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Pharmacognostic Studies on the Leaves of *Hippophae rhamnoides* L. and *Hippophae salicifolia* D. Don

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ABSTRACT

High altitude herbal medicines offers remedy for many diseases, particularly for which no medicine is available. As high altitude plants grow under stressful situations and exposed to high UV radiations, they are reported to have immense potential. Various scientific studies conducted on *Hippophae* species (Elaeagnaceae) during the last decade confirm its medicinal values. The present study was carried out with a view to lay down standards which could be useful to detect the authenticity of these medicinal plants, further the microscopical and physiochemical evaluation were studied to reveal the differences among *Hippophae rhamnoides* L. and *Hippophae salicifolia* D. Don. For the microscopic observation, free hand cross sections of the leaves of *Hippophae rhamnoides* and *Hippophae salicifolia* were stained with phloroglucinol and hydrochloric acid (1:1) and studied according to standard methods. Physiochemical analysis was carried out as per WHO guidelines on quality control methods for medicinal plants. The cross section of both the species of leaves were almost similar and revealed that the lower epidermal cells were completely covered with apressed stellate trichomes and abundant sunken stomata. Ash values of *Hippophae rhamnoides* leaves, showed higher ash content, compared to *Hippophae salicifolia*. The Pharmacognostic and phytochemical profile of *Hippophae rhamnoides* and *Hippophae salicifolia* are highly dependent on environmental adaptability of the plants. The present study helps in identification and differentiation of both the species of *Hippophae*.

Key words: *Hippophae rhamnoides* L., *Hippophae salicifolia* D. Don, Seabuckthorn, stellate trichomes, sunken stomata

INTRODUCTION

Plants grown in the Himalayan region is a rich repository of medicinal wealth, occupying an important place in *vedic* treatise, due to its wide range of altitudes, topography and climatic conditions, medicinal species found in this part of India is more commonly used by the local communities, since time immemorial for curing various ailments of human kind (Li *et al*., 2006). Leaf morphological and physiological characteristics are extremely variable across environmental

gradients in most of the woody plant due to their adaptable nature. The pharmacognostical study of the plant material is mainly concerned with the description, identification including history of use, commerce, collection, preparation and mainly for quality control purpose. To ensure reproducibility and quality of plant based drugs, authentication is essential (WHO, 1992). The present pharmacognostic study on two Seabuckthorn species provides the preliminary pharmacognostic profile and standards of these plants which has shown multi-dimensional pharmacological activities. According to World Health Organization, the macroscopical and microscopical description of medicinal plant is the main parameter for establishing the identity and purity assessment of medicinal plants.

Hippophae genus (Elaeagnaceae) consists of five species, based on morphological variations viz., *H. rhamnoides* L., *H. salicifolia* D. Don, *H. neurocarpa* Liu and He, *H. tibetana* Schlecht and *H. goniocarpa*. The main species of Seabuckthorn distributed in India are *H. rhamnoides, H. salicifolia* and *H. tibetana*. Seabuckthorn is a naturally growing thorny, deciduous bush, indigenous to the Himachal Pradesh, is fast emerging as an important crop due to its nutritional and medicinal properties. With the commercialization of *H. rhamnoides* products, demand of raw material has increased manifold (Ting *et al*., 2011). In India it grows mainly at high altitude, cold arid conditions of Ladakh, Himachal Pradesh. *H. rhamnoides* possess characteristic features like terminal and lateral thorns. The thorn per 10 cm was observed 4-6 per 10 cm in young plants. The plants rapidly spread by rhizomatous roots and suckers which quickly spread and colonize in the adjacent areas. The root is light-colored and thick (Kritikar and Basu, 2003).

Various scientific studies conducted on *H. rhamnoides* during the last decade confirm its medicinal and nutritional values. The important phytochemicals present in this plant is flavonoids, carotenoids, fatty acids etc., showing various biological activities (Li *et al*., 2006). More than 100 different kinds of phyto-nutrients and bioactive substances are present in the leaves and berries including vitamins, fatty acids, free amino acids and minerals. The vitamin-C content is 5-10 times higher than any other known fruit or vegetable (Zu *et al*., 2006). Hippophae possesses common know biological activities like antitussive, carminative (Li and Jie, 2010). Leaves are used in gastro intestinal and dermatological disorders and have been applied as compress form in rheumatoid arthritis (Suryakumar and Gupta, 2011).

The plant Seabuckthorn has shown multi-dimensional therapeutic activity including immuno-modulatory, neuro-modulatory, anti-oxidant, anti-inflammatory and anti-stress roles (Dubey *et al*., 2002). The immuno-modulatory property of *H. rhamnoides* is well established in several studies. The ripe fruits of *H. rhamnoides* contain malic acid, oxalic acid, phospholipids and vitamins like A, B complex, C, E and K. Other nutrients like fat, protein, organic acids and flavonoides are also found (Gupta *et al*., 1990). Several recent studies showed that *H. rhamnoides* contains biologically active substances which can enhance the immunity and reduces the cardiovascular disorder which may be due to the presence of 5-HT in the peel of stem and fruits which is rare occurrence in plant kingdom (Dubey *et al*., 1990). The Phytoconstituents extracted from the *H. rhamnoides* strengthens nonspecific immunity (Agrawal and Goel, 2002; Goel *et al*., 2005). The plant also showed a potent effect on age related deterioration of cognitive functions (Agrawal *et al*., 2000).

Thirty five hypertensive patients received the *Hippophae* extract for eight weeks, the total flavones present in the *H. rhamnoides*, prevented supine exercise induced increase in heart rate, blood pressure and plasma catecholamine concentration (Zhang *et al*., 2001). *H. rhamnoides* oil

also possesses hepato-protective activity (Gao *et al*., 2003). Sea buckthorn leaves can be used as food additives and can be utilized for the development of useful natural compounds, since they show antioxidant and α-glycosidase activity (Kim *et al*., 2011). The aqueous leaf extract has shown significant healing property in burn wounds and has a beneficial effect on the different phases of wound repair and also it has anti-depressant activity (Upadhyay *et al*., 2011; Batool *et al*., 2011). Thus, this plant has a wide therapeutic application in the prevention and management of various diseases.

H. salicifolia is restricted to Himalayan region at an altitude of 1500-3500 m. They are reported to be one of the best species of the genus *Hippophae*, since they yield a high quality fruit. The plant can withstand temperatures from -43-40°C, grows in areas with mean annual temperatures ranging from 4.7-15.6°C and with annual precipitation ranging from 250-800 mm. Hippophae plants are highly adaptable to various soil types. They can grow in hill and gully tops, where the water content is very low (only 15%). They can also survive in valley or gully bottoms with 1.15 salts content. *H. salicifolia* is deciduous shrub or a small tree with thorns. The plant bears foliage from April to November, flowers during June-July for a week and fruits formed during mid August to April. Female plants of *H. salicifolia* bear red, yellow or orange coloured fruits which is usually 1 cm across (Basistha *et al*., 2010).

All the species and subspecies of Seabuckthorn possess similar morphological characters (Guofu *et al*., 2006) which makes the identity and collection of the plants difficult. The review of literature revealed that, no pharmacognostic studies have been conducted on this plant. Hence the present pharmacognostic studies of two species i.e., *H. rhamnoides* and *H. salicifolia* D. Don has been undertaken with the objective to establish pharmacognostic and phytochemical standardization of the leaves, so that authentic plant material could be explored for its therapeutic claims.

MATERIALS AND METHODS

Collection of plant material: The plant material proposed for the study was collected in the month of September 2011 from Ladak district of Kashmir by Dr. D.P. Attrey, Former Director, Defense Institute of High Altitude Research [formerly Field Research Laboratory (FRL)], Leh, India. The plant specimen was identified by Prof. N.K. Dubey, Professor in Plant Taxonomy, Department of Botany, Faculty of Science, Banaras Hindu University, Varanasi, India. A voucher specimen (HR0706/SH18 and HR0707/SH19) has been preserved in the laboratory for future reference.

Instruments used: Photographs of different magnifications were taken with a Leica-camera inbuilt, inverted binocular microscope. Bright field was used for observations.

Pharmacognostic studies

Macroscopic and microscopic studies: The gross morphological character was described based on the shape, size, colour and surface of the leaves. For the microscopic observation, transverse sections of leaves were stained with phloroglucinol and hydrochloric acid, observed through microscope and were studied following standard methods (O'Brian *et al*., 1964; Esau, 1964). Coarse powder of mesh size 60 was cleared with sodium hydroxide and mounted in glycerin medium after staining with phloroglucinol and hydrochloric acid to study the powder characteristics of the leaf.

Physiochemical analysis: The dried powdered leaf material was subjected to physicochemical analysis including fluorescence analysis (Kokoski *et al*., 1958), moisture content, total ash, water soluble ash, acid insoluble ash and extractive values to determine the quality and purity of the plant materials (WHO, 1992).

Preliminary phytochemical screening: The leaves were shade-dried, coarsely powdered with a mechanical grinder and passed through a 40-mesh sieve. The sieved powder material was stored in an air-tight container and kept at room temperature till further study. The dried powder material was extracted with hexane, chloroform, ethyl acetate, methanol and water by cold maceration. The solvents were completely removed under reduced pressure using vacuum evaporator. The presence of various phytoconstituents like, alkaloids (Dragendorff's test), steroids and terpenoids (Libermann- Burchard test), tannin and phenolic compounds (Ferric chloride test), flavonoids (Shinoda test), amino acids (Ninhydrin test) etc., were detected following methods developed by (Kokate, 1986; Harborne, 2005).

RESULTS

Macroscopy

Hippophae rhamnoides: Leaves alternate; petiolate; leaf blade abaxially silvery white suffused with brown or yellow, adaxially dark greyish green, linear or linear-lanceolate, 2-8×0.2-0.8 cm, narrowed at base, abaxially with white and brown stellate scales, margin revolute, apex sub obtuse (Fig. 1).

Hippophae salicifolia: Leaves blade abaxially whitish with usually reddish brown midrib, adaxially green, linear-oblong, 4.2-6.2×0.6-1.2 cm, abaxially tomentose, adaxially stellate-hairy, margin usually revolute (Fig. 1).

Anatomical characters: The cross sections of both species leaves were almost similar and revealed the presence of same type of cells. They are dorsiventral with two layers of palisade cells below the upper epidermis, the palisade cells are absent in the mid-rib region. The transverse section of the leaves of *H. rhamnoides* and *H. salicifolia* showed a layer of upper and lower epidermis covered with thin cuticle. The epidermal cells of the adaxial surface are slightly bigger

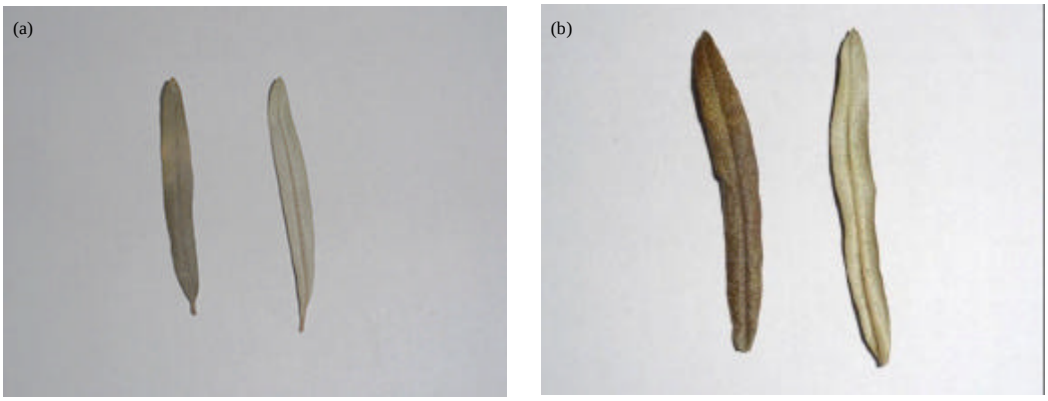

Fig. 1(a-b): Macroscopy of the dried leaves (a) *Hippophae rhamnoides* and (b) *Hippophae salicifolia*

in size than the abaxial region. The lower epidermal cells were completely covered with pressed stellate trichomes. Both *H. rhamnoides* and *H. salicifolia* leaves had sunken stomata in both the epidermis. The silvery appearance of the leaf at the ventral surface is due to the dense covering of the trichomes which is the characteristic identity of the *Hippophae* leaves. The midrib portion of the leaf composed of 5-6 layers of collenchyma cells below the upper epidermis which is followed by xylem vessels which stained pink with phloroglucinol-HCl, due to the presence of lignin. Three to four layers of phloem cells are found beneath the xylem vessels and Six to seven layers of spongy parenchyma is seen above the lower epidermis (Fig. 2). Small difference was observed in the shape of the midrib. *H. rhamnoides* showed a heart shaped midrib, whereas the shape of the midrib of *H. salicifolia* is concave. Lower epidermis of the lamina show densely covered trichomes with sunken stomata, vascular strands and loosely packed spongy parenchyma.

Powder microscopy of two species leaves revealed the presence of different types of trichomes (Fig. 3). stellate, peltate and a combination of both stellate-peltate trichomes, broken fragments of epidermis with palisade cells and broken xylem vessels.

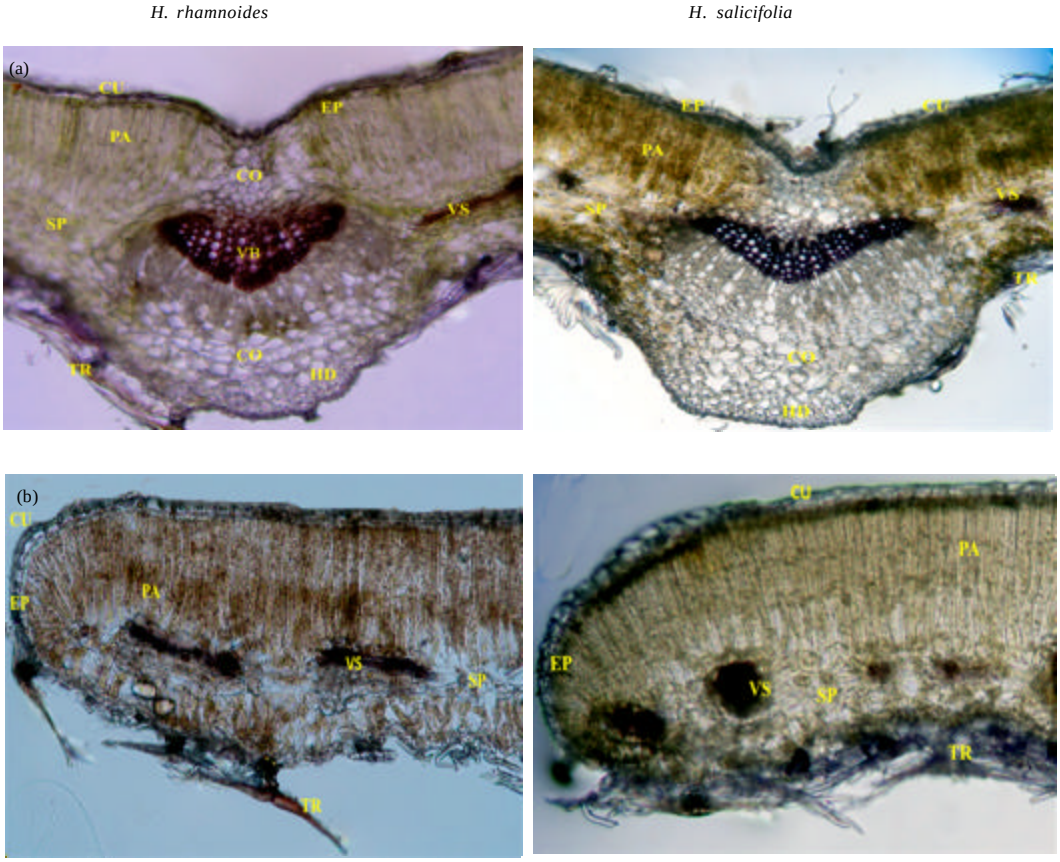

Fig. 2(a-b): Transverse section of the leaf through the midrib and lamina (a) Transverse section of the leaf through the midrib and (b) Transverse section of the leaf through the lamina, EP: Epidermis, CO: Collenchyma, TR: Trichome, HD: Hypodermis, VB: Vascular bundle, LV: Lateral vein, VS: Vascular strand, SP: Spongy parenchyma, SS: Sunken stomata and CU: Cuticle

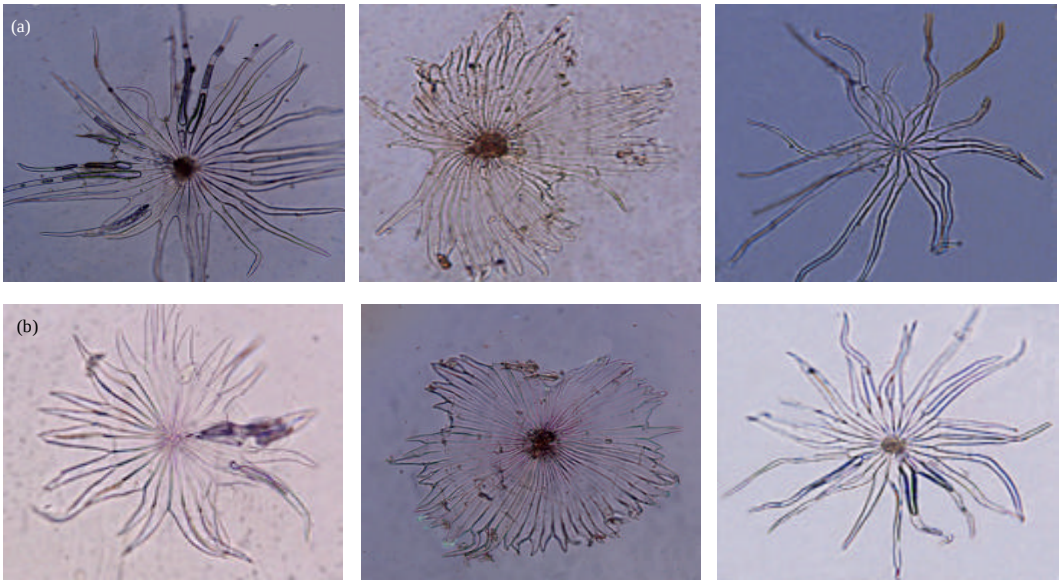

Fig. 3(a-b): Different types of Trichomes observed in powder microscopy of the leaf (a) *H. rhamnoides* and (b) *H. salicifolia*

Table 1: Preliminary phytochemical analysis of *H. rhamnoides* and *H. salicifolia*

	H. rhamnoides					*H. salicifolia*				
Phytoconstituents	Hexane	Chloroform	Ethyl acetate	Methanol	Water	Hexane	Chloroform	Ethyl acetate	Methanol	Water
Steroids	+	+	+	+	+	+	+	+	+	+
Phenolic compounds	-	-	+	+	+	-	-	+	+	+
Saponins	-	-	-	+	+	-	-	-	+	+
Flavonoids	-	-	-	+	+	-	-	-	+	+
Coumarin glycosides	-	-	+	+	+	-	-	+	+	+
Alkaloids	-	-	-	-	-	-	-	-	-	-
Proteins	-	-	-	+	+	-	-	-	+	+
Carbohydrates	-	-	-	+	+	-	-	-	+	+

+: Present, -: Absent

Preliminary Phytochemical screening: Preliminary phytochemical screening of various extracts of the leaves of *Hippophae* revealed the presence of steroids, terpenoids, saponins, flavonoids, phenolic compounds and carbohydrates (Table 1).

Physicochemical analysis: The physicochemical parameters such as ash values, loss on drying (moisture content), extractive values, fluorescence analysis and foaming index were measured and are depicted in Table 2. The total ash value of *H. rhamnoides* (80) is less when compared to *H.salicifolia* (85.60). The leaves of *H. salicifolia* contain less of water soluble ash (4.09) when compared to *H. rhamnoides* (10). Water soluble ash signifies the physiological content of the leaf. The results of extractive values are shown in Table 3. Powder characteristics of the leaves when treated with various chemicals were observed under the UV and day light, explores the presence

Table 2: Extractive values of the leaves of *H. rhamnoides* and *H.salicifolia*

	Extractive values %w/w	
Solvents	*H. rhamnoides*	*H. salicifolia*
Hexane	2.34	0.65
Chloroform	3.32	2.98
Ethyl acetate	4.62	3.58
Methanol	20.66	19.72
Water	19.98	16.79

Table 3: Physiochemical characters

	Values % w/w	
Parameters	*H. rhamnoides*	*H. salicifolia*
Total Ash	80	85.60
Acid-insoluble ash	10	1.75
Water soluble ash	10	4.09
Moisture content	7.77	7.12
Foaming index (No unit)	500	250

Table 4: Fluorescence analysis of *Hippophae* sp. leaf powder

Hippophae sp.	Treatment	UV	Day light
H. rhamnoides	Powder		Green
	Powder+ 1N NaOH	Green fluorescence	Brown
	Powder + 1N HCl	Greenish yellow	Colourless
	Powder + 50 % HNO_3	Greenish yellow	Yellow
H. salicifolia	Powder		Green
	Powder+ 1N NaOH	Green fluorescence	Yellowish brown
	Powder + 1N HCl	Greenish yellow	Colourless
	Powder + 50 % HNO_3	Greenish yellow	Yellow

of nature of Phytoconstituents present in the leaves. Green fluorescence was observed under the UV light, when the leaf powder was treated with NaOH signifying the presence of flavonoids (Table 4).

DISCUSSION

A major drawback of herbal medicine is lack of standardization and quality control. The main importance is with respect to quality control for correct identification of the species concerned, whether in the fresh, dried or powdered state (Springfield *et al*., 2005). The misclassification of species and the mistaken substitution is a real danger in the preparation and administration of herbal medicine (Elizabeth, 2004). In the present study microscopical evaluation and physiochemical analysis of two *Hippophae* species were carried out. Microscopic evaluation of *H. rhamnoides* and *H. salicifolia* leaves revealed that, they look similar in the type of cells except slight difference like shape of the midrib and the presence of hypodermis. Powder microscopy of the leaves showed the presence of different types of trichomes which is characteristic identity of the *Hippophae* species. The dense covering of stellate trichomes can be a good cover of shelter in preventing the high temperature of the leaves, sunken stomata and the dense epidermal cells cover; can effectively reduce the water loss showing the plant to be high drought resistant property. The

characters available in the powder are much fewer than the potentially available characters in whole specimens. The difference is attributable to the damage of the plant cell wall during preparation, causing distortion in tissues arrangements and patterns normally found in the untreated plant samples. The characters available in the powder form of the specimens are potentially useful for distinguishing the samples even in mixture (Jayeola, 2009). The powder microscopy of both the species revealed the presence of three different types of trichomes, stellate, peltate and combination of both the types- Stellate-peltate trichomes which are characteristic feature of the *Hippophae* sp. Preliminary phytochemical analysis of all two species of Seabuckthorn indicated the presence of steroids, terpenoids, flavonoids, coumarin glycosides, phenolic compounds and saponins. The analysis of ash values revealed that, the total ash, acid insoluble ash and water-soluble ash are present in different quantities in both the species. The ash content of *H. salicifolia* (85.60% w/w) is higher than *H. rhamnoides* (80% w/w). The extractive values are useful to evaluate the chemical constituents present in the crude drug and also helpful in estimation of specific soluble constituents in a particular solvent. Extractive values of the leaves of two species showed that the methanolic and water extractive values were more when compared with other solvents.

The present study was undertaken with a view to lay down standards which could be useful to detect the authenticity of these medicinal plants. Microscopic study and physicochemical standards can be useful to substantiate and authenticate the drug. Seabuckthorn, an underutilized and neglected plant of the cold arid region is a goldmine with an untapped potential. Efforts towards preparation of food products from Seabuckthorn have suitably highlighted its immense potential commercially.

CONCLUSION

The pharmacognostic and the phytochemical profile of *H. rhamnoides* and *H. salicifolia* are highly dependent on environmental adaptability of the plants. The present study is helpful in identification of the *H. rhamnoides*, *H. salicifolia* and various sub species of this plant.

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