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Physicochemical, Phytochemical and Pharmacognostic study of *Limonium stocksii*, a halophyte from Gujarat

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ABSTRACT

The halophyte *Limonium stocksii* (Boiss) Kuntze belongs to the family Plumbaginaceae. In the present work, pharmacognostic studies of this medicinal halophyte was attempted which included physicochemical, phytochemical, macroscopic, microscopic studies and organoleptic evaluation. The plant powder characteristics were also elucidated. The physicochemical analyses were done by using WHO recommended parameters such as loss on drying, ash values (total ash, water soluble ash, acid insoluble ash, sulphated ash, carbonated ash, nitrated ash), extractive values. The qualitative phytochemical analysis revealed the presence of saponins, steroids and coumarins in maximum amount in crude as well as in various solvent extracts. The morphological studies exhibited the organoleptic and surface characteristics of the plant and its parts. The microscopic study showed the presence of various characteristics of whole plant like palisade tissue, metaxylem, protoxylem, phloem showed companion cells, bicollateral type of vascular bundles, parenchyma, rosette calcium oxalate crystals and paracytic stomata. The pharmacognostic characters enlisted in this study will help in identification of the crude drug; the standardization parameters laid down will ensure the efficacy of drug and also distinguish the drug from its adulterants. The distinguishing characters will also be helpful for the preparation of monograph of this halophytic plant.

Keywords: *Limonium stocksii*, halophyte, physicochemical analysis, phytochemical analysis, macroscopy, microscopy, powder analysis.

INTRODUCTION

Herbal medicines are safe, inexpensive and have no adverse effects. They are effectively being used to treat many diseases but lack of acceptability still persists because of lack of documentation and stringent quality control. They are also prone to adulteration and substitution which puts a doubt on their efficacy. Therefore it is of almost importance to lay down proper quality control measures of herbal drugs. There are many modern methods but still most simple, reliable and easy method is pharmacognostic study. Correct identification and quality assurance of the staring material will help to maintain the reproducible quality of herbal drugs and contribute to its safety and efficacy [1].

Halophytes are a group of higher plants that grow in saline conditions [2]. They are characterized by diverse morphological, anatomical, ultra-structural, pheonological, physiological and biochemical adaptations. They are able to survive in natural habitats like salt marshes, coastal beaches, salt deserts, inland and alkali saline soils. Halophytes are categorized in different groups like succulent halophytes, non-succulent halophytes, shrubby halophytes, facultative halophytes, strand species [3]. They are able to tolerate salt concentration, are good sources for human food including vegetables, pickles, salads, fodder for camels, sheep, goats, wood for building material, biofuel, chemicals, landscaping, dune, etc [4].

Halophytes, distributed from coastal regions to inland deserts, have traditionally been used for medicinal and nutritional purposes. They synthesize many bioactive molecules and are well equipped with powerful antioxidant system [5]. A number of halophytic medicinal plants are used to treat a variety of diseases. For eg. the stem of *Reaumuria vermiculata* showed antioxidant activity, anti-inflammatory and anticancer activity [6]; aerial part of *Crithmum maritimum* showed antioxidant and antimicrobial activity [7]; leaf of *Cressa cretica* showed anti-inflammatory and antipyretic activity [8]; aerial part of *Suaeda nigra* showed antioxidant and synergistic antioxidant activity [9]; leaf of *Suaeda fruticosa* showed antioxidant, anti-inflammatory and analgesic activities [10].

Limonium stocksii (Boiss) Kuntze belongs to the family Plumbaginaceae. It is commonly known as sea lavender. *Limonium* genus comprises 120 to 150 species of herbs and shrubs, perennial, low-branched, salt secreting, woody shrub. It is found in different climatic regions from artic to tropical, particularly

with salt rich steppes, marshy places and sea coasts [11]. Some species of *Limonium* showed antioxidant activity [12, 13], cardioprotective activity [14]; antimicrobial [11]; hepatoprotective activity [15, 16]; antioxidant activity, anti-inflammatory and anticancer activity [17].

The objectives of the present study was to delineate the physicochemical, phytochemical, fluorescence, macroscopic and microscopic characterization of leaf and stem of *Limonium stocksii*.

MATERIALS AND METHODS

Plant collection

The halophytic plant *Limonium stocksii* (Boiss) Kuntze was collected in August, 2017 from Porbandar, Gujarat, India. The plant was washed thoroughly was tap water, shade dried and homogenized to fine powder and stored in closed container for further studies.

Physicochemical analysis

The physicochemical parameters like loss on drying, total ash, acid-insoluble ash, water-soluble ash, sulphated ash and extractive values were determined as per WHO guidelines [18]. The solvents used were petroleum ether (PE), toluene (TO), ethyl acetate (EA), methanol (ME) and water (AQ). The details of the procedure followed is as described earlier [19].

Phytochemical analysis

The qualitative phytochemical tests of crude powder was carried out to identify different phytoconstituents [20]. The phytoconstituents like alkaloids, flavonoids, phenols, saponins, tannins, cardiac glycosides, steroids, phlobatanins, triterpenes, anthocyanins, etc. The details of the procedure followed is as described earlier [19].

Fluorescence analysis

Fluorescence study of different plants powder was performed as per [21]. A small quantity of the plants powder was placed on a grease free clean microscopic slide and 1-2 drops of freshly prepared reagent solution were added, mixed by gentle tilting of the slide and waited for a few minutes. Then the slide was placed inside the UV chamber and observed in visible light, short (254 nm) and long (365nm) ultra violet radiations. The colours observed by application of different reagents in different radiations were recorded.

Pharmacognostic study

Macroscopic study

The macroscopic studies were carried out using organoleptic evaluation method. The arrangement, size, shape, base, texture, margin, apex, veination, colour, odour, taste of leaves and stem were observed [22]. Macroscopic and microscopic characters were studied as described in quality control method [22]. Photographs at different magnifications were taken by using digital camera.

Microscopic study

Microscopic study was carried out by preparing thin sections of stem and leaf. The thin sections were further washed with water, stained with safranin, fast green and mounted in glycerine for observation and confirm its lignifications (10x, 40x) [23].

Powder Microscopy

The powder microscopy of the whole plant powder was studied using standard procedure by capturing the images of different fragments of tissues and diagnostic characteristic features were recorded [23].

RESULTS

Physicochemical analysis

The values of various physicochemical parameters evaluated including extractive values of *L. stocksii* plant are given in Table 1. The loss on drying of dry powder of plant was 10%. The total ash of whole plant powder was 11.83%, while water soluble ash and acid insoluble ash was 2.83% and 1.83% respectively. The sulphated ash of whole plant powder was 18.83%.The nitrated ash of whole plant powder was 16.67%. The carbonated ash of whole plant powder was 18.66%. The maximum soluble extractive value was found in methanol (14.12%) and minimum soluble extractive value was found in petroleum ether (1.52%). The water soluble extractive value was 20.12%.

Table 1: Physiochemical analysis of *L. stocksii*

No.	Parameters	% value (w/w) whole plant
1	Loss on drying	10.25
2	Total ash	11.83
3	Water soluble ash	02.83
4	Acid insoluble ash	01.83
5	Sulphated ash	18.83
6	Nitrated ash	16.67
7	Carbonate ash	18.66
8	Petroleum ether soluble extractive value	1.52
9	Toluene soluble extractive value	2.30
10	Ethyl acetate soluble extractive value	2.19
11	Methanol soluble extractive value	14.12
12	Water soluble extractive value	20.12

Phytochemical analysis

The qualitative phytochemical screening of the crude powder of *L. stocksii* plant is given in Table 2. In the crude powder of whole plant, saponins were present in maximum amount followed by alkaloids, phenols and quinones. Other phytoconstituents like phlobatannins, steroids, cardiac glycosides, triterpenes, anthocyanins, coumarins, leucoanthocyanins were present in trace amount; flavonoids and tannins were absent (Table 2).

The qualitative phytochemical analysis of the plant *L. stocksii* in different solvent extracts is given in Table 2. In PE solvent extract, alkaloids, saponins steroids and triterpenes were present in trace amount remaining phytoconstituents were absent (Table 2). In TO solvent extract, saponins and steroids were present in moderate amount; alkaloids, flavonoids, triterpenes and coumarins were present in trace amount while remaining phytoconstituents were absent. In EA solvent extract, alkaloids was present in maximum amount followed

by saponins and coumarins; flavonoids, steroids and triterpenes were present in trace amount while remaining phytoconstituents were absent. In ME solvent extract, saponins, steroids and triterpenes were present in maximum amount followed by coumarins; tannins were present in trace amount while remaining phytoconstituents were absent. In AQ solvent extract, flavonoids and coumarins were present in maximum amount followed by alkaloids. Saponins, steroids and triterpenes were present in trace amount while remaining phytoconstituents were absent.

Table 2: Qualitative phytochemical analysis of *L. stocksii* plant

Sr. No.	Phytochemicals	Whole plant					
		Crude powder	Different solvent extracts				
			PE	TO	EA	ME	AQ
1	Alkaloids						
	(1)Mayer's reagent	+	-	-	-	-	-
	(2)Dragondroff's reagent	++	+	+	-	-	++
	(3)Wagner's reagent	+	+	+	+++	-	-
2	Flavonoids	-	-	+	+	-	+++
3	Tannins	-	-	-	-	+	-
4	Phlobatanins	+	-	-	-	-	-
5	Saponins	+++	+	++	++	+++	+
6	Steroids	+	+	++	+	+++	+
7	Cardiac glycosides	+	-	-	-	-	-
8	Triterpenes	+	+	+	+	+++	+
9	Anthocyanins	+	-	-	-	-	-
10	Phenols	++	-	-	-	-	-
11	Coumarins	+	-	+	++	++	+++
12	Leucoanthocyanins	+	-	-	-	-	-
13	Quinones	++	-	-	-	-	-

(+++) more amount, (++) moderate amount, (+) less amount, (-) absent

Fluorescence analysis

The fluorescence characters of powdered drugs of plants play a very important role in the determination of quality and purity of the drug material. In the present study, dried powder of all the three plants was treated with a number of different reagents which showed characteristic fluorescence at 254 nm and 365 nm wavelength (Table 3). The plant powder showed different colours at both the wavelengths.

Table 3: Fluorescence analysis of *L.stocksii*

Sr No.	Treatment	Visible light	Under UV light short wave length (254 nm)	Under UV light long wave length (365 nm)
1	1 N NaOH(aq)	Green	Black	Green
2	1 N NaOH(alco)	Green	Black	Green
3	Ammonia	Light green	Black	Green
4	Petroleum ether	Green	Green	Yellowish green
5	50% HCl	Dark green	Black	Dark green
6	50% H_2SO_4	Green	Black	Light green
7	Ethyl acetate	Green	Brown	Green
8	Ethyl alcohol	Green	Brown	Light green
9	Methanol	Green	Black	Yellow
10	50% KOH	Brown	Black	Dark green
11	50% HNO_3	Brown	Black	Black
12	Acetic acid	Dark green	Black	Brown
13	Iodine in water (1%)	Yellowish green	Black	Light green
14	$FeCl_3$	Blackish green	Black	Light black

Pharmacognostic study

Macroscopic characteristics

L. stocksii is a shrubby halophyte. The organoleptic and macroscopic characteristics of the plant is given in Table 4 and Fig. 1.

Table 4: Organoleptic features of *L. stocksii*

Characters	Observation	
Part	Leaves	Stem
Arrangement	Opposite	-
Size	1-3 cm long and 0.5-1 cm wide	10-15 cm long and 0.2-0.3 cm thick
Shape	Spatulate	-
Color	Green	Greenish pink
Odour	Characteristic	Characteristic
Taste	Salty	Salty
Appearance	Smooth fleshy	Herbaceous
Margin	Entire	-
Apex	Rounded	-
Base	Symmetrical	-
Petiole	Sessile	-
Texture	Glabrous and fleshy	Rough
Veination	Reticulate	-
Outer surface	Smooth	Rough

a) Leaf **b) Stem**

Figure 1: Macroscopic study of *Limonium stocksii*(Boiss)Kuntze

Leaf

The leaf was simple, green in colour, phyllotaxy was opposite, shape was spatulate, margin was entire, base was symmetrical, apex was rounded, veination was reticulate, petiole was sessile, outer surface was smooth, appearance was smooth and fleshy, the odour was characteristic and taste was salty. The average size of leaf was 1-3 cm long and 0.5-1 cm wide (Fig. 1a and Table 4).

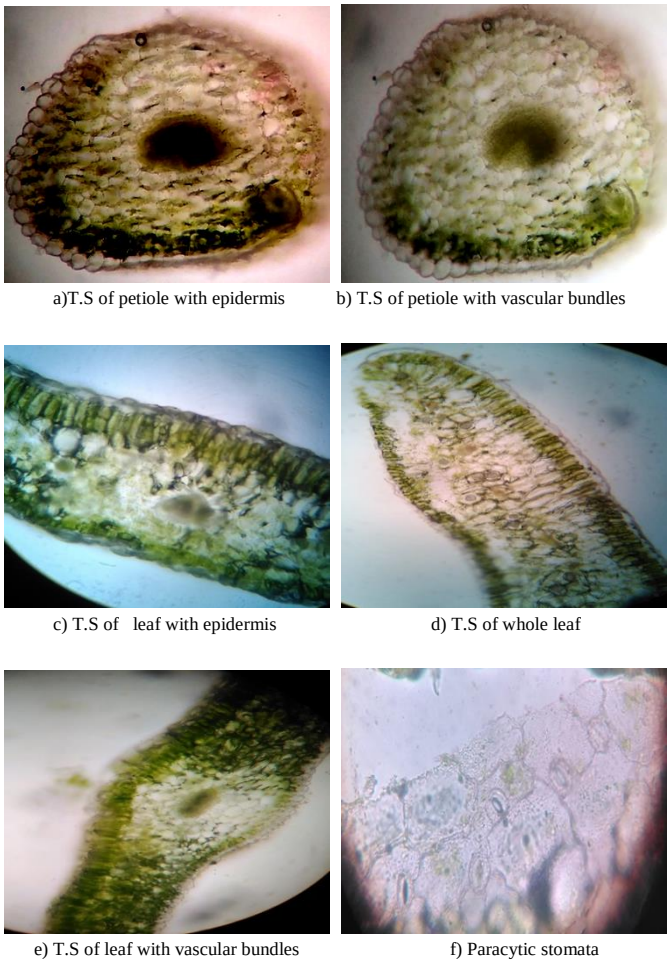
Stem

Stem was greenish pink in colour, leafy branches, herbaceous. Outer surface was rough. The average size of the stem was 10-15 cm long and 0.2-0.3 cm thick (Fig. 1b and Table 4).

Microscopic characteristics

Petiole

The transverse section of *L. stocksii* petiole is shown in Fig. 2a. The epidermis was single layered, ground tissue was not differentiated into cortex and endodermis. Ground tissue was parenchymatous, vascular bundles were arc shaped, concentric with conjoint collateral close type, i.e. xylem surrounded by the phloem (Fig. 2b).



a)T.S of petiole with epidermis b) T.S of petiole with vascular bundles

c) T.S of leaf with epidermis d) T.S of whole leaf

e) T.S of leaf with vascular bundles f) Paracytic stomata

Figure 2: Microscopic study of leaf of *L. stocksii*

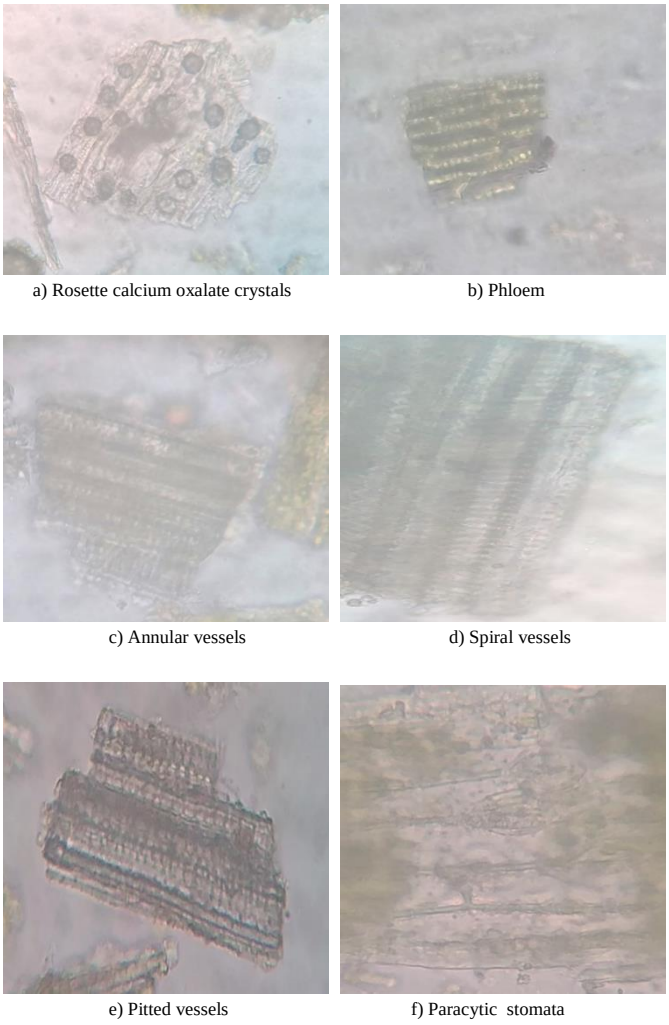
Leaf

The transverse section of *L. stocksii* leaf is shown in Fig. 2. The leaf lamina was isobilateral in nature. The upper epidermis and lower epidermis were single layered (Fig. 2c). The palisade tissue was single layered on the upper and lower surface, it was covered with thin cuticle layer (Fig. 2d). Ground tissue was parenchymatous, vascular bundles were arc shaped, concentric with conjoint collateral close type, i.e. xylem surrounded by the phloem (Fig. 2e). The paracytic stomata were present on lower epidermis (Fig. 2f).

Stem

The transverse section of *L. stocksii* stem is shown in Fig. 3. The epidermis was single layered with thick cuticle. The cortex region consisted 4-6 layers with chlorenchymatous tissue (Fig. 3a). Vascular

bundles were bicollateral, conjoint and close type, phloem surrounded by xylem from both the surface (Fig. 3b). The xylem was well developed, consisted of vessels, metaxylem, protoxylem and xylem parenchyma, phloem consisted of sieve tubes, companion cell and phloem parenchyma (Fig. 3c). Pith was well developed and made up of thick sclerenchymatous tissue (Fig. 3d).



a) Rosette calcium oxalate crystals b) Phloem

c) Annular vessels d) Spiral vessels

e) Pitted vessels f) Paracytic stomata

Figure 3: Powder study of *L. stocksii*

Powder microscopy of the whole plant

The crude powder of the *L. stocksii* plant was green in colour, taste was salty and odour was characteristic. The powder microscopic characteristics are shown in Fig. The specific characteristics of powder determined by microscopic investigation showed rosette calcium oxalate crystals, phloem, annular vessels, spiral vessels, pitted vessels, paracytic stomata, etc.

DISCUSSION

The medicinal plants occupy significant position for dominant sources of drug discovery; Standardization of a crude drug and correct identification of plant is crucial to maintain efficacy. Standardization parameters include physicochemical, phytochemical, fluorescence analysis, macroscopic, microscopic and powder study of the plant. Evaluating all these parameters will ensure and help in maintaining quality, purity and efficacy of the plant drug for its various uses. It will prevent the plant drug from adulteration and substitution intentionally or unintentionally [24].

The physicochemical parameters like loss on drying, total ash, acid insoluble ash, water soluble ash, loss on drying, and sulphated ash were determined. The values were in accordance to those reported earlier [3]. It is important to evaluate the physical constants of plant crude drugs since they help in identifying adulterants and or improper handling of the plant material [25]. The results of loss on drying for this plant were about 10% indicating that the drying process was efficient. Loss on drying for *Diplazium esculentum* was 10.8% [26] and for *Limonium brasiliense,* it was about 14% [27]. This is an important parameter, since it measures the efficiency of plant drying process which in turn is indicative of the stability of the drug during storage time [28]. It also implies that the phytoconstituents present in the plant are retained and the plant can be stored for a longer time; it will not encourage the growth of decay causing microorganisms [29]. Extractive values in a particular solvent give an idea about the purity of the crude drugs; exhausted or adulterated drugs can be easily identified. The extractive values indicate the nature and amount of chemical constituents present in the crude drug and also give an idea regarding their solubility in a particular solvent [30]. In *L. stocksii* plant the extractive value was maximum in methanol solvent (14.12) and minimum in petroleum ether (1.52).

The crude powder of *L. stocksii* plant was rich in saponins; while its solvent extract, ME extract was rich in saponins, steroids and triterpenes; EA extract was rich in alkaloids and AQ extract was rich in flavonoids and coumarins. These preliminary studies give an idea regarding the use of the plant for a particular biological activity. Saponins are reported for hypoglycemic activity [31], antioxidant activity [32], cytotoxic and antimicrobial activity [33] and anticancer activity [34]. Alkaloids are reported for antiplasmodial activity [35], antiprotozoal activity [36], antimicrobial activity [37]. Flavonoids are reported for antioxidant activity [38], anticancer activity [39], antimicrobial activity [40], antibacterial activity [41], anti-inflammatory activity [42]. Similar phytochemical screening have been reported for many other plants [43-44]. *L. stocksii* plant can be tried as a drug for the above mentioned biological activities.

Fluorescence analysis is a necessary parameter for first line standardization of crude drugs. Light rich in short wavelengths is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substances which are not visibly fluorescent in day light. The fluorescence analysis is useful and a necessary parameter for qualitative assessment of crude drugs. It is an important parameter for pharmacognostic evaluation of crude drugs [45]. Similar fluorescence analysis is reported for other plants for eg. *Terminalia bellerica* [46]; *Tinospora cordifolia* [47], *Trichosanthes cucumerina* [48].

The macroscopic evaluation showed *L. stocksii* plant to be green in colour, shape of leaves was spatulate, apex was symmetrical and stem was greenish pink in colour and herbaceous. The microscopic evaluation showed leaf lamina was isobilateral, palisade parenchyma on the upper and lower surface, ground tissue was parenchymatous. Vascular bundles were arc shaped, concentric with conjoint, collateral, close type, paracytic stomata present. In the stem single layered epidermis, cortex was chlorenchymatous, vascular bundles were bicollateral, conjoint and close type, pith was made up of sclerenchymatous tissue. The powder study showed rosette calcium oxalate crystals, phloem, annular vessels, pitted vessels, paracytic stomata, etc.

The pharmacognostic studies are essential and has to be performed for all the medicinal plants which are being studied for any particular activity. They act as reference standard and are diagnostic features of that particular plant. Similar studies are reported for many other plants by other researchers [49-52].

CONCLUSION

The physicochemical, phytochemical, macroscopic and microscopic characteristic along with the powder studies are diagnostic features of *L. stocksii* plant will help in authentication and identification of crude drug of *L. stocksii*. These parameters can be used as reference standard of this plant and also help in preparation of a monograph. They will also help in maintain the quality and efficacy of the drug.

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