcinogen and anti-swelling, therefore cures some kinds of skin cancers. The ability of carotenoids, β-carotene and cantaxanthin to act as anti-cancerogenic, anti-mutagenic and anti-swelling substances gave an opportunity of using them for safeguarding from some kinds of skin cancers (Kartangi *et al,* 1984; Mashkovski, 1997; Neels and De. Leenheer, 1983; Santamaria *et al,* 1980; Sergeev 1998; Sergeev *et al,* 1983; Shlyankevich *et al,* 1993). Oily substances of carotenoids (seabuckthorn, dog-rose and etc.) have proven to be effective remedies against bum, frostbites, ulcers, skin cancers and various gynecological illnesses (Akulinin 1958; Chugaeva *et al,* 1964; Gatin 1963; Goodvin 1980; Kushinskaya 1965; Loginov *et al,* 1983; Mashkovski 1997; Rahimv *et al,* 1983).

In twenty-five studies of the relation between carotene and lung cancer risk, 24 studies have found a significant decline in risk with consumption of high B-carotene intakes or plasma levels. In cases of stomach cancer, out of 15 studies, 8 studies have found a significant risk reduction, associated with intake of p-carotene, and 6 studies showed a non-significant risk reduction. However, no definite relationship was found for β-carotene against colorectal, prostate or breast cancer (Diplock *etal,* 1998; Frei 1994; van Poppel and Goldbohm, 1995; Wiseman and Halliwell, 1996).

VITAMIN E (TOCOPHEROLS AND TOCOTRIENOLS)

Both pulp and seed oils of seabuckthorn are rich in fat-soluble bioactive substances and vitamins, like vitamin E, also known tocopherols. Vitamin E also known as tocopherols in seabuckthorn oil is much higher than other nutrient oils. Total tocopherol content varied from 5 mg/100g in fresh fruit of Finnish seabuckthorn (Kallio *et al,* 2002,) to 1-12 mg/100g in Azerbaijan (Mekhtiev *et al,* 1979). Subsp. *turkestanica* growing in Xinjiang (China) had4-mg/100 g tocopherols in the seeds and 17 mg/100 g in dry pulp (Chen *et al,* 1990). Fresh soft parts of fruits of seabuckthorn cultivars raised in the Altai region, Russia, had 5-18 mg/100 g tocopherols (Zhang *et al,* 1989; Shapiro *et al,* 1978;, Piironen *et al,* 1986; Yang *etal,* 2001; Kallio *et al,* 2002). In another study, seeds of ssp. *rhamnoides* had a higher tocopherol and tocotrienols (29 mg/100g) than ssp. *mongolica* (25 mg) and ssp. *sinensis* (13 mg) (Yang *et al,* 2001; Kallio *et al,* 2002). However, soft part of fruit of ssp. *sinensis* had 12 mg/100mg tocopherols and tocotrienols, which is 2-3 folds higher than other species (40 and 50 mg in ssp.

rhamnoides and *mongolica,* respectively), indicating the ssp. *sinesis* fresh fruit is a better source of tocopherols and tocotrienols.

Pulp oil part has generally been richer in a-tocopherol than the seeds (Baoru *et al,* 2001). All the four tocopherol isomers have been found in the seeds and soft parts of seabuckthorn berries, a-Tocopherol constitutes up to 96 per cent of the total tocopherols in fruit pulp oil (Jablczynska *et al.,* 1994), being clearly the major isomer. In seed, y-tocopherol (up to 40 per cent of total tocopherols) is another dominating isomer, in addition to a-tocopherol (Lian *etal.,* 2000; Vang *et al,* 2001; Kallio *et al.,* 2002).

As compared to fruit pulp and seeds, their oils have attracted attention of most of the studies for the occurrence of vitamin E. Lu Rongsen (1993) found the lowest seed oil vitamin E content in *H. solicifolia* (46.9 mg/100 g oil) and the highest in *H. rhamnoides* subsp. *tnrkestanica* (159 mg/100 g oil). The highest vitamin E content in pulp oil was found in *H. rhamnoides* subsp. *sinensis* (248 mg/100 g oil). Kallio *et al.* (2002) estimated 100-300 mg/ 100g the total content of tocopherols and tocotrienols in the seed oils of subspecies *mongolica, sinensis* and *rhamnoides,* whereas in the soft part oil, the total value of tocopherols and tocotrienols was typically 400-700 mg/ 100g in subsp. *sinensis,* significantly higher than the corresponding values in the *mongolica* and *rhamnoides* (100-200 mg/ 100g oil). The total content of tocopherols and tocotrienols in the fresh berries was at peak in mid September.

Method of oil extraction also influences the vitamin E content and composition *e.g.* hexane extracted seed oil of ssp. *sinensis* had maximum value of 216 mg/ 100g tocopherols and a minimum value of 29 mg in freon extracted method (Xin *et al,* 1997). The proportions of different tocopherol isomers also differed, *i.e.* a-tocopherol constitutes 42-47 per cent, and y-tocopherol 40-43 per cent, in pressed, hexane-and $C0_2$-extracted seed oils, respectively, whereas the corresponding value for a-tocopherol in freon-extracted oil was estimated to be only 2 per cent. The freon-extracted oil had p—(15 per cent), y-(59 per cent) and dd-tocopherols (18 per cent) at higher proportions. Habitat conditions also influence the oil composition. In subsp. *sinensis,* oils from juice and press residue of berries of seabuckthorn growing in Jiaoko County, Shanxi, China growing in sloppy land with adequate sun shine had higher content of tocopherols (220 mg/100 g and 688 mg/100 g in juice oil and press residue oil respectively) than shady slopes and river banks (150-170 mg/100 g in juice oil and 440-540 mg/100 g in press residue oil) (Wei and Guo, 1996).

FLAVONOIDS

Contents of Flavonoids

Seabuckthorn fruit and leaves are very rich source of flavonoids. Flavonoids are found in all parts of seabuckthorn, *i.e.* leaves (3.8-4.0 per cent), fruits, juice and seeds. Shapiro *et al.* (1978) have estimated 420-552 mg/100g flavonoids fresh fruits of various seabuckthorn populations. Seabuckthorn growing in west Pamirs had flavonoids 310-1238 mg/100g dry wt in leaves and 168-859 mg/100g in crude fruits (Glazunova *et al.,* 1984). Studies found that the juice and dried fruit residue contained flavonoid of 0.2 per cent and 0.55 per cent, respectively in seabuckthorn from western Sichuan, China (Xiao Zhuyin *et al,* in Lu Rongsen, 1992, p.14).

Several workrs have studied chemical composition of flavonoids (Tsybikova *et al,* 1972; Tsybikova and Rasputina, 1979; Potapova *et al,* 1983; Bao *et al,* 1997). The main flavonoids identified in seabuckthorn are leucocyanidin, catechin, flavonol and trace flavanone. From flavonol, the isorhamnetin, quassin and camellin could be isolated.

Composition of Flavonoids Fruit

Potapova *et al.* (1980) and Glazunova *et al.* (1982) have isolated the same compounds, *i.e.* isorhamnetin-3|3-D-glucopyranosido-6b-D-glucopyranoside, quercetin-3-f}-D-glucopyranoside (isoquercitrin), quercetin-3-galactoglucoside and rhamnetin, quercetin, miristin, rutin, narcissin and isorhamnetin-7-O-glucoside in fruits of *H. rhamnoides.* The contents of quercetin and kaempherol in seabuckthorn fruits changed significantly during maturation, but in different ways. The overall mean of quercetin decreased 29 per cent in 19 days. In contrast to quercetin, the content of kaempherol increased significantly (33 per cent) in 5 cultivars (Jeppsson and Gao, 1999).

Oil

Mekhtiev *et al.* (1991) identified isorhamnetin, quercetin and isorhamnetm-3-O-p-glucopyranoside in the fruit oil of seabuckthorn.

Leaves

In the leaves of seabuckthorn, Rasputina *et al.* (1975,1976) have found isorhamnetin, and flavonoid glycosides isorhamnetin-3-0-(i-D-glucopyranoside, isorhamnetin-7-O-cc-L-rhamnopyranoside, isorhamnetin-3-[0-|3-D-glucopyranosyl]-7-0-<x-L-rhamnopyranoside, and kaempferol-3P-glucopyranoside (astragalin).

Mukhamed'yarova and Chumbalov (1977) have identified quercetin-3-p-D-glucopyranoside (isoquercitrin), isorhamnetin-3|3-D-glucopyranosido-6b-D-glucopyranoside and probably quercetin-3-galactoglucoside. Recently, Rongfu *et al.* (2003) also estimated flavone in Chinese seabuckthorn. The total content of flavone in different forms of seabuckthorn growing at Huairou, Beijing, varied from 0.11 to 0.54 per cent. After hydrolysis, three types of glucosides, *i.e.* quercetin, kaempferol and isorhamnetin were obtained. For *H. tibetana,* the content of of isorhamnetin was maximum and about 64 per cent of the total flavone. In *H.salicifolia* and *H.rham.* ssp. *yunnaensis,* quercetin was the main component and it was 84 per cent and 66 per cent of the total flavone. In *H. gyantsensis,* both quercetin and isorhamnetin were higher (47 per cent and 39 per cent respectively of the total flavone). However, different flavones were in almost same content in ssp. *sinensis.* It was estimated that while quercetin and isorhamnetin are known to occur in fruit pulp, kaempferol is also found in high amount in the leaves. Chinese form of ssp. *sinensis* was richer than introduced Russian forms in kaemferol. Flavone in main Chinese form of ssp. *sinensis,* was maximum during May-July. It decreased from September onwards and at minimum during autumn.

Other Phenolics

Since the phenolic acids show a high antioxidant activity as flavonoids, the total phenolics were measured in by same studies. The overall mean of the phenolics in three culitivar varied from 114 mg/ 100g to 244 mg/100g (Gao *et al,* unpublished data). Other phenolic compounds with antioxidant activity are derivatives of cinnamic acid; *e.g.* caffeic acid, chlorogenic acid, and ferulic acid (Rice-F,vans and Miller, 1996). Chlorogenic and other phenol compounds can facilitate the biosynthesis of gastric acid, stimulate gastric juice secretion, combine with taurine and participate in diuretic action and improve the functioning of capillaries and at hypophysis level, regulate the thyroid function (In: Mingyu *et al.,* 1998).

Biological Properties of Flavonoids

While biological activity of plant extract from various plant parts, containing total flavonoids have been carried out, there is no study on individual compound of flavonoids. They have been found to possess very strong anti-oxidant activity. It has been found that fla vonoids improve the immunity of the body, lower the osmosis of the capillary wall and prevent oxidation of vitamin C. Flavonoids have been found in controlling arteriosclerosis, reducing cholesterol level, turning hyperthyroidism into euthyroidism and eliminatkig inflammation. They have also been found effective against tumor and radiation damage and sustain the activity of other biologically active compounds, for example, the anti-tumor activity of leucocyanidin, the improvement of X-ray effectivness in tumor treatment by catechin and anti-tumor and anti-radiation damage effect of quassin (In Mingyu ef *al,* 1998).

Anti-oxidant Activity of Phenolic Extracts/Flavonoids

Scavenging effect of total flavonoid of seabuckthorn on oxygen radicals was studied by Ju ef *al.* (1989). Their observation on the measurement of chemiluminescence (CL) showed that 1 pg flavonoids/ ml effectively inhibited the CL response of PMA-stimulated human polymorphonuclear leukocytes (PMN). Seabuckthorn flavonoid at 1.7 pg/ml, significantly decreased the concentration of active Oxygen radicals of PMA-stimulated PMN. Seabuckthorn flavonoids at the concentration of 0.03-3.00 pg/ml remarkably reduced superoxide in xanthine/xanthint oxidase. A concentration of 3 pg/ml was found effective for the scavenging of hydroxyl radical produced by Fenton's reaction. Gao *et al.* (In Gao, 2005) also studied antioxidant activity of different extracts from seabuckthorn. The crude extracts of 50 per cent EЮH from all 3 tested cultivars of seabuckthorn showed high capability to reduce the 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation radicals. Trolox Equivalent Antioxidant Capacity (TEAC) ranged 19.8 to 25.2 pmol/g. The antioxidant activity was highly correlated with the contents of total phenolics and ascorbate (r = 0.78 to 0.98).

In a study of ascorbate-ferric ion induced lipid peroxidation, all crude extracts showed very high antioxidant activity of seabuckthorn (In Gao, 2005). The inhibitory effects, against metal ion induced lipid peroxidation at the concentration of 2 mg/ml fresh weight, were up to 79.0-98.8 per cent. Although there was high content of ascorbate (7-15 pM in the assay) in the aqueous extracts, there was no significant difference between the aqueous and ascorbate-free extracts. In contrast, from the assays of free radical scavenging and AMVN induced lipid peroxidation, the lipophilic extracts were found more effective than the aqueous and phenolic extracts against metal ion induced lipid peroxidation. The inhibitory effects of lipophilic extratcs varied from 71.7 to 79.6 per cent.

Cardiovascular Diseases

A number of studies have been carried out on the effects of flavonoids of seabuckthorn on cardiovascular diseases, as some flavonoid compounds are known to have positive inotropic effects (Shizhong, 1979). The recent studies on total flavonoids of *Hippophae* (TFH) reveal that they have a positive inotropic effect, improve cardiac performance, decrease peripheral resistance and do not increase heart rate. TFH has a curative effect on angina pectoris and also anti-arrhythmic effect. However, high doses of TFH given in a rat, weaken the myocardial contractive power (Jialiang *et al.,* 1982), therefore appropriate dose need to be standardized. Qiuyan *et al.* (1989) studied the effect of seabuckthorn extract (SE) (extracted by alcohol), which included flavonoids like isorhamneitin and quercetin, on the white rates. It was found that SE had considerable anti-myocardial ischemia, antihyperlipemia and anti-fat-liver effects. This could improve the myocardial b100d supply and decrease the myocardial oxygen consumption. SE also decreased the b100d lipid and liver lipopexia in the experiments on chickens by decreasing serum TC, B-LP and liver TC.

Bingwen *et al.* (1989) used 2 per cent TFH solution prepared from seabuckthorn leaves, while adjusting dose at 50 mg/kg, which improved cardiac contractive function of rats. Bingwen *et al.* (1993) also studied the effect of total flavonoids (TFH) on the white rats' cardiac function. It was found that

TFH strengthens the function of cardiac or myocardial contraction and diastrolization, regulate b100d pressure, and decreases the peripheral resistance. Further studies showed that TFH could improve the contractility of the extracorporeal papillary muscles of guinea pigs. Their observations showed that TFH could remit the angina and improve the mechanocardiography and the ischemic eletrocardiogram. In this regard, its curative effect was found better than that of isosorbide dinitrate. Fengming *et al.* (1989) studied the extracorporeal cors of white rats with arrhythmia. They observed that TFH could increase the time of the ventricular contraduction, decrease the heart rate and attenuate the myocardial contractility. Further, TFH could slightly increase the refractory stage (or period) of the extracorporeal left-atrium function and counteract distinctly the action of the allorhythmia of the extracorporeal right atrium in guinea pigs. Some flavonoids of seabuckthorn reduce the serum cholesterol level (Bukshtynov *et al.,* 1985).

Wound Healing

Leaf drugs of seabuckthorn, containing flavonoids, improved the wound healing after chemical bums and wounds (Tsybikova *et al.,* 1991). Gupta *et al.* (2001) used 20 pi of sterile solution of seabuckthorn leaf flavonoids for healing of cutaneous wounds in male albino rats. As compared to seed oil and control, flavonoid accelerated the healing and collagen biosynthesis. In another study, Gupta ef *al.* (2005) administered 30,100 and 200-mg/kg flavone orally in 2 per cent aqueous gum acacia suspension for 7 days. The dose of 30 mg/kg exhibited significant (p< 0.01) reduction in wound area by 51 per cent, whereas increased dose of 100 mg/kg showed reduction by 31 per cent (p< 0.01), as compared to control.

Tumors

Polyphenolic compounds of seabuckthorn possess an antioxidant activity. They inhibit the growth of tumors and exhibit a radioprotective action, while the most potent antitumor compounds were leucoanthocyanins. They also promote the antitumor effect during X-ray treatment and increase the resistance of the organism to X-rays. Thus miristicin, rutin, (-)-epicatechin, (-)-epigallocatechin, (+)-catechin and others inhibited the tumor growth, *e.g.* sarcoma 180, lymphosarcoma, Ehrlich tumor etc. in 65-75 per cent (Vermenichev, 1973).

Neurodegenerative Disorder

Free radicals have also been implicated in the development of neurodegenerative disorder such as Parkson's disease and Alzheimer's disease (Ames *et al,* 1995). Seabuckthorn fruit and leaves are quite rich in flavonoids, which neutralize these free radicals.

Food Industry

Leaf catechins and flavonoids (17.79 mg per cent and 53.2 per cent correspondingly) were proposed for application in food industry (Belykh, 1999).

METALLOTHIONEIN (MT)

Metallothionein was first time found in equine kidney as a protein, rich in Cd, Zn and S (Margoshes and Vallee, 1957). This protein is also rich in cysteine. Metallothionein acts as detoxying agent for heavy metals and as free radical scavenger for most toxic radical, hydroxyl radical (HO). The molecular weight of 6 kd, high Cd content and UV absorption spectrum revealed the new protein to be metallothionein, which must help the plant in reducing oxidative stress due to extreme environmental conditions. Metallothionein inhibits the erythrocyte hemolysis, stress induced ulcer and diabetes. In view of the high anti-oxidant activity, which is 7-8 times higher than human serum, it can be commercially utilized in sufficient quantity from seabuckthorn (In Rong *et al,* 2001).

OTHER BIOACTIVE SUBSTANCES OIL

Oil of seabuckthorn berries is the most valuable product of this plant, as it possesses anti-oxidant, wound healing, anti-ulcer, anti-tumor and curing cardiovascular etc. properties. These physiological functions of seabuckthorn fruit oil have been reviewed by Yang and Kallio (2005) in this book. Generally, the oil content of seabuckthorn fruit is low (about 4 per cent), whereas "as ssp. *turkestanica* in western Pamirs, Tajikistan (Gachechiladze *et al.,* 1981) is quite rich in oil of fresh fruits (6.8-13.7 per cent).

The fruits with high oil content of 17.8 per cent have been found in the West Sayan (Ekinov and Simakov 1968) and in the Western Pamirs forms with 17.5 per cent in the Vanch river valley (Korovina *et al,* 1993). The highest oil containing forms are typical of the forms of the Middle Asian climatype, where the oil content on the average does not fall below 6.0-6.65 per cent. In the Siberian climatype, raw fruits contain from 1.6 to 8.0 per cent oil. In the Caucasian ecotype, the fruits with the high oil content of 10.5 per cent-11.9 per cent, which is not inferior to the Siberian climatype, have been found in the Black Sea (Igoshina *et al,* 1987) and Central Caucasian forms (Igoshna *et al,* 1987; Lobzhanidze, 1991).

In Lahaul valley, Indian Himalayas, total oil in fresh fruits varied from 2.9-4.6 per cent in H. *rhamnoides* ssp. *turkestanica* and much lower of 2 per cent in *H. salicifolia* (Singh and Singh, 2004). Morphological characteristics have been observed to have a significant effect on the oil content (Korzinnikov *et al,* 1981; Dalgatov *et al,* 1985). In the natural populations of seabuckthorn growing on several sites at different altitudes in the Western Pamirs, smaller and less juicy fruits were found to contain more oil than bigger and juicier fruits (Korzinnikov *et al,* 1981).

On dry weight basis, oil in soft part varies from a minimum of 4-12 per cent in subsp. *sinensis* growing in Hebei Province to maximum of 30-34 per cent in subsp. *turkestanica* growing in Xinjiang, China (Yang and Liu, 1989; Yang, 1992). Seeds of seabuckthorn has about 10 per cent oil in most of populations (Wang and Yang, 1990), although higher values (10-16 per cent) are also known (Kallio *et al,* 1999; Kallio *et al,* 2002). In Lahaul valley, Himachal Pradesh, India, oil content in the seeds of *H. rhamnoides* ssp. *turkestanica,* varied from 10.5 per cent-14.6 per cent, which was considerably higher than 9.4 per cent in H. *salicifolia.*

In Chinese forms, Lu Rongsen (2003) measured the maximum content of seed oil (17.8 per cent) in *H. tibetana* and the minimum in *H. rhamnoides ssp. gyantsensis* (7.8 per cent). The second highest content of seed oil was found in *H. neurocarpa* (13.6 per cent). The content of seed oil in the other species varied between 9.5 per cent and 12.4 per cent. The highest content of pulp oil was found (18.5 per cent) in *H. neurocarpa* and the lowest one (2.5 per cent) in *H. rhamnoides* ssp. *yunnanensis.* The contents of seed oils in the other species varied between 2.6 per cent and 5.4 per cent. Among those species that located in the areas with lower altitudes (H. *rhamnoides* ssp. *turkestanica)* contains both high contents of seed oil (12.2 per cent) and pulp oil (5.4 per cent).

The oil content in whole berries is significantly influenced by the harvesting time (Mekhtiev *et al,* 1979; Yang, and Kallio, 2002), whereas, the annual variation has been reported to be extremely small (Gachechiladze *et al,* 1981;, Yang, and Kallio, 2002). Application of inorganic fertilisers (N, P, K, in spring) increases oil content and decreases the content of carotenoids in the oil (Garanovich *et al,* 1983).

FATTY ACIDS

Fatty acids are common denominators for all life forms. We now know that not only the amount, but also the type of dietary fat plays a major role in maintaining health. The clinical importance of fatty acids is a consequence of their role in metabolic problems resulting from either their deficiency or overabundance. The human body absolutely requires the polyunsaturated EFAs linoleic acid (omega-6 fats) and alpha-Jinolenic acid (omega-3 fats). That is exactly why these fatty acids are termed "essential." Many plants produce a-linolenic acid (18:3 omega-3), but because of the small amounts of fresh vegetables consumed, it is one of the least abundant of the essential fatty acids in most diets. Besides seabuckthorn, it is also found in flax, hemp, rape (canola) seed, soybean, walnut oils, and dark green leaves and must be supplied by such foods. Table 1.5 showing association of clinical symptoms with dietary insufficiencies, indicates the central importance of a-linolenic acid. The wide range of symptoms is due to the function of ALA in critical cell processes of membrane integrity and eicosanoid local hormone production (In Lord and Bralley).

Table 1.5 Signs and Symptoms Associated with Fatty Acid Abnormalities

Signs and Symptoms	***Fatty Acid Association***
Eczema-like skin eruptions, loss of hair, liver degeneration, behavioral disturbances, kidney degeneration, increased thirst, frequent infections, poor wound healing, sterility (m) or miscarriage (f), arthralgia, cardiovascular (g), Growth retardation	Linoleic acid insufficiency

Growth retardation, weakness, impairment of vision, learning disability, poor coordination, tingling in arms/legs, behavioral changes, mental disturbances, low metabolic rate, high b100d pressure, immune dysfunction	Alpha or gamma linolenic acid insufficiency

Source: Lord and Bralley.

Table 1.6 Clinical Associations of a-linolenic Acid (ALA) (18:3 omega-3)

Sl. No	*Application*
1	Lower b100d pressure, lower b100d cholesterol, and lower risk of stroke and heart attack
2	Normalize fat metabolism in diabetes and decrease the amount of insulin required by diabetics
3	Prevent liver damage due to alcoholism and decrease withdrawal symptoms after discontinuing the habitual use of alcohol
4	Provide adjunctive treatment for schizophrenics
5	Cause weight loss by increasing metabolic rate and fat bum-off
6	Relieve premenstrual breast pain (mastalgia) and premenstrual syndrome of bloating, irritability, depression and aggressive behavior
7	Prevent drying and atrophy of tear and salivary glands (Sjogren's syndrome)
8	Prevent arthritis in animals
9	Improve the condition of hair, nails, and skin
10	Improve certain kinds of eczema
11	Slow down or stop deterioration in multiple sclerosis
12	Help treat diabetic neuropathy in Type II diabetes
13	Kill cancer cells in tissue culture without harming normal cells (x = moderate: xx = strong clinical response)

Source. Lord and Bralley.

Linoleic Acid (LA) (18:2 omega-6)

LA is by far the most abundant polyunsaturated fatty acid in most human tissues. It is one of the essential fatty acids, because it contains a double bond at the omega-6 position, which is beyond the reach of the human desaturase enzyme. Low levels indicate dietary insufficiency that leads to a variety of symptoms. Some of these symptoms result from lack of linoleic acid in membranes where it serves a role in structural integrity. Most, however, are from failure to produce the 1-series and 3-series local hormones known as prostanoids. Linoleic acid is the starting point for this pathway. Normal neonatal status of this fatty acid is marginal, if not insufficient. Fetal linoleic and cervonic acid (DHA) are correlated with maternal RBC levels (Houwelingen *et al.,* 1992). Since dietary sources (esp. com oil) are abundant, however, linoleic acid levels may be found to be above normal in some adults. Because of the need for balanced prostanoid and leukotriene synthesis, excessive linoleic acid can contribute to an overproduction of the proinflammatory 2-series local hormones. Compared to other fatty acids, linoleic acid caused growth inhibition of Helicobacter pylori, the bacterium thought to cause gastric ulcer (Khulusi *et al.,* 1995).

Seabuckthorn seed oil is very high in two essential fatty acids, Linoleic acid (30-40 per cent) and a-Linolenic acid (20-35 per cent) (Yang *et al.,* 1989,1992; Moravcov *et al.,* 1995; Shapiro *et al.,* 1978; Lagazidze *et al.,* 1984; Kallio *et al.,* 2002; Yang and Kallio, 2002; Yang and Kallio, 2002; Kallio *et al.,* 2001; Berezhnaya *et al.,* 1993; Li and Aitzetmuller, 1995; Xin and Li, 1997; Ozerinina *et al.,* 1987; Ozerinina *et al,* 1997; Hoy and Mu, 2000; Singh *et al,* 2004).

The dominating fatty acids in the soft parts of the fruit are palmitoleic acid (16-54 per cent) (Utchenko *et al,* 1995; Franke and Muller, 1983; Kallio *et al,* 1999). Generally less than 14 per cent of Linoleic acid (Utchenko et al., 1995) and less than 3 per cent of a-Linolenic acid (Zademowski *et al,* 1997) are usually found in pulp oil. Singh *et al* (2004) analyzed the fatty acids of Lahual form (semi-arid) of *H. rhamnoides* ssp. *turkestanica* and

Spiti form (arid) of *H. rhamnoides* ssp. *turkestanica* and also H. s*alicifolia* (from Lahaul) growing in Himachal Himalayas, India. Pulp oil of H. *salicifolia* is richest source of Linoleic acid (15.0 per cent of total oil) and a-Linolenic acid (1.3 per cent). However, Palmitoleic acid was a major dominating unsaturated fatty acid (46.4-37.1 per cent), being maximum in pulp oil of Lahaul fdrm (46.4 per cent). In seed oil, Spiti form is richest source of Linoleic acid (39.8 per cent) and a-Linolenic acid (25.4 per cent). The high content of palmitoleic acid, which is uncommon in the plant kingdom, distinguishes the pulp oil from the seed oil of seabuckthorn. This fatty acid has attracted an increasing interest due to its possible effects on many physiological processes (Berghaus *et al,* 1975; Yamori *et al,* 1986; Budijanto *et al,* 1992), including cholesterol and triglyceride lowering and stroke reducing effects (Yamori *et al,* 1986; Colquhoun *et al,* 1996). Palmitoleic acid is a principal constituent of skin fat and the extract is recommended for skin softening and anti-wrinkle products.

The inflammatory skin disease atopic dermatitis is characterized by dry, itchy and lichenous skin. Abnormalities in the status of polyunsaturated fatty acids have been reported in skin, plasma, umbilical cod and breast milk of mothers of children at high risk of atopic diseases (Manku *et al,* 1984; Wright, 1990; Oliwiecki *et al,* 1991; Duchen *et al,* 1998), which may be due to deficiency in intake, incorporation and metabolism of essential fatty acids (Wright, 1990; Oliwiecki *et al,* 1991). Seabuckthorn seed oil contains high content of linoleic and a-linolenic acids, whereas pulp oil is rich in palmitoleic acid (Berezhnaya *et al,* 1993; Xin, *et al.,* 1993; Johansson, *et al,*1997). By application of seed oil, the increase in the level of a-linolenic acid in plasma lipids leaded to improvement of atopic dermatitis symptoms (Baoru *et al,* 2000).

STEROLS

Sterols, the unwashed substances are also the constituents of every fat and oil. They are found along with other compounds, like carotenoids and tocoferols. Monoatomic unsaturated hydroaromatic spirits called sterols form part of these substances. Plant sterols are plant compounds with similar chemical structure and biological functions as cholesterol (Piironen *et ah,* 2000). Plant sterols contain an extra methyl, ethyl group or double bond. The most abundant plant sterols are sitosterol, campesterol and stigmasterol (Moreau *et al.,* 2002). The daily dietary intake of plant sterol is 160-400 mg among different peoples (Ahrens and Boucher, 1978; Cerqueira et *al,* 1979; Nair *et al.,* 1984; Hirai *et al.,* 1986; Miettinen *et al,* 1989; Morton *et al,* 1995; de Vries *et al,* 1997). Dietary sources of sterols include vegetable oils (especially unrefined oils), nuts, seeds and grains (Piironen *et al.,* 2000). Due to their structural similarity to cholesterol, plant sterols are well studied for their cholesterol absorption inhibition properties. In addition to their cholesterol lowering property, plant sterols may possess anti-cancer (Awad *et al,* 2003), anti-atherosclerosis (Moghadasian *et al,* 1997,1999), anti-inflammation (Bouic, 2001) and anti-oxidation activities (van Rensburg *et al,* 2000).

Composition in Seabuckthorn

Novruzov (1981) has observed that the fruits of the various forms of seabuckthorn differ by quantitative composition and qualitative content of sterols. The amount of sterols, in the fruit pulp of studied forms ranged between 0.16 and 0.76 per cent, but in the seeds, it ranged from 0.19 to 0.96 per cent. It was found that content of sterol in seabuckthorn oil is about 10 times higher than other oils.

Total sterol content in the pulp oil (soft part) of seabuckthorn fruit ranged from 1 per cent to 3 per cent (Lagazidze *et al.,* 1984; Xin *etal,* 1997; Schiller, 1989; Bat and Tannert, 1993; Yang *et al,* 2001). Juice oil, processed by centrifugation of pressed juice of subsp. *sinensis,* had 720 mg/100g sterols (calculated as betulin) (Xin *et al,* 1997). Harvesting time had a significant effect on the content and composition of sterols of the soft parts of the berries (Yang *et al,* 2001).

In plant oils, they occur in free condition and as a component of high molecular fatty acid. The most studied plant sterols are β-cytosterol and stigmasterol. In soft parts of seabuckthorn fruit, sitosterol, isofucosterol (stigmasta-5,24-dien-3P-ol or 5-avenasterol in some papers), 24-methylene cycloartanol, cycloartenol, citrostadienol, avenasterol (stigmasta-7,24-dien-3i-ol in some papers), stigmast-7-en-3i-ol (24-ethyl-cholest-7-en-3(i-ol or 7-sitosterol in some publications), campesterol, stigmastanol, and obtusifoliol have been estimated (Lagazidze *et al,* 1984; Xin *et al,* 1997; Schiller, 1989; Bat and Tannert, 1993; Yang *et al,* 2001). Stigmasterol and cholesterol have also been found in trace amount (Schiller, 1989; Bat and Tannert, 1993). Besides these sterols, several other sterols have also been found in soft part of fruit. Sitosterol make 60-90 per cent of sterols in the pulp/soft parts (Lagazidze *et al,* 1984; Xin *et al,* 1997;, Schiller, 1989; Bat and Tannert, 1993; Yang *et al,* 2001). In seeds, like soft part, same composition of sterols have been found except small difference with minor sterols (Xin *et al,* 1997; Bat and Tannert, 1993; Yang *et al,* 2001).

Seabuckthorn oil also contains sterols. In the seed oil, the total sterol content ranged from 1 to 2 per cent (Schiller, 1989; Xin *et al,* 1997; Bat and Tannert, 1993; Yang *et al,* 2001). Seed oils of *Hippophae rhamnoides* ssp. *sinensis* and *turkestanica,* extracted by Hexane (1430 mg/ 100g oil and 1238 mg/100g oil, respectively) had higher contents of sterols than the oils from *H. tibetana* (968 mg/100g oil) and *H. neurocapa* (804 mg/100g oil) (Xin *et al,* 1997). The proportion of sitosterol of the total sterols was found to be lowest in the seeds of *H.*

tibetana (64 per cent) among the species/subspecies studied (the highest being up to 76 per cent) (Xin *et al.,* 1997).

The occurrence of β-cytosterol in the seabuckthorn oil was reported first by Shnaydman and Fondarenko (1964) and Ghugaev *et al.* (1964). Besides β-cytosterol, the considerable amount of stigmasterol was also found in sterol fraction of seabuckthorn oil (Kukina *et al.,* 1986; Mironov and Vasilev, 1980). Jamyansan (1973) has isolated β-cytosterol (68 per cent), lygnosterin (20.5) and several constituents of unidentified sterols found in the sterol fraction of seabuckthorn oil (1.5 per cent of sum total). Salenko *et al.* (1983) have found that sterol part of unwashed residue of the seabuckthorn oil consists of 35 per cent of fatty spirit and 65 per cent of mixture of sterols and triterpenes of sum total. The later fraction contatins β-cytosterol (11,24-methyl-encycloartenol) β-amirin, α-amirin, erytrodio, uvaol, cytosadienol, 24-ethylcholesten, 7-en-3-p-ol and 4 unidentified compounds. There are cycloarthenol, lupeon, obtuzifoliol, olen, ursol acid, aldehyds of cholesterin. Kukina *et al.* (1986) found side by side with above-mentioned compounds in the leaves. In seabuckthorn fruit oil, the content of P-cytosterol amounts to 40-60 per cent of the sum total of sterols, whereas in freon oil extracted from leaves, it amounts to only 13.6 per cent.

Biological Properties Ulcer; Bums and Wound Healing

Studies have found during the trials on the effect of lipid fraction of seabuckthorn fruits in cases of stomach ulcer and bums that the major effecting constituent of it was β-cytosterol and to a lesser degree, 24 methylencycloartenol and cytrostagiend (Salenko and Skuridin, 1983). Jiang Zhenyi *et al.* (1989) of the Second Army Medical University of China, observed that healing effect of seabuckthorn seed oil on the white rats' gastric ulcer, induced by acetic acid and chronic reserpinization was better than the cimetidine. They isolated the active principle from seabuckthorn seed oil and found that β-Sitosterol- β-D-Glucoside (in seed oil) was a compound responsible for healing the gastric ulcer. Other studies also agree to this conclusion (Loginov *et al.,* 1983; Salenko *et al.,* 1982). Pomeraseva (1986) explains the wound healing activity of seabuckthorn oil is due to the presence of triterpene and polyphenols. Therefore, biological activity of seabuckthorn oil is related mainly to its unwashed part. Biological activity of the constituents of seabuckthorn oil has been studied in some cases only. In his study of the various parts of fraction of seabuckthorn oil, Chugaev *et al.* (1964) has determined that the sterols are the main effecting substances during medical treatment of burns and stomach ulcer.

Arteriosclerosis and Platelet Aggregassion

Among the sterols, β-sitosterol is considered one of the active compounds used to prevent and cure the arteriosclerosis. The most studied sterols are β-sitosterol and stigmasterol, the biological activity of which includes anti-artereosclerosis, healing of bums and stomach ulcer. β-sitosterol also inhibits the platelet aggression (b100d clotting) (Zhao *et al.,* 1990). It also reduces plasma total and low-density lipoprotein cholesterol level by mechanisms affecting both absorption and synthesis of cholesterol (Field *et al.,* 1997; Jones *et al.,* 1997).

TANNINS

Seabuckthorn leaves have been found to contain high content of polyphenols, including tannins (10-12 per cent) (Guseinova, 1956; Novruzov *et al,* 1983), whereas fruits contained only 0.13 per cent (Abutalybov *et al,* 1978). Therefore, seabuckthorn plant leaves have been proposed as the prospective source for dyeing and tanning substances (Guseinova, 1956; Muradyan and Musaelyan, 1989). Fruits, pulp and juice were found to be poor in tannins (0.02, 0.02 and 0.004 per cent, correspondingly) (Aslanov, 1982; Novruzov *et al,* 1983). Tannin contents in leaves and green shoots were found to be ranging from 13-15 per cent to 20-35 per cent, respectively in the majority samples of wild populations and cultivars studied (Klimakhin *et al*.,1996; Sheichenko *et al.* ,1995,1996; Shipuiina *et al,* 1996), while in the stems, its content has not exceeded 5 per cent. Some hydrolyzable tannins of seabuckthorn, *e.g.* strictinin, were recently obtained synthetically using stereoselective esterification reaction (Khanbabaee ef *al,* 1997).

Composition of Tannins

Sheichenko *et al.* (1987,1997) have found that the main constituents of hydrophilic part of the leaf extracts are monomeric type of hydrolyzable tannins (gallo-and ellagi-tannins) identified with earlier known strictinin (I) and isostrictinin (Π) etc. The compound firstly called hipporhamnin, is obviously identical to casuarinin. Casuarinin was found to be the main component of the tannin fraction (Sheichenko *et al.,* 1995). The Japanese studies have identified in seabuckthorn leaves, collected from Chendgu, China, two flavonoids hyperine and tiliroside, as well as 12 hydrolyzable tannins, the structure of which have been described as l,2,6-tri-0-galloyl-(3-glucose, strictinin, isostrictinin, pedunculagin, casuarinin, casuarictin, hippophaenins A and B (both new compounds), tellimagrandin I [st/HOfiym-collinin isolated from Geranium collinum Steph. by Bikbulatova and Chumbalov (1971-1973), stachyurin, castalagin and vescalagin (Yoshida *et al.,* 1991). Some hydrolyzable tannins of seabuckthorn, *e.g.* strictinin, were recently obtained synthetically using stereoselective esterification reaction (Khanbabaee *et al,* 1997).

Biological Properties

Anti-viral Activities

Russian workers have recently isolated a tannin fraction from leaves of *H. rhamnoides* (Tolkachev *et al,* 19*97). A* chemotherapeutic study of dry purified tannin fraction and individual components has revealed, a high activity in respect to a wide spectrum of viruses: Influenza viruses/A (HINI, H2N2, H13N6, H3N2) and B/Singapore/222/79, Herpes simplex viruses (HSV), strain L_2, paramyxovirus infections, adenoviruses type 2 cytomegaloviruses and other viruses pathogenic for human beings and animals (Shipuiina, 1999). On the basis of the tannin fraction from seabuckthorn leaves, a new anti-viral drug Hiporamin (a dry purified tannin fraction) has been developed. It was found to be highly efficient for the treatment of various animal and human viral infections. Hiporamin anti-viral and antimicrobial activity was found to be related with its inhibition of antioxidant enzymes glutathion reductase and superoxide dismutase at the concentration $ICbMO^{-6}$ M, which seem to be the target molecules (Dubinskaya *et al.,* 1997).

Clinical studies on Hiporamin drug in a number of laboratories in Russia have revealed a strong activity in respect of viral and viral-bacterial infections: influenza (simple and complicated cases), infected with Influenza viruses A and B and other acute cases, including adenoviral infections. It was effective in case of tonsillitis, developing at the background of acute respiratory infections, of Herpes simplex infections (acute and relapsing), of Varicella zoster and cytomegaloviral infections (Shipuiina *et al.,* 1995, 1996; Gabrielyan *et al,* 1996; Kolobukhina *et al,* 1996; Merkulova *et al,* 1996, 1996; Vichkanova, 1999). The drug protected from development of viral complications. It was found to increase a number of T-helpers and normalize the ratio of cells T-helpers /T-suppressors (Kolobrukhina *et al,* 1996). In addition Hiporamin inhibits gram-positive and gram-negative bacteria, *Mycobacterium tuberculosis, Candida albicans* and definite micellar fungi (*Hiporamin, Practical Phytotherapy,* 1997).

Burns

Tsybikova *et al.* (1991) have reported about positive effects of seabuckthorn leaf extracts on the burns. Results produced are in agreement with results published by Hatano *et al.* (1990), who observed inhibitory activity of various tannins in respect of xanthine oxidase (XOD), while inhibition activity depended on the structure of tannins, *i.e.,* on degree of their galloylation and polymerization. Which may be related with radical-scavenging activity of tannins. The monomeric hydrolyzable tannins tellimagrandins I and II, casuarictin, characteristics for seabuckthorn leaves, being more effective than the oligomers.

Hepato-protection

Hydrolyzable tannins of seabuckthorn, like casuarinin, casuarictin, tellimagrandins I and II, polygalloylglucoses and catechins, gallic and ellagic acids have shown significant antihepatotoxic action, inhibiting glutamic-pyruvic transaminase (Hikino *et al,* 1985).

VITAMIN K (PHYLLOQUINONE)

Naturally produced by the bacteria in the intestines, vitamin K plays an essential role in normal b100d clotting and helps promote bone health.

Without sufficient amounts of vitamin K, hemorrhaging can occur. Deficiencies may appear in infants, or in people who take anticoagulants or antibiotic drugs (*Complete Book of vitamins and Minerals,* 2000; *Dietary Reference Intakes,* 2001). Vitamin K (phylloquinone) is called the coagulation vitamin, because it plays a catalytic role in forming of prothrombin and hence it promotes normal coagulations of the b100d during the injuries of b100d vessels. In seabuckthorn, vitamin K content varies from 0.65-1.3 mg/100gm of fresh fruit (Shapiro *et al,* 1986) to 59-64 mg/100g in seed oil, which are higher than many horticultural crops. Under conditions of Belorussia, fresh fruits of Sayan forms contain 0.576-0.847 mg per cent and fruits of Altay varieties contain 0.444-1.403 mg per cent of phylloguinone. Vitamin K was estimated 0.15 mg/100 g in Mangolian seabuckthorn fruit (Jamyansan and Badgaa, 2005). Shapiro *et al.* (1986) viewed that phylloquinone content is a stable indicator of fruit quality, not much influenced by geographic and climatic factors.

5-HYDROXYTRYPTAMINE (5-HT)

Of the chemical neurotransmitter substances, serotonin is perhaps the most implicated in the etiology or treatment of various disorders, particularly those of the central nervous system, including anxiety, depression, obsessive-compulsive disorder, schizophrenia, stroke, obesity, pain, hypertension, vascular disorders, migraine and nausea (Grahame-Smith, 1977; Ahlman and Kjellstrom, 1981). Serotonin (5-hydroxytryptamine, 5-HT) is widely distributed in animals and plants, occurring in fruits, nuts, vertebrates, and venoms. A number of congeners of serotonin are also found in nature and have been shown to possess a variety of peripheral and central nervous system activities. Of particular interest over the years is the psychotomimetic activity displayed by several serotonin-related compounds such as N,N-dimethyl tryptamine, 5-hydroxy-N,N-dimethyltryptamine (bufotenine) and 4-phosyphoryloxy-N, N-dimethyl-tryptamine (psilocybin). Although serotonin may be obtained from a variety of dietary sources, endogenous 5-HT is synthesized in situ from tryptophan through the actions of the enzymes tryptophan hydroxylase an aromatic L-amino acid decarboxylase. It is found in three main areas of the body, *i.e.* the intestinal wall (where it causes increased gastrointestinal motility); b100d vessels (where large vessels are constricted); and the central nervous system (CNS). Both dietary and endogenous 5-HT are rapidly metabolized and inactivated by monoamine oxidase and aldehyde dehydrogenase to the major metabolite, 5-hydroxy-indoleacetic acid (5-HIA A).

The most widely studied effects have been those on the CNS. The functions of serotonin are numerous and appear to involve control of appetite, sleep, memory and learning, temperature regulation, mood, behavior (including sexual and hallucinogenic behavior), cardiovascular function, muscle contraction, endocrine regulation, and depression. Peripherally, serotonin appears to play a major role in platelet homeostasis, motility of the GI tract, and carcinoid tumor secretion. This represents quite a broad spectrum of pharmacological and psychological effects, considering the fact that the average human adult possesses only about 10 mg of 5-HT.

Plasma 5-HT is derived largely from the enteroendocrine cell in the gastrointestinal tract. The evidence of the relationships between 5-HT and these pathological conditions has been obtained mostly from studies of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the plasma of humans (Feldamn, 1978). The most reliable laboratory test to detect a carcinoid tumor is the repeated determination of the urinary 5-HIAA (Strodel *et al,* 1983). Inaddition, whole-b100d 5-HT is increased in patients with metastatic midgut carcinoid tumors (Grahame-Smith, 1977; Ahlman and Kjellstrom, 1981).). Abnormalities in 5-HT neurotransmission have been implicated in the pathogenesis of diverse psychiatric disorders, such as depression, anxiety, and schizophrenia, and various classes of psychotherapeutic agents have prominent effects of different aspects of synaptic transmission mediated by 5-HT.

The behavioral alterations that occur by drugs, which affect 5-HT receptors, are diverse. One of the first behaviors in which the role of serotonin was identified was in the control of the sleep-wakefulness cycle. Studies in both animals and humans suggest that serotonin serves a role in aggression. Several human studies report a correlation between low levels of 5-HIAA and violent suicides. Clinical effects of 5-HT-related drugs often exhibit a delay in onset. This fact has sparked interest in the idea of changes in 5-HT receptor density and sensitivity after chronic drug treatment. The ability to treat anxiety, depression, aggression, compulsivity, and other behavioral dysfunctions may lie in the ability to simultaneously block multiple 5-HT receptor populations. Recently a number of studies on the subject have published (Kreiss and Lucki, 1995; Kirby *et al.,* 1997; Lucki, 1997; Reneric and Lucki, 1998; Price, 1998).

The peel of stem and fruit of seabuckthorn contains serotonin. In Russia, Kryuchkov *et al.* (1998) estimated 1.1-2.6 mg/100g serotonin in seabuckthorn fruit. 5-hydroxytryptamine (5-HT) isolated from seabuckthorn bark inhibited tumor growth (Pukhalsskaia, 1958; Sokoloff *et al,* 1961). Anti-tumor activity of seabuckthorn bark extract has also been found by Indian scientists (Ambaye *et al,* 1962).

β The leaves and fruits of seabuckthorn contain certain coumarins, which strengthen the function of b100d circulation in capillaries, anti-vitiligo, anti-tumorigenesis and regulate the disorders of gall bladder. There is trierpene, like ursolic acid, which has effect like that of adrenocorticol hormone (ACH) and control tire the action of Na+ and Cl- and cures the diseases like hypocorticoidism (bronzed skin), heal wounds, ulcer and inflammation (In: Mingyu *et al,* 1998).

I-AMYRINOLEYLALCOHOL ACID

Leaves of seabuckthorn contain (i-amyrinoleylalcohol acid, which dilates the cardiac and cerebral vessels, facilitate the b100d circulation and also slightly lower the b100d vessels (In Xiaoyan *et al,* 1986).

BETAIN

Seabuckthorn accumulate betain, an anti-ulcer compound in high amount. It varied from 19.9 to 190 mg/100 g in seabuckthorn cultivars growing at Urals Curative Plants Garden, Russia. When fruit

is used as jam, it preserved to 79-85 per cent of betain and when frozen, it maintained 46 per cent after 6 months (Kryuchkov *et al.,* 1998). Betain quantity in seabuckthorn fruits varied from 512 to 897 mg per cent in Altay cultivars and from 728 to 1389 mg per cent in East Sayan forms (Shaprio *et al,* 1986).

FOLATE

Folate is a water-soluble vitamin B known to have several benefits to human health, such as prevention of neural tube defect in babies, an action against cardiovascular diseases caused by elevated plasma homocysteine and certain forms of cancer. Seabuckthorn fruits have been found to be a rich source of folate although not much data is available, the only information given in a paper by Shyrko *etal.* (1989), in a cultivar "Aromatnaja" which showed intermediate folate content (39 pg/100 g fresh weight) along with nine other selected Swedish berries (ranging from 11 pg/100 g in blueberry to 96 pg/100 g in rose hips).

ORGANIC ACIDS

Organic acids and sugars are the major portion of the soluble solid fraction of the fruit pulp of seabuckthorn fruit. Fruit juice of seabuckthorn is quite rich in organic acid, which has been found to remit the toxicity of some antibiotics and barbitals, preventing teratogenesis and damages from X-rays (In Mingyu *et al.,* 1998). Presence of vitamin C, organic acid and tannic acid in the fruit of seabuckthorn make it an ideal sources for the production of several beverages particularly health protection juices. Chinese species showed the highest content of organic acid (4.1 to 9.1 per cent) (Kallio *et al,* 1999; Lu Rongsen, 2003; Ma *et al,* 1989; Zhang *et al,* 1989), followed by subspecies *rhamnoides,* 4.2-6.5 per cent (Kallio *et al,* 1999, Raffo *et al,* 2004) and 2.1-3.2 per cent in Russian forms (Kallio *et al,* 1999). Kallio *et al* (1999) observed that the organic acid in different subspecies, about 90 per cent of the total acidity is represented by malic and quinic acids in Chinese, Russian and Finnish berries, malic acid being a major constituent as compared to quinic acid in subsp. *sinensis* and *rhamnoides,* unlike Russian forms. Mladin *et al.* (2001) estimated value in the range of 2.6-4.1 per cent organic acid in the fruits of 10 genotypes of seabuckthorn in Romania.

Dolgacheva and Aksenova (2001) estimated 1.2-5.7 per cent organic acid in the seabuckthorn cultivars growing at M. V. Lomonosov State University, Moscow. The seabuckthorn populations of the Siberia, Russia, are characterized by sweet, sour-sweet taste without bitterness (Trofimov, 1976; Zakharova, 1986) due to low contents of organic acid (1.0-1.67 per cent). However, there are also forms with a rather significant amount of organic acids, reaching to 4.0-4.2 per cent (Shishkina, 1978; Prokofiev *etal,* 1977). In the Middle Asian region, the content of organic acids varied from 1.0 to 2.8 per cent (Doroganevskaja, 1964; Shaprio, 1980), from 1.36 per cent to 4.2 per cent in the North Caucasus (Shaprio, 1980). In the seabuckthorn fruits of the east European climatype, the amount of acids varied from 2.10 to 3.69 per cent, as compared to the Danube ecotype (Shaprio, 1980; Lebeda and Duzhurenko, 1990) and from 0.97 to 3.73 per cent, as compared to the fruits of the Baltic ecotype (Kondrashov, 1979). Therefore, the content of organic acids in the seabuckthorn fruits within the studied forms varied from 0.97 per cent to 4.2 per cent.

SOLUBLE SOLIDS

Soluble solids represent an important fraction of the seabuckthorn juice, because of the high concentration of organic acids typical of its berry. While a great variation in the ranges has been reported by different studies, Chinese seabuckthorn berries possess the highest content, varying from a minimum of 5.6 to a maximum of 22.7°Brix (Vaher and Koel, 2003; Zheng and Song, 1992; Kallio *et al,* 1999), while Russian and Altai genotypes had intermediate values (Tang and Tigerstedt, 2001; Piirand Kelt, 1998). In general, there is a decline in soluble solids during maturation of berries. Total soluble solids varied from 8.7 to 14.1°Brix and increased in general with maturation of fruits (Singh *et al,* 2001) in a stand of *H. rhamnoides* ssp. *turkestanica* growing in Lahaul valley, Himalayas.

SUGARS

Although, seabuckthorn berries are not considered rich in sugars, however, sugar is an important ingredient of seabuckthorn fruit as it plays a useful role in determining the sweetness of its juice and in fact the sugariacid ratio has been reported to constitute the major promoter of taste of seabuckthorn fruit juice (Tang, 2002). The average content of sugar in fruits is 2.00-3.26 per cent and in the sweetest Russian forms, it can go up to 7.0 per cent (Trofimov, 1976). The sugar is composed of glucose (1.27-1.80 per cent), fructose (0.71-2.33 per cent), and saccharose (0.07-0.30 per cent) (Rast. Resursy, 1988). Dolgacheva and Aksenova (2001) estimated 1.3-4.7 per cent sugar in the seabuckthorn cultivars growing at M.V. Lomonosov State University, Moscow. The content of sugar has been studied to a greater extent in reference to the seabuckthorn fruits of Siberian (Shishkina, 1978) and Caucasian climatype (Eliseev, 1976). Under the introduction conditions, the Scherbinka-1 cultivar, selected seedling of the East-Sayan ecotype, accumulates up to 11.5 per cent of sugar (In: Seabuckthorn, 1985). In the Middle Asia climatype, the content of sugar in the fruits of the east Kazakhstan and the Tien-Shan ecotypes varied in the range of 3.4-6.6 per cent (Doroganevskaja, 1964; Shishkina, 1970). Mladin *etal.* (2001) estimated a value in the range of 0.6-1.0 total sugar in the fruit of 10 genotypes of seabuckthorn in Romania.

The total soluble sugar content highly vary among subspecies and also within the same subspecies (Bieniek *et al.,* 2001), depending upon genotype intrinsic differences and different harvest time of the berries (Tian, 1985; Raffo *et al,* 2004). The total reducing sugars estimated in berry raw juice of subspecies *sinensis* varied from 0.32 per cent to 12.7 per cent (Ma *et al,* 1989; Tang *et al.,* 2001). Glucose and fructose have been estimated to be the major soluble sugars (90 per cent). Kallio *et al.* (1999) reported that ssp. *sinensis* and *mongolica's* raw juice have a very similar sugar profile, being glucose the most abundant, followed by fructose. The same observation has been obtained by Beveridge *et al.* (2002) in subsp. *rhamnoides* and by Bounous and Zanini (1988) in *subsp.fluviatilis.*

PECTIN

Pectin content of the seabuckthorn fruits is low. Shishkina *et al.* (1985) estimated in Siberian seabuckthorn (var. Katun) pectin value ranging from 0.69 to 1.22 per cent. Other studies also found the similar data (Arkhipova and Chudinova, 1993). Mladin *et al.* (2001) estimated a lower value in the range of 0.23-0.60 per cent pectin in the fruit of 10 genotypes of seabuckthorn in Romania.

PROTEINS AND AMINO ACIDS

Amino acids are the building blocks of proteins and muscle tissue. All types of physiological processes like energy, recovery, muscle strength gains and fat loss, as well as mood and brain function are intimately and critically linked to amino acids (Reeds, 2000). For at least 60 years, it has been the convention to divide amino acids into two categories: indispensable (or essential) and dispensable (or nonessential) (Womack and Rose, 1947). Essential are those amino acids, which we must include them in our diet because your body can't make them on its own. If we don't ingest them, we will not be experiencing optimal health and may have a disease caused by that deficiency, *i.e.* arginine, histidine, methionine, threonine, valine, isoleucine, lysine, phenylalanine, tryptophan, leucine. Then the non-essential amino acids are those amino adds, which under normal conditions, our body can manufacture these amino acids, so we don't have to ingest each of these, *i.e.* alanine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, proline, serine, tyrosine. However, if our body system is stressed, out of balance, or diseased, these amino acids become essential and we must get them from food or supplements, *i.e.* conditionally essential, arginine, glycine, cystine, tyrosine, Proline, glutamine and taurine. The physiological and metabolic roles of these amino acids are given in Table 1.7.

Table 1.7 The Involvement of Amino Acids in Physiological and Metabolic Function

System	*Function*	*Product*	*Precursor*
Intestine	Energy generation	ATP	Glu, Asp, Glutamine
	Proliferation	Nucleic acids	Glutamine, Gly, Asp
	Protection	Glutathione	Cys, Glu, Gly
		Nitric oxide	Arg
		Mucins	Thr, Cys, Ser, Pro
Skeletal muscle	Energy generation	Creatine	Gly, Arg, Met
	Peroxidative protection	Taurine (?)	Cys
Nervous system	Transmitter synthesis	Adrenergic	Phe
		Serotinergic	Try
		Glutaminergic	Glu
		Glycinergic	Gly
		Nitric oxide	Arg
	Peroxidative protection	Taurine (?)	Cys
Immune system	Lymphocyte proliferation	(?)	Glutamine, Arg, Asp
	Peroxidative protection	Glutathione	Cys, Glu, Gly
Cardiovascular	B100d pressure regulation	Nitric oxide	Arg
	Peroxidative protection (?)	Red cell glutathione	Cys, Glu, Gly

Source. Reeds, 2000.

High values of protein and free amino acids increase the biological value of seabuckthorn fruit. Crude protein in fresh fruits in ssp. *sinensis* was estimated to 1.2 per cent by Zhiben and Yansheng (1987). In Indian Himalayas, total protein level in fresh fruits of *H. rhamnoides* ssp. *turkestanica* was in the range of 2.1-3.4 per cent, which was higher than *H. salicifolia* (1.2 per cent) (Singh and Singh, 2004). In Indian Himalayas, protein content in the seeds, varied from 32.1-19.2 per cent in *H. rhamnoides* to 25.5 per cent in *H. tibetana* and 20.3 per cent in *H. salicifolia* (Singh *et al.* 2005). Russian seabuckthorn seeds contain 30 per cent protein (Solonenko and Shishkina, 1983,1989; Solonenko *et al.,* 1991), the variation range being from 26.8 to 32.8 per cent. Protein content in the pulp varies from 0.79-1.64 per cent, depending on the cultivar. The majority of seabuckthorn proteins are well-ingested albumins and globulins. As studied in Russian forms, globulins (53.7-56.0 per cent) and albumins (33.1-38.4 per cent) are important proteins, which contain all the proteinaceous amino acids, including irreplaceable, lysin, threonine, methionine, valine, leucine, isoleucine, phenylalanine and tryptophane. Replaceable amino acids are as follows: histidine, arginine, aspartic and glutamic acids, proline, serine, glycine, alanine, tyrosine and traces of cysteine (Solonenko and Shishkina, 1983). Solonenko *et al.* (1987) had found that free amino acids were represented by all the proteinaceous amino acids varying to 94.5-155.5 mg/100g.

Qi (1998) compared some cultivars of ssp. *mongolica* and *turkestanica* to a Chinese form and found that several free amino acids were present, varying from 14 to 17, whereas number of essential amino acids varied from 7 to 9. Similar results were found by other studies (Chen *et al.,* 1991; Zheng and Song, 1992) and also showing that about 50 per cent of the total amino acids are represented by Essential amino acids. Zheng and Song (1992) found the total content of amino acids ranges from 37 to 75 mg/ g, of which 13 to 28 mg/g are represented by essential amino acids. Results showed by Chen *et al.* (1988) are in agreement with these findings. Solonenko and an unidentified amino acid of non-protein origin was identified by Stralsjo *et al.* (2003) in the seeds of several representatives of the family of *Eleagnaceae,* including *Hippophae.* Togong (1988) estimated 18 amino acids, 8 being essential amino acids in the fruit juice of ssp. *sinensis, i.e.* threonine (6.2 mg/100g), valine (2.9 mg), methionine (1.2 mg), leusine (1.9 mg), lysine (3.5 mg), tryptophan (0.5 mg), isoleucine (0.1.0 mg) and phenylalacine (3.2 mg).

DRY MATTER

In the ripe seabuckthorn fruits, dry matter is represented by fat and non-lipid fractions. Generaly dry matter constitutes about 15 per cent of the total weight of the fruit. It is known that small-fruited seabuckthorn contains more dry substances than large-fruited one (Korzinnikov *et al.,* 1981). Gachechiladze *et al.* (1981) and Glazunova *et al.* (1991b) observed that the fruit moisture is affected by the moisture conditions of their growth areas. Dolgacheva and Aksenova (2001) estimated 7.2-25.2 per cent dry matter in the seabuckthorn cultivars growing at M. V. Lomonosov State University, Moscow. In more humid conditions of the North Caucasus and the Black Sea coast, the content of dry matter in fruits, was equal to 17.6 and 19.9 per cent, respectively (Eliseev, 1976,1985). However, forms of the Western Pamirs were found to accumulate up to 53 per cent of dry matter. Abutalybov *et al.* (1983) identified that seabuckthorn fruits collected in the valley of the Girdymanchay, Azerbaijan, contain dry substance of 18.6 per cent. Dry matter of 27.1-20.3 per cent was estimated in *H. rhamnoides* ssp. *turkestanica,* which was much higher than 15.0 per cent in *H. salicifolia,* both growing in Lahaul valley, Himalayas (Singh and Singh, 2004). Mladin *et al.* (2001) estimated the values in the range of 15.6-25.2 per cent dry matter in the fruit of 10 genotypes of seabuckthorn in Romania.

CONCLUSION

It can be summarized that during abnormal physiological conditions *e.g.* stress, ischemia, acidosis and tissue damage, various free radicals are released, resulting into abnormal metabolic functioning, leading to various pathological disorders. In order to control these disorders, suitable anti-oxidant therapies are required. Seabuckthorn is one of the plant species, which has attracted global attention due to strong anti-oxidant activities of its fruit and leaves extracts, which are quite rich in antioxidants like vitamin C, E, carotenoids, flavonoids, metallothionein and superoxide dismutase (SOD). The plant is also rich in other bioactive substances like sterols and tannins etc.

There are positive effects of the seabuckthorn oil on the lipid peroxidation, immune fuction, mucosa and skin. The seabuckthorn oil showed promising effect on the cardiovascular diseases, like inhibiting platelet aggregation and LDL-oxidation. Animal and *in vitro* studies have also indicated anticancer and anticarcinogenic effects of its oil. These studies also need to be conducted in human beings on large scale. The positive effects of seabuckthorn fruit oil on the plasma lipid levels and the formation of atherosclerosis and thrombus are needed to be studied further. As seabuckthorn has many medicinal properties, further research is needed to be conducted on the identification of more active principals and their mode of mechanism.

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