FOOD SCIENCE & TECHNOLOGY | REVIEW ARTICLE

Bioactive profile, health benefits and safety evaluation of sea buckthorn (*Hippophae rhamnoides* L.): A review

Touseef Ahmed Wani¹, S.M. Wani*, Mukhtar Ahmad¹, Mudasir Ahmad¹, Adil Gani¹ and F.A. Masoodi¹

Abstract: Sea buckthorn (*Hippophae rhamnoides* L.) contains a large number of versatile compounds with antioxidant and hence medical properties that have been reported from time to time. Intensive work on the medical properties of sea buckthorn has concluded incredible results like the effects on atherosclerosis, anti-visceral obesity, platelet aggregation, inflammation, adverse stressful situations, and that of liver injury. Only a few studies have been conducted on the safety evaluation of the plant extract but importantly no study has deemed it unsafe for animal or human consumption till date. Considering the fact that no significant changes have been observed in organ weight/body weight ratios, of any vital organ studied (except liver and kidney in 1 g/kg and 2 g/kg body weight doses, respectively) and biochemical and hematological parameters in different animal trials with an lethal dose for 50% reduction of population (LD₅₀) of >10 g/kg when given orally, there is scope for further investigations regarding its safety in the daily diet as a protective medicine.

Subjects: Bioscience; Food Science & Technology; Health and Social Care

Keywords: Sea buckthorn; *Hippophae rhamnoides*; antioxidant activity; medical value; safety evaluation

1. Introduction

Sea buckthorn (*Hippophae rhamnoides* L., family: Elaeagnaceae) is a thorny, deciduous shrub that grows widely at high altitudes of 7,000–15,000 foot of the northwest Himalayan region, native to Eurasia. It is also been domesticated and used in traditional medicine in several countries, which has long been used for relieving cough, aiding digestion, invigorating blood circulation, and alleviating pain since ancient time (Rousi, 1971; Zheng, Dong, & Yu, 1997). Recently, sea buckthorn has been planted as a new berry crop for obtaining important bioactive compounds. Its good adaptability,
rapid growth, ability to act as protection against wind and sand drift, assistance in soil and water conservation, and improvement of soil by efficient nitrogen fixation allow sea buckthorn to be widely used in vegetation and restoration of degenerated ecosystems (Chen & Chen, 2003; Hou, Bai, & Cao, 1995; Li, 2004; Ruan & Li, 2002; Ruan, Xie, & Li, 2000; Wei, Yu, & Zhu, 1998). Sea buckthorn with diverse uses such as, controlling soil erosion, a source of horse fodder, nutritious foods, drugs, and skin-care products, also contains bioactive compounds with antioxidant properties that are preferred over the synthetic antioxidants simply because the latter have quite often been found to be carcinogenic (Fan, Ding, & Gu, 2007; Rodriguez-Meizoso et al., 2006).

Oil from sea buckthorn has shown effectiveness in skin therapy for sunburns, chemical burns, radiation burns, and eczema (Goel et al., 2002; Seven et al., 2009; Yang et al., 2000; Zeb, 2004b). Furthermore, sea buckthorn oil has shown positive results in treating health problems related to damaged mucous membranes of the gastrointestinal tract including mouth ulcers, gastric ulcers, and stress ulcers (Suleyman, Buyukokuroglu, et al., 2001; Suleyman, Demirezer, et al., 2001; Xu et al., 2007). Of particular interest, the berries, the oil, and the seeds of sea buckthorn have been shown to possess antiatherogenic, hypocholesteromic, hypotensive, and anti-inflammatory properties (Eccleston et al., 2002; Ganju et al., 2005; Wu, Yu, Ma, Li, & Liu, 1994; Yang, 1995; Zhang, 1987) and could therefore be successfully exploited to prevent or treat cardiovascular disease.

Sea buckthorn has been used mostly in the Tibetan and Chinese traditional medicines for so long, which is supported by a lot of both ancient and recent literature on the use of different parts of sea buckthorn, such that the plant is also commonly known as Chinese medicinal plant. The fact that all parts of the plant possess bioactive compounds with antioxidant properties, the literature on its antioxidant activity, impact on the medical lines, and its safety evaluation are reviewed in the present manuscript.

2. Bioactive compounds and antioxidant properties

Bioactive substances like vitamins (A, C, E, riboflavin, folic acid, and K), carotenoids (α, β, δ-carotene, and lycopene), flavonoids, organic acids (malic acid and oxalic acid), sterols (ergosterol, stigmasterol, lanosterol, and amyrins) and some essential amino acids have been found in all parts of the plant (Häkkinen, Kärenlampi, Heinonen, Mykkänen, & Törrönen, 1999; Upendra et al., 2008). In general, the major components of the seed are vitamin C, large amount of carotenoids and vitamin E, flavonoids and kaempferol, fatty acids, triacylglycerol, phytosterols, sugar, organic acids, proanthocyanidins, and phenolic compounds (Fan et al., 2007; Li, Beveridge, & Drover, 2007). The ripe fruit has been reported to be a source of exceptionally high contents of vitamins (A, C, E, and K), carotenoids, flavonoids, and organic acids (Geetha, Sai Ram, Singh, Ilavazhagan, & Sawhney, 2002). Oil from sea buckthorn contains several bioactive components such as vitamin E, vitamin K, carotenoids, and β-70 sitosterol (Zeb, 2004a). Oil extracts obtained from the berries of sea buckthorn in recent studies have been found to be rich in monounsaturated fatty acids (MUFA) (Yang & Kallio, 2001), tocopherols, tocotrienols (Kallio, Yang, Peippo, Tahvonen, & Pan, 2002), carotenoids, and other bioactive compounds (Guliyev, Gul, & Yildirim, 2004). The leaves of sea buckthorn are rich in kaempferol-3-O-β-D-(6‴-O-coumaryl) glycoside, 1-feruloyl-β-D-glucopyranoside, isorhamnetin-3-O-glucoside, quercetin-3-O-β-D-glucopyranoside, quercetin-3-O-J-D-glucopyranosyl-7-O-α-L-rhamnopyranoside, and isorhamnetin-3-O-rutinoside. Nine fractions, four monomeric flavan-3-ols, catechin, epicatechin, gallatecatechin, and epigallocatechin, along with two dimeric procyanidins, catechin(4α-8)catechin and catechin(4α-8)epicatechin, have been reported from the extracts of sea buckthorn seeds (Fan et al., 2007; Kim, Kwon, Sa, & Kim, 2011; Nitin, Upadhyay, Kumar, & Gupta, 2010) (Table 1).

The assays like 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP), which are often used to test the antioxidant activity, have revealed that the antioxidant activity of seed and root extracts is better than that of leaf and stem extracts (Michel, Destandau, Le Floch, Lucchesi, & Elfakir, 2012; Nitin et al., 2010). Gallic acid, which is also present in sea buckthorn, has been reported to be the most effective antioxidant (Pandurangan, Bose, & Banerji, 2011). The antioxidant potential of
aqueous extract of sea buckthorn leaves varies within the range of 76.44–88.82% while as the total polyphenols vary in the range of 67.91–88.69 GAE/g (Wani, Wani, Shah, & Masoodi, 2013). Sea buckthorn leaf evaluation using maceration, soxhlet, and subcritical water extraction techniques showed the antioxidant potential of 86.35, 133.31–255.87, and 164.03–343.86 Trolox equivalents per gram (TE/g), respectively, while as the respective total phenolic content was reported to be 28.35, 43.77–77.85, and 60.22–86.70 mg/g (Kumar, Dutta, Prasad, & Misra, 2011). The phenolic rich fraction (PRF) of sea buckthorn leaves showed a total phenolic content of 319.33 mg gallic acid equivalents (GAE) per gram while as in the berries, it ranged from 21.31 to 55.38 mg GAE/g on dry weight basis. It showed the highest antioxidant activity of 93.54% and the lowest of 80.38% with no correlation between the total phenolic content and the antioxidant activity. The DPPH radical scavenging activity of sea buckthorn leaf extract (50% effective concentration (EC50) = 1.81 μg/mL) is higher than the butanol fraction (EC 50 = 1.86 μg/mL), and quercetin-3-O-β-D-glucopyranoside. It showed stronger reducing power (OD 700 = 1.83, and 1.78, respectively), with the highest amount of phenolic compounds (477 mg GAE/g) contained in the butanol fraction (Ercisli, Orhan, Ozdemir, & Sengul, 2007; Kim et al., 2011; Maheshwari, Yogendra Kumar, Verma, Singh, & Singh, 2011). The EC50 values of sea buckthorn seed oil from the hydrogen peroxide, superoxide radical, and hydroxyl radical scavenging assays were 2.63, 2.16, and 0.77 mg/ml, respectively (Ting et al., 2011). Taken together, sea buckthorn seed oil, leaf, branches, and root extracts have significant potential as natural antioxidants and could be used potentially for food additives and the development of useful natural compounds (Table 2).

### Table 1. Antioxidant compounds present in different parts of sea buckthorn

| Part of the plant | Antioxidant compounds                                                                 | References       |
|------------------|---------------------------------------------------------------------------------------|------------------|
| Leaves           | Kaempferol-3-O-β-D-(6″-O-coumaryl) glycoside, 1-feruloyl-β-D-glucopyranoside, isorhamnetin-3-O-glucoside, quercetin-3-O-β-D-glucopyranoside, quercetin-3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside, and isorhamnetin-3-O-rutinoside | Nitin et al. (2010) |
| Fruit            | Carotenoids, flavonoids, and organic acids                                           | Geetha et al. (2002) |
| Seed             | Catechin, epicatechin, gallocatechin, and epigallocatechin                              | Kim et al. (2011) |
|                  | Catechin(4α-8)catechin and catechin(4α-8) epicatechin                                 | Fan et al. (2007) |
| Oil              | Carotenoids and β-sitosterol                                                          | Zeb (2004a)      |
|                  | MUFA                                    | Yang and Kallio (2001) |
|                  | Tocopherols and tocotrienols                                                        | Kallio et al. (2002) |

### Table 2. Phenolic content and antioxidant properties of sea buckthorn

| Type of extract        | Phenolic content | Antioxidant potential | References      |
|------------------------|------------------|-----------------------|-----------------|
| Leaves (Aqueous extract)| 67.91–88.69 GAE/g| 76.44–88.82%          | Wani et al. (2013) |
| Leaves (Butanol fraction)| 477 GAE/g       | -                     | -               |
| Leaves (maceration)    | 28.35 mg/g      | 86.35 TE/g            | Kim et al. (2011) |
| Leaves (soxhlet)       | 43.77–77.85 mg/g| 133.31–255.87 TE/g    | Kumar et al. (2011) |
| Leaves (subcritical)   | 60.22–86.70 mg/g| 164.03–343.86 TE/g    | -               |
| Leaves (PRF)           | 319.33 GAE/g    | -                     | Maheshwari et al. (2011) |
| Berries (PRF)          | 21.31–55.38 GAE/g| 80.38–91.54%          | -               |
3. General medical properties

The branches of sea buckthorn contain (-)-epigallocatechin and ursolic acid that exhibit anti-inflammatory effects and the leaves are used in the treatment of diarrhea, gastrointestinal, and dermatologic disorders (Yasukawa, Kitanaka, Kawata, & Goto, 2009). The leaf extracts of sea buckthorn are rich in flavonoids, tannins, and triterpenes (Kallio et al., 2002), which as well as the extracts of its branches are used to treat colitis and enterocolitis in humans and animals in Mongolia (Tsybikova, Raspoutine, Zalykeeva, Darzhapova, & Kundanova, 1983). The leaves have also been applied as compress form in rheumatoid arthritis in the Middle Asia. Flowers of sea buckthorn have been used as skin softener in Tajikistan. The extracts of whole fruit, fruit pulp, pulp oil, and seed oil of ripe fruits have been reported to be useful in treating various diseases such as gastric ulcers (Suleyman, Demirezer, et al., 2001; Xing et al., 2002), skin disorders (Yang et al., 2000), coronary heart disease (Eccleston et al., 2002), radiation-induced oxidative damage (Goel, Gupta, Gupta, Garg, & Bala, 2005), wound healing (Gupta & Flora, 2005), thrombosis, and platelet aggregation in Indian and Tibetan medicine (Cheng et al., 2003).

Sea buckthorn can increase the production of plasma leptin and of neuropeptide Y in children with functional dyspepsia. The overall effect of sea buckthorn is improvement of gastric emptying, gastric mobility, gastrointestinal digestive function, and promotion of children’s growth (Xiao, Qiu, Yue, Cai, & Mo, 2013). Sea buckthorn berry oil is reported to play a potential role in treating atopic dermatitis and thrombosis (Cheng et al., 2003; Yang et al., 2000). Juice, syrup, and oil of the fruits have been used as pain killer, to heal wounds, in ulcer and other diseases of the stomach, disantheria, cancer, and as a metabolism regulator in traditional medicine. The freshly pressed juice is used in the treatment of colds, febrile conditions, and exhaustion (Yang et al., 2000). Oil from seeds and fruits is used in the treatment of eczema, lupus erythematosus, chronic wounds that are difficult to heal, inflammatory diseases, erosion of the cervix uteri, in the treatment of burns and frozen parts of the body, and in ophthalmology. Besides, they are used in the treatment of keratitis, trachoma, injuries or burns of eyelid, and conjunctivitis (Gülüyev et al., 2004). Isorhamnetin isolated from sea buckthorn has been investigated for its cytotoxicity and its influence on human hepatocellular carcinoma cells. The cytotoxic effects of isorhamnetin showed dose and time dependency against hepatocellular carcinoma cells (IC_{50} = 74.4 μg/ml) after treatment with isorhamnetin for 72 h. The cytotoxicity of isorhamnetin on tumor cells depends on cellular accumulation of the drug that permeates the cell membrane into the cell (Teng, Lu, Wang, Tao, & Wei, 2006). Cytotoxicity studied in murine macrophages induced by tertiary-butyl hydroperoxide was controlled up to >95% using a pretreatment of the cells with sea buckthorn extract of concentration as low as 25 μg/ml (Kumar et al., 2011). Sea buckthorn leaf tea supplementation suppressed body weight gain in a dose-dependent manner and significantly reduced visceral fat, plasma levels of leptin, triglyceride and total cholesterol, and ala-nine aminotransferase activity as compared to high-fat-fed control mice studied for six weeks (Lee et al., 2011). The leaf tea supplementation also decreased hepatic triglyceride and cholesterol concentrations, and lipid accumulation, whereas fecal lipid excretion elevated. The hepatic cytochrome P450 2E1 activity and hepatic and erythrocyte lipid peroxides were significantly lowered with the leaf tea supplements. The hepatic and erythrocyte superoxide dismutase and catalase activities were also increased with the leaf tea supplements in a dose-dependent manner induced by high-fat feeding, which indicate that sea buckthorn leaf tea supplementation has potential anti-visceral obesity properties and antioxidant activity mediated by the regulation of lipid and antioxidant metabolism in high-fat diet-induced obese mice.

4. Anti-inflammatory properties

Sea buckthorn leaf extract acts as an immunosuppressant during the acute phase of inflammation by selectively inhibiting T-cell activation as a treatment against adjuvant-induced arthritis in rats (Ganju et al., 2005). The inducible nitric oxide synthase (iNOS) induced by microbial products like lipopolysaccharide accounts for the sustained generation of nitric oxide (NO) and is well known for its role in immunological responses such as inflammation and autoimmunity. Many flavonoids and phenylethanoids are antioxidants protecting biological system against oxidative stress. This is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free
radicals, quenching singlet and triplet oxygen or decomposing peroxidase (Rice-Evans, Miller, & Pagana, 1996; Wang et al., 1996). Sea buckthorn leaf extract increased cell viability against dexamethasone (control) and NO scavenging activity as compared to four (amla, baheera, sacred basil, and shankhpushpi) extracts of known medicinal value. Pretreatment of macrophages with sea buckthorn leaf extract showed the maximum significant inhibition as compared to different extracts (Padwad et al., 2006). Treatment of cells with sea buckthorn leaf extract reduced iNOS expression with respect to the lipopolysaccharide-treated cells, whereas dexamethasone-treated cells showed almost a minimal expression (Wang et al., 1996). Sea buckthorn berries caused a reductive effect on C-reactive protein (marker of inflammation and a risk factor for cardiovascular diseases) in a study carried out as a randomized, double-blind, placebo-controlled trial, over an observation period of 90 days (Larmo, Alin, Salminen, Kallio, & Tahvonen, 2008). The branches of sea buckthorn contains (-)-epigallocatechin and ursolic acid that exhibit anti-inflammatory effects (Yasukawa et al., 2009).

5. Effects on adverse stressful situations
Alcoholic leaf extract of sea buckthorn (70% ethanol) has been analyzed to inhibit hypoxia-induced cytotoxicity, mitochondrial integrity, reactive oxygen species (ROS) production, and DNA damage better than vitamin C (Narayanan et al., 2005). In a study of effect of dry sea buckthorn leaf (aqueous lyophilized) extract, untreated rats exposed to cold-hypoxia-restraint (C–H–R) up to fall of rectal temperature (Trec) 23°C, blood hexokinase (HK) activity decreased, liver phosphofructokinase (PFK) activity increased, and muscle PFK activity decreased (Saggu & Kumar, 2007). During cold exposure of 20°C for 8 h in rat, liver glycolysis activated at the regulatory step catalyzed by PFK/fructose-1,6-biphosphatase (Churchill et al., 1994). Acute hypoxic exposure of 7,000 m was found to increase heart HK activity in rats (Bass et al., 1989). Another study by Saggu and Kumar (2008) on the effect of sea buckthorn dry leaves (aqueous lyophilized extract) showed improvement in anaerobic generation of energy during C–H–R exposure and post-stress recovery, enabling the organism to adapt successfully in such a severe stressful situation (Table 3).

### Table 3. Medical 6 properties of the different parts of sea buckthorn

| Part of the plant | Used for the treatment of | References |
|-------------------|--------------------------|------------|
| Leaves            | Rheumatoid arthritis     | Suleyman, Demirezer, et al. (2001) |
|                   | Over weight, visceral fat, leptin, triglyceride, and total cholesterol | Lee et al. (2011) |
|                   | Inflammation             | Padwad et al. (2006) |
|                   | Hypoxia-induced cytotoxicity and DNA damage | Narayanan et al. (2005) |
|                   | Cold-hypoxia-restrain    | Saggu and Kumar (2007, 2008) |
|                   | Cytotoxicity             | Kumar et al. (2011) |
| Branches          | Inflammation and diarrhea | Yasukawa et al. (2009) |
|                   | Colitis and enterocolitis | Tsybikova et al. (1983) |
| Fruit             | Gastric ulcers           | Xing et al. (2002) |
|                   | Skin disorders           | Yang et al. (2000) |
|                   | Cardiovascular diseases  | Eccleston et al. (2002), Larmo et al. (2008) |
|                   | Radiation-induced oxidative damage | Goel et al. (2005) |
|                   | Wound healing            | Gupta and Flora (2005) |
|                   | Thrombosis and platelet aggregation | Cheng et al. (2003) |
| Seed and berry oil| Dermatitis and thrombosis | Cheng et al. (2003), Yang et al. (2000) |
|                   | Eczema, lupus erythematosus, chronic wounds, inflammatory diseases, erosion of the cervix and uterus, keratitis, trachoma, and conjunctivitis | Guliyev et al. (2004) |
6. Safety evaluation
In rats exposed to C–H–R, aqueous extract of sea buckthorn leaf administered for 14 days (single oral dose of 1 g/kg and 2 g/kg once daily) and 30 days (single oral dose of 100 mg/kg once daily) showed that the maximal effective adaptogenic dose of the extract was 100 mg/kg body weight. No significant changes were observed in organ weight/body weight ratios, of any vital organ studied (except liver and kidney in 1 g/kg and 2 g/kg body weight doses, respectively) and biochemical and hematological parameters of the sub-acute drug-treated animals in comparison to control rats. In acute toxicity study, LD₅₀ of the extract was observed to be >10 g/kg when given orally (Saggu et al., 2007). Safety and efficacy of supercritical CO₂-extracted sea buckthorn seed oil on burn wound model revealed no adverse effects in acute and sub-acute oral toxicity (Upadhyay et al., 2009). Sub-chronic toxicity and efficacy study of sea buckthorn oil showed no significant changes in various physiological, hematological, biochemical (AST, ALT, ALP, uric acid, urea, and creatinine) and histopathological parameters in three groups of New Zealand white rabbits (0.5, 1, and 1.5 ml/kg body weight) with no mortality in any of the treatment groups (Rashid et al., 2011). Aqueous extract of sea buckthorn fruit administered in four groups of rats (0, 100, 250, or 500 mg/kg in a single dose/day for 90 days) showed no treatment-related change in mean body weight, organ/body weight ratio, histological, hematological, and biochemical parameters (Tulsawani, 2010).

7. Scope for further investigations
Analysis of the published literature on sea buckthorn reveals a lot of health-promoting properties in vitro and in vivo. However, the safety concerns regarding the use of sea buckthorn extract in the daily diet is still in its early days. Although only a few studies have been performed on the safety evaluation of different sea buckthorn extracts on different trial animals, yet no study has deemed its use deleterious for human consumption to the best of the knowledge of the authors. Therefore, further studies on the safety evaluation of sea buckthorn extracts find a great scope and are needed to authenticate the area of incorporation of sea buckthorn extracts in the daily diet of human beings as a protective medicine.

8. Conclusion
Different parts of sea buckthorn plant have been widely used in traditional medicine in various countries (India, China, Nepal, Pakistan, Myanmar, Russia, Britain, Germany, Finland, Romania, France, etc.) of the world. Among the various studies conducted on the effects of different parts of the plant are cardiovascular effects, effect on atherosclerosis and platelet aggregation, effect on diabetes, anti-inflammatory effect, antitumor effect, effect on clinical diseases of the liver, anti-visceral obesity, antiulcerogenic effect, effect on adverse stressful situations, etc. The different pharmacological effects of sea buckthorn are attributed to the various bioactive components with antioxidant properties of which sea buckthorn is a good source. These bioactive components include kaempferol, isorhamnetin, quercetin, catechin, epicatechin, gallocatechin, epigallocatechin, procyanidins, carotenoids, organic acids, etc. Sea buckthorn offers an excellent source of functional and therapeutic food whose safety evaluation although is in its infancy, yet the trails till date deem its use safe.
References

Bass, A., Ostodal, J., Prochazka, J., Pelouch, V., Samanek, M., & Stejskalova, M. (1989). Intermittent high altitude induced changes in energy metabolism in the rat myocardium and their reversibility. Physiol Bohemoslov, 38, 155–161.

Chen, Y. M., & Chen, Y. Q. (2003). Mechanism of hydrolysis and soil and water conservation effect of artificial sea buckthorn forest in Loess Hilly Region. Acta Botanica Boreali Occidentalia Sinica, 23, 1357–1361.

Cheng, J., Kondo, K., Suzuki, Y., Ikeda, Y., Meng, X., & Umemura, K. (2008). Inhibitory effects of total flavones of Hippophae Rhamnoides L. on thrombosis in mouse femoral artery and in vitro platelet aggregation. Life Sciences, 72, 2263–2271. http://dx.doi.org/10.1016/j.lfs.2007.12.037

Churchill, T. A., Cheetham, K. M., Simpkin, S., Green, C. J., Gupta, R., & Flora, S. J. (2005). Therapeutic value of Hippophae Rhamnoides L. berries grown in Turkey. Boreali Occidentalia Sinica, 49, 237–242.

Eccleston, C., Baoru, Y., Tahvonen, R., Kallio, H., Yang, B., Peippo, P., & Pan, R. (2002). Comparison of cold stress, cold hardiness and soil and water conservation effect of artificial sea buckthorn forest in Loess Hilly Region. Acta Botanica Boreali Occidentalia Sinica, 23, 1357–1361.

Ercisl, S., Orhan, E., Ozdemir, O., & Sengul, M. (2002). The genotypic effects on the chemical composition and antioxidant activity of sea buckthorn (Hippophae rhamnoides L.) berries grown in Turkey. Scientia Horticulturae, 115, 27–33. http://dx.doi.org/10.1016/j.scienta.2007.07.004

Fan, J., Ding, X., & Gu, W. (2007). Radical-scavenging proanthocyanidins from sea buckthorn seed. Food Chemistry, 102, 168–177. http://dx.doi.org/10.1016/j.foodchem.2006.05.049

Ganju, L., Padwad, Y., Singh, R., Karon, D., Chanda, S., Chopra, M. K., ... Sawhney, R. C. (2008). Anti-inflammatory activity of seabuckthorn (Hippophae rhamnoides) leaves. International Immunopharmacology, 8, 1675–1684. http://dx.doi.org/10.1016/j.intimp.2005.03.017

Geetha, S., Selvam, M., Singh, V., Ilovazhagan, G., & Sawhney, R. C. (2002). Anti-oxidant and immunomodulatory properties of seabuckthorn (Hippophae rhamnoides)—An in vitro study. Journal of Ethnopharmacology, 79, 373–378. http://dx.doi.org/10.1016/S0378-8741(01)00406-8

Goel, H. C., Gupta, D., Gupta, S., Garg, A. P., & Bala, M. (2005). Protection of mitochondrial system by Hippophae rhamnoides L. against radiation induced oxidative damage in mice. Journal of Pharmacy and Pharmacology, 57, 135–143. http://dx.doi.org/10.1111/j.1368-8302.2004.7158.x

Goel, H. C., Prasad, J., Singh, S., Saggar, R.K., Prem Kumar, I., & Sinha, A. K. (2003). Restoration of a herb preparation of Hippophae rhamnoides, RH-3, against whole body lethal irradiation in mice. Phytomedicine, 9, 15–25. http://dx.doi.org/10.1078/0944-7113-00077

Gulyaev, V. B., Gul, M., & Vildirim, A. (2004). Hippophae rhamnoides L.: Chromatographic methods to determine chemical composition, use in traditional medicine and pharmacological effects. Journal of Chromatography B, 812, 291–307. http://dx.doi.org/10.1016/j.jchromb.2005.03.017

Gupta, R., & Flora, S. J. (2005). Therapeutic value of Hippophae rhamnoides L. against subchronic arsenic toxicity in mice. Journal of Medicinal Food, 8, 353–361. http://dx.doi.org/10.1089/jmf.2005.8.353

Hakkinen, S. H., Kärenlampi, S. O., Heinonen, I. M., Mkyskinen, H. M., & Törnrosen, A. R. (1999). Content of the flavonoids quercetin, myricetin, and kaempferol in 25 edible berries. Journal of Agricultural and Food Chemistry, 47, 2274–2279. http://dx.doi.org/10.1021/jf9811065

Hou, X. L., Bao, S., & Cao, Q. Y. (2005). Contrast study on soil infiltration capacity and anti scourability in Robinia pseudoacacia, Caragana microphylla and Hippophae rhamnoides woodlands. Journal of Soil and Water Conservation, 9, 90–95.

Kallio, H., Yang, B., Peippo, P., Tahvonen, R., & Pan, R. (2002). Triacylglycerols, glycerophospholipids, tocopherols, and tocotrienols in berries and seeds of two subspecies (ssp. sinensis and mongolica) of sea buckthorn (Hippophae rhamnoides). Journal of Agricultural and Food Chemistry, 50, 3004–3009. http://dx.doi.org/10.1021/jf011556o

Kim, J. S., Kwon, Y. S., So, Y. J., & Kim, M. J. (2011). Isolation and identification of phenolic compounds from Seabuckthorn (Hippophae rhamnoides) leaves for the comparative evaluation of antioxidant activity. Food Chemistry, 127, 1309–1316. http://dx.doi.org/10.1016/j.foodchem.2011.01.088

Kumar, M. S. Y., Dutta, R., Prasad, D., & Misra, K. (2011). Subchronic studies on risk factors for coronary heart disease in humans. The Journal of Nutritional Biochemistry, 13, 346–354. http://dx.doi.org/10.1016/j.jnutbio.2007.04.004

Larmo, P., Alin, J., Salminen, E., Kallio, H., & Tahvonen, R. (2010). Effects of sea buckthorn berries on infections and inflammation: A double-blind, randomized, placebo-controlled trial. European Journal of Clinical Nutrition, 62, 1123–1130. http://dx.doi.org/10.1038/ejcn.2010.158

Lee, H. I., Kim, M. S., Lee, K. M., Park, S. K., Seo, K. I., Kim, H. J., ... Lee, M. K. (2011). Anti-visceral obesity and antioxidant effects of powdered sea buckthorn (Hippophae rhamnoides L.) leaf tea in diet-induced obese mice. Food and Chemical Toxicology, 49, 2370–2376. http://dx.doi.org/10.1016/j.fct.2011.06.049

Li, M. (2004). The function of protection bio-diversity of sea buckthorn in semi-arid region. Hippophae, 17, 17–22. http://dx.doi.org/10.1007/s11010-005-7636-2

Maheshwari, D. T., Yogendra Kumar, M. S., Verma, S. K., Singh, V. K., & Singh, S. N. (2010). Antioxidant and hepatoprotective activities of phenolic rich fraction of Seabuckthorn (Hippophae rhamnoides L.) berries. Food and Chemical Toxicology, 49, 2422–2428. http://dx.doi.org/10.1016/j.fct.2011.06.061

Michel, T., Destandau, E., Le Floch, G., Lucchesi, M. E., & Elfokir, C. (2012). Antimicrobial, antioxidant and phytochemical investigations of seabuckthorn (Hippophae rhamnoides L.) leaf, stem, root and seed. Food Chemistry, 131, 754–760. http://dx.doi.org/10.1016/j.foodchem.2011.09.029

Narayanan, S., Ruma, D., Gitika, B., Sharma, S. K., Pauline, T., Ram, M. S., ... Banerjee, P. K. (2005). Antioxidant activities of seabuckthorn (Hippophae rhamnoides L.) leaves. Food and Chemical Toxicology, 43, 3443–3448. http://dx.doi.org/10.1016/j.fct.2005.06.076

Negro, F., Boado, R., Inzé, C., & Van Montagu, M. (2000). Calcium uptake and vacuolization during early embryogenesis in Arabidopsis thaliana. Journal of Cell Science, 113, 4243–4250. http://dx.doi.org/10.1242/jcs.113.171

Nitin, K., Upadhyay, M. S., Kumar, Y., & Gupta, A. (2010). Antioxidant, cytotoxic and antibiotic effects of seabuckthorn (Hippophae rhamnoides L.) leaves. Food and Chemical Toxicology, 48, 3443–3448. http://dx.doi.org/10.1016/j.fct.2009.11.033

Padwad, Y., Ganju, L., Jain, M., Chanda, S., Karon, D., Kumar Baraneeju, P., & Chand Sawhney, R. (2006). Effect of leaf extract of Seabuckthorn on lipopolysaccharide induced hyperglycemia and diabetes in mice. Journal of Agribusiness, 5, 207–213. http://dx.doi.org/10.1080/14785580600544102

Page 7 of 9
inflammatory response in murine macrophages. *International Immunopharmacology*, 6, 46–52.  

Pandurangan, N., Bose, C., & Banerji, A. (2011). Synthesis and antitoxicogenic activities of sea buckthorn flavone-3-ols and analogs. *Bioorganic & Medicinal Chemistry Letters*, 21, 5328–5330.  

Rashid, A., Raisuddin, A., Kumar, N. D., Abhinav, J., Amit, T., Gaurav, M., ... Asem, B. (2011). Safety and efficacy study of intramuscularly administered sea buckthorn (Hippophae rhamnoides L.) oil as depot formulation. *International Journal of Drug Development & Research*, 3, 356–365.  

Rice-Evans, C. A., Miller, N. J., & Pagana, G. (1996). Structure–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology & Medicine*, 20, 933–956.  

Rodriguez-Meizoso, I., Marín, F. R., Herrero, M., Sánchez, F. J., Reglero, G., Cifuentes, A., & Ibáñez, E. (2006). Subcritical water extraction of nutraceuticals with antioxidant activity from oregano. Chemical and functional characterization. *Journal of Pharmaceutical and Biomedical Analysis*, 41, 1560–1565.  

Ruan, C. J., Xie, Q. L., & Li, D. Q. (2000). Function and benefit of sea buckthorn improving eco environment of Loess Plateau. *Journal of Environmental Protection*, 5, 30–31.  

Ruan, C. B., Xie, Q. L., & Li, D. Q. (2000). Function and benefit of sea buckthorn improving eco environment of Loess Plateau. *Journal of Environmental Protection*, 5, 30–31.  

Saggu, S., Divekar, H. M., Gupta, V., Sawhney, R. C., Banerjee, P. K., & Kumar, R. (2007). Adapogenic and safety evaluation of seabuckthorn (Hippophae rhamnoides) leaf extract: A dose dependent study. *Food and Chemical Toxicology*, 45, 609–617.  

Saggu, S., & Kumar, R. (2007). Possible mechanism of adapogenic activity of seabuckthorn (Hippophae rhamnoides) during exposure to cold, hypoxia and restraint (C–H–R) stress induced hypothermia and post stress recovery in rats. *Food and Chemical Toxicology*, 45, 2426–2433.  

Saggu, S., & Kumar, R. (2008). Effect of seabuckthorn leaf extract on circulating energy fuels, lipid peroxidation and antioxidant parameters in rats during exposure to cold, hypoxia and restraint (C–H–R) stress and post stress recovery. *Phytotherapy*, 15, 437–446.  

Siddiqui, M. S., Sawhney, R. C., & Gupta, A. (2009). Safety and healing efficacy of Sea buckthorn (Hippophae rhamnoides L.) seed oil on burn wounds in rats. *Food and Chemical Toxicology*, 47, 1146–1153.  

Suleyman, H., Demirezer, L. O., Buyukokuroglu, M. E., Akcay, M. F., Gepdiremen, A., Banoglu, Z. N., & Gocer, F. (2001). Antilulcerogenic effect of Hippophae rhamnoides L. *Phytotherapy Research*, 15, 625–627.  

Teng, B. S., Lu, Y. H., Wang, Z. T., Tao, X. Y., & Wei, D. Z. (2006). In vitro anti-tumor activity of isorhamnetin isolated from Hippophae rhamnoides L. against BEL-7402 cells. *Pharmacological Research*, 54, 186–194.  

Ting, H. C., Hsu, Y. W., Tsai, C. F., Lu, F. J., Chou, M. C., & Chen, W. K. (2011). The in vitro and in vivo antioxidant properties of seabuckthorn (Hippophae rhamnoides L.) seed oil. *Food Chemistry*, 125, 652–659.  

Tsybikova, D. T., Raspoutine, D. B., Zolykeeva, D. N., Darzhabopova, G. Z., & Kundanov, L. L. (1983). A study of leaves and the oil cake of seabuckthorn; biology, chemistry and pharmacology of seabuckthorn (pp. 107–109). Novosibirsk.  

Tulsawani, R. (2010). Ninety day repeated gavage administration of Hippophae rhamnoides extract in rats. *Food and Chemical Toxicology*, 48, 2483–2489.  

http://dx.doi.org/10.1016/j.fct.2010.06.018  

Upadhyay, N. K., Kumar, R., Mandota, S. K., Meena, R. N., Siddiqui, M. S., Sawhney, R. C., & Gupta, A. (2009). Safety and healing efficacy of Sea buckthorn (Hippophae rhamnoides L.) seed oil on burn wounds in rats. *Food and Chemical Toxicology*, 47, 1146–1153.  

Upendra, K. S., Sharma, K., Sharma, N., Sharma, A., Singh, H. P., & John, A. K. (2009). Microwave assisted efficient extraction of different parts of Hippophae rhamnoides for the comparative evaluation of antioxidant activity and quantification of its phenolic constituents by reversephase high performance liquid chromatography (RP-HPLC). *Journal of Agricultural and Food Chemistry*, 56, 376–379.  

Wang, P. F., Kang, J. H., Zheng, R. L., Yang, Z. H., Lu, J. F., Gao, J. J., & Jia, Z. (1996). Scavenging effects of phenylpropanoid glycosides from Pedicularis on superoxide anion and hydroxyl radical by the Spin trapping method(95)02255-4. *Biochemical Pharmacology*, 51, 687–691.  

http://dx.doi.org/10.1016/S0006-2952(95)02255-4  

Wani, T. A., Wani, S. M., Shah, A. G., & Masoodi, F. A. (2013). Optimizing conditions for antioxidant extraction from Sea Buckthorn leaf (Hippophae rhamnoides L.) as herbal tea using response surface methodology (RSM). *International Food Research Journal*, 20, 1677–1681.  

Wei, T. X., Yu, X. X., & Zhu, J. Z. (1998). Litter interception of forests in southwestern Shanxi province. *Journal of Beijing Forestry University*, 20, 1–6.  

Wu, J., Yu, X. J., Mo, X. L., Xi, X. G., & Liu, D. (1994). Electrophysiologic effects of total flavones of Hippophae rhamnoides L. on guinea pig papillary muscles and cultured rat myocardial cells. *Zhanqiao Yao Li Xue Bao*, 15, 341–343.  

Xiao, M., Qiu, X., Yue, D., Cai, Y., & Mo, Q. (2013). Influence of hippophae rhamnoides on two appetite factors, gastric emptying and metabolic parameters, in children with functional dyspepsia. *Hellenic Journal of Nuclear Medicine*, 16, 38–43.  

Xing, J., Yang, R., Dong, Y., Wang, B., Wang, J., & Kallio, H. P. (2002). Effects of sea buckthorn (Hippophae rhamnoides L.) seed and pulp oils on experimental models of gastric ulcer in rats. *Fitoterapia*, 73, 644–650.  

http://dx.doi.org/10.1016/S0367-326X(02)00221-6  

Xu, X., Xie, B., Pan, S., Liu, L., Wang, T., & Chen, C. (2007). Effects of sea buckthorn procyanidins on healing of acetic acid-induced lesions in the rat stomach. *Asia Pacific Journal of Clinical Nutrition*, 16, 234–238.  

Yang, C. (1995). *A clinical study of reducing fat and regulating blood lipids of big ramishu* for the comparative evaluation of antioxidant activity and quantification of its phenolic constituents by reversephase high performance liquid chromatography (RP-HPLC). *Journal of Agricultural and Food Chemistry*, 43, 13–17.  

Yang, R., & He, H. P. (2001). Fatty acid composition of lipids in sea buckthorn (Hippophae rhamnoides L.) berries of different origins. *Journal of Agricultural and Food Chemistry*, 49, 1939–1947.  

http://dx.doi.org/10.1021/jf001059s
Yang, B., Kalimo, K. O., Tahvonen, R. I., Mattila, L. M., Katajisto, J. K., & Kallio, H. P. (2000). Effect of dietary supplementation with sea buckthorn (Hippophae rhamnoides) seed and pulp oils on the fatty acid composition of skin glycerophospholipids of patients with atopic dermatitis. The Journal of Nutritional Biochemistry, 11, 338–340. http://dx.doi.org/10.1016/S0955-2863(00)00088-7

Yasukawa, K., Kitanaka, S., Kawata, K., & Goto, K. (2009). Anti-tumor promoters phenolics and triterpenoid from Hippophae rhamnoides. Fitoterapia, 80, 164–167. http://dx.doi.org/10.1016/j.fitote.2009.01.006

Zeb, A. (2004a). Chemical and nutritional constituents of sea buckthorn juice. Pakistani Journal of Nutrition, 3, 99–106.

Zeb, A. (2004b). Important therapeutic uses of sea buckthorn (Hippophae): A review. The Journal of Biological Sciences, 4, 687–693.

Zhong, M. (1987). Treatment of ischemic heart diseases with flavonoids of Hippophae rhamnoides. Chinese Journal of Cardiology, 15, 97–99.

Zeb, A. (2004a). Chemical and nutritional constituents of sea buckthorn juice. Pakistani Journal of Nutrition, 3, 99–106.

Zeb, A. (2004b). Important therapeutic uses of sea buckthorn (Hippophae): A review. The Journal of Biological Sciences, 4, 687–693.

Zenz, Z. H., Dong, Z. H., & Yu, J. (1997). Modern study of traditional Chinese medicine (p. 2423). Beijing: Beijing University of Traditional Chinese Medicine, Xue Yuan Press.