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Pharmacognostic and phytochemical studies of *Ipomoea pes-caprae*, an halophyte from Gujarat

Rokad Nilam, Pande Jyoti and Chanda Sumitra

Abstract
Ipomoea pes-caprae (L.) commonly called as beach plant belongs to the family Convolvulaceae and is a salt tolerant plant. It is a fast growing halophytic herb. The plant and its parts are used as traditional medicine for treating different diseases. In the present work, various quality control parameters were evaluated to lay down standardized reference parameters of *Ipomoea pes-caprae*. The microscopic study of leaf and stem showed abundant large cells of palisade tissues, paracytic stomata, conjoint collateral open vascular bundles throughout the transverse section. Powder microscopy study revealed the presence of spiral vessels, border pitted xylem vessels, paracytic stomata, multicellular trichomes, etc. The physicochemical properties such as loss on drying, total ash, acid insoluble ash, water soluble ash, sulphated ash and extractive values of leaf and stem were carried out which were all in the permissible limits. The qualitative phytochemical analysis showed the presence of more amount of phyto constituents like alkaloids, phenols, steroids, tannins, flavonoids in leaf and stem. The parameters evaluated will serve as pharmacognostic standards for *Ipomoea pes-caprae* leaf and stem. They will also help in correct identification and authentication of the plant and it can maintain its therapeutic efficacy.

Keywords: *Ipomoea pes-caprae;* pharmacognosy; qualitative phytochemical analysis; physicochemical analysis; powder study; leaf; stem

1. Introduction
Ipomea pes-caprae is valuable medicinal plant which belongs to Convolvulaceae family [1]. It is a climbing herbaceous plant which have heart shaped leaves and funnel shaped flowers [2]. It is known by many names like bayhops, railroad vine, coast morning glory, goat's-foot morning glory, salsa-dapraia etc. It is a beach plant which is found in tropical and subtropical regions of the world [3] and is a salt tolerant plant. All parts are medicinally useful and are generally used in folk and traditional medicines for treating different diseases like stomach pain, fever and rheumatoid arthritis, etc [4]. The plant shows many biological activities for eg. anti-inflammatory activity [5]; collagenase inhibitory activity [6]; antinociceptive activity [7]; anticancer activity [3]; antibacterial and antioxidant activities and the ability to inhibit plant growth [4]; antioxidant activity [8, 9]; anti sunscreen activity [10]; antifungal activity of flowers [11].

The therapeutic ability of the plants is not part specific. It varies from plant to plant but all parts are endowed with medicinal properties. It may be root, rhizome, stem, flower, leaf, fruit or seed. Different parts of the plant show different biological activities. Hence it is very essential and important to perform pharmacognostic studies of medicinal plants [12]. Before the plant can be taken up as drug alone or in formulation with other compounds, it is of utmost importance to lay down standardization parameters which will enable to maintain the authenticity and quality of the drug and prevent it from being adulterated and/ or substituted. Pharmacognostic studies of different parts is reported for many medicinal plants. Some of the examples are pharmacognostic studies are *Tephrosia purpurea* root [13]; *Ferula sumbul* root [14]; rhizome of *Smilax domingensis* [15]; *Mangifera indica* leaf [16]; *Diplazium Esculentum* leaf [17]; *Cissus quadrangularis* stem [18]; *Argyreia pilosa* stem [19]; fruit of *Helicteres isora* [20]; flowers of *Woodfordia fruticosa* [21] and *Aerva lanata* [22].

In the present work, an attempt has been done to lay down pharmacognostic, phytochemical and physicochemical parameters of *Ipomoea pes-caprae*, an halophytic plant from Gujarat.

2. Materials and methods
2.1 Plant collection
The plant *Ipomea pes-caprae* L. was collected in August, 2017 from Porbandar, Gujarat, India. The leaves and stem were separated, washed thoroughly was tap water, shade dried and homogenized to fine powder an d stored in closed container for further studies.

2.2 Pharmacognostic studies

Macroscopic studies

Macroscopic studies were carried out using organoleptic evaluation method. The shape, size, colour, odour, taste, base, texture, margin, apex, veination, arrangement, of leaves and stem of plants were observed [23]. Macroscopic and microscopic characters were studied as described in quality control method [24]. Photographs at different magnifications were taken by using digital camera.

Microscopic studies

Microscopic studies were carried out by preparing thin sections of leaf and stem. 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). The powder microscopic studies were also carried out and the specific diagnostic characteristic features were recorded [24].

2.3 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 [25] in dried powder of different plants. The details of the procedure followed is as described earlier [26].

2.4 Qualitative phytochemical analysis

Qualitative analysis for the detection of phytoconstituents in the powdered leaf and stem was carried out by standard methods [27, 28]. Alkaloids were detected by using three reagents viz. Dragondroff's reagent, Mayer's reagent and Wagner's reagent separately. The tests were scored positive on the basis of orange precipitate, creamish precipitates and brown precipitate respectively. Flavonoids were detected by alkaline reagent test. The crude powder of leaf and stem was treated with a few drops of diluted sodium hydroxide. Formation of intense yellow colour which turned colourless on addition of few drops of diluted HCl indicated the presence of flavonoids. Tannins were detected by $FeCl_3$ test. The crude powder of leaf and stem was treated with alcoholic ferric chloride ($FeCl_3$) reagent. Appearance of blue colour indicated the presence of tannins. Cardiac glycosides were detected by Keller-kiliani test. The crude powder of leaf and stem was treated with 5% $FeCl_3$ and glacial acetic reagent to which few drops of concentrated H_2SO_4 was added. Appearance of greenish blue colour within few minutes indicated the presence of cardiac glycosides. Steroids were detected by Liebermann-Burchard test. The chloroformic solution of the crude powder of leaf and stem was treated with acetic anhydride and a few drops of concentrated H_2SO_4. Appearance of blue green ring indicated the presence of steroids. Anthocyanins were detected by the appearance of blue colour on treatment of crude powder of leaf and stem with NaOH. Saponins were determined by Frothing test; the formation of stable froth upon vigorous shaking of crude powder of leaf and stem with distilled water indicated a positive test. Triterpenes were detected by the addition of concentrated H_2SO_4 to the chloroform extract of crude powder of leaf and stem. Appearance of reddish brown ring indicated the presence of triterpenes. Leucoanthocyanins were detected by the addition aqueous extract of crude powder of leaf and stem to isoamyl alcohol. Appearance of red colour in upper layer indicated the presence of leucoanthocyanins. Quinones were detected by the addition of aqueous extract of crude powder of leaf and stem to concentrated HCl. Formation of yellow precipitation indicated the presence of quinones. Coumarins were detected by the addition of aqueous extract of crude powder of leaf and stem to 10% NaOH. Formation of yellow colour indicated the presence of coumarins.

3. Results

3.1 Organoleptic and macroscopic characteristics of *Ipomea pes-caprae*

Organoleptic and macroscopic characteristics of *Ipomea pes-caprae* leaf and stem are given in Table 1 and Fig. 1.

Leaves

The leaf was simple, alternate, obcordate, margin entire, apex emarginate, veination reticulate, odour and taste characteristic. The average leaf size was 5-7 cm in length and 7-9 cm in width (Fig.1 and Table 1).

Stem

The stem was green, herbaceous, prostrate, cylindrical, up to 30 cm height bearing numerous branches, 0.3-0.4 cm in thickness; odour and taste was characteristic. Outer surface was smooth (Fig.1 and Table 1).

3.2 Microscopic characteristics

Petiole

The transverse section of *I. pes-caprae* is shown in Fig. 2. The petiole was kidney shaped towards the distal end (petiole) and crescent shaped towards the laminal side. The upper and lower epidermis was single layered (Fig. 2a). The hypodermis was 5-6 celled with collenchymataous tissue. Ground tissue was parenchymatous, vascular bundles were present in 'arc' shape and three in number; the size of the vascular bundles varied from centre to leaf margin i.e. large too small. They were centripetally arranged i.e. xylem surrounded by the phloem (Fig. 2b).

Leaf

The transverse section of *I. pes-caprae* leaf is shown in Fig. 2. Leaf lamina was dorsiventral in nature. The upper epidermis was single layered, covered with a single layer of cuticle (Fig. 2c). The palisade tissue was single layered on upper surface; the mesophyll layer was small 3-5 celled (Fig. 2d). Transverse section passing through the mid rib region showed vascular bundles present towards the ventral surface. Some parenchymatous cells were surrounded by centrally located collateral vascular bundles (Fig. 2e). The paracytic stomata were present in lower epidermis. The stomata were surrounded by two parallel small subsidiary cells, whereas the guard cells were comparatively larger in size (Fig. 2f).

Stem

The transverse section of *I. pes-caprae* stem is shown in Fig. 3 The epidermis was single layered, thick walled with narrow and elongated cells. The epidermis was surrounded by cuticle (Fig. 3 a). The transverse section of stem was cylindrical in shape, the cortex consisted of 4-6 layers, vascular bundles were surrounded by polygonal lignified parenchyma and chollenchyma cells (Fig. 3b).Vascular bundles consistsed of secondary phloem with sieve tubes, companion cells and phloem paranchyma and secondary xylem consisted of lignified trachea, tracheids, few vessels and xylem fibre. Fibers were pitted, elongated and moderately thickened (Fig. 3c). Pith was very large and consisted of thin walled sclerenchymatous cells (Fig. 3 f).

3.3. Powder microscopy

Leaf

The crude powder of *I. pes-caprae* was dark green in colour, fine with characteristic odour and taste. The powder microscopy characteristics are shown in Fig. 4. The specific characteristics determined from the powder study under microscopic investigation showed spiral vessels, bordered pitted vessels, paracytic stomata, annular vessels, etc.

Stem

The crude powder of *I. pes-caprae* was green in colour with characteristic odour and taste. The powder characteristics are shown in Fig. 5. The specific characteristics determined from the powder study under microscopic investigation showed multicellular trichome, elongated parenchyma, sclerenchymatous tissue, spiral vessels, etc.

3.4 Physicochemical analysis

The physicochemical parameters of *I. pes-caprae* plant evaluated is given in Table 2. The loss on drying of leaf dry powder 90 % while that of stem was 93 %. The total ash was 14.5 % in leaf and 12.5% in stem, while water soluble ash and acid insoluble ash was 9.3 % and 1 % in leaf and 6.3 % and 0.16 % in stem respectively. The sulphated ash was 16 % in leaf and 15.3 % in stem. The extractive values of leaf and stem are given in Table 2. The maximum soluble extractive value was found in methanol solvent extracts; it was 15.74 % in leaf and 13.22 % in stem. Minimum soluble extractive value was found in petroleum ether extracts; it was 1.11% in leaf and 1.49 % in stem. The water soluble extractive value was 19.75 % in leaf and 12.71 % in stem.

3.5 Qualitative phytochemical analysis

The results of qualitative phytochemical screening of the crude powder of *I. pes-caprae* leaf and stem are given in Table 3. In leaf, alkaloids, steroids and triterpenes were present in maximum amount followed by flavonoids, phenols and leucoanthocyanins (Table 3); tannins, saponins, cardiac glycosides, anthocyanins were present in trace amount; other phytoconstituents were absent. In stem, phenols were present in maximum followed by moderate amount of other all phytoconstituents except anthocyanins, coumarin and quinones which were absent (Table 3).

4. Discussion

The pharmacognostic analysis of *I. pes-caprae* plant characters helps in establishing its botanical identity. The standardization of the herbal medicines is necessary to assure the quality of the drug like traditional and allopathic medicine quality control. Standardization parameters will also help in checking and preventing substitution and adulteration of foreign material, which is nothing but mixing or substituting the original drug material with other spurious, substandard, defective, spoiled, useless other parts of the same or different plants [29] which can occur in herbal medicine especially in plants which are short supplied or highly in demand. Hence pharmacognostic studies are very important. According to WHO [30], standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment [30, 16].

Pharmacognostic study involves organoleptic evaluation, macroscopic characterization, microscopic characterization, powder studies, phytochemical analysis, physiocochemical analysis, etc. In the present work, the leaf showed specific microscopic characters like thin walled epidermis with cuticle, 2-3 layers of cortex, conjoint collatral with arc shape vascular bundles, etc. The stem showed specific microscopic characters such as thick walled epidermis, a few layers of cortex, conjoint collatral close type of vascular bundles with thin continuous cylindrical sclerenchymatous cells.

The powder analyses for various phytochemical constituents revealed maximum amount of alkaloids, steroids and triterpenes in leaf while phenols were maximum in stem. The physicochemical parameters like ash values, acid insoluble ash, water soluble ash, loss on drying and sulphated ash were found with in the limits of standard. The extractive value was maximum in methanol solvent in both leaf and stem. Such pharmacognostic studies have been done for various other medicinal plants such as *Terminalia bellerica* leaf and stem [31]; *Limonia acidissima* L. leaf and stem [32]; leaf and stem of *Pterocarpus santalius* [33]; Different parts of the plant i.e. leaf, root, stem and seed of *Putranjiva roxburghii* [34]; stem bark of *Detarium Microcarpum* [35].

Fig 1: Macroscopic study of *Ipomea pes-caprae*

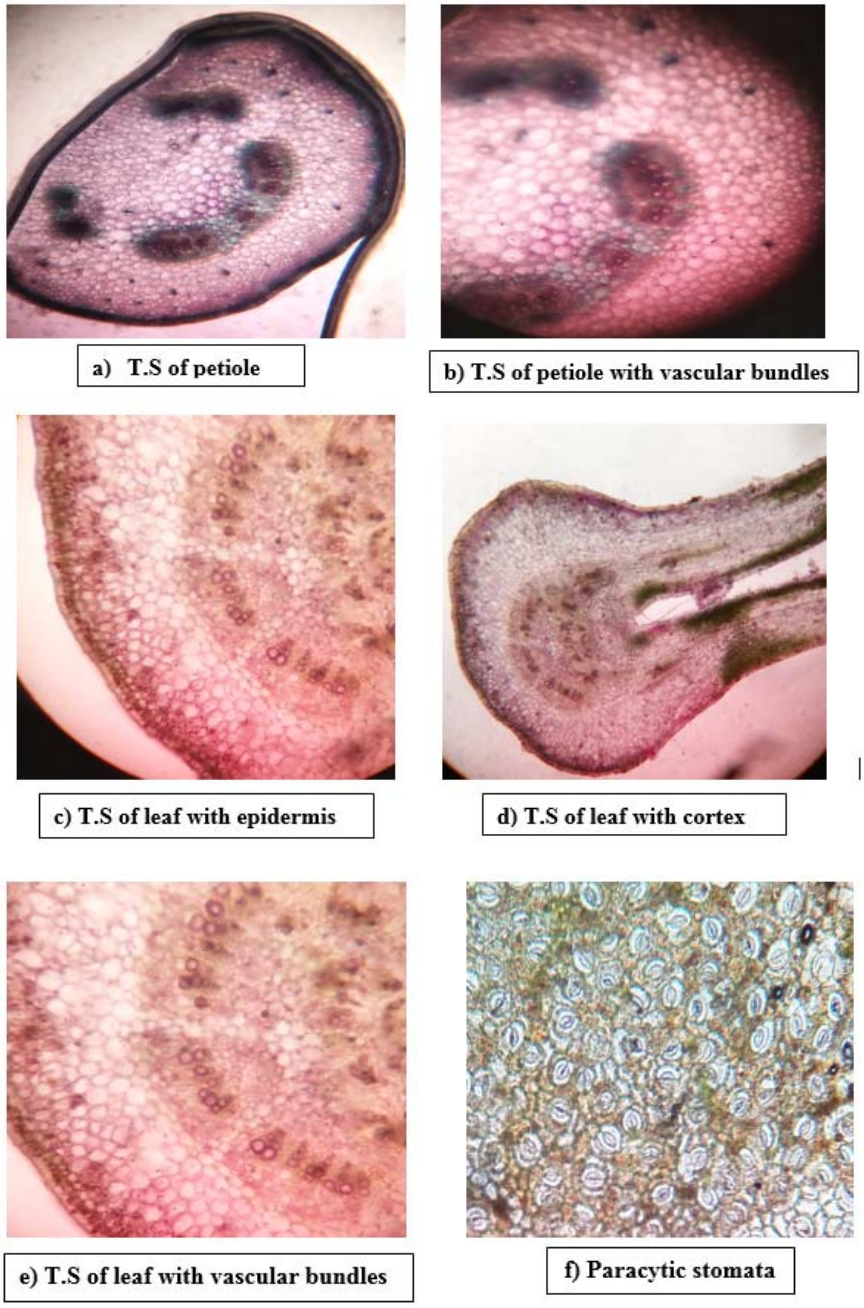
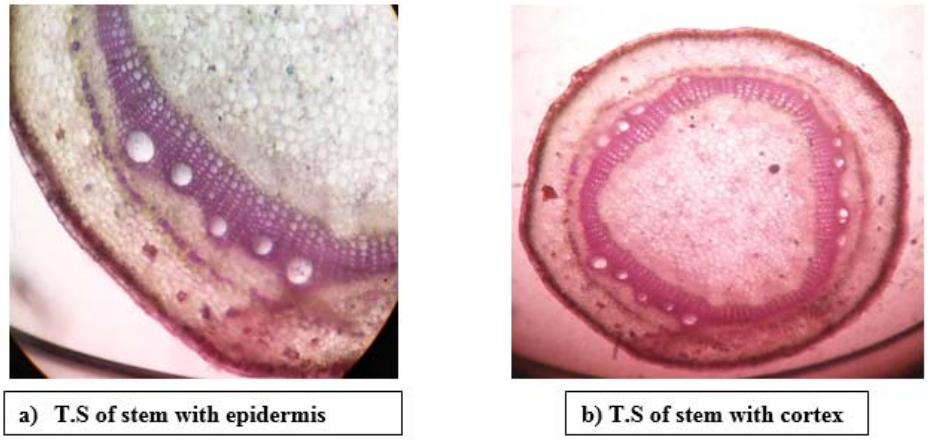


Fig 2: Microscopic study of leaf of *Ipomea pes-caprae*



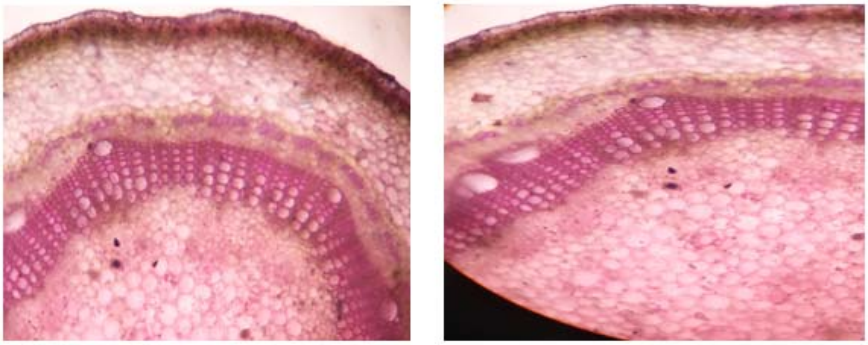


Fig 3: Microscopic study of stem of *Ipomea pes-caprae*

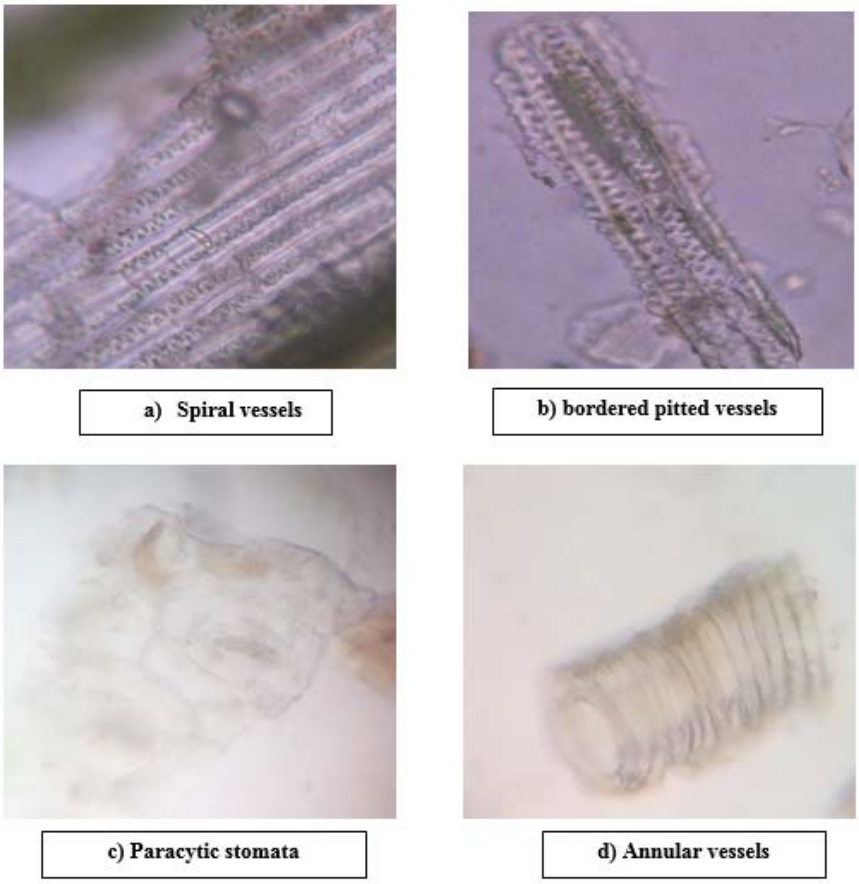
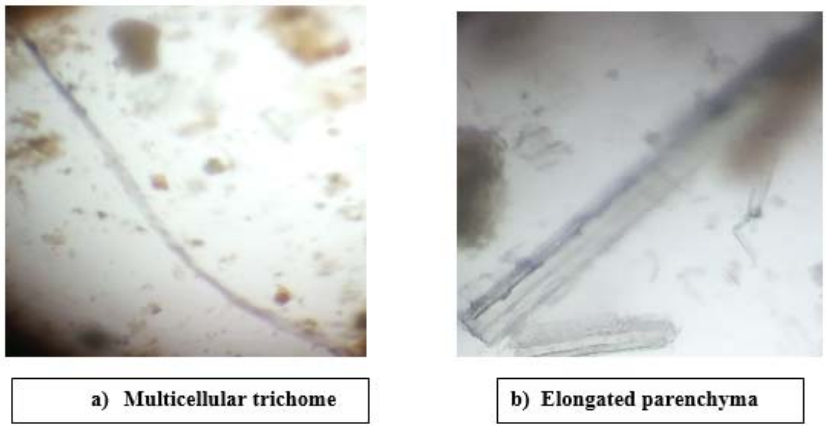


Fig 4: Powder study of *Ipomea pes-caprae* leaf



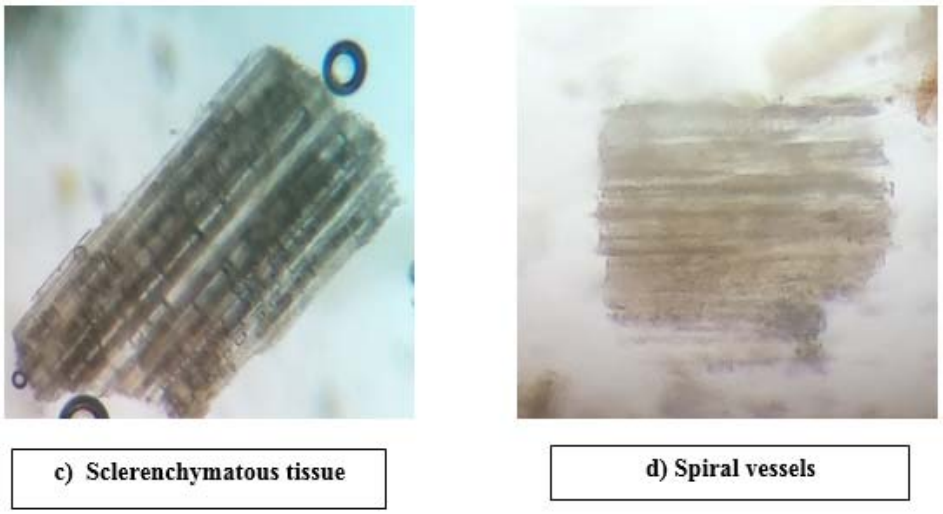


Fig 5: Powder study of stem of *Ipomea pes-caprae*

Table 1: Organoleptic features of *Ipomea pes-caprae*

Characters	Observation	Observation
Part	Leaves	Stem
Arrangement	Alternate	-
Size	5-7 cm long, 7-9 cm wide	0.3-0.5 cm thickness,30 cm height
Shape	Obcordate	Cylindrical
Color	Yellowish green	Green
Odour	Characteristic	Characteristic
Taste	Characteristic	Characteristic
Appearance	Smooth	Herbaceous
Margin	Entire	-
Apex	Emarginate	-
Base	Symmetrical	-
Petiole	Long	-
Texture	Smooth	Smooth
Veination	Reticulate	-
Outer surface	-	Light green color Smooth surface

Table 2: Physiochemical parameters of *Ipomea pes-caprae*

No.	Parameters	% value (w/w) Leaf	% value (w/w) Stem
1	Loss on drying	90	93
2	Total ash	14.5	12.5
3	Water soluble ash	9.3	6.3
4	Acid insoluble ash	1.0	0.16
5	Sulphated ash	16	15.3
6	Petroleum ether soluble extractive value	1.11	1.49
7	Toluene soluble extractive value	1.98	2.3
8	Ethyl acetate soluble extractive value	2.02	2.18
9	Methanol soluble extractive value	15.74	13.22
10	Water soluble extractive value	19.75	12.71

Table 3: Qualitative phytochemical analysis of *Ipomea pes-caprae*

No.	Phytochemicals	Leaf	Stem
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

5. Conclusion

I. pes-caprae is traditionally used to treat various diseases and has many therapeutic uses and therefore deserves to be correctly identified and maintain the quality of crude drug. Various parameters like microscopic studies, powder studies, phytochemical and physicochemical parameters evaluated in the present work will aid in identifying the plant and prevent it from getting adulterated. Presence of maximum amount of certain phytoconstituents like alkaloids, steroids and triterpenes in leaf and phenols in stem will help the researchers to use this plant in certain type of diseases where these phytoconstituents are required.

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