Antioxidant and Antibacterial Activity of *Hippophae rhamnoides* Methanolic Leaf Extracts from Dry Temperate Agro-climatic Region of Himachal Pradesh

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**ABSTRACT**

The present study was carried out to evaluate the antioxidant and antibacterial activity of methanolic leaf extract of *Hippophae rhamnoides* L. (Sea buckthorn, SBT) leaves under *in vitro* conditions. The chemical composition of the methanolic leaf extract was quantified in terms of total phenol and flavonoid contents. The *Hippophae rhamnoides* methanolic leaf extract exhibited potent antioxidant activity determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and then by 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), assays. The scavenging capacity by DPPH and ABTS of the extracts was found to increase with every unit increase of *Hippophae rhamnoides* leaf extracts reaching 68% at 30 μg mL⁻¹ concentration. Further, the extract was evaluated for antibacterial activity against three human pathogenic bacteria *E. coli*, *A. protophormial* and *M. luteus*, however, it was maximum against *E. coli*. The study shows that the methanolic extract of *Hippophae rhamnoides* leaves have marked antioxidant and antibacterial activities.

**Key words:** *Hippophae rhamnoides*, antioxidant, antibacterial, 2, 2-diphenyl-1-picrylhydrazyl, 2, 2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

**INTRODUCTION**

Bio-based products involved in therapeutic and curative applications in human health is on the rise for the last few decades due to the side effects of synthetic compounds used in medical and nutritional applications (Gupta *et al.*, 2011). There are numerous reports regarding beneficial effects of fruits, vegetables and other plant derived products on the human health because of the presence of various bioactive molecules in them (Michel *et al.*, 2012; Crozier *et al.*, 2009). These substances are secondary metabolites, biosynthesized within plants, mainly phenolic compounds including flavonoids, phenolic acids and tannins having strong antioxidant and antibacterial activity (Saleem *et al.*, 2010). Dietary intake of such phytochemicals may be an important strategy for inhibiting or delaying of pathological conditions caused by free radicals either formed by cellular metabolism, exogenous chemicals or due to stress and is capable of oxidising biomolecules which may cause many diseases (Upadhyay *et al.*, 2010). The biomolecules obtained from the natural plants are in big demands because they have no or less side effects, toxicity of food by synthetics and cosmetic preservatives (Darbre *et al.*, 2002).
Hippophae rhamnoides belongs to the family Elaeagnaceae commonly known as Sea buckthorn (SBT), a thorny nitrogen fixing actinomycetes deciduous shrub producing yellow orange berries is native to Europe and Asia (Suryakumar and Gupta, 2011). In India Hippophae rhamnoides is widely distributed in cold Himalayan region at an altitude of 2500-4000 m. All parts of the plant are rich source of bioactive substances which have been simultaneously investigated for their therapeutic potential and phytochemical contents. It has been used in the traditional system of medicine for the treatment of cough, skin diseases, gastric ulcers, asthma and lung disorders for ages (Mingyu et al., 1991; Zeb, 2004; Ranjith et al., 2006). It was also reported that Hippophae rhamnoides leaf has no cytotoxicity or adverse effect after oral administration (Chawla et al., 2007; Ganju et al., 2005; Geetha et al., 2005). For the last few years, different Hippophae rhamnoides extracts from berries, oil and leaf have been scientifically investigated for various pharmacological activities such as anti-inflammation, immune-modulation, radio protective and tissue regeneration. However, little or no efforts have been made to evaluate its antioxidant potential and antibacterial activity against human pathogenic bacteria. Hence, the objective of present study was to evaluate the total phenolic contents vis-à-vis antioxidant activity and antimicrobial properties of methanolic leaf extract of Hippophae rhamnoides under in vitro conditions.

MATERIALS AND METHODS
Collection of plant material: The leaves of Hippophae rhamnoides were collected from Kukumseri area in the Lahaul Valley of Himachal Pradesh during August- September, 2011. The sample was authenticated by Seabuckthorn Scientist, CSK HPKV Palampur, HP.

Preparation of extract: Fresh leaves were cleaned thoroughly and air dried under shade in clean environment. The leaves (25 g) were then powdered and used for extraction purpose on weight: volume basis. The methanolic extract of powdered Hippophae rhamnoides leaves were prepared by mixing the leaves in methanol and macerated overnight with stirring using a magnetic stirrer. Filtered using muslin cloth and coarse filter paper, the residue was discarded and the methanolic extract thus obtained was dried in vacuo. The dried extract was then stored in an air tight container.

Determination of total phenolics and flavonoids content
Total phenolics contents: Total phenol content was determined by using Folin-Ciocalteu reagent as per the method described by Makkar (2003).

Total flavonoids contents: Total flavonoids content was determined by the method of Cheng et al. (2003).

Determination of antioxidant activity
DPPH assay: The free radical scavenging activity of Hippophae rhamnoides leaf methanolic extracts on DPPH radical was determined by the method of Sharma and Bhat (2009) with some modifications.

ABTS assay: ABTS assay was conducted by following the method described by Re et al. (1999) with some modifications. Firstly, to produce the radical cation ABTS⁺, 7 mM L⁻¹ ABTS diammonium salt and 2.4 mM L⁻¹ potassium persulfate were mixed in a volume ratio of 1:0.5 and the reaction
mixture was allowed to stand in the dark for 12-16 h at room temperature. The solution was then
diluted by mixing 1 mL ABTS solution with 20 mL methanol to obtain an absorbance of 1.10±0.02
units at 734 nm using UV-spectrophotometer (Smart Spec 3000, Bio-Rad, USA). Fresh ABTS
solution was prepared for each assay. The assay solution was prepared by taking different aliquot
of methanolic *Hippophae rhamnoides* leaf extract mixed with ABTS solution to a final volume of
3 mL and the absorbance was measured at 734 nm after 2 h using UV-spectrophotometer. The
standard curve prepared with ascorbic acid used as standard was linear.

**Antibacterial activity:** Antibacterial activity of *Hippophae rhamnoides* leaf extracts along with
tetracycline used as standard was evaluated against *Escherichia coli* (MTCC 739),
*Arthrobacter protophormial* (MTCC 2682) and *Micrococcus luteus* (MTCC 106). Sensitivity of
different bacterial strains to *Hippophae rhamnoides* extract was measured in terms of zone of
inhibition using agar-well diffusion assay method and paper disc diffusion method (Ayandele and
Adebiyi, 2007).

**RESULTS**

**Total phenolic and flavonoid contents:** Methanolic extract of *Hippophae rhamnoides* leaves
were found to be rich in total phenolics and flavonoids contents expressed as 34.6 mg of gallic acid
equivalent and 18.1 mg of Quercetin equivalent/100 g dry leaf powder (Table 1).

**Antioxidant activity using (DPPH) radicals:** Methanolic extracts of *Hippophae rhamnoides*
leaves exhibited potent antioxidant activity when analyzed by DPPH and ABTS assays and the
percent radical scavenging activity is presented in Table 2. The scavenging capacity of the extracts
was found to increase with every unit increase of *Hippophae rhamnoides* leaf extracts reaching
68% at 30 μg mL⁻¹ concentration.

The change in *Hippophae rhamnoides* leaf extract concentration was found to affect the percent
inhibition of both DPPH and ABTS radical cation. The highest inhibition percent was achieved with
increase in extract concentration, which can be explained by its high polyphenol contents as shown
in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total phenols (mg gallic acid equivalent/100 g dry leaf)</th>
<th>Total flavonoids (mg quercetin equivalent/100 g dry leaf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic leaf extract</td>
<td>34.6±0.21</td>
<td>18.1±0.31</td>
</tr>
</tbody>
</table>

Values are Mean±SEM

<table>
<thead>
<tr>
<th>Concentration (μg mL⁻¹)</th>
<th>Scavenging activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPPH</td>
</tr>
<tr>
<td>5</td>
<td>10±0.21</td>
</tr>
<tr>
<td>10</td>
<td>23±0.25</td>
</tr>
<tr>
<td>15</td>
<td>32±0.29</td>
</tr>
<tr>
<td>20</td>
<td>42±0.36</td>
</tr>
<tr>
<td>25</td>
<td>54±0.38</td>
</tr>
<tr>
<td>30</td>
<td>68±0.39</td>
</tr>
</tbody>
</table>

Values are Mean±SEM
Table 3: Determination of antibacterial activity in methanolic extract of sea buckthorn leaf using agar-well diffusion and disc diffusion assay

<table>
<thead>
<tr>
<th>Sample (mg mL⁻¹)</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td></td>
<td>Well diffusion</td>
</tr>
<tr>
<td>0.78</td>
<td>9.2</td>
</tr>
<tr>
<td>1.56</td>
<td>12.0</td>
</tr>
<tr>
<td>3.13</td>
<td>18.2</td>
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<tr>
<td>6.25</td>
<td>20.0</td>
</tr>
<tr>
<td>12.5</td>
<td>22.0</td>
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<tr>
<td>25.0</td>
<td>24.0</td>
</tr>
</tbody>
</table>

**Antibacterial activity:** The *Hippophae rhamnoides* methanolic leaf extracts exhibited broad spectrum activity against human bacterial pathogens. The antibacterial activity of methanolic extract of *Hippophae rhamnoides* leaves assessed against three bacterial strains viz., *Escherichia coli* (MTCC 739), *Arthrobacter protophormial* (MTCC 2582), *Micrococcus luteus* (MTCC 106) in terms of zone of inhibition (mm) is presented in Table 3. The *Hippophae rhamnoides* methanolic leaf extract was found most effective against *E. coli* in which a maximum zone of inhibition (24 mm) was observed at a concentration of 25 mg mL⁻¹ whereas inhibition (8 mm) for *M. luteus*.

**DISCUSSION**

This study was aimed at evaluation of antioxidant activity and antibacterial potential of sea buckthorn, a plant commonly used in the traditional medicine system around the world to validate its scientific base in various applications (Michel et al., 2012). Phenols are the major plant compounds with antioxidant activity, which is believed to be mainly due to their redox properties, that plays an important role in adsorbing and neutralizing the free radicals, quenching singlet and triplet oxygen, or decomposition of the peroxides (Long et al., 2000). In the present study, total phenolics and flavonoids contents were determined to analyze the chemical composition of methanolic extracts of *Hippophae rhamnoides* leaves. Results showed that phenolic and flavonoids compounds were present in considerable amount in the *Hippophae rhamnoides* leaf extract. This shows that the *Hippophae rhamnoides* leaf extracts possess antioxidant properties that can help in restoring the health of humans by causing inhibition of oxidative damage diseases. The information about the total phenolic levels in *Hippophae rhamnoides* leaves supplement the view point of various workers who demonstrated polyphenols as one of the important contributors to the antioxidant and free-radical scavenging activities of various plant extracts. Our findings are in conformity with other studies on different medicinal plants and herbs (Kevers et al., 2007; Sreramulu and Raghunath, 2010).

The free radical scavenging activity of *Hippophae rhamnoides* leaf extracts was studied by their ability to decolourize the stable ABTS and DPPH free radicals, which provides information on the reactivity of compounds with a stable free radical (Badami et al., 2003). The results of this study showed that *Hippophae rhamnoides* leaf extracts are effective in scavenging ABTS and DPPH radicals, though the ABTS and DPPH radical scavenging abilities of the extracts were significantly less than those of ascorbic acid. This indicates that the extracts have the proton-donating or scavenging ability and could serve as free radical inhibitors or scavengers, acting possibly as
primary antioxidants. Results obtained from this assay further supported the validity of DPPH and ABTS assay and reconfirms the antioxidant potential of the *Hippophae rhamnoides* leaf extracts. Significant antioxidant activity showed by *Hippophae rhamnoides* leaf extract provide a scientific validation for the traditional use of these plants in traditional medicine system, however, work on isolation and identification of active compounds and its efficacy needs further investigations.

The *Hippophae rhamnoides* leaf extracts showed marked antibacterial activity against *E. coli* followed by *A. protophormia* and *M. luteus*, however, the tetracycline showed higher effectiveness over the *Hippophae rhamnoides* extracts. Phenol constituents of the plant extracts have shown potent antimicrobial properties in different studies (Kaur and Arora, 2008; Klancnik et al., 2009). The observed antibacterial activity could be attributed to the presence of phenol constituents in the *Hippophae rhamnoides* leaf extract. Further, antibacterial activity observed in the present study justified the traditional uses of *Hippophae rhamnoides* for wound healing, skin disorders and other infectious conditions (Upadhyay et al., 2010). The Higher sensitivity of the gram-positive bacteria to antibiotics has been attributed to their cell wall composition that contains peptidoglycan in the outer layer, a poor permeability barrier than negative bacteria like *E. coli* (Scherrer and Gerhardt, 1971). The well diffusion test exhibited more antibacterial activity than disc diffusion method. This could be due to better diffusion of the extract through medium in the wells, whereas time is required for the movement of substance from disc to the medium and subsequent diffusion.

The involvement of phenolic contents has been attributed for the higher antibacterial and antifungal activities of crude seed extract of *H. salicifolia* (Gupta et al., 2011; Patro et al., 2001) also reported that higher antioxygenic activity of *Hippophae rhamnoides* berry is due to higher contents of flavonoids. However, it is observed that *Hippophae rhamnoides* fruit juice with a few phenolic compounds also exhibited good antioxidant capacity but their contribution to the antioxidant effect is very low as compared to ascorbic acid (Rosch et al., 2003). The antibacterial activity of chloroform, ethyl acetate, acetone and methanol extracts of *Hippophae rhamnoides* seeds was also studied (Rosch et al., 2003; Negi et al., 2005). Similarly, Chauhan et al. (2007) showed antioxidant and antibacterial activities of aqueous extract of seabuckthorn seeds. Seed oil of *Hippophae rhamnoides* possesses several strong antioxidative and antimicrobial properties, which are due to high content of tocopherols and carotenoids present in the oil (Chen et al., 1990). In addition, antiviral and other biological activities of *Hippophae rhamnoides* leaf extracts have also been documented by Shipulina (2001).

Flavonoids are one of the major secondary metabolites synthesized by seabuckthorn. Though they are known to be synthesized by plants in response to microbial infection but are effective antimicrobial against a wide array of microorganisms (Sabir et al., 2005). The microbial activity is probably due to the ability of flavonoids to form complex with extracellular and soluble proteins of the bacterial cell walls by nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Therefore, their antimicrobial mode of actions may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins and many more. However, there evidence is also therefore direct inactivation of microorganisms by these compounds.

CONCLUSION

In conclusion, the present observations support that *Hippophae rhamnoides* is a potential source of antioxidants, their precursors and bioactive antimicrobial agents, which could be used as a natural preservative and in the development of nutraceutical formulations to overcome the
adverse effects of synthetics. This study also provide a scientific support to the ongoing studies exhibiting the effectiveness of sea buckthorn leaves in wound healing, inflammatory and free radical mediated diseases. Further investigation on the isolation and identification of active component(s) may lead to chemical entities with potential clinical uses.

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REFERENCES


