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RESEARCH ARTICLE

Assessment of antioxidative potential of different plant parts of *Hippophae rhamnoides* and *H. salicifolia* from Kinnaur and Lahaul (Himachal Pradesh)

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Abstract The present study was carried out to investigate the comparative account of antioxidative properties of various parts (leaf, bark, fruit and seed) of *Hippophae rhamnoides and H. salicifolia* from Himachal Pradesh by employing the DPPH assay and pyrogallol autoxidation assay. The stated assays were performed by involving the methanol and distilled water extracts of the plant parts. The findings from the adopted assays reveled all tested plant parts of the *Hippophae* genotypes to possess substantial antioxidative properties. In general, methanol proved to be a better solvent for extraction of the antioxidant components as evident from substantially stronger antioxidant activities of the methanolic extracts than those of the water extracts. There were quantitative variations regarding the antioxidative potential of different plant parts. Thus, the seed extracts exhibited significantly higher antioxidant properties than other tested plant parts. Likewise, the species/population specific differences were observed. For example, the antioxidant potential of leaf extracts was found to be in the following descending order: *H. salicifolia*>*H. rhamnoides* Kinnaur population >*H. rhamnoides* Lahaul population. The observations strengthen the importance of *Hippophae* leaf, bark, pulp and seed as a potent source of natural antioxidants with implications for nutraceutical and pharmaceutical product development.

Keywords: Antioxidative properties, DPPH radical, Pyrogallol autoxidation, Sea buckthorn, Superoxide dismutase

Introducation

Seabuckthorn plants are a rich source of biologically active compounds (Michel *et al.* 2012). These include several vitamins (A, C, E, K, riboflavin, folic acid), carotenoids (carotene, lycopene), phytosterols (ergosterol, stigmasterol, amyrins), organic acids (malic acid, oxalic acid), polyunsaturated fatty acids (linolenic and linoleic acids), and some essential amino acids (Upendra *et al*. 2008). In addition, flavonoids like leucocyanidin, catechin, flavanol, and flavanone are major components of seabuckthorn leaves and fruits. Different parts (seeds, pulp, fruits, and pomace) of seabuckthorn also contain valuable medicinal oils. Actually, medicinal properties of seabuckthorn have been ascribed to its rich phytochemical diversity (vitamins, flavonoids, carotenoids, fatty acids etc.). Several of these seabuckthorn phytochemicals have been tested to have a potent antioxidant activity (Rosch *et al*. 2003). Their antioxidant potential can be utilized for several important purposes like as an aid to patients undergoing cancer therapy (Xu 1994), a long-term therapy for reduction of cardiovascular risk factors (Xu 1994, Tabassum 1998), treatment of gastrointestinal ulcers (Nuzov 1991, Zhou, 1998), antimutagenic (Nersesian1990), immunological, antitumor (Yu 1993), protection against radiation (Agrawal and Goel 2002), antioxidant in coronary heart diseases (Rice-Evans and Miller 1994, Eccleston *et al.* 2002), internal and topical therapy for a variety of skin disorders (Zhao 1994) and as a liver protective agent (Zao *et al.* 1987). However, it is important to emphasize that the biochemical enrichment of a major set of these discussed medicinal compounds very much rely on the plant growth conditions. This makes it crucial to identify the best ecotypes and environmental settings for growth of those ecotypes, which could be useful for a medicine and pharmaceutical industry. On the similar lines, in the present study, the antioxidant potential of *H. rhamnoides* and *H. salicifolia* populations from Kinnaur and Lahaul was evaluated employing

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routine DPPH and pyrogallol autoxidation based assays.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) is used as a free radical to evaluate antioxidant activity of some natural products/biochemicals, where the degree of its discoloration is attributed to hydrogen donating ability of test compounds, which is indicative of their free radical scavenging potential (Shimada *et al.* 1992). Also, DPPH· can trap other radicals easily, however, in that case it does not dimerize. The advantage of this method is that DPPH is allowed to react with the whole sample and given sufficient time; DPPH can react slowly with weak antioxidants (Prakash 2001). Furthermore, the method offers advantages of being rapid, simple, and inexpensive and provides firsthand information on the overall antioxidant capacity of the test compounds/extracts.

Marklund and Marklund (1974) described another simple and rapid method for the assay of superoxide dismutase, based on the ability of the enzyme to inhibit the autoxidation of pyrogallol. Thereafter, this method has also been widely used to evaluate the antioxidant capacities of biochemical for scavenging the superoxide anion radicals originating from the autoxidation of pyrogallol. Now, the improved pyrogallol autoxidation assay is regarded as the most reliable, cheap, and convenient method to estimate the antioxidant scavenging activity on superoxide anion radical (Li 2012). Recently, due to its rapidity and wide applicability, it has also been used in the determination of other antioxidants such as polyphenols (Liu *et al*. 2007), phenolic acids (Wu *et al.* 2007), tannins (Gu *et al.* 2008), flavonoids (Li *et al.* 2008), anthraquinones (Zou *et al*. 1997), polysaccharides (Zhong *et al.* 2010) and even various nutritional additives (Scorei *et al.* 2005). Zhang *et al.* (2016) assessed and observed that pyrogallol autoxidation could be successfully used to determine the antioxidant capacity of ascorbic acid and rutin, which correspondingly suggests the feasibility of its use to measure the superoxide anion radical scavenging activity. However, pyrogallol autoxidation cannot be used to evaluate the superoxide anion scavenging activity of catechin and gallic acid, although their good antioxidant capacity was confirmed by the 1, 1-diphenyl-2-picrylhydrazyl assay. The trend in antioxidant activity obtained by using the DPPH and pyrogallol method is comparable to trends found using other methods. These methods are unique in allowing use of methanol and water as extraction solvents with no interference in the reaction of the sample with DPPH and pyrogallol. This is useful as using methanol as well as water facilitates the extraction of a variety of antioxidant compounds from the plant tissue, and does not limit the compound types that can be extracted and tested.

In the present study, the antioxidative potential of methanol and water extracts of different plant parts namely, leaves, fruits, bark and seeds of *Hippophae rhamnoides* and *H. salicifolia* from Kinnaur and Lahaul (Himachal Pradesh) have been evaluated employing the DPPH and pyrogallol autoxidation assays. The results confirm earlier studies with other seabuckthorn populations, where free radical inhibition was reported by leaf and fruit methanolic extracts (Geetha *et al.* 2002). But very less data on these aspects is available for *Hippophae spp* of Kinnaur and Lahaul regions.

Materials and methods

Preparation of Extracts

Different plant parts of two species of seabuckthorn namely, *Hippophae rhamnoides* L. and *H. salicifolia* D. Don were collected from natural population growing at Pooh (2700-2800 m asl) Kinnaur and Pattan valley (2700-3000 m asl) Lahaul(H.P.). The powdered seabuckthorn leaves, fruits, seeds and bark were extracted with methanol and distilled water. The powder was soaked in above solvents (1:20 w/v) at room temperature. After 24 h, the supernatant was decanted off. The supernatant was filtrated and filtrate dried till a solid mass was obtained. Finally, the dried extracts were dissolved in methanol and distilled water. Dissolved extracts were stored at 4^0C till further analysis.

DPPH Scavenging Activity

The DPPH (1, 1- diphenyl-2-picrylhydrazyl) scavenging activity was determined using the method described by Blois (1958). A 0.1 mM solution of DPPH was prepared by using methanol. Two ml of this solution was added to 2 ml of extract (concentration series; 10-60 µg/ml) and the mixture

was kept in dark for 20 min. The absorbance of the samples and control solutions were determined at 517 nm. The % DPPH radical scavenging activity was calculated as follows:

$$DPPH\ radicalscavenging\ activity\ (\%) = \frac{A_{C-T}}{A_C} x100$$

Where C, Control; T, Treatment

Pyrogallol autoxidation assay

Pyrogallol in a basic solution rapidly autoxidizes forming superoxide radical and lemon-yellow colored product which can be quantified spectrophotometrically by absorbance measurement at 325 nm. The reaction mixture contained 2.8 ml of Tris-hydroxymethyl aminomethane (pH 8.0, added with 1 mM EDTA) and 100 µl of 60 mM pyrogallol (prepared in 1 mMHCl). To assess the effect of various leaves, fruits, seeds and bark extracts of seabuckthorn, these were appropriately added to the reaction mixture. The change in absorbance could be taken as index of free radical generation (Marklund and Marklund, 1974). The free radical scavenging ability was calculated as:

$$Inhibitionofpyrogallolautoxidation\ (\%) = \frac{\Delta A_{325\ C} - \Delta A_{325\ T}}{\Delta A_{325\ C}} x\ 100$$

Where C, Control; T, Treatment

Results

DPPH radical scavenging activity:

In this assay, the DPPH radical serves as the oxidizing substrate, which can be reduced by an antioxidant compound to its hydrazine derivative via hydrogen donation. The extracts were assessed against DPPH radicals to determine their free radical scavenging properties. The DPPH assay revealed substantial scavenging (antioxidant) effects of both methanol and water extract of different plant tissues from different *Hippophae* species and populations. The radical scavenging activity was found to be invariably greater in case of the methanol extracts as compared to the water extracts at all the tested concentrations. The radical scavenging activity increased with increasing concentration of both methanol and water extracts, confirming the validity of extraction procedure as well as DPPH assay. Comparison of radical scavenging activity of methanol and water extract showed that higher scavenging effect was observed for methanol leaf extract especially in lower concentrations in all studied plant parts. At 10 µg/ml methanolic leaf extract concentration, the radical scavenging activity was found to be 36.7, 36.2 and 56.2% in *H. rhamnoides* (Kinnaur population), *H. rhamnoides* (Lahaul population) and *H. salicifolia* (Kinnaur), respectively. With the increase in concentration, the activity increased and at 60 µg/ml concentration, it became almost similar in all genotypes. A 92, 92.2 and 93.5% radical scavenging activity was evident at 60 µg/ml methanolic leaf extract in *H. rhamnoides* (Kinnaur), *H. rhamnoides* (Lahaul) and *H. salicifolia* (Kinnaur) respectively (Fig. 1A). In case of leaf water extract, the values for radical scavenging activity were 23.3 and 86.9% in *H. rhamnoides* (Kinnaur), 26.9 and 78% in *H. rhamnoides* (Lahaul) and 36.3 and 89.3% in *H. salicifolia* (Kinnaur) at 10 and 60 µg/ml, respectively (Fig. 1B). In comparison, the methanolic extract proved more effective for scavenging radical. In case of the bark extracts, same pattern was recorded with higher radical scavenging activity in methanol extract in all three studied plants. A 59.8, 93% (*H. rhamnoides* Kinnaur), 62.9, 94.6% *(H. rhamnoides* Lahaul) and 35.6, 93.3% (*H. salicifolia*) radical scavenging activity was detected at 10, 60 µg/ml concentration of methanolic extract, respectively (Fig. 1C). The scavenging effect of water extract was 42.6, 92% in *H. rhamnoides* Kinnaur, 37.6, 93.5% in *H. rhamnoides* Lahaul population and 24, 92.3% in *H. salicifolia* at 10, 60 µg/ml bark water extract, respectively (Fig. 1 D).

As water extract showed the low reducing power than methanol extract, the radical scavenging activity of fruit methanolic extract increased with increasing concentration of both methanol and water extracts but no significant differences observed among the studied genotypes at all concentrations (Fig. 2A, B). The DPPH reducing power due to seed extract in methanolic extract was 82.7 and 91.5% (*H. rhamnoides* Kinnaur), 74.4 and 92% (*H. rhamnoides* Lahaul) and 61.2 and 94.2% (*H. salicifolia*) at 10 and 60 µg/ml, respectively (Fig. 2C). These values for DPPH reducing power with methanolic extract were again more than the values obtained for water extract with all described types of plants (Fig. 2C,

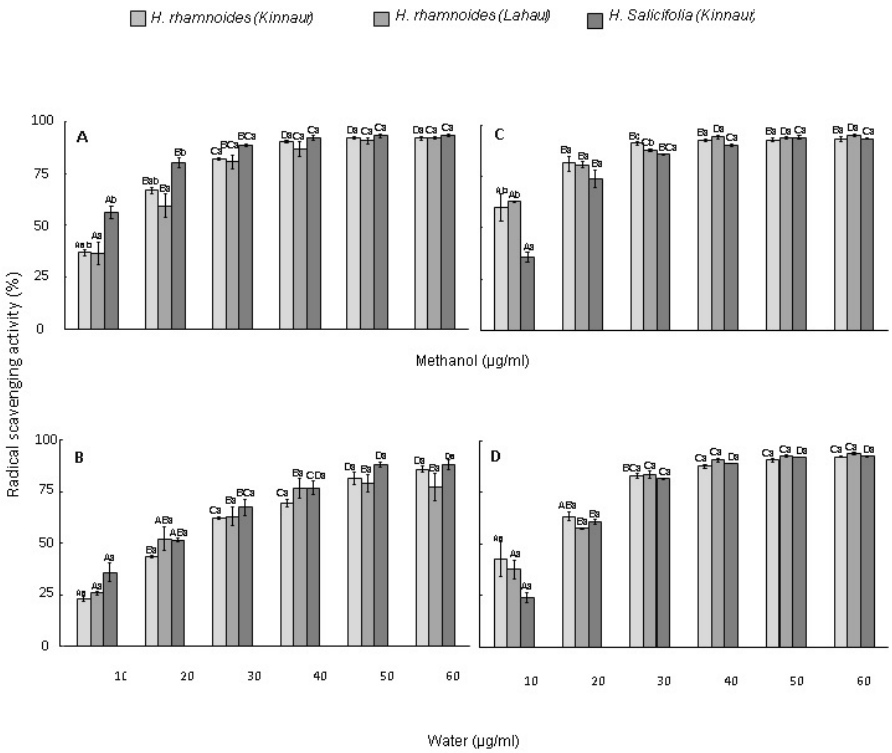

Figure 1: DPPH radical scavenging activity of leaf (A. Methanol; B. Water) and bark (C. Methanol; D. Water) extracts of *H. rhamnoides (*Kinnaur and Lahaul populations) and *H. salicifolia*. Data are presented as mean ± SE n=3. Upper case letters represent significant differences among different treatments within a genotype and lower case letters represent significant differences among *Hippophae* genotypes at same treatment (Tukey's test; $p<0.05$)

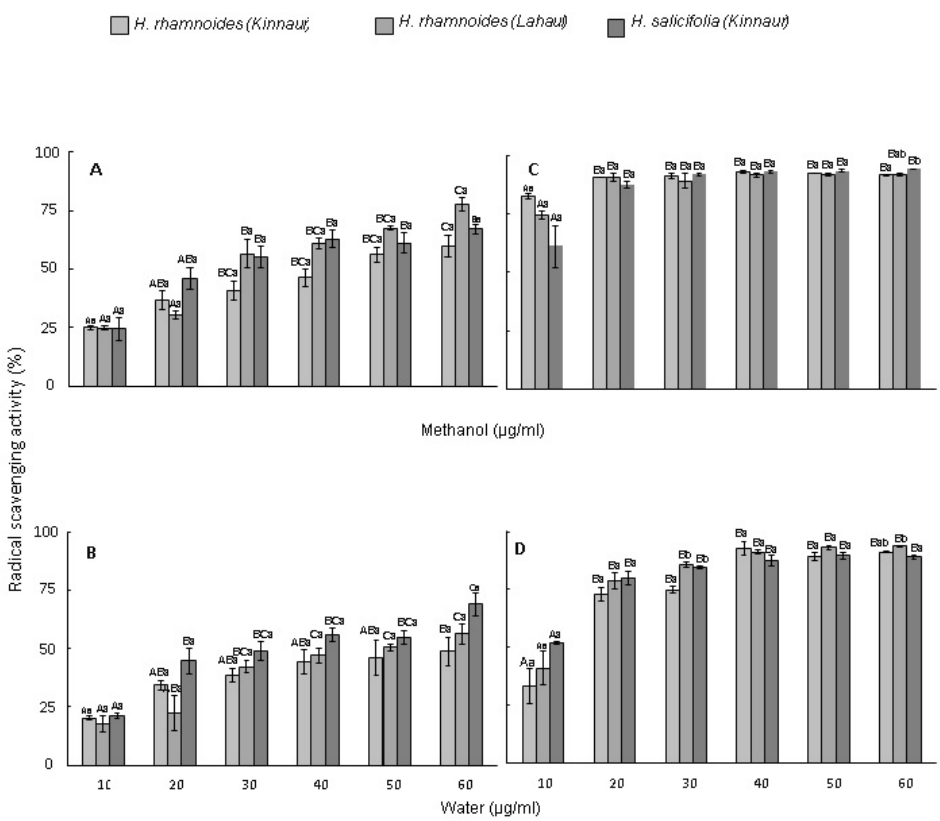

Figure 2: DPPH radical scavenging activity of fruit (A. Methanol extract; B. Water extract) and seed (C. Methanol extract; D. Water extract) extracts of *H. rhamnoides (*Kinnaur and Lahaul populations) and *H. salicifolia*. Data are presented as mean ± SE n=3. Upper case letters represent significant differences among different treatments within a genotype and lower case letters represent significant differences among *Hippophae* genotypes at same treatment (Tukey's test; $p<0.05$)

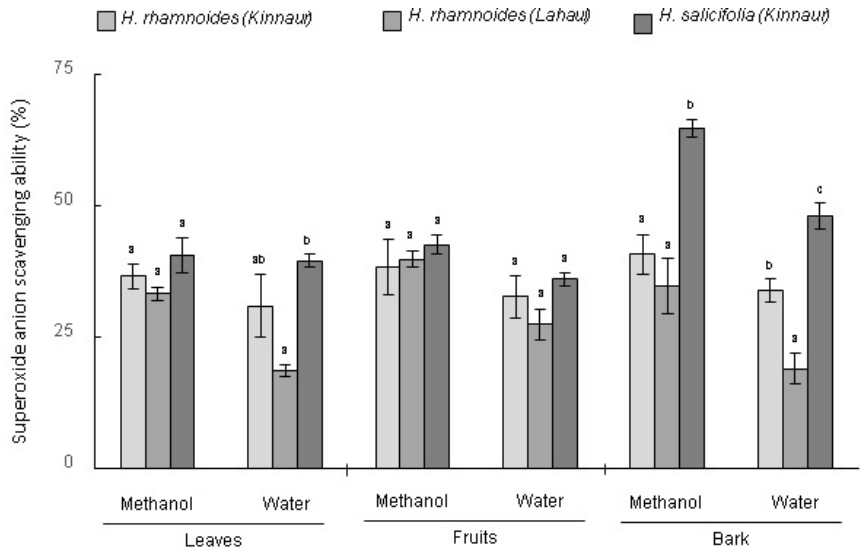

Figure 3: Effect of methanol and water extracts of leaves, fruits and bark of *H. rhamnoides (*Kinnaur and Lahaul populations) and *H. salicifolia* on pyrogallol autoxidation within 1 minute. Data are presented as mean ± SE n=3. Lower case letters represent significant differences among *Hippophae* genotypes at same solvent (Tukey's test; $p<0.05$)

D).

The antioxidant potential of studied plants was determined to be in the following descending order in case of leaf methanolic extract: *H. salicifolia*>*H. rhamnoides* (Kinnaur) >*H. rhamnoides* (Lahaul). In case of bark and seed methanolic extracts(10 µg/ml), these values were higher in *H. rhamnoides* populations as compared to *H. salicifolia* and due to bark water extract the scavenging effect was almost similar in all three types of studied plants. Comparison among different plant parts showed that reducing power was highest in seed followed by bark, leaves and fruit especially in lower concentration of extracts.

Pyrogallol autoxidation assay

To monitor the free radical scavenging properties of different plant part extracts of *Hippophae* species/populations, a pyrogallol autoxidation assay was adopted. The A_{325} nm value of the reaction mixture was measured for 1 min. The inhibition of pyrogallol autoxidation, a measure of free radical (superoxide anion) scavenging, was observed at 1 minute in all studied extracts. For example, inhibition of pyrogallol autoxidation was 38.3 (*H. rhamnoides* Kinnaur), 39.8 (*H. rhamnoides* Lahaul) and 42.4% (*H. salicifolia*) when 100 µl methanolic fruit extract was added to the assay. In case of the addition of fruit water extract, these inhibition values were less (Fig. 3).

Due to the bark extracts, the inhibition of pyrogallol autoxidation was observed to be 40.7, 34.7, 64.7% (methanol extract) and 33.8, 19, 48% (water extract) (Fig. 3). The inhibition of pyrogallol autoxidation was observed to be 36.6, 33.2 and 40.6% (leaf methanol extract) and 30.9, 18.7 and 39.5% (leaf water extract), respectively in *H. rhamnoides* (Kinnaur), *H. rhamnoides* (Lahaul) and *H. salicifolia.* In a comparative analysis, the inhibition of pyrogallol autoxidation was highest in *H. salicifolia* extracts when compared to other counterparts.

In brief, the DPPH assay and pyrogallol autoxidation assay revealed strong antioxidative properties of the studied plant parts of two *H. rhamnoides* populations and *H. salicifolia*. The antioxidative properties were species/population-specific, plant part-specific and extract-specific.

Discussion

From the study it was found that the seed had significantly higher antioxidant properties than other studied plant parts. This result is in agreement with data reported by Sharma *et al.* (2008) and Michel *et al.* (2012) in *H. rhamnoides* where seeds had more antioxidant capacity than leaves. High antioxidant activity in the seeds of *H. salicifolia* was also reported by Saikia and Handique (2012). As phenolic compounds possess a high potential to scavenge radicals by their ability to donate a hydrogen atom from their phenolic hydroxyl groups (Sawa *et al.* 1999), higher antioxidant properties of seeds may be due to its higher phenolic contents. The seabuckthorn extracts were

also tested for pyrogallol autoxidation assay. It was designed specifically to determine free radical-scavenging activity for superoxide dismutase (Marklund and Marklund, 1974). All the studied extracts inhibited the rate of autoxidation indicating that extracts having antioxidant properties. Marklund and Marklund (1974) noted the inhibition of pyrogallol autoxidation in the presence of enzyme superoxide dismutase.

The comparison of antioxidant potential of studied plants showed the following order in case of leaf extract: *H. salicifolia*>*H. rhamnoides* Kinnaur >*H. rhamnoides* Lahaul population. Ranjith *et al.* (2006) estimated the vitamin C and polyphenols content in the three sea buckthorn species (*H. rhamnoides*, *H. salicifolia* and *H. tibetana)* from India and showed vitamin C content in *H. salicifolia* to be 10-fold greater than that in other species. A higher phenolic content in *H. salicifolia* as compared to *H. rhamnoides* has also been confirmed. Such a difference in contents might explain the higher antioxidative potential observed in case of *H. salicifolia.* Variation within same species could be because of genetic diversity in varied habitats.

This study has demonstrated the comparative account of antioxidative properties of various solvent extracts of leaf, bark, fruit and seed of *Hippophae spp*. Methanol was found to be a better solvent for extraction of antioxidants that showed strong antioxidant activities compared to the water extract. This could be extraction of varied antioxidative compounds with different solvents. Truong *et al.* (2019) observed the highest levels of bioactive molecules (phenolics, flavonoids, alkaloids, and terpenoids) in methanolic extracts. So the result confirming the extraction solvents affect the antioxidant activities of the plant extract. Thus, the *Hippophae* leaf, bark, pulp and seed can be considered as a potent source of natural antioxidants and can be exploited for developing nutraceutical and pharmaceutical products.

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