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PHARMACOGNOSTIC STUDY OF THE FRUIT AND SEED OF SEABUCKTHORN (*HIPPOPHAE RHAMNOIDES* SUBSP. *TURKESTANICA* ROUSI) – A SUPER PLANT OF THE COLD DESERT OF INDIA

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ABSTRACT: *Hippophae rhamnoides* subsp. *turkestanica* Rousi. (seabuckthorn), a multipurpose plant growing in the cold desert of the Union Territory of Ladakh, India. The plant samples were collected from Skurbuchan, located at 34° 26' 07" N and 76° 42' 38" E, along the Indus River bank in the Union Territory of Ladakh, India. The samples are shade-dried. The microscopic observations are examined under different magnifications. The physicochemical properties, such as LoD, ash value, water and alcohol soluble extractive value, and acid insoluble ash, as prescribed in the Ayurvedic Pharmacopoeia of India have also been calculated. The T.S. of fruit pulp reveals a pericarp with an inner endocarp, middle mesocarp, and outer epicarp. The mesocarp contains parenchyma cells with orange material, while the epicarp consists of elongated epidermal cells. The pericarp also has oil droplets and vascular bundles. The seed test a has a hypodermal layer of thick-walled sclereids, followed by a thick endosperm and cotyledon epidermis layer. The powder microscopy also supports the features. Physicochemical analysis shows values recorded in %w/w. The fruit pulp showed a slightly higher moisture content. The total ash and acid-insoluble ash values were relatively higher in the seed (8.5% and 1.25%) than in the fruit pulp (2.75% and 0.25%). However, the alcohol and water extractive values are 54% and 59% for fruit pulp and 15% and 12% for seed. The results obtained from the pharmacognostic studies could be useful to identify and authenticate crude drugs and ensure their quality and purity.

INTRODUCTION: The genus *Hippophae* L. (Elaeagnaceae) is a thorny, deciduous, hardy shrub/small tree distributed across the dry temperate regions of Asia and Europe,¹⁻² and known by various epithets like sea buckthorn, sallow thorn, sand thorn, or sea berry³.

Due to its economic, environmental, nutritional, and medicinal importance, sea buckthorn has gained global attention and has been domesticated in several parts of the world, including the USA, Canada, Russia, etc.⁴.

Hippophae is a drought and cold-resistant plant; its complex root system is associated with soil enrichment by fixing atmospheric nitrogen, which plays a vital role in soil enrichment and land reclamation⁵⁻⁶. Sea buckthorn is used extensively in the traditional medicinal system⁷, and researchers from across the world have reported a

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variety of pharmacological activities, including anti- atherogenic, anti-microbial, anti-cancer, anti-oxidant, anti-stress, cytoprotective, hepato-protective, immunomodulatory, radioprotective, and tissue regeneration ⁸⁻¹³. The pharmacological effects have also been documented in the ancient Greek literature of Theophrastus and Dioscorides ¹⁴, classical records including Jing Zhu Ben Cao from the Qing Dynasty and Sibü Yidian from the Tang Dynasty, and ancient Tibetan medical books, such as "The *RGyudBzi*" (The Four Books of Pharmacopoeia) ^{14, 15}. For many decades, sea buckthorn has been used in central and

southeastern Asia. The berries of this plant are one of the most nutritious and vitamin-rich in the plant kingdom ^{16, 17}. The fruit and seeds are good sources of bioactive compounds, including vitamins, carotenoids, organic acids, *etc* **Table 1**. Owing to these properties, sea buckthorn is known as "*The Super Plant*," "*Super Healthy Fruit*," "*Gold Mine*," "*Gold of the Cold Desert*" and "*Holi fruit*" ^{17, 18}. The present paper discusses the pharmacognostic study to lay down reliable parameters for the identification and authentication of its fruit and seed-based drugs.

TABLE 1: PHYTOCHEMICAL COMPOSITION OF FRUIT AND SEED OF SEABUCKTHORN ^{10, 19-20}

Plant part	Phytochemical constituents
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, dulcic 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.

MATERIAL AND METHOD:

Sample Collection and Authentication: The fruits 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. Seeds are separated from the fruits and allowed to dry at room temperature. The shade-dried fruit pulp and seed arethen preserved in the Raw Drug Museum of RARI, Jammu.

Section Cutting: The dried samples were soaked for softening as per the procedure mentioned in API (Ayurvedic Pharmacopoeia of India) ²¹.

Free-hand sections of fruit pulp and seed were cut with a sharp razor blade 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 fruit pulp and seeds was prepared separately using an electric grinder and then sifted through a 60 No. sieve as per the standard procedure ²². The sifted powders were then used for the powder microscopy and physicochemical study **Fig. 1**.

Powder Analysis: The microscopic features were studied using an Olympus CX41 trinocular microscope attached to a desktop by 10MP Magnus Magcam DC10, and macroscopic features were examined under a stereomicroscope Olympus SZ2- ILST connected by 5.1MP Magnus Magcam DC5. The Macroscopic observations, powder microscopy, and photographs of the sample were carried out under different magnifications ²³.

Physicochemical Analysis: Physicochemical parameters such as foreign matter, total ash value, acid insoluble ash, loss on drying, water and alcohol soluble extractive value, were computed as per the Ayurvedic Pharmacopoeia of India (API) guidelines.

Organoleptic Characterization: Organoleptic characteristic such as colour, taste, odour and texture of the fruit pulp and seed powder were studied.

RESULTS AND DISCUSSIONS:

Macroscopic Study:

Fruit: The fruits of *Hippophae rhamnoides* subsp. *turkestanica* Rousi. are oval or round in shape. The colour of fruit ranges from yellow to red, with

many transitional shades like deep yellow, yellowish orange, orange, and reddish orange. However, reddish-orange is the most prominent colour found in this plant ²⁴. The average size of fruits is 0.62 cm in length and 0.46 cm in width, with 0.12–0.26 cm peduncle **Fig. 1**.

Seed: The Seeds of Seabuckthorn also show variation in both colour and size. However, it is usually dark brown in colour, oval or elliptical in shape, and size varies from 0.2 cm to 0.45 cm in length and 0.15–0.2 cm in width respectively. Distinct variation in colour and shape has been reported in different accessions collected from the Union Territory of Ladakh ^{4, 24} **Fig. 1**.

Microscopic:

Fruit Pulp: The T.S. of the fruit shows pericarp consisting of outer epicarp, middle mesocarp, and inner endocarp. The Epicarp comprises of

epidermis consisting of tangentially elongated cells covered with cuticle, the mesocarp occupies the major part of the region.

It is formed of delicate, thin parenchyma, polygonal, oval, and cuboidal cells filled with orange content. A ring of vascular bundles is present in this region. The endocarp comprises of thin wall tangentially elongated cells. Oil droplets are scattered throughout the pericarp **Fig. 2**.

Seed: The T.S. of the seed shows epidermis of testa consisting of long, thick-walled palisade-like sclereids followed by a layer of hypodermis composed of short, thick-walled sclereids (bearer cells). Tegmen is represented by layers of disintegrated cells followed by the epidermis of the cotyledon. Below this are 5-6 rows of thick-walled, compactly arranged cells. The radicle region is positioned toward the micropyle **Fig. 3**.

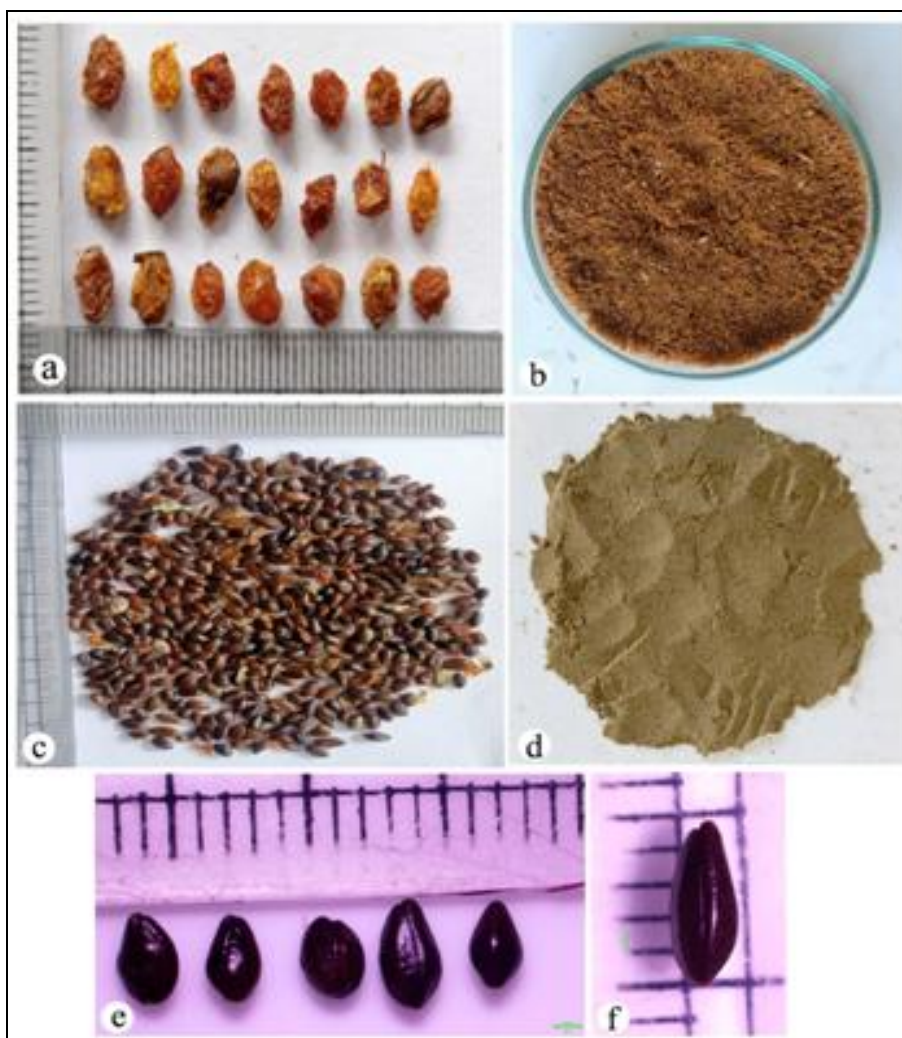


FIG. 1: HIPPOPHAE RHAMNOIDES SUBSP. TURKESTANICA ROUSI. FRUIT PULP (A) AND ITS POWDER (B); SEEDS (C) AND ITS POWDER (D); MAGNIFIED SEEDS (E, F).

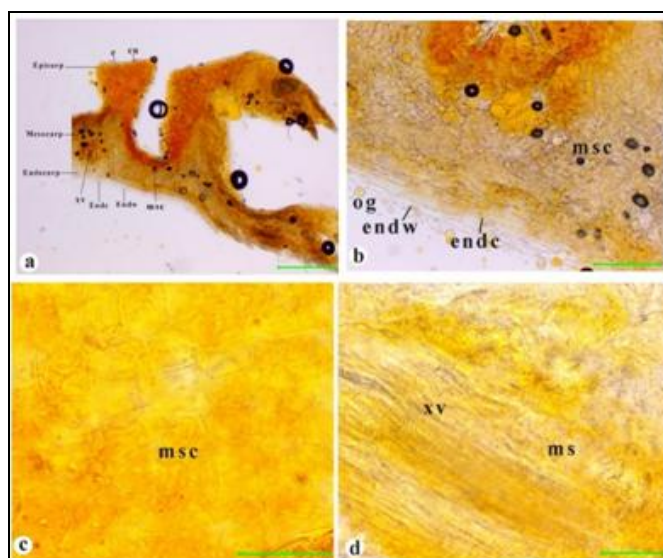


FIG. 2: T.S OF THE FRUIT PULP OF *H. RHAMNOIDES* SUBSP. *TURKESTANICA* ROUSI. ABBREVIATIONS: CU (CUTICLE); E (EPIDERMIS), XV (XYLEM VESSEL); MSC (MESOCARP); ENDW (ENDOCARP WALL); ENDC (ENDOCARP CELL).

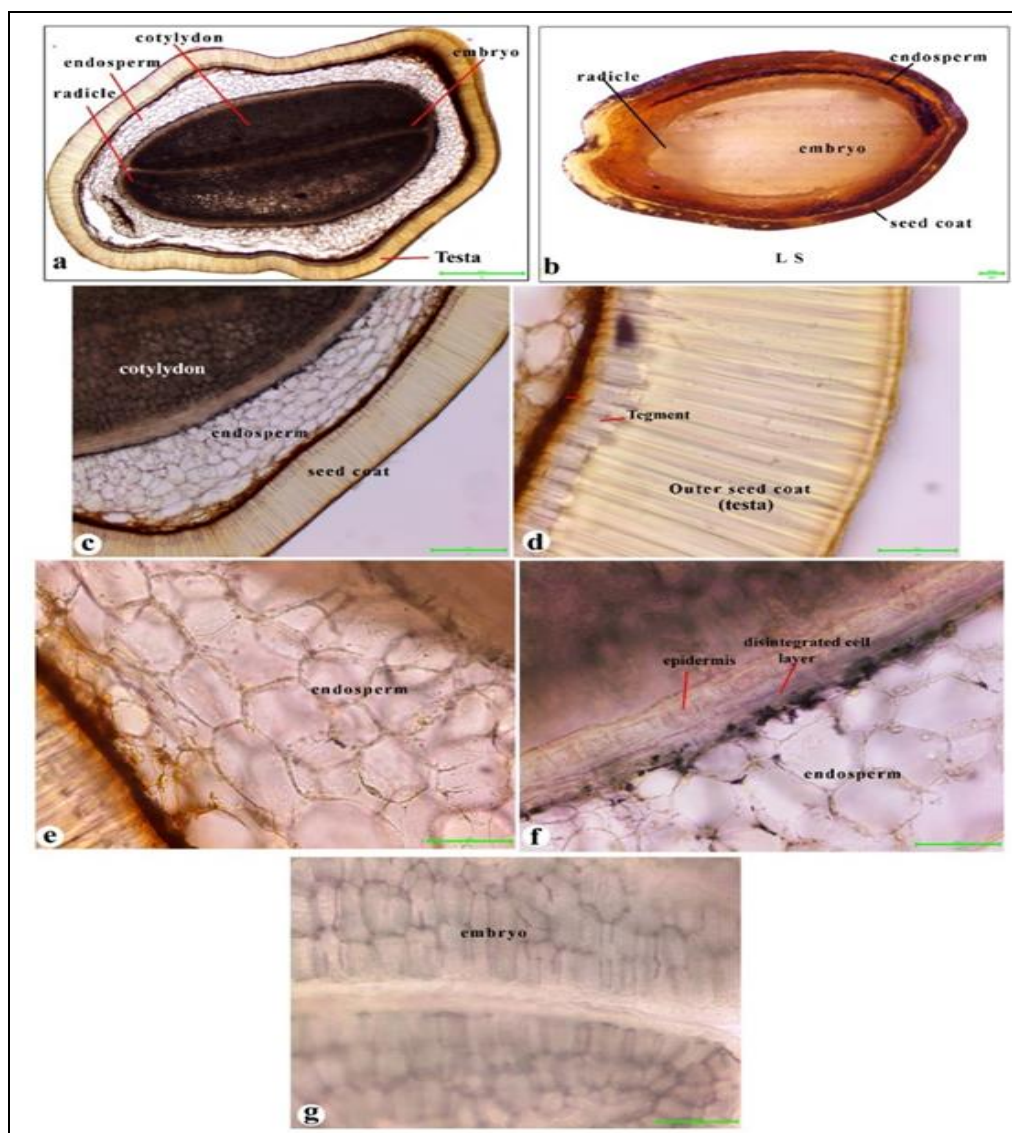


FIG. 3: T.S. (A, C-G) AND L.S. (B) OF THE SEED OF *H. RHAMNOIDES* SUBSP. *TURKESTANICA* ROUSI. SHOWING DIFFERENT PARTS OF THE COTYLEDON

Powder Analysis:

Organoleptic Study: The study of characteristic features of fruit pulp and seed powder of *Hippophae rhamnoides* L. is tabulated in **Table 2**.

TABLE 2: ORGANOLEPTIC CHARACTERS OF FRUIT PULP AND SEED POWDER OF SEABUCKTHORN

S. no.	Organoleptic characters	Fruit pulp	Seed
	Colour of powder	Dark orange	Golden
	Odour	Sweet	Sweet smell
	Taste	Slightly bitter	Not characteristic
	Texture	Intermediate	Smooth

Powder Microscopy:

Fruit Pulp: The powder of fruit pulp consists of fragments of cells of mesocarp filled with oil droplets, oil droplets, cells of the outer layer of endosperm, fragments of tangentially elongated epidermal cells, and vessels. It also shows the presence of lysigenous canals, palisade sclereid, peltate hairs and rhomboidal crystals of calcium oxalate **Fig. 3**.

Seed: The powder of the seed shows fragments of endosperm parenchyma cells of the outer covering of the cotyledon, cells of the endosperm, outer palisade sclereids of the seed coat, abundant oil droplets, the prismatic and rhomboidal crystals of calcium oxalate **Fig. 4**.

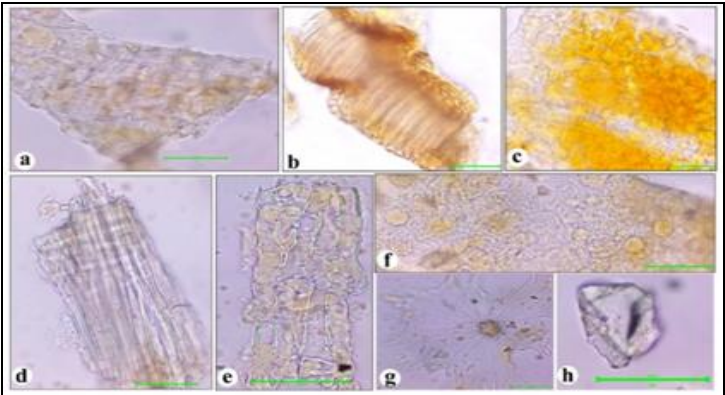


FIG. 4: PHOTO-MICROGRAPHS OF POWDER OF *H. RHAMNOIDES* SUBSP. *TURKESTANICA* ROUSL. FRUIT PULP. FRAGMENTS OF MESOCARP WITH OIL DROPLET (A, C); ANNULAR VESSEL (B); PALISADE SCLEREID (D); LYSIGENOUS CANAL (E); CELLS OF EPIDERMAL LAYER (F); PELTATE HAIR (G); RHOMBOIDAL CRYSTAL (H).

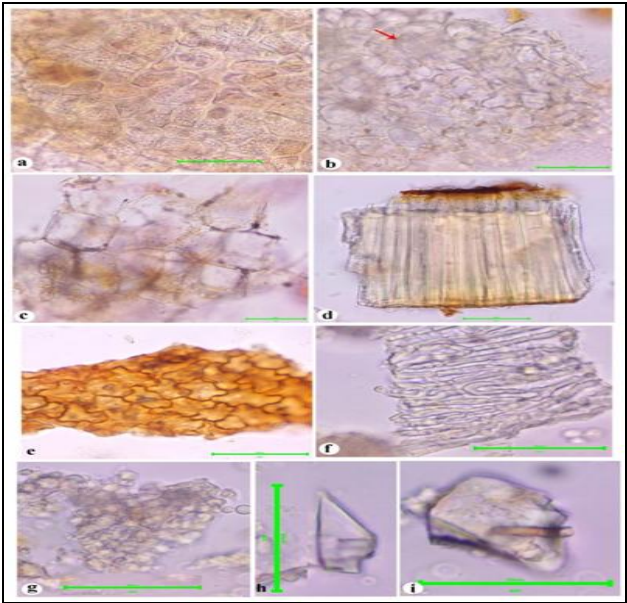


FIG. 5: PHOTO-MICROGRAPHS OF POWDER OF SEABUCKTHORN SEEDS. THE OUTER LAYER OF EMBRYO (A-B); CELLS OF ENDOSPERM (C); OUTER PALISADE SCLEREID (D); PARENCHYMA CELL OF OUTER COVERING OF COTYLEDON (E); LYSIGENOUS CANAL (F); OIL DROPLETS (G); PRISMATIC AND RHOMBOIDAL CRYSTALS (H-I)

Physicochemical Analysis: The physicochemical properties of the fruit pulp and seed of *H. rhamnoides* subsp. *turkestanica* Rousi. are presented in **Table 3**. Foreign matter was found in negligible amounts in both samples. The fruit pulp showed a slightly higher moisture content, as indicated by a greater loss on drying (0.44%) compared to the seed (0.13%). The result of ash analysis shows that the total ash and acid-insoluble ash values were relatively higher in the seed as compared to the fruit pulp. However, the alcohol and water extractive values for fruit pulp are 54%

and 59%, respectively, which are significantly higher than those of the seed **Table 3** and **Fig. 5**. The higher value of total ash and acid insoluble ash value of the seed indicates the presence of a higher concentration of impurities than in the fruit pulp. Similarly, the elevated values of alcohol and water extractive values of fruit pulp indicate the occurrence of more polar and soluble constituents. These properties may contribute to the plant's physiological adaptations to the harsh environmental conditions of high-altitude regions such as Ladakh.

TABLE 3: PHYSICOCHEMICAL PARAMETERS OF FRUIT PULP, AND SEED OF *H. RHAMNOIDES* SUBSP. *TURKESTANICA* ROUSI

S. no.	Physicochemical Parameters	Results of physicochemical analysis of <i>H. rhamnoides</i> subsp. <i>turkestanica</i> .	
		Fruit pulp	Seed
1	Foreign matter	<0.5%	<0.5%
	Values in % w/w		
2	Loss on drying	0.44	0.13
3	Total ash value	2.75	8.5
4	Acid-insoluble ash value	0.25	1.25
5	Alcohol-soluble extractive value	54.0	15
6	Water-soluble extractive value	59.0	12

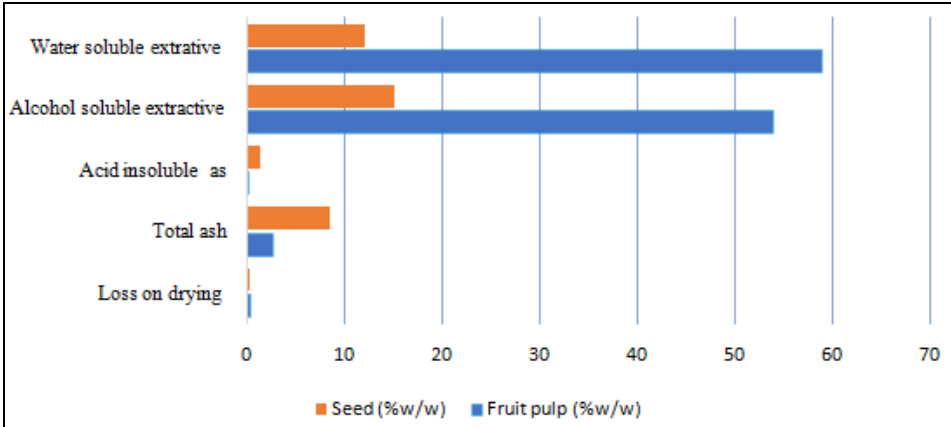


FIG. 6: BAR DIAGRAM DEPICTING THE PHYSICOCHEMICAL PARAMETERS AND THEIR VALUES OF SEABUCKTHORN FRUIT PULP, AND SEED

Seabuckthorn (*H.rhamnoides* subsp. *turkestanica* Rousi.) is a valuable plant, familiar for its various therapeutic properties, environmental, economic, and nutritional benefits. Across the world, research continues to explore its potential in various health applications. The present study aims to determine the macro- and microscopic characteristics as well as the physicochemical properties of the fruit and seed. The findings will support quality control, ensure purity, and aid in the identification and authentication of the raw drug or its powdered form, even when incorporated into formulations. This will also help to distinguish it from adulterants and guide future research.

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CONFLICT OF INTEREST: The authors declare there are no competing interests.

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