Hippophae Genetic Resources of North East India: Diversity, Traditional Uses and Antioxidant Potential

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ABSTRACT

Hippophae salicifolia D. Don., have been located, identified and collected from the Arunachal Pradesh and Sikkim, the two Himalayan states of North Eastern Region of India. This is the first authentic collection of *Hippophae* species in Arunachal Pradesh which was hitherto unreported. Extensive collection was also made in Sikkim particularly at Lachen and Lachung valleys. The collected accessions were enumerated giving information on plant morphology, habit, habitat, ecology, distribution and topography using GPS. The detail characteristics of plant morphology, leaf, fruit and seed were recorded on a descriptor data sheet. Phenology of the species was studied and recorded. Phenology was comparable in *Hippophae salicifolia* species found in Arunachal Pradesh and Sikkim. Information was collected on traditional uses of *H. salicifolia* through interviews with the local inhabitants. Quantitative biochemical evaluation of the fruits and evaluation of antioxidant properties of leaf and fruit of selected accessions was done. Quantitative biochemical characterization of ripe fruits was made for acidity percentage, calculation of juice content and residue percentage using a standardized protocol. Antioxidant activity of the fruit pulp and seeds was measured separately using total phenolic content, reducing power and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays. The extraction yield and total phenolic contents were significantly higher in seed extracts. Methanolic extract of seeds showed the highest reducing power and DPPH-radical scavenging activity.

Keywords: *Hippophae salicifolia,* diversity, antioxidant potential, North-East India, Arunachal Pradesh and Sikkim Himalayas.

INTRODUCTION

North East (NE) India, comprising of eight states namely, Arunachal Pradesh, Assam, Meghalaya, Manipur, Mizoram, Nagaland, Tripura and Sikkim, houses a large number of wild and indigenous medicinal and economic plants with less or no assessment concerning its distribution, diversity and uses. Seabuckthorn *(Hippophae* spp., Family: *Elaeagnaceae*) is one such group, which is well described and characterized from other habitats of India, particularly Western Himalayas but without satisfactory information from NE India. Reports are available on the morphological and biochemical variations in natural populations of seabuckthorn in Himachal Pradesh and Ladakh region of Jammu and Kashmir (Dwivedi *et al.,* 2009; Khosla *et al.,* 1994; Sankhyan *et al.,* 2004; Singh, 1994; Singh 1998; Singh and Dogra, 1995). A study was also conducted to determine the variations in morphological and biochemical characters of natural populations of seabuckthorn growing in Harsil and Gangotri area of Uttaranchal located in Garhwal Himalayas (Yadav *et al.,* 2006). However, there is not much data available on the distribution, population, local uses and genetic variations of the species that grow in NE India except an ecological report from North Sikkim (Basistha *et al.,* 2010).

Seabuckthorn is an important multi-use wild plant species with its nutritional and medicinal attribute. It is a deciduous thorny shrub to small tree. It prefers to grow in low humid, alluvial gravel, wet landslips and riverside with brown rusty-scaly shoots (Lu, 1992). In recent years, seabuckthorn is gaining importance in large scale cultivation in different regions of the globe for its fruits and also due

to its wide adaptability that fulfills the afforestation requirements. Seabuckthorn has been planted as orchards for commercial purposes and for greening of hilly regions in countries such as Russia and Germany (Lu Rongsen, 1992). Seabuckthorn is primarily valued for its golden-orange fruits, which are very rich in vitamins A, B_p B_{12}, C, E, K and P; flavonoids; and oil rich in essential fatty acids. It has been shown to have potent antioxidant activity, mainly attributed to its flavonoids, vitamin C, or both vitamins E and C (Li and Schroeder, 1996; Rosch, 2004; Varshney and Tyagi, 2004). The leaves, apart from being very good fodder, are used in the preparation of herbal tea and cakes (Xing, 2003).

Seabuckthorn seed oil has been used in Asia for treating various skin conditions. It is rich in carotenoids, tocopherols, rare fatty acids and phytosterols. It effectively combats wrinkles, dryness and other symptoms of malnourished or prematurely aging skin and is utilized in anti-aging skin cream and lotions (Parimelazhagan *et al.,* 2004). Triacylglycerol, a major constituent of seabuckthorn seed oil extensively is used in a variety of cosmetic formulations. Seabuckthorn is traditionally used in the treatment of gastric ulcers which has been confirmed through laboratory studies (Xing, 2002). Seed oil is very rich in an Omega-7 series fatty acid (palmitoleic acid), which can act as a replacement for other fatty acids in cell membranes (Methew *et al.,* 2014). Biochemical studies have already contributed to the taxonomy of some sections of *H. rhamnoides* (Kallio *et al.,* 2002).

Variability in the genus *Hippophae* is huge, and its conservation and scientific exploration for crop improvement are necessary for which a comprehensive set of morphological descriptors that accommodate all possible variations is required (Lian and Chen, 1993; Lian *et al.,* 1995, 1998). The variability of seabuckthorn in India is vast and the country has the potential to emerge as a leader in the improvement and commercial exploitation of this crop (Mathew *et al.,* 2014). Several efforts to characterize *Hippophae* germplasm using morphological characters had made use of 30 quantitative and 7 qualitative characters. These studies had clustered *H. rhamnoides* and *H. tibetana* together, and *H. salicifolia* in a different but closer cluster (Hyvonen, 2003). However, Rousi (1971) and Huang (1995) pointed to a closer association between *H. rhamnoides* and *H. salicifolia* than between *H. rhamnoides* and *H. tibetana.* DNA-based markers have proved to be efficient in bringing out exact genetic distances among and within the species in *Hippophae* and were reported to be superior to morphological methods (Bartish *et al.,* 2000).

In the present work, we aimed to explore the availability of seabuckthorn in Arunachal Pradesh and Sikkim and to study their population distribution, traditional uses as well as diversity in terms of morphology and biochemical composition.

MATERIALS AND METHODS

Field survey and collection of Hippophae sample

Intensive field surveys were conducted in Arunachal Pradesh and Sikkim during the year 2009-2012. *Hippophae* grown areas were identified with the help of local information. However, there was no local information available on the availability of any *Hippophae* species in Arunachal. We undertook repeated and intensive field trips in selected areas of Arunachal and Sikkim. Physical information of the collection sites viz., map location, geographical position and altitude using GPS were recorded on the spot. Individual plants were considered as accession and marked. From each accession shoot tips, leaves stem cuttings and ripe fruits were collected for analysis. The accessions were enumerated giving information on sex and habit and serially numbered giving prefix 'GU-AP' for Arunachal and 'GU-SK' for Sikkim collection. Photographs were taken of *Hippophae* population, habitat and individual plants including leaf, fruits, plant size and branching pattern.

Morphological and biochemical characterization using descriptors

Various data on morphology of each accession were recorded as per the descriptor data sheet designed for the "National Network Programme on *Hippophae* (2009-12)" funded by DBT, New Delhi. Both qualitative characters and quantitative characters on plant morphology, leaf and fruits were recorded. Accessions wise geographic information was recorded using GPS. Plant height, canopy and inter-branch length of each accession was documented during collection. The leaf length, leaf width, peduncle length, color and phyllotaxy was recorded in the data recorder. The fruit colour and shape, seed colour and shape were also recoded. The number of fruits per 10 cm branching, average fruit weight, juice percentage, acidity percentages was determined. Phenology of the species was studied. The analysis of fruit juice quality, including total soluble solids content (TSS) and titratable acidity (TA) were performed following the procedures mentioned by Saleem *et al.* (2007).

Ethno-botanical information

Information on local uses of seabuckthorn was collected by making interview with the local inhabitants of *Hippophae* growing areas of Arunachal Pradesh and Sikkim. Both elderly as well as young people were interviewed.

Antioxidant activity of seabuckthorn

To evaluate the antioxidant potential of various parts of *Hippophae salicifolia,* three different aspects were studied that included determination of the total phenolic contents, determination of reducing power and DPPH-radical scavenging activity. Extracts of leaf, bark, pulp and seed were prepared using various solvents like methanol, acetone, chloroform and petroleum ether.

1) Determination of the total phenolic contents: The amounts of phenolic compounds in the extracts were determined following Folin-Ciocalteu method (Waterman and Mole, 1994) with certain modifications. The experiment was carried out in triplicate and the content of total phenolic compounds was calculated using a standard curve prepared with Gallic acid.

2) Determination of reducing power: The reducing power was determined using method described by Yildirim *et al.* (2001). Substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

3) DPPH-radical scavenging activity: The free radical scavenging activity was evaluated by DPPH method (Shyur *et al.,* 2005) with slight modifications. The test was performed in 3ml reaction mixture containing 2 ml of 0.1 mM DPPH-methanol solution, 0.9 ml of 50 mM tris-HCl buffer (pH 7.4) and 0.1 ml of test extract at different concentrations or catechin (standard reference). The mixture was incubated at room temperature for 30 minutes and then the absorbance was measured at 517 nm. Radical scavenging activity was represented as % inhibition of DPPH radical and was calculated by the following formula:

$$\% \text{ inhibition} = (\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}} / \text{Absorbance}_{\text{Control}}) \times 100$$

IC_{50} is defined as the concentration of substrate that causes 50% loss of the DPPH activity (Molyneux, 2004). IC_{50} of reference (catechin) was used for comparison to IC_{50} of the plant extracts.

RESULTS AND DISCUSSION

Morphology and distribution

The *Hippophae* species collected from Arunachal Pradesh and Sikkim were identified as *Hippophae salicifolia* D. Don., based on the morphological characters and confirmed with the help of available text and by comparing with the authentic specimen at the Herbarium of Botanical Survey of India, Eastern Circle, Shillong. The species was found to grow at high altitudinal areas (between 2007 m

and 2997 m) of North Sikkim and Tawang district of Arunachal Pradesh. Plants were found in small clusters on road side, amid forest and river side. The habit of the plant showed variation from small shrubs (60 cm height) to large tree (> 9 meter height). The species was found to be dioecious with distinct male and female plants. Plants were found thorny and the shrubs were found to be thornier than the trees. Large trees were almost thorn less. Branched thorns were also recorded. Yellow to orange yellow berries were found in clusters attached to the stem. Both major and minor branches of the stem bear large number of fruits and about 10 cm long portion of a branch bear 10-90 numbers of fruits. The general attributes of the species regarding distribution, habitat, local name, plant habit and detailed plant morphology were recorded and presented in the Table-1. Plant population, tree habit and fruiting plants are shown in Plate-1. Phenology of the species have been studied and recorded. Phenology is comparable in the *Hippophae salicifolia* species found in Arunachal Pradesh and Sikkim (Table 2).

The biochemical parameters of the fruits viz., acidity percentage, total soluble solids (TSS)-acidity ratio, juice percentage and residue percentage showed a wide range of variability. The acidity in juice ranged from 5.30% to 7.48% and TSS - acidity ratio ranged between 1.02 and 1.76. The residue percentage ranges from 14.39 % to 21.45%. The juice yield ranged from 74.3% to 84.29% (Table 3).

(a)

(b)

(c)

(d)

(e)

Plate 1. *Hippophae salicifolia* D. Don.

a) Natural population on the bank of the river Nyamjang-chhu, Sikkim.
b) A large tree in Sikkim. c) Fruiting tree in Arunachal. d) A fruiting branch (Sikkim).
e) Close up view of fruits (Sikkim).

Traditional Uses

It was observed that *Hippophae salicifolia* is known to the local inhabitants of *Hippophae* growing areas in Arunachal Pradesh and Sikkim. The species is locally known as 'Taru' and 'Tarubo' in Sikkim and 'Tarusen' in Arunachal Pradesh. However, the traditional and local uses of seabuckthorn were insignificant in these areas. Plants were generally considered as weed and were not much of importance in Arunachal Pradesh. However, in Sikkim, the local inhabitants were using the ripe berries for preparation of home-made juice. The juice was mainly used as cleansing liquid for metal utensils. The juice was also used for dying of wools. Very rarely the juice mixed with water was consumed as sour drink. In both the states, the old trees were seen to cut for firewood.

Table 1. Summary of general attributes of *Hippophae salicifolia* D. Don. in Arunachal Pradesh and Sikkim Himalayas

Descriptors	***Attributes***
Common name	Seabuckthorn
Local Name	Taru, Tarubo (Sikkim), Tarusen (Arunachal)
Scientific name	*Hippophae salicifolia* D.Don
Collection area	Arunachal (Zimithang), Sikkim (Lachen and Lachung)
Altitude	2007-2997m
Accessions recorded	200 Nos. (18 Arunachal, 182 Sikkim)
Male	75 accessions
Female	125 accessions
Habit - Tree	77 accessions (24 male & 53 Female)

Habit - Shrub	123 accessions (51 male & 72 Female)
Habitat	Road side, amid forest and river side.
Roadside	67 accessions (30 trees, 37 shrubs)
Riverbanks	110 accessions (39 trees, 71 shrubs)
Amid forest	23 accessions (14 trees, 9 Shrubs)
Thorns	Both male and female plants bear thorns, 5-12 cm long.
Plant height	Ranges from 60 - 900 cm approx.
Leaf length	4 -9.8 cm
Leaf width	1 - 1.9 cm
Fruits	Yellow to orange yellow berries in clusters attached to the stem.
Fruit length	0.6- 1.0 mm
Fruit width	0.6 - 0.9 mm
Fruit weight (100)	17.94 - 34.31 g
Seeds	Seeds are brown and oval shaped.
Seed weight (1000)	10 - 16 g
Juice percentage	70 - 88%.

Table 2. Phenology of *Hippophae salicifolia* in N-E India

Season	***Attribute***
December - February	Leaf shading
March - April	Leaf flashing
May - June	Flowering
June - September	Fruiting
October - December	Fruit ripening

Table 3. Quantitative biochemical traits of fruits of 10 selected accessions of *Hippophae salicifolia* D. Don grown in Arunachal and Sikkim

Accessions No.	***Juice percentage (%)***	***Residue percentage (%)***	***Fruit acidity (% citric acid)***	***Fruit TSS acidity ratio***
GU-SK-01	78.21±0.03	21.45±0.02	5.48±0.14	1.62±0.01
GU-SK-02	81.26±0.02	13.33±0.03	5.90±0.08	1.48±0.02
GU-SK-05	78.24±0.03	14.39±0.04	5.38±0.04	1.02±0.04
GU-SK-06	82.18±0.04	18.82±0.04	5.44±0.03	1.68±0.02
GU-SK-10	70.30±0.02	16.39±0.05	5.74±0.02	1.34±0.02
GU-SK-52	84.29±0.13	19.42±0.07	7.48±0.13	1.74±0.02
GU-SK-58	83.26±0.04	17.34±0.09	5.30±0.04	1.54±0.10
GU-SK-94	83.38±0.02	20.83±0.06	6.14±0.02	1.63±0.03

GU-AP-14	83.47±0.01	12.37±0.02	5.47±0.05	1.28±0.02
GU-AP-03	86.32±0.03	17.63±0.05	5.38±0.02	1.36±0.03

Values represent means ± SE of three replicates Antioxidant properties

Total phenolic content: Plants contain many phenolic compounds which act as reducing agents and antioxidants (Bursal and Koksal, 2011). In *Hippophae salicifolia,* high amount (98.7 mg/g) of phenolic compounds was recorded in methanolic leaf extracts followed by acetone, chloroform and petroleum ether extracts. Methanolic extracts of barks also showed 85.3 mg/g phenolic content while pulp showed 123.5 mg/g. The seed extracts were found to possess high phenolic content compared to leaf, bark and pulp extracts. The methanolic seed extract was found to possess high amount of phenolic content with 156.7 mg/ g followed by acetone 117.6 mg/g. Chloroform and petroleum ether extracts showed 53.9 mg/g and 42.3 mg/g respectively. We achieved similar results while analyzing various *Hippophae* samples of Arunachal and Sikkim (Saikia and Handique, 2014).

Reducing power: The reducing ability may serve as a significant indicator of potential antioxidant activity (Meir *etal.,* 1995). In the present study, various solvents extracts of different parts of *Hippophae* were examined for reductive capabilities through the potassium ferricyanide reduction method. The pulp and seed extracts exhibited higher reducing ability than the leaf and bark extracts. The methanolic extract showed the highest activity, followed by the acetone, chloroform and petroleum ether extracts. When compared to the ascorbic acid, the methanolic fraction showed higher activity ($P<0.05$) at all concentrations. The correlation coefficient ($R2$) between the total phenolic content versus reducing power leaf, bark, pulp and seed extracts of *H. salicifolia* was found to be 0.93, 0.95, 0.96 and 0.98, respectively. These correlation coefficients imply that the reducing power of different extracts was positively correlated with their corresponding antioxidant activities.

DPPH radical scavenging activity: Various solvent extracts of *H. salicifolia* showed potential free radical scavenging activity. Methanolic extracts of different plant parts showed highest radical scavenging activity followed by the acetone, chloroform and petroleum ether extracts. The seed and pulp extracts exhibited significantly higher antioxidant properties than leaf and bark extracts. This result was also correlated with reducing power assay where pulp and seed extracts showed higher activity than the leaf and bark extracts. The antioxidant potential of the extracts of various plant parts could be arranged in the order of: seed > pulp > leaf > bark extracts. We had similar results in several other samples of *H. salicifolia* (Saikia and Handique, 2014). These results were also in accordance with the earlier findings where seed extract showed more antioxidant capacity than the leaf extracts (Sharma *et al.,* 2008; Michel *et al.,* 2012).

CONCLUSION

The results of the present study revealed that only one species of seabuckthorn, *Hippophae salicifolia* D. Don is grown in Arunachal Pradesh and Sikkim. The plants are found at the altitude of2007-2997 meter amid forests, road sides and riversides. The habit of the plant ranges from shrub to tall trees. The species is still under-utilized in Arunachal Pradesh and Sikkim. However, natural population of seabuckthorn is gradually diminishing in these areas due to habitat destruction for various development activities.

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