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Pharmacognostic evaluation of stem and leaf of *Hippophae rhamnoides* subsp. turkestanica Rousi. a multipurpose taxon

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ABSTRACT

Background: *Hippophae* L. belongs to the family Elaeagnaceae. It is a thorny, deciduous, hardy shrub/small tree wildly growing varied conditions. According to the available literatures, it is widely used in traditional medicinal systems. The plant comprises of pharmacological activities including anti-atherogenic, anti-cancer, anti-microbial, anti-stress, cytoprotective, hepatoprotective, immunomodulatory, radioprotective, and tissue regeneration. **Objective:** The focus of the study was to determine the pharmacognostic characters and physicochemical analysis of the stem and leaf of *Hippophae rhamnoides* subsp. *turkestanica* Rousi. **Materials and Methods:** The plant parts, i.e., stem and leaves, were collected from Skurbuchan, located at 34° 26' 07" N and 76° 42' 38" E in the Union Territory of Ladakh, India. The macroscopic features were studied under stereomicroscope whereas, the microscopic observations and powder microscopy were carried out using trinocular microscope under different magnifications. The physicochemical properties such as LoD, Ash value, water and alcohol soluble extractive value, and Acid insoluble ash were determined as prescribed in the Ayurvedic Pharmacopoeia of India. **Results:** The study reveals that the transverse section (T.S.) of the stem shows the xylem as the dominant tissue, with laticiferous canals and fiber patches in the cortical area. The leaf's epidermis consists of tangentially elongated cells with a thin cuticle and stellate hairs on both surfaces. The midrib contains phloem with laticiferous cells and a xylem arranged in a semicircular pattern. The Powder microscopy also supports the features, including palmate and stellate hairs, glandular trichomes, vessels with thickening, and rhomboidal calcium oxalate crystals. The results of the physicochemical parameters were recorded in % w/w and the lowest is reported in the stem and maximum in the case of leaf. The value of loss on drying is more or less similar (0.03% & 0.06), whereas the alcohol and water extractive values are recorded higher in leaf than in the stem. Total ash content in stem and leaf is 1.25% and 1.5%, and the acid-insoluble ash value is also comparatively higher in leaf. **Conclusion:** The study will help to identify and authenticate crude drug/raw materials or those in powder form, even when added to a formulation as ingredients from adulteration, and also form a base for future studies on formulations.

Keywords: *Hippophae rhamnoides*, Macro-Microscopic, Pharmacognosy, Physicochemical, Seabuckthorn.

INTRODUCTION

Hippophae L. belongs to the family Elaeagnaceae. It is a thorny, deciduous, hardy shrub/small tree wildly growing at an altitude of 0-5000 m asl [1-3]. The plant is adapted to harsh climates and has the potential to grow in a wide range of temperatures ranging from -40°C to +40°C and acidic soil as well [4-6]. The genus *Hippophae* L. comprises 7 species and 11 subspecies, distributed across the dry temperate regions of Asia and Europe [1-2] and referred to as Seabuckthorn, sallowthorn, sandthorn or seaberry [7]. The plant has a complex root system forming a symbiotic relationship with a free-living nitrogen-fixing bacteria "Frankia" and fixes atmospheric nitrogen twice as much as soybean [8].

In India, three out of the seven species, *H. rhamnoides* ssp. *turkestanica* Rousi*, H. salicifolia* D. Don and *H. tibetana* Schlecht. are naturally distributed in cold deserts and other regions of Himalaya abounded in Union territory of Ladakh (erstwhile Jammu and Kashmir state), Lahaul and Spiti of Himachal Pradesh, higher altitudes of Uttarakhand, Sikkim and Arunachal Pradesh in North East [9-11]. 67% of the distribution is in Ladakh, followed by Uttrakhand, Himachal, and the least in North East [6,12-13]. According to Li [14], the ancient Greek writings of Theophrastus and Dioscorides discuss the use of *Hippophae* L. in medicine. Historical texts, including Jing Zhu Ben Cao from the Qing Dynasty and Sibu Yidian from the Tang Dynasty also documented its pharmacological effects. As early as 900 A.D, it was utilized as a medicinal plant in Tibet. The ancient Tibetan medical books, i.e., "*The RGyud Bzi*" (The Four Books of Pharmacopoeia) [1, 15-16], have mentioned the usage of seabuckthorn in medicine.

The plant has a vast array of significant chemical components, each of which are found in distinct plant parts (Table 1). Researchers from across the world have reported on a variety of pharmacological activities including anti-atherogenic, anti-cancer, anti-microbial, anti-stress, cytoprotective, hepatoprotective, immunomodulatory, radioprotective, and tissue regeneration (Table 2). According to [17] seabuckthorn berries are one of the most nutritious and vitamin-rich in the plant kingdom. Therefore, it is also known as "The Super Plant," "Super Healthy Fruit," "Gold Mine," and [13] "Gold of the Cold Desert" [18]. Worldwide, research has been done on *Hippophae* L. from a variety of perspectives including its use in agriculture, ornamental plants, medicine, nutrition, and cosmetics [9, 11, 19]. The present paper discusses the much-desired pharmacognostic study to lay down reliable parameters for the identification of its different parts in pharmaceutical based drug industry.

Table 1: Parts of the *Hippophae* L. and their phytochemical composition [12, 15, 20]

Plant part	Phytochemical Composition
Leaf	Flavonoids, carotenoids, free and esterified sterols, triterpenols, isoprenols; vitamin E, catechins, elagic acid, ferulic acid, folic acid, calcium, magnesium and potassium.
Fruits	Vitamins (A, B, C D, E, folic acid), Carotenoids (β-carotene, lycopene, lutein and zeaxanthin), flavonoids (isorhamnetin, quercetin, isorhamnetin-3-betad- glucoside; isorhamnetin-3-beta-d-glucosaminide; kaempferol, etc.), organic acids, amino acids, micro and macronutrients
	Oleanolic acid, ursolic acid, 19-alpha-hydroxyursolic acid, dulcioic acid, 5-hydroxymethyl-2-furancarbox-aldehyde, cirsiumaldehyde, octacosanoic acid, palmitic acid and 1-Ohexadecanolenin
	palmitic acid, oleic acid (omega-9), palmitoleic acid (omega-7), linoleic acid (omega-6), and linolenic acid (omega-3); phytosterols and β-Sitosterol,
Seeds	Vitamins (E, K), essential fatty acids (omega-3 and 6),β-Sitosterol, phyto-sterols oleic acid, linoleic acid and linolenic acid.
Bark	Phenolic compound.

Table 2: Major phytochemicals in *Hippophae rhamnoides* L. and their medicinal properties

S. No.	Phytoconstituents	Medicinal properties	References
1.	Carotenoids	Antioxidant activities and help in the synthesis of collagen and epithelial growth.	[21]
2.	Tocopherols	Antioxidant activity minimizes lipid peroxidation and helps in pain relief	[22]
3.	Vitamin C	Antioxidant activity and sustain integrity of cell membrane, enhancement of collagen synthesis	
4.	Vitamin K	Wound healing, prevention of bleeding; anti-ulcer effect.	[23]
5.	Vitamin B complex	Stimulate cell and nerve regeneration	
6.	Phytosterols	Regulates inflammatory process, improves microcirculation in the skin, Acts as anti-cancer, anti-ulcer, and anti-atherogenic,	[24]
7.	Polyphenolic compounds	Antioxidant activity, cardioprotective, cytoprotective, wound healing	[25]
8.	Polyunsaturated fatty acids (PUFA)	Act as anti-tumor, Immunomodulatory, and neuroprotective,	[26]
9.	Organic acids	Minimize the risk of stroke, heart attack, and arthritis; act as anti-cancer and wound healing.	
10.	Coumarins and triterpenes	Regulate the appetite, memory, learning, and sleep.	[27]

MATERIALS AND METHODS

Sample Collection and Authentication

The aerial parts including stem and leaves of *Hippophae rhamnoides* subsp. *turkestanica* Rousi. were collected from Skurbuchan, located at 34° 26' 07" N and 76° 42' 38" E in the Union Territory of Ladakh, India. All the morphological data of the plant was generated and validated from eflora of China and available literature [1, 28]. The shade dried samples are preserved in the Raw Drug Museum of RARI, Jammu bearing accession numbers RARI-JM-273 & 274.

Section Cutting

The dried samples were soaked for softening as per API (Ayurvedic Pharmacopeia of India) [30]. Free hand sections of stem, leaf, were cut with a sharp razor blade. The sections were then cleared in distilled water and studied under the Olympus CX41 trinocular microscope. No stains were used; the scale-bars represent photographs of magnifications of the image.

Preparation of Powder

Fine powder of stem and leaf was prepared separately using a grinder and then sifted through 60 No. sieve as per the standard procedure [29]. The sifted powders were used for the powder microscopy and physicochemical study (Figure 1).

Powder Analysis

The microscopic features were observed using an Olympus CX41 trinocular microscope attached to HP desktop by Magnus Magcam DC10 (10MP) and macroscopic features were studied under stereomicroscope Olympus SZ2-ILST connected by Magnus Magcam DC5 (5.1MP). The Macroscopic observations, powder microscopy, and photographs of the sample were carried out under different magnifications. The physicochemical analysis was carried out by standard method (as per API).

RESULT

Macroscopic Study

Stem: *Hippophae* L is a woody and highly branched plant. The stem of both mature and immature plants shows wide colour variations. It is generally grayish or silver in color. According to Tamchos S [3], the stem of matured plants growing in Ladakh has seven different colours usually brownish, dark brown, light brown, reddish, silvery, grayish, and ashy. The stem is branched and possesses thorns, which are either branched or un-branched. The average thorn size is 0.5 ranging from 0.2 – 1.4 cm (Figure 1a-b).

Leaf: *Hippophae rhamnoides* L. is a deciduous plant and it sheds its leaves at the commencement of winter in November. The leaf size ranges from 1.1 – 4.2 cm in length and 0.2 – 0.5 cm in width. The leaves are alternately arranged; sub-sessile or petiolated, lanceolate with obtuse or acute tip, and entire margins. The upper and lower surfaces of leaves are covered with scales that give a shiny appearance during the day. The leaves of this plant show varied colour [3, 30]. However, the upper surface (adaxial) is mostly dark green, whereas the lower surface is light green (Figure 1c-d).

Microscopic

Stem: The T.S. of mature stem is more or less circular and wavy in outline. The outer most layer consists of brown-colored cork followed by 2-4 layers of phelloderm. The cortex region is composed of 5-7 cell layers and embedded with laticiferous canals underneath the crests, Pericycle is represented by a discontinuous band of patches of fibers. Phloem region is narrow loaded with fibers. Xylem occupies the major part of the stem. Vessels form a complete ring in the peripheral region of the xylem. The inner region of the secondary xylem is composed of xylem fibers. The primary xylem is located towards the pith. Medullary rays form distinct vertical bands of cells. The central region is occupied by parenchymatous pith cells. It consists of abundant brownish resin filled cells. Pitted parenchyma cells are also present in the pith region (Figure 2).

Thorn: The T.S of thorn is simple and circular, formed of fibers and parenchyma cells. The outer layer comprises of epidermis, which is covered by a thick layer of cuticle. The epidermal cells are tangentially elongated. It is followed by 2-3 layers of hypodermis, composed of cells filled with brownish-red content. The rest of the ground tissue is composed of fibers, except the central portion, which is parenchymatous, and the cells are filled with brown content. Many pitted parenchyma cells are also present in the centre region (Figure 3).

Leaf: The T.S of leaf passing through the midrib is convexly protruding at the lower side and narrowly sunken on the upper side, both upper and lower epidermii are composed of tangentially elongated cells and covered with a thin cuticle. Stellate hairs are present on both surfaces. Meristele of mid rib region is broadly embedded in the parenchymatous ground tissue and consists of radially placed xylem in rows, arranged in the semicircle and an arc of phloem containing abundant laticiferous cells. In lamina region, the epidermis is covered by a thick layer of cuticle and the mesophyll region is parenchymatous. The mesophyll comprises of both spongy and palisade parenchyma. The spongy parenchyma cells are loosely arranged towards the lower epidermis whereas the palisade mesophyll parenchyma cells are arranged toward the outer epidermis (Figure 4).

The T.S of petiole is more or less circular. The epidermal cells are covered by a thick cuticle. The epidermis bears stellate hairs. It is followed by the parenchymatous cortical region. The vascular bundles are present in the centre and consist of xylem in radial rows arched by phloem (Figure 5).

Powder Analysis

Organoleptic Characters

The study of characteristic features of the powder of *Hippophae rhamnoides* L. are tabulated in Table 3.

Powder Microscopy

Stem: The powder of the stem consists of laticiferous canal cells, thick fibers, brown content-filled cells, cork cells, fragments of parenchyma cells, vessels with pitted and annular thickening, and rhomboidal crystals of calcium oxalate. The powder also consists of fibers, hypodermal cells, and parenchyma cells of thorn (Figure 6).

Leaf: The powder of the leaf consists of laticiferous canals, cortical cells of the petiole, and fragments of parenchyma cells. It also consists of sessile glandular trichomes; stellate and peltate hairs, pitted and annular thickened vessels, rhomboidal and prismatic crystals of calcium oxalate (Figure 7).

Physiochemical Analysis

The results of the physicochemical analysis of the stem and leaf of the *Hippophae rhamnoides* subsp. *turkestanica* Rousi. are summarized in Table 4. The foreign matter present in the samples is less than 0.5%. Loss on drying of both the parts shows a negligible difference. This indicates that the maximum moisture content was released during the process of drying, and it can be less prone to pathogen contamination. The Total ash value and the acid-insoluble ash value is slightly higher in the leaf than in the stem. Whereas, the aqueous and alcohol extractive values are significantly highest in leaf (11% w/w & 24% w/w) as compared to stem (3% w/w & 5% w/w), this indicates that the leaf contains higher alcohol and water-soluble constituents than the stem. (Table 4).

Table 3: Organoleptic characters of powder of *Hippophae rhamnoides* L.

S. No	Plant part	Organoleptic characters			
		Colour of powder	**Odour**	**Taste**	**Texture**
1	Stem	Woody colour	Not characteristic	Not characteristic	Rough
2	Leaf	Olive	Sweet	Not characteristic	Smooth

Table 4: Physicochemical parameters of stem and leaf of *Hippophae rhamnoides* L.

S. No.	Physicochemical Parameters	Result of *H. rhamnoides* subsp. turkestanica Rousi.	
		Stem	**Leaf**
1	Foreign matter	< 0.5%	< 0.5%
2	Loss on drying	0.03 (% w/w)	0.06 (% w/w)
3	Total ash value	1.25 (% w/w)	1.5 (% w/w)
4	Acid-insoluble ash value	0.25 (% w/w)	1.25 (% w/w)
5	Alcohol-soluble extractive value	3.0 (% w/w)	11 (% w/w)
6	Water-soluble extractive value	5.0 (% w/w)	24 (% w/w)

Figure 1: *Hippophae rhamnoides* subsp. *turkestanica* parts and their powder. Stem (a-b); leaves (c-d)

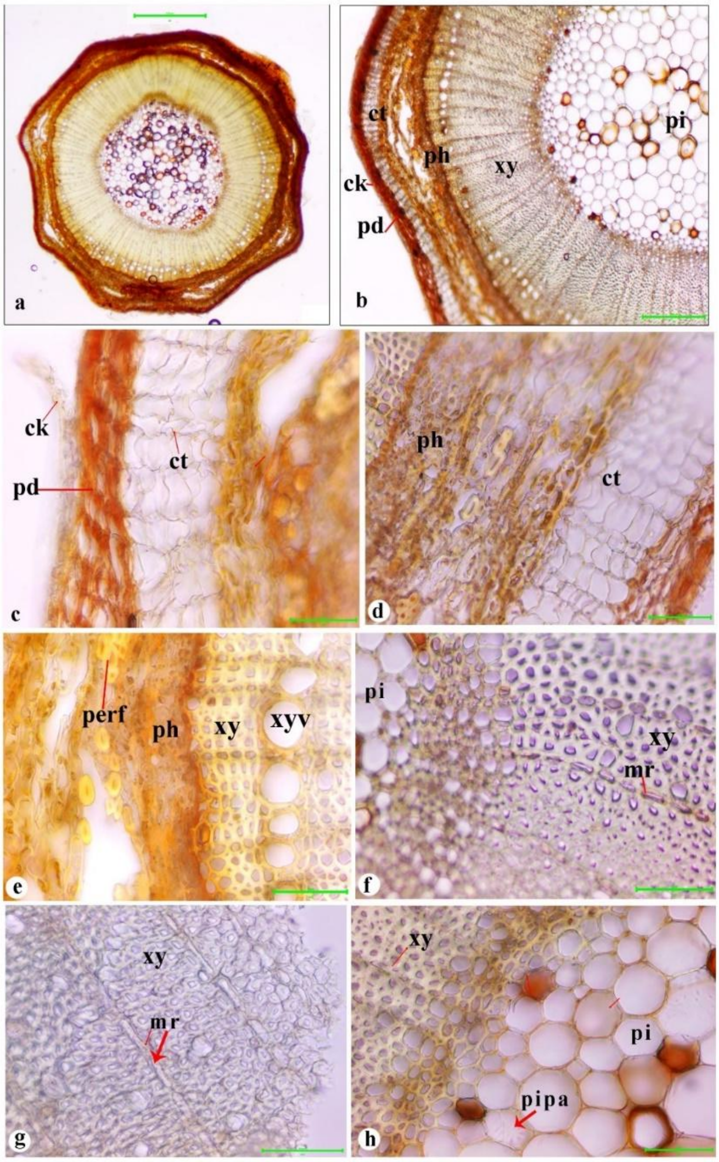

Figure 2: T.S of the mature stem of seabuckthorn. At 4x (a); 10x (b) and 40x (c-h). Abbreviations, ck (crock); pd (phelloderm); ct (cortex); perf (pericyclic fiber); ph (phloem); xy (xylem); xyv (xylem vessel); mr (medullary ray); pi (pith); pipa (pitted parenchyma)

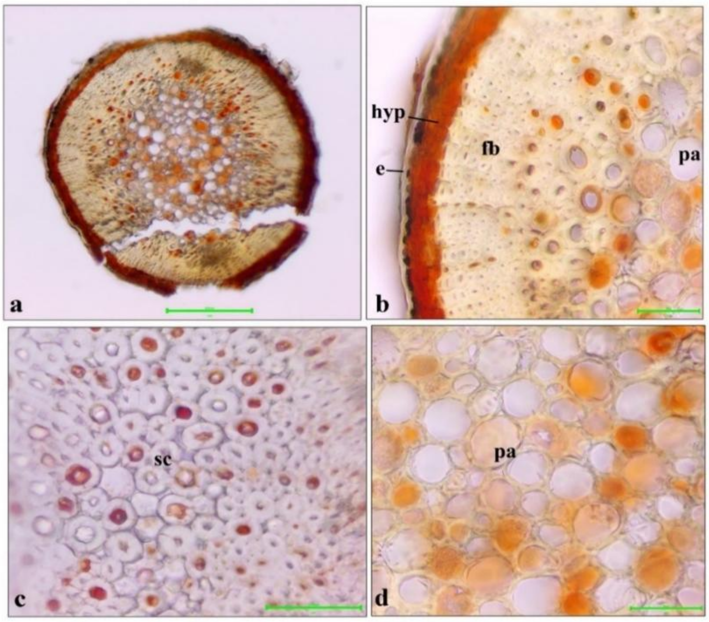

Figure 3: T.S of the thorn of *Hippophae rhamnoides* subsp. *turkistanica* Rousi. at different magnification 10x (a) and 40x (b-d). Abbreviations: e (epidermis); hyp (hypodermis); fb (fiber); sc (sclereids); pa (parenchyma)

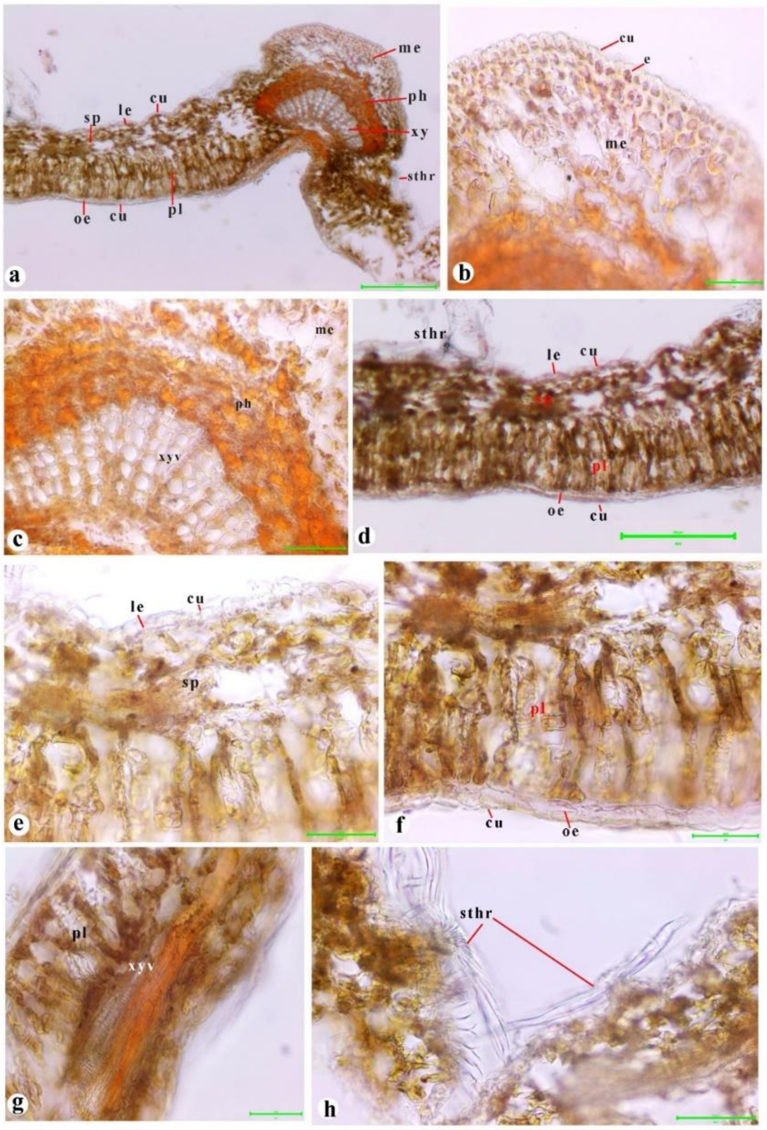

Figure 4: T.S of leaf of *Hippophae rhamnoides* subsp. *turkestanica* Rousi. at 4x, 10x and 40x. Abbreviations: cu (cuticle); le and oe (lower and outer epidermis); sp (spongy parenchyma), pl (palisade parenchyma); me (mesophyll); ph (phloem); xy (xylem); xyv (xylem vessel); sthr (stellate hair)

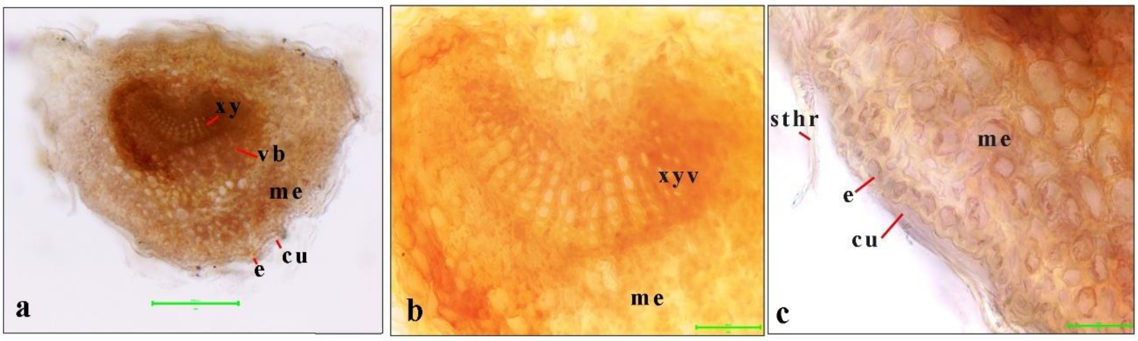

Figure 5: T.S of the petiole of the leaf of seabuckthorn**.** Abbreviations: cu (cuticle); e (epidermis); me (mesophyll); xy (xylem); sthr (stellate hair)

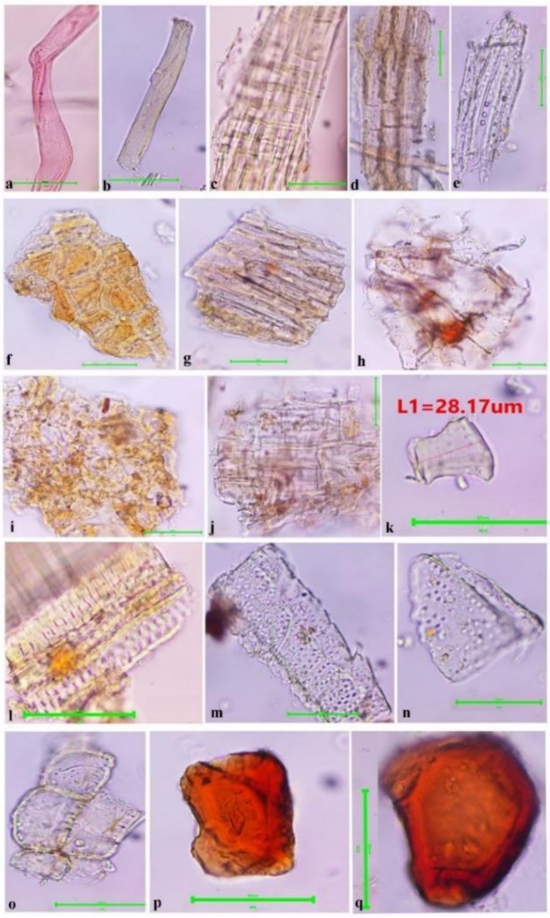

Figure 6: Photo-micrographs of powder of stem. Laticiferous canal (a); fiber (b); cork cell (c); hypodermal cell (d-e); parenchyma cell (f-i); rhomboidal crystal (k); pitted and annular vessel (l); xyel fiber (m-n) pitted paremchyma (o); tannin filled cell (p-q)

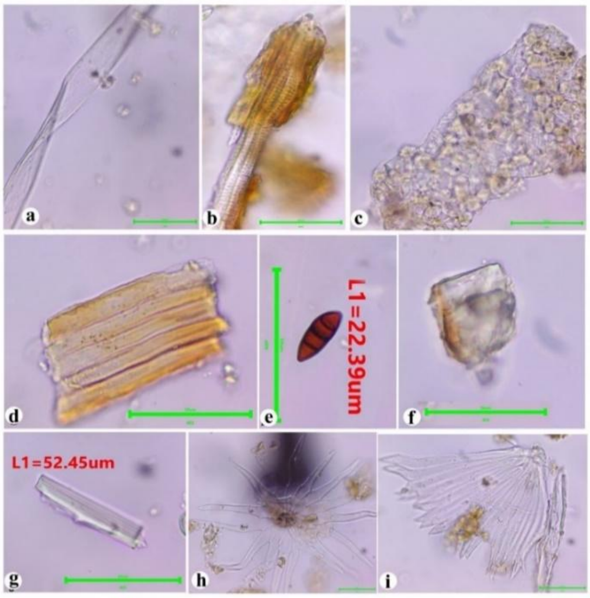

Figure 7: Photo-micrographs of powder of seabuckthorn leaves. Laticiferous canal (a), xylem vessel (b); cortical cells of petiole (c), fiber (d); glandular trichome (e); rhomboidal and prismatic crystal (f-g); stellate hairs (h-i)

DISCUSSION

The present study gives a detailed pharmacognostic study of *H. rhamnoides* subsp. *turkestanica* Rousi., growing in the cold desert Himalaya of Ladakh. It highlights the morphological, anatomical, and physicochemical characteristics of the plant. Morphologically, the plant shows considerable variation in stem colour, ranging from brownish to silvery and ashy; which may reflect its environmental adaptations, particularly in high-altitude regions like Ladakh [3]. The deciduous nature of plant, presence of branched/unbranched thorns, and its leaves structure further supports its xerophytic nature of the *Hippophae rhamnoides* [3,30].

Anatomical analysis reveals well-developed secondary tissues in the stem, which include laticiferous canals, abundant xylem, and resin-filled cells, features indicative of vigorous structural integrity. The thorn and leaf structures show adaptive characters such as thick cuticle, stellate hairs, and arrangement of vascular tissue suitable for arid conditions. The leaf mesophyll shows a typical dorsiventral differentiation, supporting effective photosynthesis in cold desert regions like Ladakh.

The powder microscopy confirms characteristic features such as calcium oxalate crystals, cork fragments, pitted vessels, and stellate hairs useful in the authentication of raw drug. Based on organoleptic observations like colour, texture, and odor, the powder of stem and leaf has also been differentiated.

The physicochemical analysis reveals that the leaf consists of higher aqueous (11% w/w) and alcohol (24% w/w) extractive values as compared to the stem (5% w/w & 3% w/w), which suggest that leaf contains higher amount of alcohol- and water-soluble phytoconstituents. Low foreign matter and minimal moisture content in both parts emphasize its stability and suitability for their storage.

These findings support the therapeutic potential of *H. rhamnoides* subsp. turkestanica and aid in quality control in herbal preparations by providing an initial baseline for the pharmacognostic study.

CONCLUSION

Seabuckthorn (*Hippophae rhamnoides* subsp. *turkestanica)* is a valuable plant recognized for its diverse therapeutic properties and nutritional benefits. Ongoing researches continues to explore its potential in various health applications. The present study is an attempt to determine the macro-and microscopic features and physicochemical properties of its stem and leaf. The results will assist in differentiating the plant and help in the identification and authentication of crude drug/raw standardization of the single drug or as a powder form, even when added to a formulation as ingredients from adulterant and future studies.

Conflict of interest

The authors declared no conflict of interest.

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