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Microscopic, pharmacognostic and phytochemical screening of *Epiphyllum oxypetalum* (dc) haw leaves

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Abstract
Epiphyllum oxypetalum (DC.) Haw is a widely used medicinal and ornamental plant that belongs to cactus species. It is universally known as Queen of the night, Night blooming Cereus or Lady of the night due to the resemblance of its beautiful flowers to the Lotus and the fact that it blooms at night, much like most other white flowers. The macroscopic and microscopic evaluation was performed along with an analysis of physicochemical and phytochemical properties of the powdered leaves. The arrangement of different cells, vascular bundle, sclerenchyma, pith etc was studied using transverse section analysis. Under ordinary and UV light of short and long wavelength, different colours were observed using fluorescence analysis. Natural bioactive compounds present in the leaves were evaluated using different solvent extracts, which indicated the presence of tannins, alkaloids, phenols, flavonoids, sterols, saponins, carbohydrates and proteins. Physicochemical analysis like loss on drying, ash values, water solubility, acid insolubility and pH values helped in the identification and quality control of *E. oxypetalum* plant extract.

Keywords: *Epiphyllum oxypetalum;* phytochemicals; pharmacognostic; transverse section; fluorescence analysis.

1. Introduction
E. oxypetalum Haw. Is commonly known as Brahma Kamal or Nishagandhi in India, Bakawali or Bunga Raja in Malaysia, Kadupul in Sri Lanka, Gekka Bijin in Japan and Wijaya Kusuma in Indonesia. This is the native of southern Mexico but seen in major part of North America and South East Asia. It is an important medicinal plant belonging to the family Cactaceae and is a night blooming cereus, usually known as Orchid cactus, Queen of night or Lady of night (Dandekar *et al.*, 2015) [5]. The name *oxypetalum* refers to the acute shape of the petals. The origin of this species is still not known clearly. In India it is treated as a sacred plant known by the name Brahma Kamal, which is, in fact, a false Brahma Kamal (Dandekar *et al.*, 2015) [5]. The botanical name of the true Brahma Kamal is *Saussurea obvallata* (DC.) Edgew. and is an inhabitant of the Himalayas in India. *E. oxypetalum* belongs to Cactaceae and *S. obvallata* belongs to Asteraceae family.
E. oxypetalum is cultivated mainly as an ornamental plant and is now being explored for its antibacterial and nutritious properties (Jeelani *et al.*, 2018) [9]. Bentham and Hooker classification of *E. oxypetalum* is given in Table 1 (Mahmad *et al*, 2017) [13]. The phylloclade of the plant is found to have anti-bacterial activity (Chen, 1967) [4]. Different bioactive components extracted from the plant have been used to treat numerous diseases caused by pathogenic micro-organisms in Asia, Africa, Latin America and Middle East Countries (Upendra and Khandelwal, 2012) [19]. This epiphytic plant is covered with hair bristles and has fibrous roots. The stem is branched and tapers towards the end. The flowers are 6-8 inches wide. The plant is used to treat respiratory problems like cough, phlegm, shortness of breath and also has a potential to neutralize blood clotting. Flowers are used for quick healing of wound abscesses (Chen, 1967) [4]. This medicinal plant contains a large variety of compounds having therapeutic and antioxidant properties which might be used as a lead for the development of novel drugs (Winrow *et al.*, 1993) [21]. Most of the medicinal plants contain alkaloids, tannins, saponins, terpenoids, flavonoids and phenolic compounds which are believed to be originated naturally in the plant as a self-defence against different pathogens and pests (Jeeva *et al.*, 2006) [10]. Dandekar and coworkers had done the GC-MS analysis of this plant extract and found that steroids were the major phytoconstituents present in the extracts (Dandekar *et al.*, 2015) [5]. The production of Ag nanoparticles using leaf extract of *E. oxypetalum* gave stable results. The synthesized Ag NPs exhibited antibacterial property

against both Gram-positive and Gram-negative bacteria (Paralikar, 2014) [15].

The leaves of *E. oxypetalum* have been found to have natural bioactive compounds, which are reflected from their diverse medicinal properties. Due to the picturization of the plant for its ornamental purpose, little information is available in the literature about this plant's bioactive constituents and much more attention is needed to explore the plant for other possible medicinal activities. Therefore, the present research involves anatomical and structural evaluations, fluorescence analysis and screening of phytochemicals and pharmacognostic characterization of *Epiphyllum oxypetalum* leaves. This analysis delivers the scientific data for the appropriate identification of standards and use of *E. oxypetalum* for various applications in the medicinal field.

Table 1: Taxonomy of *Epiphyllum oxypetalum*

Kingdom	Plantae
Phylum	Angiosperms
Class	Magnoliopsida
Order	Caryophyllales
Family	Cactaceae
Genus	Epiphyllum
Species	*E. oxypetalum*
Binomial name	*Epiphyllum oxypetalum*

Material and Methods

Pharmacognostic Studies

Collection of the plant material

Fresh leaves of *Epiphyllum oxypetalum* were collected in March 2018 from Bangalore district, Karnataka, India. The plant species were identified and authenticated by Dr. P Santhan, in-house taxonomist, R&D center, Natural Remedies Pvt. Ltd., Bangalore. The plant specimen is deposited in the herbarium of Natural Remedies Pvt. Ltd., Bangalore under voucher no: Bot.1106. The leaves were washed and dried under sunlight for seven days, powdered and preserved in a container and stored in a dry place.

Extraction of the plant material

Powdered leaves of *Epiphyllum oxypetalum* were packed in a Soxhlet extractor and extracted using methanol, water, petroleum ether and ethanol. This extract was used for different phytochemical tests.

Reagents and Chemicals

Analytical grade chemicals and reagents used in the present study were purchased from *s. d. Fine Chemicals*, India. Ethanol, methanol, petroleum ether, acetic anhydride, sulphuric acid, hydrochloric acid, Fehling solution A and B, ferric chloride, phenolphthalein indicator, potassium hydroxide, sodium carbonate, sodium thiosulphate, safranin dye and chemical reagents such as Wagner's reagent, Mayer's reagent, Dragendorff's reagent, Anthrone reagent, Molisch's reagent, Benedict's reagent, Ninhydrin reagent, Barfoed's reagent, Biuret reagent were used for different tests. Olympus Upright phase contrast microscope with DP20-5I Digital camera installed, (magnification ranges from 4x, 10x, 40x, 100x.) and UV-Vis Spectrophotometer (Shimadzu UV-1201) were used for the analysis.

Organoleptic evaluation: Sensory parameters of the leaves such as colour, odour and taste were studied by organoleptic evaluation.

Pharmacognostic evaluations

Macroscopic evaluation

Macroscopic assessments of leaves such as the occurrence of petioles and different characters of lamina (base, texture, venations etc) were carried out on the collected sample. The plant was taxonomically described according to the data collected from various sources (Kokate *et al.*, 2006) [12].

Microscopic evaluation

a) Powder Microscopic Examination

The powdered leaf was mixed with warm water and soaked for half an hour. Excess water was removed and the semisolid solution was taken on the slide and examined under a microscope (Ishtiaq *et al.*, 2016) [7].

b) Transverse section cutting

Authentication of the internal structure of the leaf was done by identifying internal structures such as epidermis, collenchyma, vascular bundles and its arrangement, sclerenchyma, crystals etc. For this purpose, a transverse section of the leaf by freehand sectioning technique was prepared. Fresh leaves were collected from Bangalore area. The midrib of the leaf including a small portion of lamina was cut using a sharp razor to obtain a transverse section of the leaf. These sections were stained using Safranin dye (1% solution). After staining for a few minutes, these sections were shifted to a watch glass comprising plain distilled water and the excess stain was removed. The slides were seen under a microscope and were photographed to know the internal structure.

Stomatal number

It is the average number of stomata present per square millimetre of the epidermis of the leaf. Stomatal index is the percentage of the ratio of a number of stomata per unit area on the leaf surface (S) to the total number of stomatal and epidermal cells (E) in the same unit area. $Stomatal\ index = \frac{S}{(E+S)}\ x\ 100$ E is the number of epidermal cells

Fluorescence analysis

Various chemical constituents present in the leaves exhibit the property known as fluorescence. It is a significant tool for screening the compounds that display different colours under UV and visible light. Ultraviolet fluorescence analysis of powdered leaf was carried out by treating them with different reagents including both acidic and basic solvents. In this typical analysis procedure, 0.2g of dried powdered leaves was taken in a test tube and 2 mL of the reagent was added. This mixture was then observed under UV (λ = 254 and 366 nm) and visible light (Schoor *et al.*, 2015) [17].

Phytochemical analysis

The phytochemical analysis of the leaf extract gives a general idea about the nature of chemical constituents present in the leaf and experiments were done according to the prescribed standard procedures (Ishtiaq *et al.*, 2016) [7] (Aslam and Afridi, 2018) [2].

Test for carbohydrate

Molisch's test: 1 mL of the extract and 2 mL of α-naphthol were mixed. 2 mL of concentrated H2SO4 is added along the sides of the test tube. The appearance of purple coloured ring at the interface of test solution and acid layer indicates carbohydrates.

Benedict's test: 1 mL of the extract and 2 mL of Benedict's reagent were mixed. Formation of brick red precipitate shows the presence of carbohydrates.
Barfoed's test: 2 mL of leaf extract and 2 mL of Barfoed's reagent were taken in a test tube. This test tube was immersed in a boiling water bath for 5 minutes. Brick red precipitates indicate carbohydrates.
Fehling's test: Extracts were dissolved in 5 mL of distilled water and filtered. The filtrate was hydrolyzed with dilute HCl. Fehling A and B solution was added into it. Formation of red precipitate gives a positive result.

Tests for proteins
Biuret test: 3 mL extract is mixed with 1 mL NaOH (40%) and 0.5 mL $CuSO_4$ solution. Presence of protein is confirmed by the blue colour in the test tube.
Millon's test: To 2 mL extract in test tube, 1 mL Millon's reagent is added to see the presence of brick red colour which confirms the presence of protein.
Ninhydrin test: 1 mL extract was mixed with 2 mL Ninhydrin reagent and the test tube is heated in a boiling water bath. The appearance of a violet colour directs the presence of protein.

Test for tannins
Ferric chloride test: 2 mL of the extract was mixed with 3 drops of $FeCl_3$ solution. The appearance of bluish black or greenish black colour specifies tannins**.**
Gelatin test: To 2 mL of the leaf extract, few drops of gelatin solution was added. Precipitation of gelatin directs the presence of tannins.

Test for Phenols
Ferric chloride Test: 1 mL of extract is mixed with 3 drops of $FeCl_3$ solution to form bluish black precipitate indicating the presence of phenols.

Test for alkaloids
Wagner's test: 1 mL of the extract and 3 drops of Wagner's reagent were mixed, development of brownish black colour shows a positive test for alkaloid.
Mayer's test: 1 mL of the extract and 3 drops of Mayer's reagent were mixed, the appearance of white colour indicates the presence of alkaloid.
Dragendorff's Test: 1 mL of the extract and 3 drops of Dragendorff's reagent were mixed, development of brownish red colour specifies the presence of alkaloid.

Test for flavonoids
Ferric chloride test: 2 mL of the extract was mixed with 0.5 mL of $FeCl_3$ solution. Development of intense green colour specifies flavonoids.
Alkaline reagent test: 5 drops of dilute NaOH was added to 2 mL of the extract. Formation of yellow colour which changes to colourless on addition of dilute HCl directs the presence of flavonoids.
Lead acetate test: Formation of yellow precipitate by the addition of lead acetate (10%) solution to the extract indicates the presence of flavonoids.

Tests for sterols
Liebermann Burchard's Test: 2 mL of the extract, 2 mL of chloroform and 2 mL of glacial acetic acid were mixed. 2 mL of concentrated H_2SO_4 is added along the sides of the test tube forming a green colour in the chloroform layer that shows the presence of sterols.
Salkowaski test: 1 mL of the extract is mixed with 1 mL of chloroform and 1 mL conc. H_2SO_4 is added along the side of the test tube. Formation of a reddish colour shows the presence of sterols.

Tests for saponins
Bromine water test: 2 mL of bromine water was added to 1 mL of the extract, formation of yellow precipitates shows saponins.
Foam test: 1 mL of the extract is shaken vigorously with 10 mL of water. Saponins presence was indicated by the formation of foam which persisted for 5 minutes.

Physicochemical analysis
Powdered leaf samples were used for the following physicochemical analysis i.e., loss on drying, total ash, water soluble and acid insoluble ash content, pH, acid value, total phenolic content, total carbohydrate content, total flavonoid content and total tannin content.

Loss on drying value
1 g of powdered leaves were placed in a crucible and dried in an oven at 100 ºC to constant weight. The loss on drying was calculated using Eqn (1) (Junejo *et al.*, 2015) [11].

$$percentage\ of\ loss\ on\ drying\ = \frac{weight\ loss\ in\ gram}{Initial\ weight\ in\ gram}\ x\ 100 \quad (1)$$

Total ash value
2 g of powdered leaf sample was placed in a previously weighed silica crucible and the crucible was heated gradually to 500 °C. The colour changes from green to black and finally to white indicate the absence of carbon. The ash along with the crucible was cooled in a desiccator and weighed. Ash value is calculated Eqn (2) (Upreti *et al.*, 2013) [20].

$$percentage\ of\ total\ ash\ value\ = \frac{Initial\ weight - Final\ weight}{Initial\ weight}\ x\ 100 \quad (2)$$

Water soluble ash content
The ash obtained from the above method was mixed with 10 mL of distilled water, boiled for 5 minutes, cooled and filtered. The insoluble matter collected on an ash less filter paper was transferred to a previously weighed silica crucible and ignited. The residue obtained was cooled, weighed and percentage of water-soluble ash was calculated using Eqn (3) (Upreti *et al.*, 2013) [20].

$$percentage\ of\ water\ soluble\ ash\ = \frac{weight\ of\ residue\ ash\ in\ gram}{Initial\ weight\ in\ gram}\ x\ 100 \quad (3)$$

Acid insoluble ash content: The ash obtained from the above method was mixed with 10 mL of dilute HCl, boiled for 5 minutes, cooled and filtered. The insoluble matter was collected on an ash less filter paper, transferred to previously weighed silica crucible and ignited. The residue obtained was cooled weighed and percentage of acid insoluble ash was calculated using Eqn (4) (Upreti *et al.*, 2013) [20].

$$percentage\ of\ acid\ insoluble\ ash\ = \frac{weight\ of\ residue\ ash\ in\ gram}{Initial\ weight\ in\ gram}\ x\ 100 \quad (4)$$

pH value
0.5 g of leaf powder and 5 mL of distilled water were taken in a small conical flask and mixed thoroughly. With the help of a pH meter (Systronics Ltd), pH of the solution was measured at room temperature.

Acid Value

0.5 g leaf powder was dissolved in 25 mL of an equal volume of ethanol (95%) and petroleum ether. After filtering using *Whatmann* No.1 filter paper, this solution was titrated against 0.1 M KOH solution using phenolphthalein indicator. The acid value is calculated using Eqn (5).

$$Acid\ value = \frac{5.61\ n}{W} \quad (5)$$

where *n* is the number of mL of 0.1 M KOH required and *W* is the weight in grams of leaf powder (Parimelazhagan, 2016) [16].

Total Phenolic Content

5 mL of the extract was taken, and aliquots of 0.2-2 mL were pipetted out. The volume in each tube was made up to 3 mL with water and 0.5 mL of Folin–Ciocalteu (FC) reagent was added. After 3 min, 2 mL of Na_2CO_3 (20%) was added and was placed in a boiling water bath for one minute, cooled, and absorbance was read at 650 nm against blank. A standard curve for catechol was prepared using different concentrations of catechol for comparison.

Total Carbohydrate Content

5 mL of sample was taken in a boiling tube and hydrolysed in a boiling water bath for three hours with 5 mL of HCl (2.5 N) and cooled to room temperature. This solution was neutralized with solid sodium carbonate until the effervescence ceases. The volume was made up to 100 mL and centrifuged. The supernatant liquid was collected, 0.5 and 1 mL aliquots were taken for analysis. Standards were made using 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard. The volume was made up to 1 mL in all the tubes including the sample tubes using distilled water. 4 mL of anthrone reagent was added and test tubes were heated for eight minutes in a boiling water bath. This was cooled rapidly and absorbance was read at 630 nm and calculations were done using Eqn (6) (Jagannathan *et al.*, 2010) [8].

$$Amount\ of\ carbohydrate\ present\ in\ 100\ mg\ of\ leaf = \frac{mg\ of\ glucose}{volume\ of\ test\ sample}\ x\ 100 \quad (6)$$

Total Flavonoid Content

5 mL extract was mixed with 10 mL of H_2SO_4 (10%) and heated on a water bath with for 30 minutes to undergo hydrolysis. Once the initial volume was decreased to half, the mixture was cooled on ice for 15 minutes where the flavonoids are pre-evaporated. The cooled solution was filtered; the residue was dissolved in 50 mL of warm ethanol (95%) and further made to 100 mL with ethanol. 5 mL aliquot was pipetted into a 25 mL standard flask and diluted with 50% ethanol. The absorbance of the resulting solution was measured at 370 nm against 50% ethanol as blank. Flavonoid concentration was calculated using a reference curve of pure quercetin (Muhammad and Abubakar, 2016) [14].

Total Tannin Content

5 mL of leaf extract was transferred to a stoppered conical flask. To this, 25 mL of 0.1N iodine and 10 mL of 4% NaOH were added. This was mixed and kept in the dark for 15 minutes. The mixture was diluted with water and acidified with 4% H_2SO_4 (10 mL). The mixture was titrated with 0.1N sodium thiosulphate solution using starch solution as indicator. The number of mL of 0.1N $Na_2S_2O_3$ used corresponds to the sum of tannins and pseudo tannins. A blank experiment was carried using distilled water. Total tannin content was calculated using Eqn (8) (Muhammad and Abubakar, 2016) [14].

$$1\ mL\ of\ 0.1N\ Na_2S_2O_3 = 1\ mL\ of\ 0.1\ N\ I_2 solution = 0.0290\ g\ of\ tannin$$

$$Tannin\ Percentage\ (\%) = [Blank - Expt]\ x\ 0.029\ x\ 100 \quad (8)$$

Statistical analysis

Microsoft Excel 2013, was used for statistical analysis. All the results were calculated in triplicates and were represented as mean ± SEM.

Results and Discussion

Organoleptic evaluation

The organoleptic characteristics of leaves are given in Table 2. The leaves are dark to light green in colour, glabrous texture with an astringent taste. The leaf dimensions are explained in Table 3.

Table 2: Organoleptic evaluation of *E. oxypetalum* leaf

S. No	Parameters	Observation
1	Colour of the Plant	Dark green
2	Colour of the Leaf	Dark to Light green
3	Colour of the powder	Yellowish brown
4	Taste	Astringent
5	Odour	Characteristic odour
6	Texture	Glabrous
7	Apex	Acute to acuminate
8	Base	Cuneate or attenuate
9	Venation	Pinnate type of venation is observed at the apex side
10	Margins	Undulate to deeply crenate
11	Mid-rib	2-6 mm wide, stout

Table 3: Leaf dimensions of *E. oxypetalum* leaf

S. No	Leaf dimensions	Height (cm)	Width (cm)	Apex width (cm)
1	Small	12.6	4.8	2.7
2	Medium	21.9	7.5	4.4
3	Large	36.3	9.2	7.1

Macroscopic features

E. oxypetalum is an epiphytic shrub, freely branched, which grows to a height of 2-6 m tall, with aerial roots (Figure 1). Macroscopic features of the plant give an overall awareness about the leaf. The old stems and basal extension shoots are terete to 2 m or more, woody. Numerously branched, dark green in colour, the leaf is laterally flattened with lanceolate to oblong-lanceolate shape which is 15-50 x 5-12 cm.

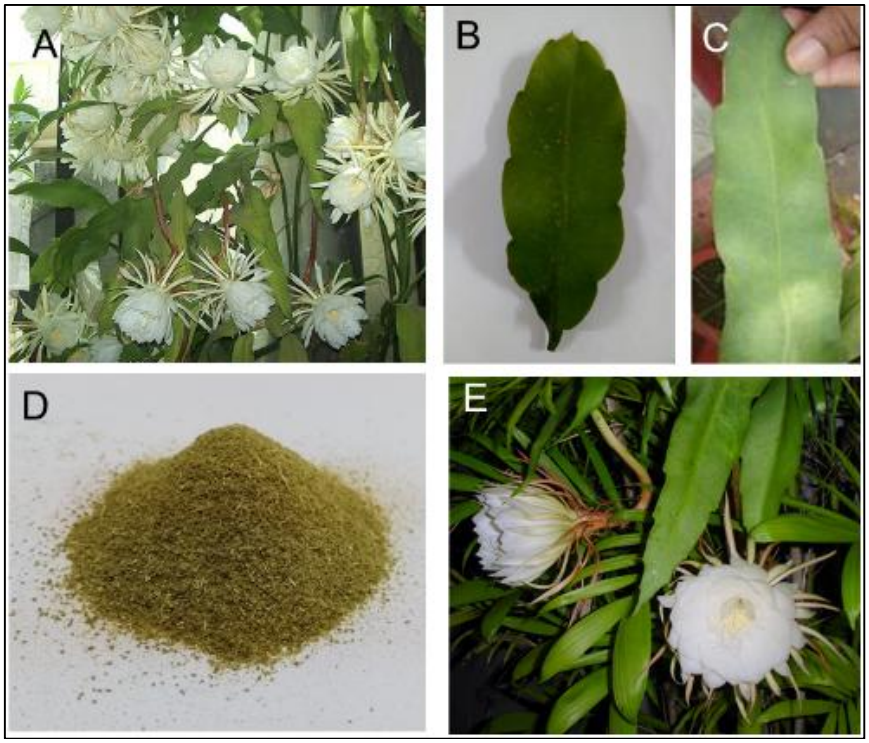

Fig 1: *Epiphyllum oxypetalum* (A) plant (B&C) medium-sized leaf (D) powdered leaf (E) flower

Leaves are glossy green on the upper and lower surface and glabrous, the leaf base is cuneate, attenuate or stalked, leaf margins are undulate to deeply crenate, leaf apex is acute to acuminate, midrib 2-6 mm wide and stout having small spineless areoles. The leaves are of characteristic odour with an astringent taste. Flowers are nocturnal, funnel-form, fragrant, 25-30 x10-27 cm. Receptacle tube 13-18 cm, the base is green, 4-9 mm in diameter, slightly angled with triangular to lanceolate scales 3-10 mm. Sepaloids are often recurved, pale green or pinkish red, linear to oblanceolate. Petaloids are white, oblanceolate to obovate, 7-10 x 3-4.5 cm. The filaments are white with 2.5-5 mm, anthers are creamish in colour with 3-3.5 mm. The style is white in colour with 20-22 cm; stigmas are 15-20 in numbers, creamish in colour and linearly narrow with 1.6-1.8 mm. Fruits appear to be rare, which is purplish red in colour, oblong, 16x5.7 cm, seeds are 2-2.5x1.5 mm.

Microscopic features

Internal anatomy of the plant can be explained by microscopic study. Transverse section of the leaves (Figure 2) depicts thick mesophyll tissue which is not differentiated into palisade and spongy.

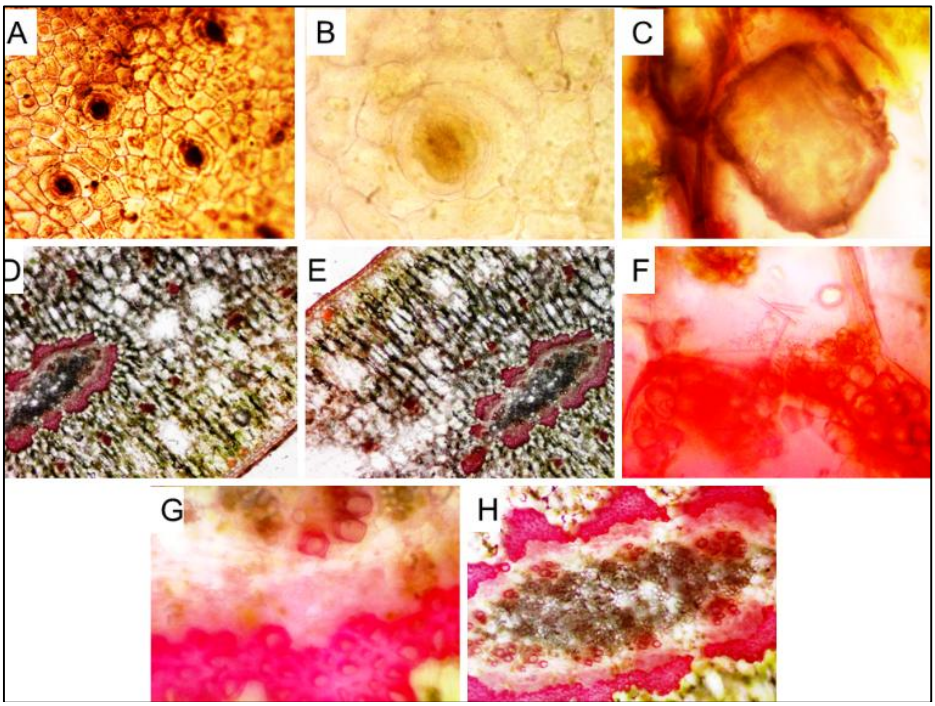

Fig 2: Transverse section of *Epiphyllum oxypetalum* leaf (A) Upper epidermis (B) Paracytic stoma (C) Cystolith crystal (D) Mesophyll with midrib vascular tissue (E) Mesophyll with upper epidermis (F) Needle-shaped crystals, starch grains (G) Xylem vessels, phloem, sclerenchyma of bundle sheath (H) Pith tissue, xylem vessels, phloem layer, sclerenchyma patches of bundle sheath.

The epidermis consists of 2-3 layers of uniform cells. Epidermis is nearly smooth with few trichomes while stomatas are paracytic and sometimes tricytic. Leaf tissue is filled with mucilage and mucilage canals. Midrib has a vascular bundle which consists of sclerenchyma sheath, beneath is phloem zone, inner to it is xylem vessels, in the middle of the vascular cylinder, pith tissue is present which comprises large thin-walled cells filled with starch grains. Mesophyll cells near the epidermis have chlorophyll pigments, inner mesophyll cells have starch grains, often star-shaped cystolith crystals found; which are about 500 -750 microns long. Some cells also have a thin rod-like crystal. Numerous small and large mucilage canals are present in the hypodermal zone and inner zone. Stomatal index was calculated using a washed and cleaned piece of leaf. Both lower and upper epidermises were peeled with the help of forceps and observed under the microscope. In the present study stomatal index was found to be 1.582 (5x100/316).

Powder study

Powder microscopic study reveals (Figure 3) that the leaf of *Epiphyllum oxypetalum* is succulent leaf with a characteristic odour. Leaf pieces with stomata, stomata are tetracytic or anisocytic type with more stomata in the abaxial side, less on the adaxial side. Often leaf tissue contains star-shaped calcium oxalate crystals; which are 50 - 100-micron diameter, nearly circular starch grains present inside the cell or scattered in the medium. Xylem vessels are with spiral wall thickening.

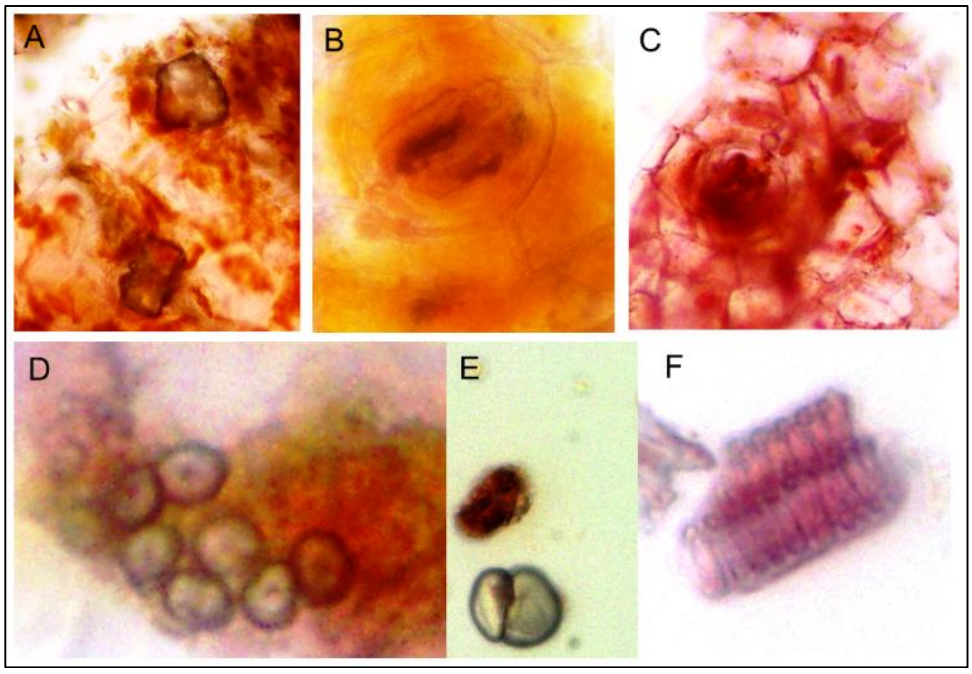

Fig 3: Powder microscopic study of *Epiphyllum oxypetalum* leaf. (A) star-shaped calcium oxalate crystals (B) tetracytic Stoma (C) anisocytic stoma (D&E) starch grains (F) xylem vessel with spiral wall thickening

Fluorescence analysis

Fluorescence analysis summarised in Table 4 gives valuable information and identification of fluorescent compounds present in the leaves of *E. oxypetalum.* Different compounds present in the leaves gives fluorescence when exposed to longer or shorter UV light (Akbar *et al.*, 2014) [1]. Figure 4 depicts the UV-Vis spectra of leaf powder in different solvents. It shows absorption at different wavelength depending on the solvents used. This confirms that the different chemical compounds present in the leaf powder reacts with the solvents and form new moieties and they shows absorption at a different wavelength.

Table 4: Fluorescence analysis of leaf powder of *E. oxypetalum*

S. No	Reagents + powder	Inferences		
		UV light wavelength (nm)		Ordinary light
		short (254)	longer (365)	
1	Powder	Dark brown	Light yellow	Light green
2	Water	Colourless	Colourless	Colourless
3	Chloroform	Colourless	Light orange	Light yellow
4	Con.H_2SO_4	Dark yellowish green	Yellow	Brown
5	Methanol	Colourless	Light orange	Light yellow
6	Ethanol	Colourless	Light orange	Light yellow
7	Dil. H_2SO_4	Colourless	Colourless	Colourless
8	$FeCl_3$	Dark yellow	Black	Yellow
9	1N NaOH	Dark yellow	Light yellowish green	Light t yellow
10	Con. HCl	Dark yellow	Light yellowish green	Light yellow
11	Dil. HCl	Colourless	Colourless	Colourless
12	10% NaOH	Dark yellowish green	Light yellowish green	Light yellow
13	Dil.NH_3	Dark yellow	Light yellowish green	Yellow
14	Con.HNO_3	Light yellow	Black	Light yellow
15	Dil.HNO_3	Colourless	Light Brown	Light yellow

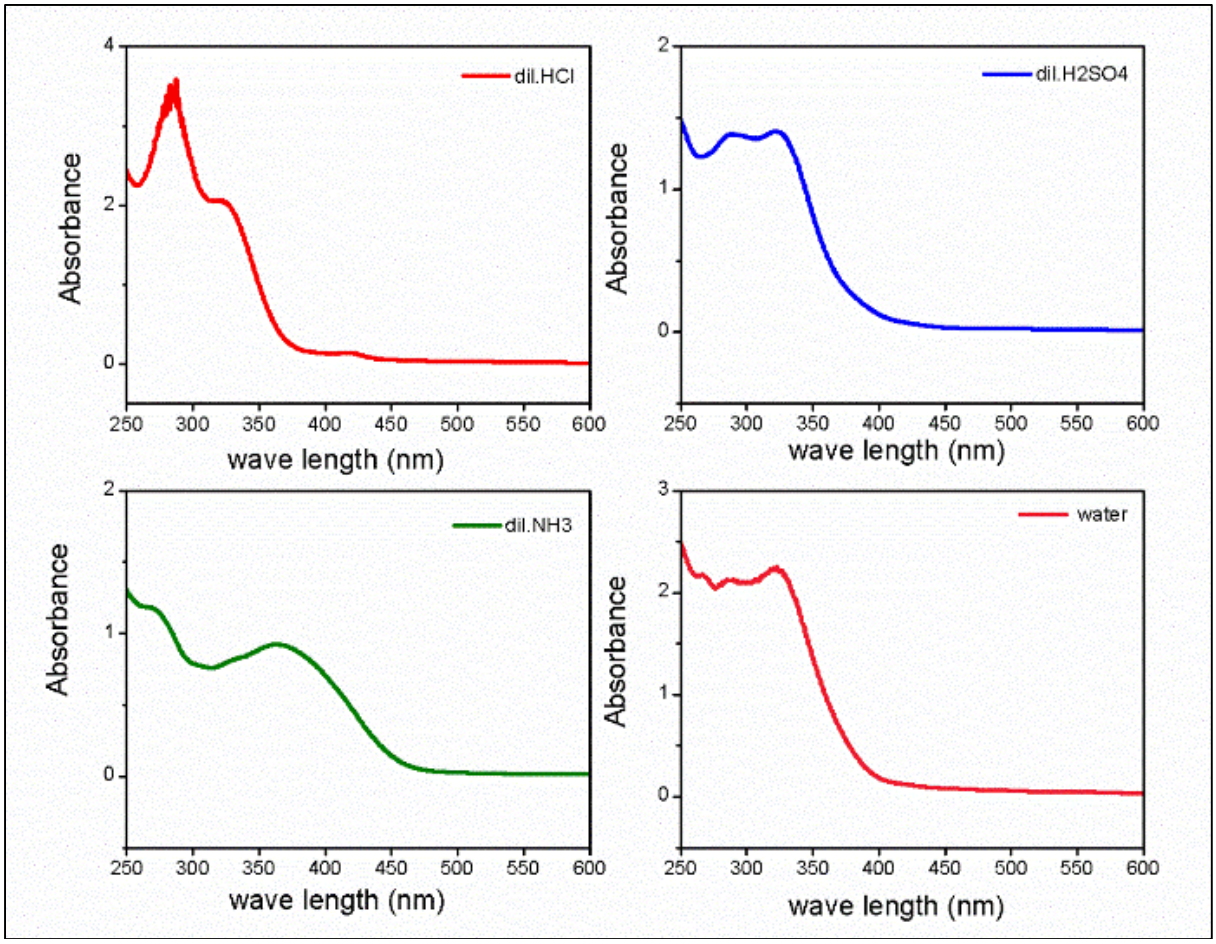

Fig 4: UV-Vis spectra of *E. oxypetalum* leaf under different solvent

Phytochemical analysis

Phytochemical analysis of the leaf extract gives a general idea regarding the nature of chemical constituents present in it. Diversified phytochemicals were found within the methanolic, aqueous, petroleum ether and ethanolic extract of the leaves of *E. oxypetalum*. The results of this analysis are summarized in Table 5. Methanolic extract was showing positive results for most of the tests except Salkowaski and Dragendorff's test under study.

Table 5: Phytochemical analysis of *E. oxypetalum* extract under different solvents

Group	Name of the Test	Inference of the leaf extract in			
		Methanol	water	Petroleum ether	Ethanol
carbohydrates	Molisch's test	+	+	+	+
	Benedict's test	+	+	+	+
	Barfoed's test	+	+	+	+
	Fehling's test	+	+	+	+
proteins	Biuret test	+	+	-	+
	Millon's test	+	+	-	+
	Ninhydrin test	+	+	-	+
tannins	Ferric chloride test	+	+	-	+
	Gelatin test	+	+	+	+
phenols	Ferric chloride	+	-	-	+
alkaloids	Wagner's test	+	+	+	+
	Mayer's test	+	-	+	+
	Dragendorff's Test	-	+	-	+
flavonoids	Ferric chloride test	+	+	+	+
	Alkaline reagent test	+	-	-	-
	Lead acetate test	+	-	-	-
sterols	Liebermann Burchard Test	+	+	+	+
	Salkowaski test	-	-	-	-
saponins	Bromine water test	+	+	+	+
	Foam test	+	+	-	+

Key: + Detected, - Not detected

Physicochemical analysis

Physicochemical analysis, acid value, loss on drying, pH value were carried out and the results were summarized in Table 6. The leaves showed the maximum value for acid value, tannin content. The recorded reading for loss on drying was moderate. The sample was found to be slightly acidic.

Table 6: Physicochemical analysis of *E. oxypetalum* leaf

Quantitative Test	Values
Loss on drying	2 ±0.10 %
Total ash value	4.6 ± 0.4 %
Water soluble ash	8.5 ± 0.5 %
Acid-insoluble ash	9.2 ± 0.2 %
pH	6.1 ± 0.5 (slightly acidic)
Acid value	13.46 ± 0.05 mL/g
Phenolic content	19.09 ± 0.08 µg/0.6 mL
Carbohydrate content	0.0237 ± 0.001mg/0.5 mL
Flavonoid content	8.728 ± 0.02 µg/mL
Tannin content	31.32 ± 0.08 %

Moisture content in the leaf part is calculated by loss on drying. Usually, excess moisture leads to microbial contamination while lower moisture content suppresses microbial degradation. The decline of the constituents of the leaf part occurs in presence of excessive moisture which helps in the hydrolysis of different constitutes (Chanda, 2014) [3]. Total ash values for the powdered leaf gives the information about the total quantity of siliceous material left over in the leaf residue. Estimation of pH values is helpful in formulating the extraction procedure for the chemical components from the leaf which helps in the product development (Gong *et al.*, 2014) [6]. The pH value for the leaves of *E. oxypetalum* showed slight acidic nature because of the different phytochemicals.

Conclusion

Present investigation reveals the microscopic, pharmacognostic and physiochemical screening of *Epiphyllum oxypetalum* leaf. Methanolic extraction was showing maximum yield. Macroscopic and microscopic findings explain the anatomy of the leaf while fluorescent analysis indicates the different fluorescent material present in presence of different solvents under different wavelength of light. Compounds which are not fluorescent by themselves are converted into some fluorescent materials or fluorescent derivatives in presence of different solvents. The distinctive characters of leaves were the presence of paracytic stoma, needle-shaped starch grains, sclerenchyma, calcium oxalate crystals and tetracytic stoma. Ash values, water soluble and acid insoluble content of the leaf gives more information about the usage of this material for the crude drug production. Screening of phytochemical constituents gives important information which may be beneficial for quality control of this medicinal plant in therapeutic uses in later studies.

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Compliance with Ethical Standards

No funding and during research there is no involvement of human participants and animals.

Conflicts of interest

The authors declare that there is no conflict of interest.

Author contributions

All of the authors had contributed equally towards this manuscript.

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