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Antioxidant and antibacterial activities of various seabuckthorn (*Hippophae rhamnoides* L.) seed extracts

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Abstract

Seabuckthorn (*Hippophae rhamnoides* L.) seeds were successively extracted with chloroform, ethyl acetate, acetone and methanol (MeOH) using a Soxhlet extractor for 8 h each. The crude extracts were screened for antioxidant and antibacterial activities. The reducing power and antioxidant activities evaluated in various in vitro models (1,1-diphenyl-2-picrylhydrazine and liposome model system) showed the highest activity for MeOH extract. The MeOH extract was also found to posses maximum antibacterial activity. The MIC values, with respect to MeOH extract for *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Listeria monocytogenes*, *Yersinia enterocolitica*, were found to be 200, 300, 300, 300, and 350 ppm, respectively. These results indicated the possibility of using seabuckthorn seeds for medicinal uses and food preservation.
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Keywords: Hippophae rhamnoides; Antioxidant activity; Antibacterial activity; Seed extract; Methanolic extract

1. Introduction

Seabuckthorn (*Hippophae rhamnoides* L., Elaeagnaceae) is a native of Eurasia and has been domesticated in several countries including India, China, Nepal, Pakistan, Myanmar, Russia, Britain, Germany, Finland, Romania and France at a high altitude of 2500–4300 m. It is known as the wonder plant and bears small orange yellow- to red-coloured fruits on two-year-old thorny twigs. The berry-like fruit develops from an ovary, or calyx tube connected to an ovary. Seabuckthorn (SBT) berries are known to be acidic with a mild sweet unique aroma. High amounts of vitamin C, flavonoids, oils and oil-soluble compounds, as well as minerals, are present in the berry (Kallio, Yang, Tahvonen, & Hakala, 2000). Berries also contain many bioactive substances and can be used in the treatment of several diseases, such as cardiovascular disease, cancer, and acute mountain sickness. For the past 50 years several medicinal preparations of SBT have been clinically used to treat radiation damage, burns, oral inflammation and gastric ulcers in China and the former Soviet republic, and more than 300 preparations have been reported in literature (Lu, 1992). In addition to medicinal use, the berries are processed into various products such as juice and marmalade, and used for flavouring of dairy products because of their unique taste (Gao, Ohlander, Jeppsson, Bjork, & Trajkovski, 2000).

Spoilage of foods due to the presence of bacterial and fungal infection has been a major concern for decades and it causes a considerable loss worldwide. The demand for non-toxic, natural preservatives has been rising with increased awareness and reports of ill-effects of synthetic chemicals present in foods. Furthermore, emergence of food-borne pathogens has lately become a major public health concern. Many compounds

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present in plants have been reported to be biologically active, antimicrobial, allopathic antioxidants and have bioregulatory properties. SBT oils, juice, leaves and bark are well known for their medicinal properties, and they have been used to treat high blood lipid symptoms, gingivitis, eye and skin ailments, and cardiovascular diseases (Liu, Wu, & Liu, 1980; Yang et al., 2000).

Proximate composition of SBT has been well documented (Gao et al., 2000). Also, there have been reports on chemical composition, characterization and health benefits of SBT fruit and its oil (Chen et al., 1990; Yang et al., 2000). But, SBT seed, a byproduct of the berry processing industry, is not yet exploited. To our knowledge, no information is available on the antioxidant and antibacterial properties of SBT seeds. Therefore, the objective of the present study was to investigate the antioxidant activity of various SBT seed extracts in in vitro models, and determine their antibacterial activity against different food-borne pathogens for their potential as a natural preservative and for nutraceutical formulations.

2. Materials and methods

2.1. Materials

SBT berries were collected from the Lahaul Spiti region (Himachal Pradesh, India) and seeds were obtained after deseeding the berries by passing through stainless steel sievers (Mesh pore size 0.042 in.). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and tannic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All solvents/chemicals used were of analytical grade and obtained from Merck, Mumbai, India.

2.2. Preparation of the SBT seed extracts

Dried seeds of *Hippophae rhamnoides* were powdered and successively extracted in a Soxhlet extractor for 8 h each with chloroform, ethyl acetate, acetone, and methanol according to the procedure of Chauhan, Negi, and Ramteke (2003). The extracts were used as such for determination of reducing power, dissolved in methanol: water (6:4 v/v) for evaluation of antioxidant capacity by the liposome model system, in dimethyl sulphoxide for scavenging activity by DPPH method; and in propylene glycol for evaluation of antibacterial activity.

2.3. Determination of total phenolics

The concentration of phenolics in the extracts was determined by the method of Negi, Jayaprakasha, and Jena (2003) and results were expressed as (+) tannic acid equivalents. Five milligrammes of each dried SBT seed extract were mixed with 10 ml mixture of acetone and water (6:4 v/v). An aliquot of the samples was mixed with 1.0 ml of 10-fold diluted Folin–Ciocalteu reagent and 2 ml of 10% sodium carbonate solution. After standing for 30 min at room temperature, the absorbance was measured at 765 nm using a GBC UV–Vis Spectrophotometer (Model Cintra-10, Victoria, Australia).

2.4. Evaluation of antioxidant activity by liposome model systems

Egg lecithin (300 mg) was sonicated with 30 ml phosphate buffer (10 mM, pH 7.4) in an ultrasonic sonicator for 10 min to ensure proper liposome formation. The various dried extracts (25–100 μg) of each sample were mixed with the sonicated solution (0.5 ml, 10 mg/ml), $FeCl_3$(0.5 ml, 400 mM), and ascorbic acid (0.5 ml, 400 mM). The antioxidative action was measured by the method of Buege and Aust (1978). The absorbance of the samples was determined at 535 nm after incubation for 1 h at 37 °C. The results were expressed as nmol of malondialdehyde (MDA) formed per mg lipid and were calculated by using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

2.5. Determination of reducing power

The determination of reducing power was performed as described by Yen and Duh (1993). Various extracts (0.48, 1.2, 2.4, 3.6, and 4.8 mg) were mixed with phosphate buffer (5.0 ml, 2.0 M, pH 6.6) and 1% potassium ferricyanide (5 ml), and incubated at 50 °C for 20 min; 5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 2500 rpm for 10 min. The upper layer of the solution (5 ml) was mixed with distilled water (5 ml) and 0.1% ferric chloride (1 ml) and the absorbance was read at 700 nm. Increase in the absorbance of the reaction mixture indicated increase in the reducing power.

2.6. Scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

Free radical-scavenging activity of each antioxidant was assayed using a stable free radical, DPPH, according to the method of Blois (1958). The reaction mixture contained 0.5 ml of 0.5 mM DPPH and 0.1 ml of dimethyl sulphoxide containing the antioxidant extract at different concentrations (10–50 μg). Finally, the total volume of the reaction mixture was adjusted to 1.0 ml by adding 100 mM Tris–HCl buffer (pH 7.4). The reaction mixture was allowed to stand for 20 min at room temperature in the dark and the radical-scavenging activity of each antioxidant was quantified by decolorization at 517 nm.

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