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PHYTOCHEMICAL, ETHNOMEDICINAL AND PHARMACOLOGICAL POTENTIALS OF SEABUCKTHORN- A MINI REVIEW

SIVARAJ ANBARASU, MANIKKAM RADHAKRISHNAN, ARMUGAM SURESH AND JERRINE JOSEPH*

Centre for Drug Discovery and Development, Sathyabama University, Chennai-600119.

ABSTRACT

Sea Buckthorn (SBT) is distributed in the plains of India. The plant parts are used traditionally for several ailments. Medicinally, it has been proven to possess various pharmacological activities such as antioxidant, antimicrobial, antifungal, metabolic disorders, immunostimulatory activity, hepatoprotectant and anticancer activity. Several studies reveal the presence of various phytochemical constituents viz., flavonoids (isorhamnetin, quercetin, myricetin, kaempferol and their glycoside compounds), carotenoids (β and δ-carotene, lycopene, Zeaxanthin), few essential amino acids, sitosterol, triterpene, fatty acids, tannin acid, 5-hydroxytryptamine, umbelliferone, antioxidant vitamins and minerals in various parts of SBT. These studies reveal that SBT is a potential source of medicinally active compounds and have various pharmacological effects; yet the anti mycobacterial activity of this plant is unexplored and not well documented to the best of our knowledge, research in this area will encourage finding therapeutic use for TB patients which will be a next wonder drug as hepatoprotectant, immunostimulatory as well as anti microbial.

KEYWORDS : Sea buckthorn, hepatoproctectant, immunostimulatory and antimicrobial

*Corresponding author

JERRINE JOSEPH
Centre for Drug Discovery and Development, Sathyabama University, Chennai-600119, India.

INTRODUCTION

Sea Buckthorn (SBT) is a deciduous, branched, spiny shrub belonging to genus *Hippophae* and family Elaeagnaceae which usually forms shrub 3 to 15 feet height although some SBT (*Hippophae rhamnoides*) in China have reached 18 m (59 feet), and others grow no higher than 50 cm (20 inches). Before 12 century BC, the ancient Greeks surprised to find some sick horses loose to die a natural death became strong and energetic again. They found the source of this magic was traced to sea buckthorn and named the shrub *H. rhamnoides* L., meaning trees that make horse shine. The plant can withstand winter temperatures of up to -40^{o} C and commonly found growing at high altitudes of 4000 to 14000 feet. SBT is dioecious plant, the male plants produces brownish flowers which produce wind-distributed pollen and female plants produce an orange berry-like fruit. The roots of the plant providing a non-leguminous nitrogen fixation role in surrounding soils, makes the plant is an optimal pioneer plant for water and soil conservation in eroded areas. The leaves are alternate, narrow and lanceolate, with a silver-gray colour. The plant are considered to be rich source of a large number of bioactive substances like flavonoids (isorhamnetin, quercetin, myricetin, kaempferol and their glycoside compounds), carotenoids (β and δ-carotene, lycopene, Zeaxanthin), few essential amino acids, sitosterol, triterpene, fatty acids, tannin acid, 5-hydroxytryptamine, umbelliferone, antioxidant vitamins and minerals.[1,2,3]. SBT has been called a wonder plant in many Asian countries, including China, India, and Pakistan. SBT is a particular and valuable plant species, currently domesticated in various parts of the world reflecting interest in its long-identified multiple uses. In India species of Hippophae grow in five states; 3 in the North-West (Lahaul-Spiti districts of Himachal Pradesh, Uttaranchal and river belts of Indus, Nubra, Shyok, Zanskar etc. of ladakh) and 2 in the North-East (Sikkim and Arunachal Pradesh) Himalaya[4]. The classification of genus Hippophae is still unclear although it has been classified into seven major species; *H. tibetana, H. salicifolia, H. rhamnoides, H. neurocarpa, H. litangensis, H. gyantsensis and H. goniocarpa*. In India, *H. rhamnoides, H. salicifolia* and *H. tibetana* have been described. Of which *H. rhamnoides L. ssp. Turkestanica* are the major one[5]. Traditionally, the berries have been used for more than 1,000 years in Tibetan and Indian systems of medicine. Chinese people were the first to use sea buckthorn as a drug and its medicinal products were recorded in Sibu Yidian medical book. Thirty chapter out of 158 chapters deal with its medicinal value mentioning the pharmacological effects on inducing the expectoration, opening the inhibited lung energy, dispersing dampness, tonifying the YIN and strengthening the YANG, to strengthen the spleen and the stomach, and to promote blood circulation and to remove blood stasis[6]. In the history of Indian and Tibetan medicine, the plant fruits are used to treat pulmonary, GI, ischemic heart disease, blood, hepatic, and metabolic disorders. In Mongolia, the Leaves and branches extracts of the SBT are used to treat colitis, enterocolitis in humans, animals. The leaves of the plant are used in Middle Asia for GI and skin disorders, topically applied to treat rheumatoid arthritis. The present review is an attempt to assess the research activities of SBT related to health benefits.

SBT BERRIES

The berries/ fruits are pearl-shaped, yellowish-orange (Figure 1), sour to taste, weighs between 270 and 480 mg and averages 350 mg (depending upon cultivar and maturity) which contains a range of healthful compounds including unsaturated fats, phytosterols, carotenoids and vitamin E and K. The berries are especially high in vitamin C and flavonoid antioxidants. It is often used in juices, jams, sauces, liqueurs and flavoring of dairy products because of their unique taste. The berries/fruits pulp and seeds have proven important traditional medicine for various clinical conditions.

Figure 1
Berries of Hipophae rhamnoides

SBT berry is rich in oil both in fruit soft parts and in seeds. Depending upon the origins and varieties of SBT, the oil content in the soft parts varies between 0.5 to 10% whereas seeds contain around 10% oil. Both seed and pulp oils combine high levels of beneficial fatty acids, natural antioxidants and vitamins as well as plant sterols[7]. The oil content in berries of two subspecies of sea buckthorn (*H. rhamnoides* L.) result showed that subsp. rhamnoides contained a higher proportion of oil in seeds (11.3% vs 7.3%), berries (3.5% vs 2.1%), and seedless parts (2.8% vs 1.7%) than the berries of subsp. *sinensis.* More linoleic acid (40.9% vs 39.1%) and less α-linolenic acid (26.6% vs 30.6%) were found in the seed oil of subsp. *sinensis* than in the seed oil of subsp. *rhamnoides.* The fatty acid composition of two subsp. of SBT berries were investigated in terms of proportion of palmitoleic acid was higher in the oil of berries of subsp. *rhamnoides* than the berries of subsp. *sinensis* (26.0% vs 21.5%), but was vice versa with α-linolenic acid (8.8% vs 11.2%) Researchers investigated the oils extracted from different parts of six sea buckthorn berry subspecies (ssp. *carpatica*) cultivated in Romania for fatty acid composition of the total lipids and the major lipid fractions viz. polar lipid (PL), free fatty acids (FFA), triacylglycerols (TAG) and sterol esters (SE) by using GC-MS. The results revealed that the pulp/peel and whole berry oils are dominating fatty acids respectively palmitic, oleic and palmitoleic of 23-40%, 20-53% and 11-27% whereas seed oils had about 65-72% of polyunsaturated fatty acids (PUFAs). The major fatty acids in polar lipid of berry pulp/peel oils contain oleic, palmitic, palmitoleic and linoleic acids respectively 20-40%, 17-27%, 10-22% and 10%-20%, whereas in seeds PLs, polyunsaturated fatty acids prevailed. Comparing with the other lipid fractions, the sterol esters had the highest contents of saturated fatty acids. The fatty acid profiles of the free fatty acids fractions were relatively similar to those of triacylglycerols[8,9]. Another study explored the GC-MS profiling of fatty acids composition in canadian grown cultivars *Hippophae rhamnoides* L. (ssp. *mongolica*) seeds and pulp of berries indicated that the seed oil contained linoleic and α-linolenic acids at 33–36% and 30–36%, respectively, while the pulp oil contained palmitoleic acid at 32–42%[10] Ranjith et al., (2006) collected the sea buckthorn berries from *H. rhamnoides, H. tibetana*, and *H. salicifolia* from the cold deserts of the Himalayas (Lahaul, Ladakh, and Spiti; India) and characterized in terms of the FA, carotenoid, tocopherol, and tocotrienol composition in their pulp oil. *H. rhamnoides* and *H. tibetana* contained the highest amount of the lipophilic carotenoids and tocols. *H. salicifolia* berries had higher amounts of lipophobic constituents such as vitamin C. The highest level of flavonols (quercetin derivatives) was found in sea buckthorn[11,12]Uransanaa et al., (2003) analysed the protein and amino acid composition of sea buckthorn seeds (*Hippophae rhamnoides mongolica* Rouse) and were summarized about 7.2% of wild sea buckthorn berries belong to seeds and the protein in seeds composed 37.79%, while the protein in shells equal to 15.25%. The total content of essential amino acids is varies between 43.32-45.04% and nonessential

amino acids observed between 56.66-55.96% of total protein extracted at pH=1.5 and 1 1.5 respectively[13]. Seabuckthorn pulp is rich in antioxidant vitamins and source of minerals. The assessment of biochemical composition in seabuckthorn growing at Leh valley in Trans-Himalaya shown that vitamin C (275 mg/100g) content is high in the fruit than other fruits viz. mango, apricot, banana, orange, peach. Other vitamins, riboflavin, niacin, pantothenic acid, vitamin A, vitamin B-6, vitamin B-2 and Vitamin E contents were found respectively as 1.45 mg, 68.4 mg, 0.85 mcg, 432.4 IU, 1.12, 5.4 mcg and 3.54 mg per 100g pulp. Potassium element is the most abundant elements found as 647.2 mg/l in SBT pulp. Other mineral elements composition investigated that calcium content is 176.6 mg/l, iron (30.9 mg/l), magnesium (22.5 mg/l), phosphorous (84.2 mg/l), sodium (414.2 mg/l), zinc (1.4mg/l), copper (0.7 mg/l), manganese (1.06 mg/l), and 0.53 mg/l of selenium[14]. The clinical and experimental investigations of the Sea buckthorn berry have shown multiple beneficial effects. The medicinal uses include antioxidant, Liver health, immune health, wound healing, atopic dermatitis, cancer protection. It is demonstrated great promise in the treatment of the mucous membranes including ulcers and gastro-intestinal disorders as well as vaginal problems. The beneficial effects of the oils on mucous membranes have shown to promoting tissue regeneration, improving immune function and reducing lipid peroxidation. The seed of the berries also has antibacterial, antifungal properties etc. Sea buckthorn berry juice is very high in organic acids as reflected in the high levels of titratable acidity, and has low pH nearly 2.7. About 60% to 85% juice from berries can be yield by pressing. Protein levels are fairly high for a fruit juice and this probably explains the fact that sea buckthorn juice is a cloudy or opalescent product. Sea buckthorn juice was the common medicine used in ancient period. It is the best nutritional/ medicinal source for the human and other animal. The juice had pharmacological functions like making expectoration easy, good for lungs and stomach, invigorating the function of the spleen, removing blood stasis and promote blood circulation etc.[1]. Eccleston *et al*., [15] evaluated the effect of sea buckthorn juice on plasma lipids, LDL oxidation, platelet aggregation and plasma soluble cell adhesion protein concentration. The study determined that 8 weeks of supplementation with 300 ml of sea buckthorn juice daily resulted in 20% and 17% increase in plasma HDL-C and triacylglycerol (TAG) concentrations[15]. Sea buckthorn may be a hopeful drug for prevention and treatment of liver fibrosis, but further well controlled clinical trials are required. A study included 30 and 18 cirrhotic patients as the treated group (group A) and 18 control group (Group B) respectively. Group A orally taking 15 g of sea buckthorn extract and group B taking vitamin B complex one tablet, 3 times a day for 6 months. The result showed that the serum levels of TNFa, IL-6, laminin (LN) and type IV collagen in group A were significantly higher than those in the control group. The serum levels of LN, hyaluronic acid, collagen types III and IV, total bile acid decreased significantly as compared with those before and after treatment in the control group after a course of sea buckthorn treatment[16]. SBT juice decreased genotoxic effect of cisplatin at dose of 1.2 mg/kg on somatic (bone marrow) and germ (sperm) cells of mice[17]. Padmavathi *et al*., [18] suggest that Hippophae fruit is able to decrease carcinogen-induced forestomach and skin tumorigenesis[18]. The flavone of *H. rhamnoides* L. (Obtained from Beijing Jianghe Sea buckthorn Company) was used as topical administration on cutaneous wound healing to determine the efficacy in rats. The study indicated that the flavone promotes significant wound healing activity. The flavone treated wounds were found to contract much faster. The flavones treated animals showed faster epithelialization of wound in 16.33 ± 0.42 days and the control animals showed the wound epithelialization in 24.83 ± 0.31 days[19]. The antidiabetic and antioxidant effect of seabuckthorn *(H. rhamnoides L.*) fruit pulp was evaluated by orally administered the SBT to streptozotocin (STZ) diabetic rats. It produced a significant reduction in blood glucose levels and Thiobarbituric Acid Reactive Substances (TBARS) levels in the STZ- diabetic rats. Tissue glutathione (GSH) reduced significantly in diabetic rats, was brought back to near normal levels by co-

administration of sea buckthorn. In STZ-diabetic rats, the degenerative changes of pancreatic beta cells were minimized to near normal morphology[20]. The Juice of *H. salicifolia* plants growing in the sacred forests of northeast India was found to be as high as 98.78% hydrogen peroxide scavenging activity and greatest nitric oxide scavenging effect of 75.24% [21].

SBT SEEDS

Seabuckthorn (SBT) seed oil is a rich source of unsaturated fatty acids, phytosterols, carotenoids and flavonoids. The aqueous extracts of *H. rhamnoides* seeds were found to possess antibacterial activity against *Listeria monocytogenes* and *Yersinia enterocolitica* with MIC values of 750 and 1000 ppm, respectively. The antioxidant and antimicrobial effects of the extract implicate its potential for natural preservation[22]. Gupta *et al*., (2011) observed that seed extracts of seabuckthorn (*H. salicifolia* D. Don) showed significant activity against *B. subtilis*, *B. thuringiensis* and no activity was observed against gram negative bacteria but some activity was obtained against *Agrobacterium tumefaciens*. The seed extract also inhibited the growth of fungus viz. *Mucor* and *Tilletia*[23]. Metha et al., (2013) concluded that seabuckthorn seedcake possess good *in vitro* antioxidative properties and can be incorporated as a supplement in animal feed. Authors were conducted a study in three extracts viz. 100% methanolic, 70% aqua-methanolic and 100% aqueous to assess the antioxidant potential by using various *in vitro* methods such as ABTS, DPPH, nitric oxide, reducing power, hydroxyl and superoxide radical scavenging assays. The results measured through IC_{50} values, revealed that 100% methanolic extract was better scavenger of ABTS, DPPH, hydroxyl radicals and nitric oxide. However, 70% methanolic extract was better scavenger of superoxide radicals. The reducing power of the extracts was also found in a dose dependent manner and was higher in methanolic extracts (100% and 70%) [24]. The anti-atherogenic effects of super**r**critical carbon dioxide extracted SBT seed oil was evaluated in white albino rabbits fed on high cholesterol diet for 60 days. The acetylcholine-induced vasorelaxant activity was significantly decreased in cholesterol-fed animals and could be restored to that of normal values by seed oil administration. These observations suggested by Basu *et al*., [25] stated that supercritical carbon dioxide extracted SBT seed oil has significant anti-atherogenic and cardioprotective activity[25]. Upadhyay *et al*., (2009) have extracted seed oil from by using supercritical carbon dioxide extraction method and studied the safety and healing efficacy of sea buckthorn (*H.rhamnoides* L.) seed oil in experimental burn wounds in rats. The oil treatment up-regulated the expression of matrix metalloproteinases (MMP-2 and 9), collagen type-III, VEGF in granulation tissue and it also increase in reduced glutathione (GSH) level and reduced production of reactive oxygen species (ROS) in wound granulation. This study suggests that seed oil possesses significant wound healing activity and no associated toxicity or side effects[26].

SBT LEAVES

Different populations of *H. rhamnoides* L. growing in Kargil district of Ladakh region of J&K (India) were subjected to the quantification of total phenolic carotenoid and chlorophyll (a & b) contents in male and female plants. The total phenols across populations ranged from 54.4 to 86.4 mg/0.25g GAE. Chlorophylls and carotenoids varied from 7.74 to 45.02 and, between 0.0170 and 0.0399 mM/0.5g respectively. Comparative evaluations made in male and female plants at inter- and intra- population levels reveal higher contents of almost all these antioxidants in the male sex. Some populations like Barootsog, Andoo, Kanoor and Mingee were found to be promising[27]. A study was aimed to investigate the preliminary phytochemical and metals in the leaves of *H. rhamnoides L*. The results showed that the extracts contain medicinally important bioactive constituents and also heavy metals present in the plant extracts were within the permissible limits. This justifies its use in the traditional medicine for the treatment of different diseases such as asthma, heart disorders, chest pain, high cholesterol and antioxidant. SBT leaf promotes wound healing

in experimental burn wounds. The increased wound contraction in SBT treated rats might be due to an enhanced activity of fibroblasts in regenerated wound tissue. Myofibroblasts are believed to play a key role in wound contraction. The topical treatment with leaf extract augmented endogenous antioxidants and prevented the free radical- mediated tissue injury. It also played an important role in angiogenesis[28]. Gupta *et al.,*[29] conducted a wound healing preclinical study of the aqueous lyophilized extract of seabuckthorn (*H. rhamnoides* L.) leaf on cutaneous wound in albino rats. Topical application of 1.0% seabuckthorn leaf extract increases the content of hydroxyproline and protein as well as the reduction in wound area when compared with similar effects in response to treatment using povidone-iodine ointment, which significantly augmented the healing process. SBT leaf extract-treated wounds showed significant increases vitamin C, superoxide dismutase, catalase, and glutathione peroxidase activities, reduced glutathione and decreased lipid peroxide levels[19]. The anti-dengue activity of *H. rhamnoides* leaf extract was studied by Jain *et al*., (2008). The extract was able to maintain the cell viability of Dengue-infected cells at par with Ribavirin drug along with the decrease in TNF-alpha and increase in IFN-gamma. These observations suggest that the SBT leaf extract has a significant anti-dengue activity and has the potential for the treatment of Dengue[30]. The methanolic extract of sea buckthorn leaves showed high total phenolic content (TPC) of 278.80 mg GAE/g extract and antibacterial activity with low MIC value of 125 µg/ml against *Listeria monocytogenes.* The antilisterial activity of the methanolic extract of leaves can possibly be used as a biosanitizer in food industries[31]. A study showed that methanolic leaf extracts of *H. rhamnoides* exhibited activity against *E. coli* at a concentration of 25 mg mL^{-1} and *Arthrobacter protophormial,* as well as *Micrococcus luteus*. The extract also has potent antioxidant activity, which showed scavenging capacity increase with every unit increase of leaf extracts reaching 68% at 30 µg mL^{-1}concentration[32]. The antibacterial activity of Phenolic rich fraction (PRF) from sea buckthorn leaves have studied by Yogendra Kumar *et al., (*2013). The result showed PRF has potent broad spectrum antibacterial properties, which has inhibiting effect against *Escherichia coli, Salmonella typhi, Shigella dysenteriae, Streptococcus pneumoniae* and *Staphylococcus aureus*[33]. A study demonstrated that the antioxidant and antibacterial substances from leaf and bark of *H. salicifolia* by using methanol, acetone, chloroform and petroleum ether solvents. The results showed that the MIC were found to be the most effective against methanolic extracts than acetone and chloroform but petroleum ether extracts was found to have no activity against all tested organisms[34]. The antifungal activity of methanolic leaf extracts of seabuckthorn against common skin dermatophytes have studied by Verma *et al.,* (2013). The leaf extracts were obtained by soxlet extraction method and cold extraction method in methanol. An *in vitro* study showed both leaf extracts has significant antimycotic activity against *M. gypseum and T. rubrum*. The study revealed that cold methanolic extract was found to be most significant as compared to the hot methanolic extract[35]. Padwad *et al.,* (2006) studied the effect of leaf extract of Seabuckthorn on lipopolysaccharide induced inflammatory response in murine macrophages. The extract showed significant scavenging of NO radicals released by the NO donor. Treatment of macrophages with SBT leaf extract also caused a significant inhibition of iNOS activation. These observations suggest that the inhibition of net NO production by SBT leaf extract may be due to its scavenging activity and/or its inhibitory effects on iNOS activation. The study suggests that SBT leaf extract has significant anti-inflammatory activity and has potential for the treatment of inflammatory diseases[36]. Geetha *et al.,* (2008) studied the hepatoprotective effects of sea buckthorn leaf extract against carbon tetrachloride induced liver injury in male albino rats. The study showed that sea buckthorn has significant hepatoprotective effects which might be due to its antioxidant activity and can be developed as a nutraceutical or food supplement against liver diseases[37]. Maheswari et al., (2011) studied the antioxidant and hepatoprotective activities of phenolic rich fraction of Seabuckthorn leaves on carbon tetrachloride

(CCl(4)) induced oxidative stress in Sprague Dawley rats. Oral administration of phenolic rich fraction (PRF) at dose of 25-75 mg/kg body weight significantly protected from CCl(4) induced elevation in aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT) and bilirubin in serum, elevation in hepatic lipid peroxidation, hydroperoxides, protein carbonyls, depletion of hepatic reduced glutathione (GSH) and decrease in the activities of hepatic antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR) and glutathione-S-transferase (GST). The PRF also protected against histopathological changes produced by CCl(4) such as hepatocytic necrosis, fatty changes, vacuolation, etc. The study suggests that PRF has potent antioxidant activity, prevent oxidative damage to major biomolecules and afford significant protection against CCl (4) induced oxidative damage in the liver[38]. The modulation of the immune system in an individual is achieved by introducing the immunomodulatory agents, which can enhance the immunological responsiveness of an organism by interfering with its regulatory mechanism. The immunomodulatory potential of Seabuckthorn leaf extract is well documented. Ganju *et al.,* (2005) studied the immunomodulatory activity of sea buckthorn (SBT) leaf extract in adjuvant induced arthritis (AIA) rat model. Administration of SBT extract on the same day or 5 days prior to inflammatory insult into the joint, significantly reduced the inflammation as compared to the untreated animals in a dose dependent manner. These observations suggest that the SBT leaf extract has a significant anti-inflammatory activity and has the potential for the treatment of arthritis[39]. Geetha *et al.,*[40] investigated the in vitro anti-oxidant and immunomodulatory properties of seabuckthorn using lymphocyte as a model system. Alcoholic extracts of leaves and fruits of seabuckthorn at a concentration of 500 µg/ml were found to inhibit chromium-induced free radical production, apoptosis, DNA fragmentation and restored the anti-oxidant status to that of control cells. In addition, these extracts also were able to arrest the chromium-induced inhibition of lymphocyte proliferation. These observations suggest that the alcoholic extracts of leaves and fruits of seabuckthorn have marked cytoprotective properties, which could be attributed to the anti-oxidant activity[40]. Saggu *et al.*, [47] investigated the seabuckthorn leaf aqueous extract in rats for its dose dependent adaptogenic and toxicity. The maximal effective adaptogenic dose of the extract was 100mg/kg body weight. In acute toxicity study LD (50) of the extract was observed to be >10g/kg when given orally. These results indicate that sea buckthorn leaf aqueous extract possess potent adaptogenic activity with no toxicity even after sub-acute (30 days) maximal effective dose administration[41]. The cytoprotective and antioxidant properties of alcoholic leaf extract of seabuckthorn (SBT) against hypoxia induced oxidative stress in C-6 glioma cells were studied by Narayanan *et al.*,[42]. Pretreatment of cells with alcoholic leaf extract of SBT at 200 µg/ml significantly inhibited cytotoxicity, ROS production and maintained antioxidant levels similar to that of control cells. Further, the leaf extract restored the mitochondrial integrity and prevented the DNA damage induced by hypoxia[42]. The radioprotection effect of herbal preparation of *H. rhamnoides*, RH-3, against whole body lethal irradiation in mice were studied. Free radical scavenging, acceleration of stem cell proliferation and immunostimulation are the radioprotective attributes, which require further investigations[43].

CONCLUSION

In the light of the foregoing literature review the plant seabuckthorn and the wide spectrum of its plant parts medicinally usage have been assessed. The antimycobacterial potential of the plant has not been explored neither documented well. Hence this area will be of emerging interest to venture for aspiring researchers struggling to combat the drug resistance and HIV co-infection and latency issues with TB. For the past four decades no new wonder drug has been available for TB. The multifaceted therapeutic potential of SBT is promising as an immune booster, hepatoprotectant as well as antimicrobials. Hence, it is required to tap this potential into translational value.

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