DETAIL PHARMACOGNOSTIC STUDIES ON LEAVES OF *PANCRATIUM LONGIFLORUM (AMARYLLIDACEAE)*

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Abstract

Background: *Pancratium Longiflorum* is a rare herb used medicinally, mostly found growing wild. Leaves are narrow, about half an inch wide, long, arising from the base. The flowering stem is exceptionally short, about 3 cm, bearing only a single flower. The flower-tube is exceptionally long, 5-6 inch. The limb is about 3 inch long. Flowers are large for a Pancratium, about 4-5 inches across.

Objective: Present research was done for authentication and identification by detail studies of various pharmacognostic parameters of *Pancratium Longiflorum* leaves.

Material and Methods: Intensive qualitative microscopic, phytochemical analysis, macroscopic characteristics followed by TLC finger prints of different extractives studies were performed of *Pancratium Longiflorum* leaves by using various reagents and chemicals.

Results: Leaves are narrow, about half an inch wide and long with parallel venation, entire margin and acute apex. Transverse section of leaf is more or less uniform from one surface to other has Single layered Upper epidermis and Lower epidermis with equal number of stomata, Midrib contain Collenchyma and Vascular bundles showing Patches of sclerenchyma associated with xylem and phloem. The extractive value of *Pancratium Longiflorum* was about 10.77 %, in ethanol (successive). Steroids and/or triterpenoids were present in hexane, chloroform and ethanol extract of *Pancratium Longiflorum*. Alkaloids were also present in chloroform, ethanol and aqueous extracts *Pancratium Longiflorum*.

Conclusion: Study results will be serving as future standard data for diagnosis and distinguishing, *Pancratium Longiflorum* from its adulterants and substitutions also use for quality control studies of pharmaceutical preparation from *Pancratium Longiflorum* leaves. The results obtained in this study demonstrated the utility of TLC not only in establishing plant identity, but also in distinguishing with certainty between allied species.

Keywords: *Pancratium Longiflorum,* phytochemical, adulterants, species, extractive

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DOI: 10.48047/ecb/2023.12.si10.019

INTRODUCTION

The name "Pancratium" is derived from the Greek and means "all-strength", probably referring to the strength of a plant that can tolerate extreme climates. Pancratium species often inhabit extremely dry and sandy areas.

Pancratium Longiflorum is a rare herb, mostly found growing wild. Leaves are narrow, about half an inch wide, long, arising from the base. The flowering stem is exceptionally short, about 3 cm, bearing only a single flower. [(1)] The flower-tube is exceptionally long, 5-6 inch. The limb is about 3 inch long. Flowers are large for a Pancratium, about 4-5 inches across. They are white with a greenish yellow center. Petals are six, narrow, linear. Stamens are also six in number, and erect. [(2)]

The style is green, nearly equal to the limb. This plant can be grown as a house-plant in a pot. Belongs to family (Hindi name): SUDARSHAN FAMILY, as per The APG System III): AMARYLLIDACEAE and as per The Plant List): *Pancratium Longiflorum* Roxb. ex Ker Gawl. Occasional found in hilly areas at high altitudes with Flowering and Fruit July-August month. In india it is distributed in Andhra Pradesh (Vishakapatnam district), Karnataka (Bengaluru district) and Maharashtra (Akola district) and in Rajashthan (Jaipur District) [(3-4)]

MATERIALS AND METHODS

Plant material

Leaves of *Pancratium Longiflorum* were collected in the month of June 2012 from Kulis Smiriti Van, Jaipur, Rajasthan (India) by Mr. Vinod Kumar, Department of Botany, Rajasthan University, Jaipur, Rajasthan. (Herbarium No. RUBL221135 /RUBL21134/ RUBL21132/RUBL 21133)

Macroscopic, microscopic and powder characters

Free hand transverse sections of leaves were taken and transverse were cleared with chloral hydrate. The sections of plant materials were treated with phloroglucinol and a drop of concentrated hydrochloric acid to stain the lignified elements. The sections were also stained with dilute solution of iodine to study the starch grains.

Photomicrographs were taken with a Motic Monocircular, Motic DM-111Microscopic Unit. For study of the powder drug; the dried leaves were ground and passed through a sieve 60 mesh. [(5)]

Quantitative Microscopy [(6-7)]

The cleared materials were washed thoroughly and stained with safranin for quantitative microscopic studies.

i) Stomatal Number[(5)]

It is the average number of stomata per square mm of epidermis of the leaf. A minimum of ten readings were taken from different locations of the leaf and the average value was calculated.

ii) Stomatal Index[(5)]

It is the percentage in which the number of stomata to the total number of epidermal cells, each stoma being counted as one cell. Stomatal Index was calculated.

iii) Vein-Islet and Vein termination number

It is the average number of vein-islets per square mm of the leaf surface. It is determined by counting the number of vein-islets in an area of 4 sq.mm of the central part of the leaf mid way between the midrib and the margin. Vein termination number is the average number of vein let termination per sq.mm of the leaf surface. It is determined by counting the number of vein terminations in an area of 4 sq.mm of the central part of the leaf mid way between the midrib and the margin.

iv) Palisade Ratio

It is the average number of palisade cells beneath each epidermal cells of a leaf. It is determined by counting the palisade cells beneath four continuous epidermal cells. [(8)]

Histochemical colour reactions

The micro-chemical tests for histological zones were performed according to the methods given by Kay (1938), Johansen (1940), Wallis (1967) and Trease and Evans (1972).

Behaviour of powder with different chemical reagents

The dark brown powder was treated with different chemical reagents to detect the phytoconstituents with colour changes under ordinary daylight by the standard method. [(9)]

Colour and consistency of extracts

Colour and consistency of extracts were observed by standard method. [(9)]

Loss on drying (gravimetric determination)

It is the amount of volatile matter of any kind (including water) that can be driven off. One gram of each of the samples was weighed accurately, spread on shallow Petri dish and heated at a regulated temperature of 105 ± 1ºC to constant weight. The samples were weighed immediately after removing from the oven. Loss on drying is expressed as the loss in weight in percent w/w [(10)]

Determination of pH[11]

(i) pH of 1% Solution Accurately weighed 1g of air dried coarsely powdered material dissolved in 100 ml of distilled water. The pH of the water soluble portion was measured with calibrated pH meter at 25°C.

(ii) pH of 10% Solution

Accurately weighed 10g of air dried; coarsely powdered material was dissolved in 100ml of distilled water. The pH of the water soluble portion was measured with standardized pH meter at 25°C.

Determination of ash values[10-11]

(i) Total ash

About 8 g of the ground air dried material, accurately weighed, in a previously ignited and tared crucible and incinerated in a muffle furnace at a temperature not exceeding 450°C until the formation of ash. The sample was then cooled overnight and transferred to desiccator and then weighed. The percentage of total ash was determined with reference to air dried sample.

ii) Acid insoluble ash

To the crucible containing the total ash, added 25 ml of 2N hydrochloric acid and boiled for 5 min. The insoluble matter was collected on ash less filter paper and washed with hot water until the filtrate was neutral. It was ignited to constant weight and allowed the residue to cool in a desiccator, then weighed immediately. The percentage of acid insoluble ash was calculated with reference to air dried material.

(iii) Water soluble ash

To the crucible containing the total ash, added 25 ml of distilled water and boiled for 5 min. The insoluble matter was collected on ash less filter paper and washed with hot water and ignited to constant weight. The residue was allowed to cool in a desiccator and then weighed immediately. The weight of insoluble ash was subtracted from the weight of total ash and the difference in weight indicated the weight of water-soluble ash. The percentage of water-soluble ash was calculated with reference to air dried material.

(iv) Sulphated ash

About 2 g of the ground air dried material was accurately weighed, in a previously ignited and tared crucible and incinerated at a temperature not exceeding 450°C until disappearance of black particles in a muffle furnace. The ash obtained was moistened with sulphuric acid and re-incinerated till constant weight was obtained. The residue remaining was weighed and percentage of sulphated ash was calculated with reference to air dried material.

Total alcohol and water soluble extractives

About 4 g of air dried coarsely powdered material, accurately weighed to a glass toppered conical flask. Added 100 ml of alcohol or water and allowed to macerate for 24 h, shaking frequently during the first 8 h and then allowing standing for 18 h. The solvent was filtered rapidly through Whatman No.1 filter paper. 25 ml of the filtrate was evaporated to dryness in a tared flat bottom shallow dish and then dried at 105 ±1oC and weighed. The percentage of alcohol or water-soluble matter was calculated with reference to air dried drug.

Determination of successive extractive values

Extractive values of crude dugs are used to determine the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists. The successive extraction was carried out in Soxhlet apparatus with different organic solvents viz Hexane, chloroform, ethanol and then water. After exhaustive extraction, the solvent was filtered and concentrated under reduced pressure at 50-55°C. The percentages of extracts were calculated with reference to drug taken after evaporation of the respective solvents.

Qualitative phytochemical analysis [12-14]

All four extracts were subjected to qualitative photochemical tests for different constituents such as alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds and tannins, proteins and free amino acids, saponins, steroids and triterpenoids. (Rao et al.,1985; Harborne, 1988; Wagner and Bladt, 1996).

Test for alkaloids

Alkaloids were tested in various extracts. In chloroform extract, 10 mg of chloroform residue was macerated with HCl (2%). The resulting acid solution was filtered and filtrates were separately tested with following alkaloidal reagents:

· Dragendorff's reagent (potassium bismuth iodide solution)

· Hager's reagent (a saturated solution of picric acid)

· Mayer's reagent (potassium mercuric iodide solution)

· Wagner's reagent (solution of iodine in potassium iodide)

In alcoholic extract, 10 mg of ethanol residue was macerated with HCl (2%), filtered, basified with NH4OH (25%) and extracted with CHCl3. The chloroform soluble portion was evaporated,

dissolved in HCl (2%) and tested as in the chloroform extract.

In hexane extract, 10 mg of hexane residue was macerated with HCl (2%), filtered, basified with NH4OH (25%) and extracted with CHCl3. The chloroform soluble portion was evaporated, dissolved in HCl (2%) and tested as in the chloroform extract. In water extract, 10 mg of aqueous residue was dissolved in distilled water (1.5 ml), basified with NH_4OH (25%) and extracted with CHCl3.The chloroform soluble portion was extracted with HCl (10%) and the aqueous acid solution tested as in the chloroform extract.

A positive (+) reaction was recorded if the addition of reagents produced a faint turbidity; + + reaction was recorded if a light opalescent precipitate was observed; and + + + reaction as recorded if a heavy yellowish-orange precipitate with Dragendorff's reagent, a heavy yellow precipitate was observed with Hager's reagent, a heavy yellowish-white precipitate was observed with Mayer's reagent and a heavy reddish brown precipitate observed with Wagner's reagent.

Test for carbohydrates

The small quantity of extracts were dissolved separately in 5 ml of distilled water and filtered. The filtrate was subjected following tests:

Molisch test: The filtrate was treated with few drops of a-naphthol (20% in ethyl alcohol). Then about 1 ml of concentrated H_2SO_4 was added along the sides of inclined test tube and observed for formation of violet coloured ring at the interface.

Fehlings test (detection of reducing sugars): A few drops of mixture of equal parts of Fehling's solutions No. 1and No.2 were added to the filtrate of extracts and heated on a steam bath for 30 minutes and observed for the formation of brick red coloured precipitate. In most cases reduction took place near the boiling point and was shown by a brick-red precipitate of cuprous oxide. Reducing sugars include all monosaccharide's and many disaccharides (e.g. lactose, maltose, cellobiose and gentiobiose). A positive (+) reaction was recorded when a slight violet coloured ring/ precipitate was recorded; + + reaction was recorded when a medium violet coloured ring/ precipitate was observed; and + + + reaction was recorded when a heavy violet coloured ring/ precipitate was observed.

Test for glycosides and anthraquinones

Borntrager's test: Borntrager's test was used for the detection of anthraquinones in extracts. Free anthraquinones and combined anthraquinones were tested in extracts. A portion of extracts were dissolved in benzene and the benzene layer was separated. To this equal quantity of dilute ammonia solution was added and observed for the formation of reddish pink colour. A small amount of extracts were separately hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysed was extracted with benzene. The benzene layer was treated with dilute ammonia solution and observed for the formation of reddish pink colour.

A positive (+) reaction was recorded when a light pink, red or violet colour was observed; + + sign was indicated when a medium pink, red or violet colour was observed; + + + sign was indicated when a strong pink, red or violet colour was observed.

Keller- Killiani test: The extracts were dissolved in glacial acetic acid containing traces of $FeCl_3$ and filtered. To this filtrate about 1 ml of concentrated sulphuric acid was added and observed for formation of reddish brown colour which gradually becomes blue. A + sign was recorded when a light colour was observed; + + sign for a medium colour and + + + for a strong colour.

Legal test: The extracts were dissolved in pyridine and made alkaline with few drops of 10% NaOH, and then freshly prepared sodium nitroprusside was added and observed for formation of blue colour. A + sign was recorded when a light colour was observed; + + sign for a medium colour and + + + for a strong colour.

Test for flavonoids

Ammonia test: Filter paper strips were dipped in the dilute solution of various extracts, ammoniated and observed for colour changes from white to yellow. Shinoda's/Pew test: Ten milligrams of each extracts residue was dissolved in 1.5ml appropriate solvent and treated with a piece of metallic magnesium ribbon followed by addition of few drops concentrated hydrochloric acid.

A positive (+) reaction was recorded when a slight yellow or red coloration was observed: + + reaction was recorded when a medium yellow or red coloration was observed; and + + + reaction was recorded when a strong yellow or red coloration was observed.

Test for tannins and phenolic compounds

After dissolving 10 mg of powdered extracts in their respective solvents and divided separately into three parts. A sodium chloride solution was added to one portion of each test extracts. 1% gelatin solution to each second portion and the gelatin salt reagent to each third portion were added. Precipitation with the latter reagent or with both the gelatin salt reagents was indicative of the presence of tannins. Precipitation with salt solution

(control indicated a false-positive test. Positive tests were further confirmed by the addition of a few drops of dilute ferric chloride (1% $FeCl_3$) to the test extracts, which gave blue black or green black coloration.

A positive (+) reaction was recorded when a slight precipitate was recorded: ++ reaction was recorded when a medium precipitate was observed; and + + + reaction was recorded when a heavy precipitate was observed.

Test for proteins and amino acids

Small amount of extracts were dissolved in distilled water and filtered. The filtrates were subjected to following tests:

Biuret's tests: To the ammoniated alkaline filtrates, 2-3 drops of 0.02% copper sulphate were added and observed for appearance of red or violet colour.

Millon's test: To 2 ml of filtrate, 5-6 drops of Millon's reagent (1g of mercury + 9 ml of fuming nitric acid solution) were added and observed for red precipitate.

Ninhydrin test: To the filtrate, lead acetate solution was added to precipitate tannins and filtered. The filtrate was spotted on a paper chromatogram, sprayed with ninhydrin reagent and heated at 11oC for 5 minutes and observed for red or violet colour.

Xanthoprotein test: To the filtrate, a few drops of concentrated nitric acid was added by the sides of the test tube and observed for appearance of yellow colour. A + sign was recorded when a light colour was observed; + + sign for a medium colour and + + + for a strong colour.

Test for saponins

Foam test: A small amount of various extracts were extracted with petroleum ether and acetone. To the insoluble residue left after extraction, a few ml of water was added and shaken vigorously for 15 min and observed for formation of honeycomb froth that persisted for at least 30 min. A + sign was recorded when froth reached a height of 0.5 cm; + + sign with a height up to 1 cm: + + + sign with a height more than 1 cm.

Test for sterols and or triterpenes

The extracts were refluxed with alcoholic potassium hydroxide until the saponification was complete. The saponification mixture was diluted with distilled water and extracted with diethyl ether. The ethereal extract was evaporated and the unsaponifiable matter was subjected to following tests;

Libermann-Burchard test: The ether soluble residue was dissolved in chloroform and few drops acetic anhydride was added followed by few drops of concentrated sulphuric acid from the side of the tube and observed for the formation of blue to blue-red colour.

Salkowski's reaction: To the ether soluble residue, 2 ml of concentrated sulphuric acid was added and observed for the formation of yellow ring at the junction, which turns red after one minute.

Hesses reaction: The residue dissolved in chloroform and an equal quantity of concentrated sulphuric acid was then added along the side of the tube and observed for the formation of pink coloured ring, which on shaking diffused in both the layers.

Hersch's Sohn's reaction: To the residue2-3 ml of trichloroacetic acid was added, heated and observed for the formation of red to violet colour.

Sterols and or triterpenes were tested in the extracts by Salkowski test or Libermann's test or Hesses reaction or Hersch's Sohn's reaction. A + sign was recorded when a light colour was observed; + + sign for a medium colour and + + + for a strong colour.

Fluorescence analysis of powder and extracts

The powder material and extracts were examined and analysed in daylight, short and long UV light for fluorescence, according to the reported methods. [15]

Estimation of inorganic constituents

To estimate the inorganic metal content, 1 g of the completely dried powder sample was digested with concentrated nitric acid and perchloric acid (3: 1) until a clear solution was obtained. After cooling, the solution was made up to a specific volume with deionised water and analyzed in an atomic absorption spectrophotometer.

Estimation of crude fiber content

Crude fiber content is the residue remaining after digestion of sample with 1.25 % sulphuric acid and 1.25% sodium hydroxide solution under specific conditions and subsequent ignition thereof. About 2g of ground material were extracted with diethyl ether and added 200 ml of boiling dilute sulphuric acid (1.25 %) to the ether exhausted marc, in a 500ml flask. The mixture is refluxed for 30 minutes (accurately timed), filtered through a filter paper, and the residue is washed with boiling water until the effluent washing is acid free. Rinsed the residue and placed back into the flask with 200 ml of boiling sodium hydroxide solution (1.25 %) and reflux the mixture again for 30 minutes (accurately timed), then filter through a line paper filter and wash the residue with boiling water until the last washing was neutral. It is then dried at 110 °C to

constant weight and then ignited to constant weight. Cooled in a desiccator, and weigh the ash.

TLC finger prints of different extractives [16]

For thin layer chromatograms, a prepared silica gel plate of 0.25 mm thickness was used after activation of plate at 110°C for 1 hour. About 10-12 µl of each plant extract solution was gently applied on chromatographic plate in a circular and compact spot with the help of glass capillary and chromatogram was developed in a suitable solvent system using a closed developing chamber to separate the individual compounds. The developing tank was lined with filter paper in order to help saturate the atmosphere inside with the chromatographic solvent system. After the solvent had run for more than 2/3rd, the plates were withdrawn, dried sprayed with ceric sulphatesulphuric acid (65%). The hRf values were extracted for each spot.

RESULTS

Macroscopic characters of *Pancratium Longiflorum* leaves

Leaves are narrow, about half an inch wide and long. They are green, linear and with parallel venation, entire margin and acute apex. The flowering stem is short, about 3 cm, bearing only a single flower. The flower is about 3 inch long. Flowers are white with a greenish yellow center. Petals are six, narrow, linear. Stamens are also six in number, and erect. The style is green, nearly equal to the limb.

Figure 1: *Pancratium Longiflorum* leaves

Microscopic features of *Pancratium Longiflorum* leaves.

The Transverse section of leaf is more or less uniform from one surface to other as shown in Figure 2

Lamina

Upper epidermis

Single layered, contains more or less an equal number of stomata on either side and is also somewhat uniformly thickened and cutinized.

Mesophyll:

Isobilateral

It is not differentiated into palisade and spongy parenchyma but consists of only spongy cells in which chloroplasts are evenly distributed.

Lower epidermis

It is similar to upper epidermis

Midrib

Collenchyma

Thick walled cellulosic cells forming the patches of collenchyma above the lower epidermis

Vascular bundles

Vascular bundles consist of xylem lies towards the upper epidermis and phloem lies towards the lower epidermis. Patches of sclerenchyma are associated with xylem and phloem in the vascular bundle. Surrounding each vascular bundle there is compact layer of thin walled parenchymatous cells known as border parenchyma or bundle sheath.

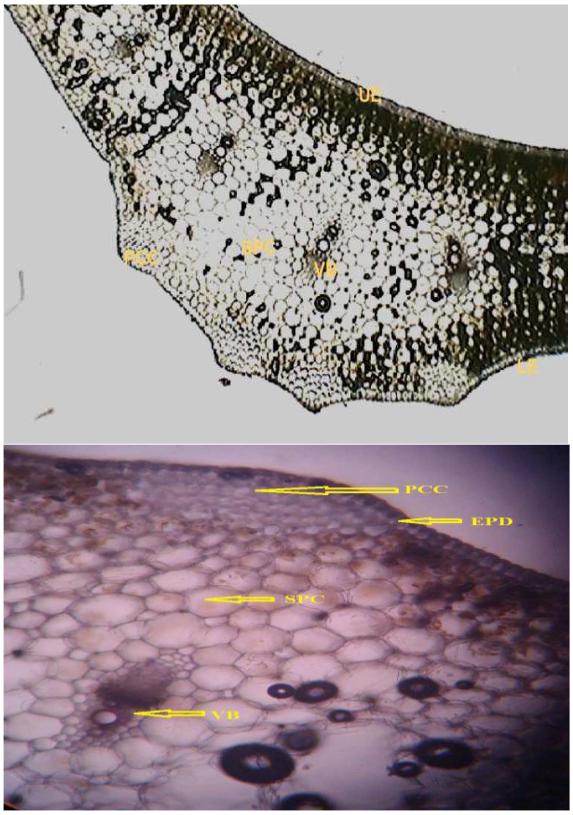

Figure 2: Transverse section of *Pancratium Longiflorum* leaves

[UE: Epidermis, SPC: Spongy parenchyma, PCC: Patch of collenchyma, VB: Vascular bundle, LE: Lower epidermis]

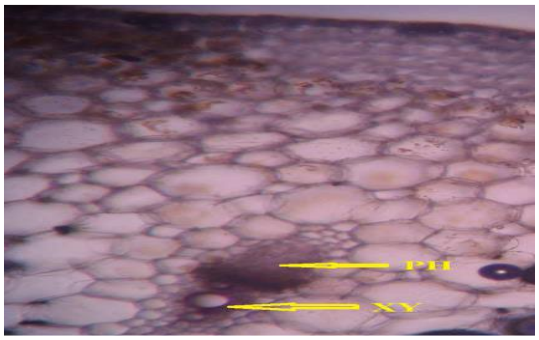

Figure 3: Enlarged portion of xylem and phloem [XY: Xylem, PH: Phloem]

Powder microscopy of *Pancratium Longiflorum* Leaves

Spongy parenchyma: Parenchyma fragments with intracellular spaces containing chloroplasts in the cell Xylem vessels: Xylem vessels associated with xylem parenchyma

Epidermis: Fragment of epidermis showing tubular elongated cells with compact arrangement

Sclerenchyma: Lignified sclerenchyma of vascular bundles forms the patch of sclerenchyma

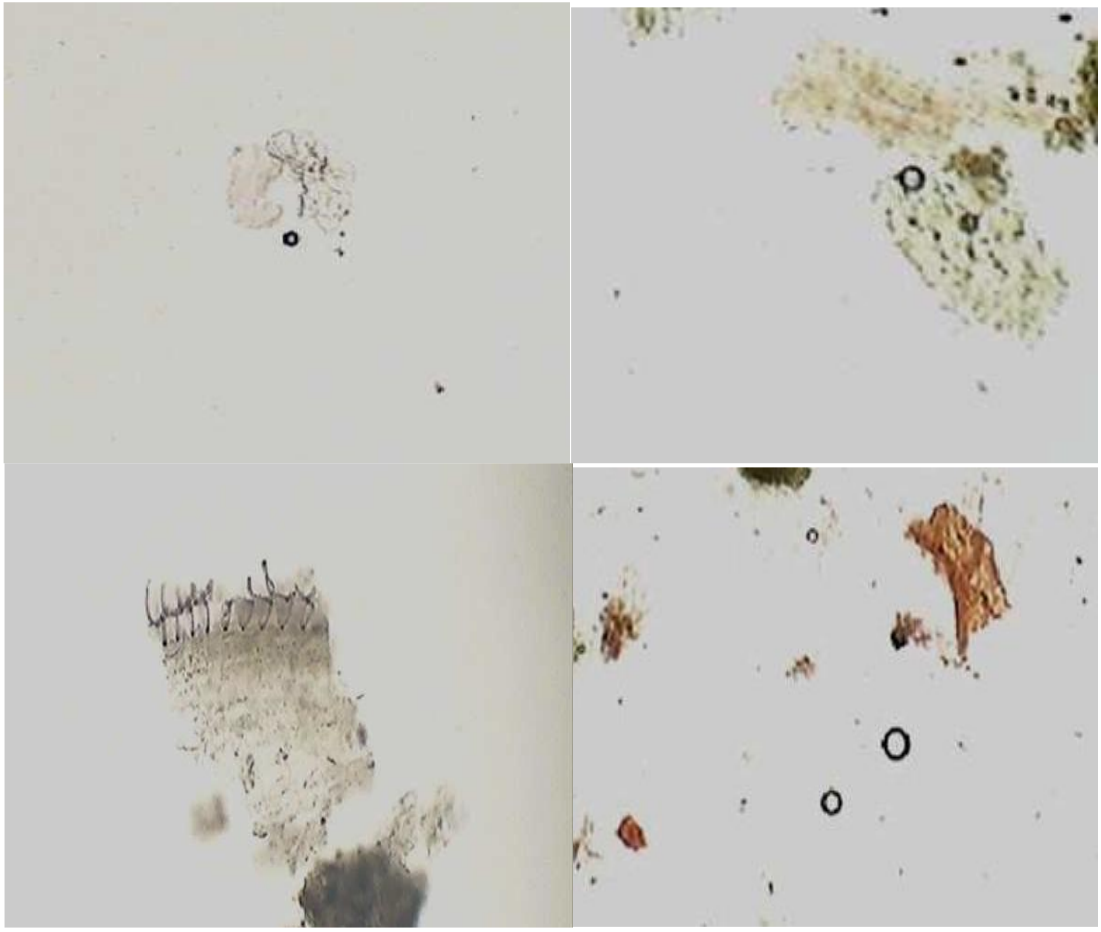

Figure 4: Powder characteristics of *Pancratium Longiflorum* leaves (a) Spongy parenchyma with chloroplast (b) Xylem vessel with xylem parenchyma (c) Epidermis (d) Patches of sclerenchyma

Quantitative microscopy of the leaves

Table 1: Determination of leaf constant of *Pancratium Longiflorum*

Sr. No.	Parameter	Range	Average
1	Stomatal Number	51-69	60
2	Stomatal index	15-17	16
3	Vein-islet number	3-5	4
4	Vein Termination Number	0	0
5	Palisade ratio	7-9	8

Table 2: Histochemical colour reactions of *Pancratium Longiflorum*

Reagent	Constituent	Colour
Conc. H_2SO_4	Saponins or Lipids	-
Weak Iodine solution	Starch	Blue
Millons reagent	Protein	Red
Dragendroff regent	Alkaloids	Orange
Phloroglucinol + HCl	Lignin	Faint pink
Mg+HCl	Flavonoids	Yellow
Iodine Solution	Cellulose	Dark blue
Sb Cl_3	Steroids, Triterpenoids	Reddish pink
Caustic alkali + HCl	Calcium oxalate	-

Table 3: Behaviour of the powder with different chemical reagents on leaves powder of *Pancratium Longiflorum*

Reagent	Colour/Precipitate	Constituent
Aq. $FeCl_3$	Reddish brown	Tannins absent
Iodine Solution	Blue	Starch present
Ammonia solution	No change	Anthraquinone glycosides absent
5% Aq. KOH	No change	Anthraquinone glycosides absent
Aq. $HgCl_2$	Orange precipitate	Alkaloids present
Aq. $AgNO_3$	White precipitate	Protein present
Conc. H_2SO_4	Reddish-pink	Steroids, lipids present
Mg-HCl	Yellow	Flavonoids present
Picric Acid	Yellow precipitate	Alkaloids present

Table 4: Fluorescence characteristics of powdered leaves of Pancratium Longiflorum

Sr. No.	Treatment with Reagents	Under Ordinarylight	Under UV light	
			Long wavelength	Short wavelength
1	Powder as such	Brownishyellow	Bright yellow	Dull yellow
2	Dry powder was placed onglass slide affixed with nitrocellulose	Bright yellow	Light yellow	Bright green
3	Powder treated with 1MNaOH in Methanol	Yellowishbrown	Dull brown	Bright green
4	Powder treated with 1N NaOH in Methanol, dried and then mounted in Nitrocellulose in Amylacetate	Reddishorange	Greenishbrown	Dark green
5	Powder treated with 1MHCl	Light yellow	Brownishgreen	Greenishyellow
6	Powder treated with 1M HCl, dried and then mounted in nitrocellulose inamylacetate	Dark yellow	Very lightgreen	Bright green
7	Powder treated with 1MNaOH in water	Brownishyellow	Dark green	Yellowishgreen
8	Powder treated with 1M NaOH in water, dried and then mounted in nitrocellulose in amylacetate	Yellowishorange	Light green	Bright green
9	Powder treated with 50%HNO_3	Dark yellow	Black	Light green
10	Powder treated with 50%H_2SO_4	Yellowishbrown	Darkness	Light green

Consistency, colour and fluorescence analysis of extracts

All the extracts obtained were examined in day light, short and long-UV to detect the fluorescent compounds by the standard method. Consistencies of extracts were also observed and the results are presented in Table 5

Table 5: Consistency, colour and fluorescence analysis of extracts of *Pancratium Longiflorum*

Parameters	Extracts			
	Hexane	$CHCl_3$	Ethanol (95%)	Aqueous
Consistency	Sticky Mass	Sticky Mass	Sticky semi solid mass	Solid mass
Colour (day light)	Yellowish brown	Brownish Black	blackish brown	Yellowish brown
Short UV	No Change	No change	Greenish Brown	Light Brown

Physicochemical study

The standard physicochemical protocols developed from the Indian Pharmacopoeia (1955) were followed for calculating moister content, pH, crude fiber content, and ash values. The values obtained are presented in Table 6.

Table 6: Physicochemical values of Pancratium Longiflorum leaves powder

Parameters	Results ± S.D.
1. Organoleptic characteristics	
Appearance	Powder
Colour	Brownish yellow
Odour	No characteristic odour
Taste	No taste
2. Loss on drying	9.16 ± 0.28
3. pH values	
pH of 1% aqueous solution	5.97 ± 0.02

pH of 10% aqueous solution	5.77 ± 0.02
4. Ash values (%)	
Total ash	7.36 ± 0.37
Water soluble ash	3.13 ± 0.11
Acid insoluble ash	0.46 ± 0.11
5. Alcohol soluble matter (%)	16.13 ± 0.24
6. Water soluble matter (%)	8.62 ± 0.72
7. Successive extractives (%)	
Hexane extract	0.88 ± 0.98
Chloroform extract	3.83 ± 0.85
Ethanol extract	10.77 ± 0.91
Aqueous extract	4.56 ± 0.51
8. Crude fiber content (%)	8.57 ± 0.46

Qualitative phytochemical analysis

The various dried extracts were tested for different constituents, such as alkaloids, glycosides, tannins and phenolic compounds, reducing sugars, protein and amino acids, steroids and /or triterpenoids and flavonoids and results are given in Table 7

Table 7: Qualitative phytochemical analysis of various extracts of *Pancratium Longiflorum*

Type of Constituent	Hexane	$CHCl_3$	Ethanol (95%)	Aqueous
Alkaloids	-	+	+ + +	+ +
Phenolic compounds	-	-	+ +	+ +
Flavonoids	-	-	+ +	-
Saponins	-	-	-	-
Steroids	+ +	+ +	+ +	-
Triterpenoids	+ +	+ +	+ +	-
Proteins	-	-	+ +	+ +
Carbohydrates	-	-	+ +	+ +
Glycosides	-	-	-	-

Estimation of Inorganic Constituents

The concentration of inorganic concentration was calculated and is presented in Table 8

Table 8: Concentration of inorganic constituents present in *Pancratium Longiflorum*

Elements	Quantity of elements (µg/g) in dried powder
Zn	55.6
Mn	17.8
Cu	11.2
Cr	12.4
Pb	9.80

Thin Layer chromatographic studies of different extractives of, Pancratium Longiflorum

The chromatographic solvent system and spray reagents used for developing or detecting resolved spots on the chromatographic plate and their hRf values are given Table 9

Table 9: TLC Finger printing of different extracts of *Pancratium Longiflorum*

Extract	Solvent system	Detection	Spots	hRf. Values
Hexane	Toluene-ethyl acetate(95:5)	Ceric sulphate -sulphuric acid (65%)	3	88; 27; 11
Chloroform	Benzene- chloroform-Diethylether (95:3:2)	Ceric sulphate -sulphuric acid (65%)	6	87; 41; 23; 17; 09; 03
Ethanol	Benzene-Acetone -Methanol(5:2:3)	Ceric sulphate -sulphuric acid (65%)	7	91; 83; 55; 38; 24;08; 04
Water	n-butanol-glacialacetic acid-water (5:1:4)	Ceric sulphate -sulphuric acid (65%)	4	93; 51; 32; 10

DISSCUTION

The present study establishes macro and microscopical characters, phytochemical screening fluorescence analysis of powder, physico-chemical values and TLC fingerprints of different extractives of leaves of *Pancratium Longiflorum*. In recent year, there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. It is due to their specific healing properties and potential action. Of course, in this view pharmacognostical evaluation of leaves *Pancratium Longiflorum* is a substantial step. As the most cost effective aid of identification of a medicinal herb, the use of microscopic characteristics has been the mainstay of classical pharmacognosy and remains a vital component of the modern monograph.

CONCLUSION

The important diagnostic characters of plants such as: *Pancratium Longiflorum* leaves are narrow, about half an inch wide and long with parallel venation, entire margin and acute apex. Transverse section of leaf is more or less uniform from one surface to other has Single layered Upper epidermis and Lower epidermis with equal number of stomata, Midrib contain Collenchyma and Vascular bundles showing Patches of sclerenchyma associated with xylem and phloem. The analysis of the anatomical features, leaves of *Pancratium Longiflorum* may help to evaluate the usefulness of these characters in establishing the botanical identity of the plant when compared with other Pancratium species, to rule out any confusion on taxonomic basis. The extractive value of *Pancratium Longiflorum* was about 10.77 %, in ethanol (successive). Steroids and / or triterpenoids were present in hexane, chloroform and ethanol extract of *Pancratium Longiflorum*. Alkaloids were also present in chloroform, ethanol and aqueous extracts *Pancratium Longiflorum*.

The macro and microscopical studies, micro chemical test, fluorescence analysis, physicochemical parameters and TLC finger printing will be served as future standard data for diagnosis and distinguishing, *Pancratium Longiflorum* from its adulterants and substitutions. The identification of many plant constituents and also for screening crude drugs .The results obtained in this study demonstrated the utility of TLC not only in establishing plant identity, but also in distinguishing with certainty between allied species.

These parameters also will be serving as standard data for quality control studies of pharmaceutical preparation from *Pancratium Longiflorum*.

SOURCE(S) OF FUNDING

None

CONFLICT OF INTEREST

None

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