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Pharmacognostical Characterization & Preliminary Phytochemical Investigation of Seabuckthorn (*Hippophae rhamnoides* L.) Leaves

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ABSTRACT: Seabuckthorn (SBT; *Hippophae rhamnoides* L.) is a thorny bush found growing wild in high altitude cold deserts of India. Present study involved pharmacognostical (Botanical and physicochemical) characterization and phytochemical investigation of SBT leaves. Botanical characterization revealed the presence of large amount of stellate trichomes. Among physicochemical parameters, total ash was found to be 6.5%, acid insoluble ash 1.5% and water soluble ash 1.1% w/w of the dried powdered leaf. Extractive values were found to be 24%, 5% and 5% for water, alcohol and ether respectively. Preliminary phytochemical screening indicated the presence of tannins, flavonoides along with various other phytochemicals. Certain characteristic parameters observed during this study may facilitate the establishment of standard quality control profile for SBT leaves. © 2011 IGJPS. All rights reserved.

KEYWORDS: Seabuckthorn; Botanical Characterization; Stellate Trichomes; Physicochemical; Extractive Values.

INTRODUCTION

Being a treasure house of various therapeutic phytochemicals, plants have been reported to be an integrated part of traditional medicinal system. 70-95% population of most of the developing countries depends on these traditional medicines for primary care. The global market for traditional medicines has been estimated worth US$ 83 billion annually in 2008, with an exponential rate of increase[1]. In order to ensure safety and efficacy of herbal products, quality control of raw material i.e. medicinal plants, is a basic and compulsory requirement[2]. Lack of standard quality control profile, is one of the major problems in the global acceptance of the herbal products[3]. There are several factors which may affect the quality of plant materials. According to WHO, first step in assuring quality, safety and efficacy of traditional medicines, is correct identification. Subsequent to this identification, standardization of medicinal plant starts which involves investigations of its various botanical, physicochemical and phytochemical parameters which help in identification, authentication and its bioactive potential[4].

Hippophae rhamnoides L., commonly known as Seabuckthorn (SBT); Family: Elaeagnaceae is a high altitude medicinal plant growing in North-West Himalayas at high altitude (7000–15,000 feet). It is thorny, nitrogen fixing deciduous shrub, native to

Europe and Asia[5]. Seabuckthorn (SBT) is a good source of a large number of nutrients, phytochemicals and bioactive substances[6,7]. SBT leaf extracts have been reported to possess many medicinal properties, such as antioxidant, anti-inflammatory, antimicrobial, neuroprotective etc[5, 8-11].

In spite of huge therapeutic potential, there is a lack of standard quality control profile on SBT leaves. In view of the above, present study was undertaken to carried out pharmacognostic and preliminary phytochemical investigation of SBT leaves.

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MATERIALS & METHODS

Plant material

Leaves of Seabuckthorn were collected from Leh area in Ladakh, J&K, India, and authenticated by National Institute of Science Communication And Information Resources (NISCAIR), New Delhi, India. Fresh leaves were used for histological determination. Shade dried leaves were used for physicochemical evaluations and phytochemical screening.

Macroscopic study

It involved studies on color, odor, taste, length, width, shape, fracture etc[2].

Microscopic study

Free hand sections of the fixed leaf material were taken and boiled with Diluted HNO_3 (1:3, 60% HNO_3: Water) for 2-3 minutes to remove the coloring matter, washed with distilled water. Further it was kept in alkaline KOH solution for 2-3 minutes. Then almost transparent peel of leaf was treated with 0.5 % safranin solution for staining purpose and mounted on a clean glass slide with glycerin and covered with cover slip. The sections were then viewed under low power (10 X) and subsequently under high power (40 X) microscope[12]. The microphotographs were taken using Nikon Phase Contrast microscope attached with Nikon Eclipse E600 camera**.** The powder of Seabuckthorn leaves was also examined for its microscopic characters. The powders were passed through sieve no. 60 and studied for their organoleptic and microscopic characteristics[13].

Physicochemical study

Various physicochemical constants like Ash values[14] (Total, Acid-insoluble and Water soluble ash), Extractive values[2] (Water, Alcohol and Ether soluble) and Loss on drying[2] were studied.

Phytochemical study

Various extracts like Petroleum ether extract, Chloroform extract, Ethyl Acetate extract, Ethanol extract and Aqueous extract were prepared using soxhlet extraction and were used for phytochemical analysis[15], fluorescence analysis and for the determination of percentage yields[2].

RESULTS & DISCUSSION

Present study was focussed on characterization of botanical, physicochemical and phytochemical parameters of Seabuckthorn leaf. In general, botanical and physicochemical characterization helps in confirmation of identity and determination of quality & purity of herbal raw materials. They also help in detection of nature of adulterants. Preliminary phytochemical studies indicate towards qualitative chemical profile of the plant material. The results of the present investigation and their discussions were presented below under following headings.

Macroscopic study

Leaves were found to be simple with undivided lamina, waxy, glandular, pinnate, entire margin and rounded or acute base, asymmetrical, lower surface covered with silvery scales, midrib prominent at the lower surface, grooved on the upper surface, lateral vein-lets, color-dark green to dull light, lower side whitish. Size of the leaf was found to be 2.5 - 4cm (length) & 4-5mm (width). Shape was lanceolate. Powdered leaf was found to be fine, green colored, pungent, astringent with slight bitter taste and irritating odor.

Microscopic study

Diagrammatic T.S. of the leaf passing through midrib was convexly elevated at the lower side and grooved at the upper side, showed centrally located meristele and isobilateral laminar extensions on its lateral sides. The cells of the lower epidermis were fully covered with stellate trichomes unlike the upper ones, where they were few in number (**Figure 1**). Powdered leaf showed plenty of stellate trichomes of various sizes and shapes, mostly fan shaped, with recurved end and some thick walled with bifurcating arms. Prismatic crystals of calcium oxalate were also observed. Epidermal cells were found to be with slightly sinuous wall and embedded with ranunaculaceous types of stomata in surface view. The upper epidermal cells were slightly bigger in size and were embedded with few stomata unlike the lower ones which showed more number of stomata (**Figure 2**).

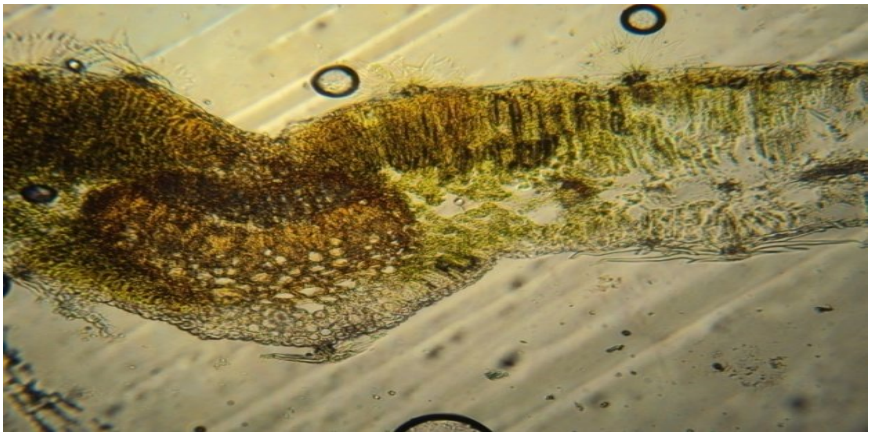
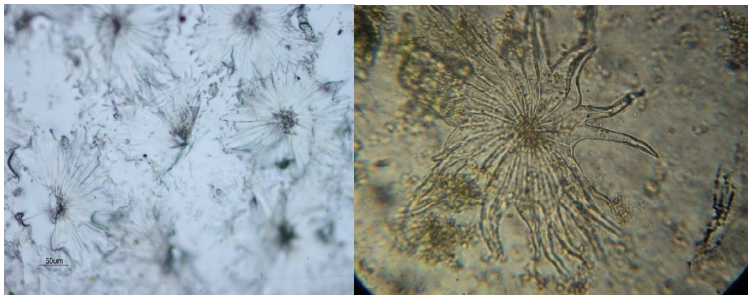


Figure 1: Transverse section of the leaf

Detailed T.S showed a layer of upper and lower epidermis covered with thin cuticle. The cells of the upper epidermis being slightly bigger in size than the lower one and bear fan shaped stellate trichomes. The lower epidermal cells were completely covered with apressed stellate trichomes. Stomata were more in the lower epidermis. Collateral meristele was located in the centre, protected at

lower side with thick walled parenchymatous cells. Lamina showed two to three rows of palisade cells underneath the upper epidermis occupying more than half the portion of the mesophyll tissue. Palisade layer lying underneath the lower epidermis was very narrow and obscure. Cells of the spongy parenchyma were embedded with rows of vascular bundles (**Figure 1**).



(A) **(B)**

Figure 2: Stellate trichome present in leaf peel (A) and in powdered leaf (B)

Stellate trichomes were much more abundant on leaf epidermis. These trichomes were covering the stomata so it was quiet hard (**Figure 3**) to calculate the Stomatal Index, so trichome density (Number of trichomes per microscopic field) was calculated and it was found to be 11-13.

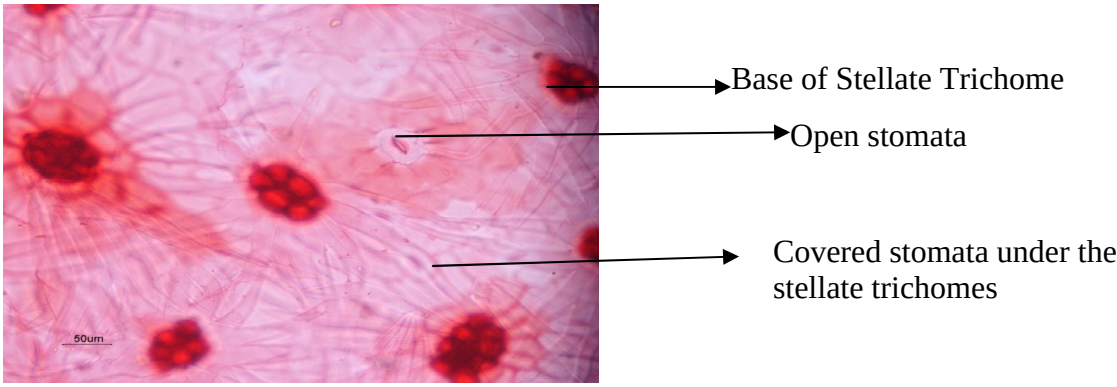


Figure 3: Stellate trichomes covering the stomata

Physicochemical study

Physicochemical parameters like ash values (total ash, acid insoluble ash and water soluble ash); extractive values (water soluble, alcohol soluble and ether soluble) and moisture content have been shown in **Table 1** & **2** respectively.

Table 1: Ash values (% w/w dried leaf powder)

Total ash	6.5
Acid insoluble ash	1.5
Water soluble ash	1.1

Table 2: Extractive values and moisture content (% w/w dried leaf powder)

Water	24
Ethanol	5
Ether	5
Moisture content	8

Phytochemical study

Results of phytochemical studies have been shown in **Table 3** & **4** respectively.

Table 3: Fluorescence analysis and Successive Extractive Values

Solvent	**Color**	**Consistency**	**UV– 366 nm**	**UV – 254 nm**	**% yield (w/w)**
Pet. Ether	Greenish black	Sticky viscous	Greenish black	Blackish green	1.4
Chloroform	Greenish brown	Smooth viscous	Black	Greenish brown	2.3
Ethyl acetate	Yellowish black	Sticky solid	Dark green	Yellowish black	2.1
Ethanol	Greenish brown	Smooth non-sticky solid	Dark green	Greenish black	1.3
Water	Brownish black	Non-sticky solid	Black	Shiny black	31.2

Table 4: Preliminary Phytochemical Screening in Successive Extracts

Test	**Pet. Ether**	**Chloroform**	**Ethyl Acetate**	**Ethanol**	**Water**
Alkaloids	+ve	+ve	+ve	-ve	-ve
Amino acids test	-ve	-ve	-ve	+ve	+ve
Carbohydrate test	-ve	-ve	-ve	+ve	+ve
Flavanoids	-ve	-ve	-ve	+ve	+ve
Glycoside test	-ve	-ve	-ve	+ve	+ve
Tannins	-ve	-ve	-ve	+ve	+ve
Steroid & triterpenoid test	+ve	+ve	+ve	-ve	-ve

+ve Present, **-ve** Absent

As summary, stellate trichomes appeared to be the characteristic feature of SBT leaves. Phytochemical screening indicated the chemical profile of SBT leaves and revealed the presence of alkaloids, glycosides, tannins, flavonoides, steroids, triterpenoides and carbohydrates.

CONCLUSION

In the present study, all the characterizations/investigations were done as per WHO/Ayurvedic Pharmacopoeia of India guidelines. Results of this study are likely to help in identification of SBT leaves and determination of their quality & purity. They may facilitate the establishment of standard quality control profile for SBT leaves.

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