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Original Article

Physicochemical Evaluation and Pharmacognostical Standardization of *Pellionia heyneana* Wedd. Leaf

Vilash V, Suja SR*, Latha PG, Aneesh Kumar AL, Ragesh R Nair and S Rajasekharan

ABSTRACT

Introduction: *Pellionia heyneana* Wedd. Leaves have long been employed as a traditional remedy by the Cholanaikan tribe of South India to treat various ailments. **Methods**: Pharmacological and physicochemical evaluation of *P. heyneana* leaf has been carried out to determine its macro and microscopic characters, and also some of its quantitative characters as per standard procedures. **Results**: The pharmacognostical evaluation of *P. heyneana* leavesrevealed the presence of characteristic microscopic features of the crude drug like cystoliths in upper epidermis, helicocytic stomata in lower epidermis, large number of peculiar shaped, huge (200-400 μm) foliar sclereids, absence of palisade tissue in the lamina *etc*. Powder microscopy showed the presence of calcium oxalate crystals, stone cells, multicellular trichomes, resinous blocks, spiral vessels, xylem fibre, starch grains, simple fibre *etc*. **Conclusions**: All the parameters evaluated in the study will aid to identify the authenticity of *P. heyneana* leaf even from the crushed or powdered form.

Key words: Cholanaikan tribe, Pharmacognosy, Helicocytic stomata, Fluorescence analysis, Powder microscopy.

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INTRODUCTION

Pharmacognostic evaluation of drugs has assumed immense significance in the realm of pharmaceutical industry due to the realization that reproducibility and quality of herbal drugs are directly related to the authenticity of the plant material. Qualitative or quantitative uniqueness of different drugs with respect to the morphological, anatomical and biochemical parameters are acknowledged as one of the reliable tools in distinguishing allied drug samples in the herbal industry. Lack of proper documentation and the adoption of stringent quality control measures continue as a major setback in the use of herbal drugs. To mitigate this, quality assurance by standardization of the drug employing different pharmacognostic parameters has become the need of the hour.[1] This is particularly important since these parameters are not easily amenable by the environment. Hence, this might help not only to authenticate and identify the drug, but also for its safe and efficacious use.

The plant *Pellionia heyneana* Wedd. is an erect or decumbent herb which belongs to Urticaceae family. It is widely distributed in Peninsular India, Sri Lanka, Cambodia, Indonesia, Thailand, and China. Traditionally, its leaves are used by the Cholanaikan tribes to enhance general health and immunity and also to treat various liver ailments.[2] The genus *Pellionia* as well as the species, *P. heyneana* is a very scarcely studied groupand there have been only a couple of attempts made to study the pharmacognostic aspects of the species. The plant leaf is very difficult to distinguish from other allied species in dried or crushed form. Thus the present study has been carried out to evaluate the detailed pharmacognostical and physicochemical properties of *P. heyneana* leaf.

MATERIALS AND METHODS

Collection of plant material

P. heyneana Wedd. plants were collected from Kallar, Thiruvananthapuram district and authenticated by the plant taxonomist of the Institute. Voucher specimen was deposited at the Jawaharlal Nehru Tropical Botanic Garden and Research Institute Herbarium (TBGT 57060 dated 10/12/2010).

Macroscopic and organoleptic characterization

The following macroscopic and organoleptic characters for the fresh leaves were noted: phyllotaxy, size, shape, colour, venation, presence or absence of petiole, apex, margin, base, lamina, texture, surface, odour and taste.[3,4]

Microscopic characterization

Anatomical studies of the leaf

Free hand transverse sections of leaf lamina and midrib were prepared, stained with safranin, mounted on glass slides using glycerine and observed under light microscope with camera attachment and photomicrographs were taken.[3]

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Quantitative leaf microscopy

Stomatal number and stomatal index

A small piece of leaf was cleared by boiling with sodium hypochlorite solution. The upper and lower epidermis were peeled separately. The peeled epidermis was placed on slide and mounted with glycerine water. Average number of stomata per mm^2 of the epidermis of the leaf (stomatal number) is calculated from the microphotographs taken using camera attached microscope.[10] Values for upper and lower epidermis were determined separatelyusing the equation:

$$\text{Stomatal index (SI)} = S \times 100 / E+S.$$

Where, S= the number of stomata per unit area and E = the number of epidermal cells in the same unit area of leaf.

Determination of vein-islet number and vein-let termination number

The vein-islet number is the average number of vein-islets per mm^2 of a leaf surface midway between midrib and margin and the average number of terminated vein-let islets per mm^2 of a leaf was taken as vein-let termination.[3]

Powder analysis

The fresh leaves were separated from the collected plants, thoroughly washed with fresh water, shade dried and powdered. The leaf powder is boiled with chloral hydrate for 5-10 min, and then stained with phloroglucinol, safranin, glycerine and iodine solution to determine the presence of lignified cells, calcium oxalate crystals, starch grains *etc*.[5,6]

Fluorescence analysis

The fluorescence character of the plant leaf powder (40 mesh) was studied both in daylight and UV light (254 and 366 nm) after treatment with different reagents like sodium hydroxide, picric acid, acetic acid, hydrochloric acid, nitric acid, iodine, ferric chloride *etc*.[7,8] The colour changes were noted using Methuen handbook of colour.[9]

Physicochemical analysis

The following physicochemical parameters of *P. heyneana* leaf powder were determined according to the quality control methods for medicinal plant materials.[10]

Determination of pH

pH 1% solution: Dissolved 1 g of the leaf powder in 100 mL of distilled water, filtered and checked the pH of the filtrate with a standardized glass electrode.

pH 10% solution: Dissolved 10 g of the leaf powder in 100 mL of distilled water, filtered and checked pH of the filtrate with a standardized glass electrode.

Determination of alcohol soluble extractive

Take 5 g of the air dried leaf powder in100 mL of ethanol in a closed flask, shaken frequently during 6 h and allowed to stand for 18 h. Filtrate is collected and evaporated to dryness in a tared flat bottom shallow dish, dried at 105°C and weighed. The percentage of alcohol-soluble extractive is calculated with reference to the air dried drug.

Determination of water soluble extractive

Methodology followed as directed for the determination of alcohol soluble extractive using water instead of ethanol.

Determination of petroleum ether soluble extractive

Methodology proceeded as directed for the determination of alcohol soluble extractive, using petroleum ether instead of ethanol.

Loss on Drying (LOD)

About 2-3 g of leaf powder is accurately weighed in a China dish and kept in a hot air oven maintained at 105°C for 5 h. After cooling in a desiccator, the loss in weight was recorded. This procedure was repeated till constant weight was obtained.

$$\text{Loss on drying (\%) LOD} = \frac{\text{Loss in weight}}{\text{Weight of the druging}} \quad 100$$

Swelling index

Leaf powder (1 g) was taken in a measuring cylinder (25 mL) and suspended in 25 mL distilled water for 1 h by thorough mixing every 10 min. After 3 h, volume in mL occupied by the plant material including any sticky mucilage was measured. The experiment was repeated three times for accuracy and the swelling index was calculated.

Foaming index

Finely divided (sieve No. 1250) plant powder 1 g was kept in to a 500 mL flask containing 100 mL of boiling water for 30 min. Then cooled and filtered into a 100 mL volumetric flask and added sufficient water to makeup the volume. The prepared decoction was poured in to 10 stoppered test tubes each 1 mL, 2 mL up to 10 mL. The volume of the liquid in each tube was adjusted to 10 mL with water. The tubes were duly stoppered and shaken in a lengthwise motion for 15 sec (two shakes per second) and allowed to stand for 15 min. The foam height in each tube was measured.

$$\text{Foaming index} = 1000/a$$

'a' is the volume of the plant decoction for foaming foam of height 1cm.

Determination of total ash

About 2-3 g weighed crude drug powder in a tared silica dish was ignited and weighed. Scattered the powder drug on the bottom of the dish and incinerated by gradually increasing the heat not exceeding dull red heat until free from carbon, cooled and weighed. The % w/w of total ash with reference to the air-dried drug was calculated.

Determination of acid insoluble ash

Boiled the ash for 5-10 min with 25 mL of diluted hydrochloric acid, collected the insoluble matter in a Gooch crucible, washed with hot water, ignited and weighed. Percentage of acid insoluble ash is calculated with reference to the air dried drug. The % w/w of acid insoluble ash with reference to the air-dried drug was calculated.

Determination of water-soluble ash

To the crucible containing the total ash, add 25 mL of water and boil for 5-10 min. Collect the insoluble matter in a Gooch crucible, wash with hot water and ignite in a crucible for 15 min at a temperature not exceeding 450°C. Subtract the weight of insoluble matter from the weight of the ash. The difference in weight represents the water soluble ash. Percentage of water soluble ash is calculated with reference to the air dried drug. The % w/w of water-soluble ash with reference to the air-dried drug was calculated.

Percentage extractive and characteristics of fractions

P. heyneana leaf powder was first extracted with hexane using Soxhlet apparatus, powder was then dried and again extracted with chloroform and finally with ethanol to get; 1. Hexane fraction (PHHF), 2. Chloroform fraction (PHCF) and 3. Ethanolic fraction (PHEF). Yield in g/100 g leaf powder of PHHF, PHCF and PHEF of *P. heyneana* leaf powder was calculated. Consistency, colour and odour were also evaluated.

Table 1: **Macroscopic and organoleptic characters of *P. heyneana* leaf**

Macroscopic parameters	Observation
Phyllotaxy	Alternate or sub opposite
Type	Simple
Lamina length	14–18 cm
Laminawidth	5.25–6.50 cm
Shape	Obliquely elliptic- oblong
Apex	Acuminate
Margin	Entire
Venation	Reticulate
Base	Cuneate
Petiole	1 ± 0.25 cm
Surface	Puberulous
Colour- Upper	Dark green
Colour- Lower	Light green
Odour	No characteristic odour
Taste	No characteristic taste

Results are out of 25 observations ± SD.

Table 2: **Quantitative leaf microscopy of *P. heyneana***

Parameter	Range	Mean ± SD
Stomatal number- Upper surface	0	0
Stomatal number- Lower surface	31 – 43	35.32 ± 6.45
Subsidiary cell length	15 - 20 µm	16.45 ± 2.8 µm
Subsidiary cell width	5 - 6 µm	5.54 ± 0.53 µm
Stomatal index- Upper surface	0	0
Stomatal index- Lower surface	8.09-13.12	11.61 ± 1.25
Vein islet number	12–18	16.34 ± 1.14
Vein let termination number	10–13	11.64 ± 1.21

Values are expressed as mean ± SD of ten values.

RESULTS

Macroscopic and organoleptic characterization

Macroscopic and organoleptic characters of the fresh leaves were noted and the results were presented in the Table 1.

Microscopic characterization of P. heyneana leaf

Anatomical studies of leaf

Transverse section of the leaf shows two distinguished regions, midrib region and laminar region.

Midrib: A prominent projection on the abaxialside (Figure 1A). Upper epidermis is of single layered large rectangular cells with outer thin layer of cuticle and some epidermal cells with cystoliths (Figure 1B). Single layered lower epidermis has small rectangular cells (Figure 1C) with uniseriate multi cellular trichomes with tapering end (Figure 1D). Ground tissue is represented with 6-8 layers of collenchyma seen below the upper epidermis and 4-6 layers above the lower epidermis.In between the collenchyma is seen the thin walled thickly packed parenchyma cells with cluster of calcium oxalate crystals. Single layers of sclerenchyma cells seen below the upper collenchyma, vascular bundles arehorseshoe shaped (Figure 1E).

Lamina: Narrow lamina, upper epidermis is single layered large rectangular cells with an outer thin layer of cuticle. Upper epidermis is followed by two layers of sclerenchymatus hypodermis. Hypodermis is followed by 5-6 layers of mesophyll tissue; palisade absent (Figure 1F). Single layered lower epidermis of small rectangular cells is seen with numerous helicocytic stomata. Large number of huge foliar sclereids (200-400 µm length) with peculiar shape was found throughout the leaf (Figure 1G).

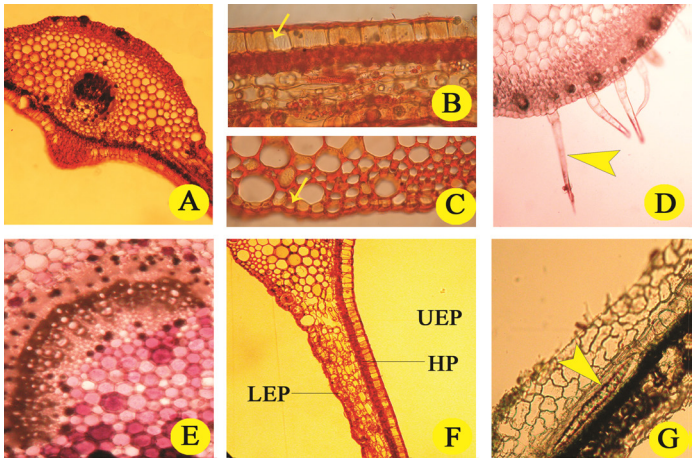

Figure 1: Leaf anatomy of *P. heyneana* leaf. A -T. S. of leaf midrib, B - T. S. of midrib showing single layered upper epidermis with large rectangular cells, C -T. S. of midrib showing single layered lower epidermis with small rectangular cells, D - Uniseriate multicellular trichomes, E - Leaf vascular bundle, F - T. S of lamina (UPE - upper epidermis, LEP - lower epidermis, HP - hypodermis) G - T. S. of lamina showing foliar sclereid in mesophyll tissue.

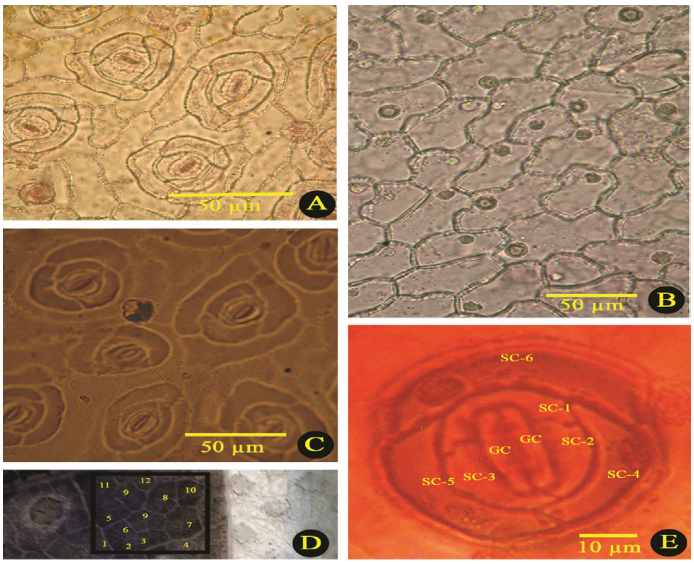

Figure 2: Quantitative leaf characteristics of *P. heyneana* A - Leaf peeling of lower surface showing helicocytic stomata, B - Leaf peeling of upper surface without stomata, C - Stomatal impression of lower surface, D - Vein inlet number in 1mm2, E - Helicocytic stomatal complex showing guard cells (GD) and subsidiary cells (SC).

Quantitative leaf microscopy

Quantitative leaf characteristics were observed and the results were shown in the Table: 2 and Figure 2.

Powder analysis

Analysis of *P. heyneana* leaf powder revealed the presence of prismatic calcium oxalate crystals, stone cells, multicellular trichomes, helicocytic stomata, epidermal cells, spiral vessels, xylem fibre, starch grains, simple fibreand abundance of special shaped large foliar sclereids (Figure 3).

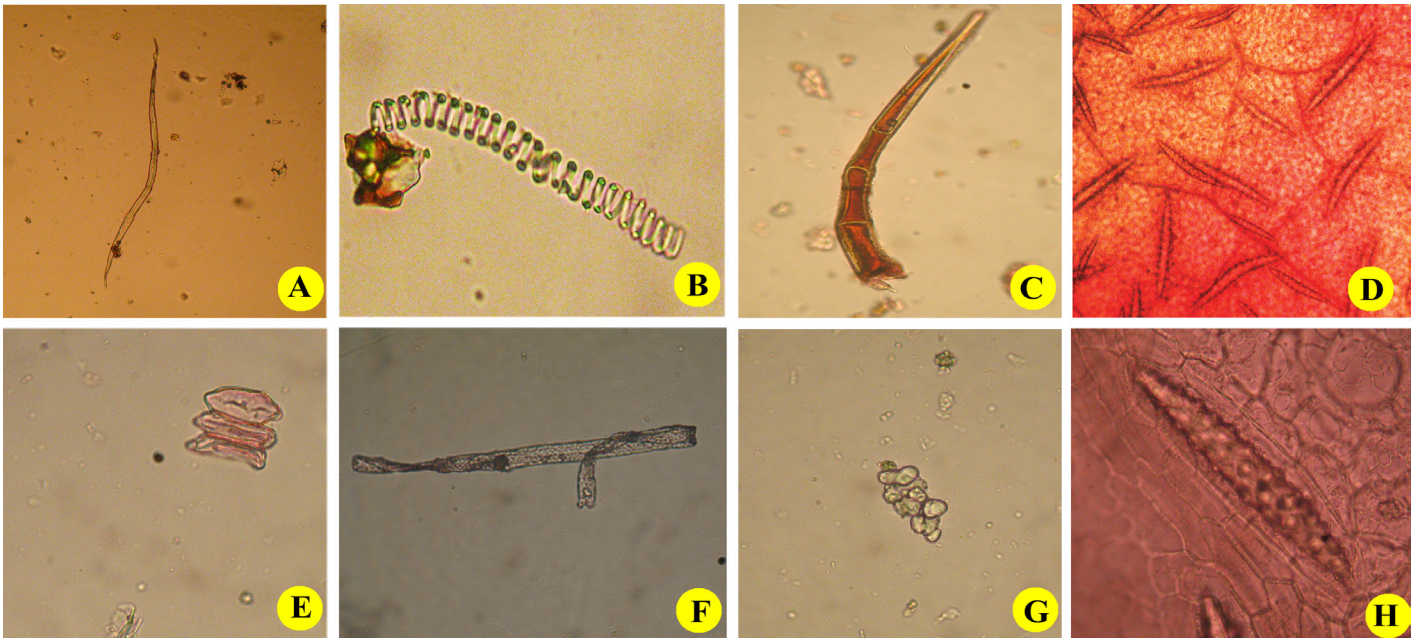

Figure 3: Powder analysis of *P. heyneana* leaf. A - Xylem fibre, B - Spiral xylem vessel, C -Multicellular trichomes, D - Foliar sclereids in leaf pealing, E - Stone cells, F - Simple fibre, G - Calcium oxalate crystals, H - Single foliar sclereid.

Table 3: Observations of *P. heyneana* leaf powder under visible light and UV (254nm and 366nm) light

Treatment	Observation (Colour developed)		
	Visible light	UV-254nm	UV-366nm
Powder alone	Olive green (25C6)	Grayish brown (6E3)	Dark brown (6F8)
1. Powder + 1 M NaOH	Yellowish brown (5E8)	Current red (10B6)*	Dark violet (15F4)
2. Powder + 1 M NaOH + Methanol	Deep green (28E8)	Reddish orange (7B7)*	Dark violet (15F3)
3. Powder + 1 M NaOH + Water	Honey yellow (5D6)	Greenish blue (20B4)*	Wax yellow (3B8)*
4. Powder + 1 M HCl	Golden blade (5C4)	Pastel blue (28D3)*	Straw yellow (3B4)
5. Powder + dil HNO_3	Light orange (5A5)	Deep green (28D3)	Reddish violet (16C8)
6. Powder + 5% Iodine	Cherry red (10D8)	Red (10A8)*	Dull violet (16E3)
7. Powder + 5% $FeCl_2$	Sahara (6C5)	Greenish grey (27B3)	Dark violet (16F4)
8. Powder + dil Ammonia	Golden	Bleu ciel (22A5)*	Dull violet (16E3)
9. Powder + Methanol	Green (27C8)	Bluish red (12A6)*	Dark violet (16F7)
10. Powder + HCl	Dark green(28F6/F5)	Violet brown (10F3)	Dark violet (15F6)
11. Powder + 1M H_2SO_4	Greyish orange (5B4)	Persian blue (23A3)*	Cream (4A3)
12. Powder + HNO_3	Orange (6A6/7)	Cactus green (28E4)*	Champagne (4B4)*
13. Powder + $K_2Cr_2O_7$	Caramel (6C6)	Olive brown (4F5)	Dark blonde (5D4)
14. Powder + 95% Ethanol	Green (28C8)	Deep orange (6A8)*	Reddish brown (8D6)
15. Powder + Toluene	Spring green (30C7)	Persian orange (6A7)*	English red (8D8)

Methuen Hand Book of Colour. *presence of fluorescence.

Table 4: Physicochemical parameters of *P. heyneana* leaf powder

pH of water solution- 1% w/v	8.15 ± 0.34
pH of water solution- 10% w/v	8.34 ± 0.26
Alcohol-soluble extractive	5.03 ± 0.24% w/w
Water-soluble extractive	11.82 ± 0.45% w/w
Petroleum ether-soluble extractive	1.25 ± 0.23% w/w
Loss on drying (LOD)	9.3 ± 3.25% w/w
Total ash	18.61 % w/w
Acid-insoluble ash	11.63 % w/w
Water-soluble ash	2.52 % w/w
Swelling index	7mL
Foaming index	200

Values are expressed as mean ± SD of six values.

Table 5: Percentage extractive and characteristics of *P. heyneana* extract and fractions

Name of the Extract/ Fractions	Consistency	Colour	Odour	Yield (g/100 g powder)
PHHF	Semi solid	Dark brown	Characteristic	1.79
PHCF	Hard	Greenish brown	Characteristic	1.42
PHEF	Solid	Golden brown	Characteristic	6.84

Fluorescence analysis

Chemical tests of *P. heyneana* leaf powder were conducted with different reagents and observed under UV- 254 nm and UV-366 nm. The results were compared with their respective observations in visible light and they were represented in Table 3.

Physicochemical analysis

Physiochemical parameters of *P. heyneana* leaf powder were evaluated and the observations are presented in Table 4.

Percentage extractive and characteristics of extract and fractions of P. heyneana leaf

Physical characteristic and percentage yield of PHHF, PHCF and PHEF were shown in the Table 5.

DISCUSSION

The macroscopic characters of leaf (Table 1), the species possess simple leaf with a mean length 14 ± 4.5 cm and breadth 5.25 ± 1.25 cm. The plants, in general have an obliquely elliptic oblong entire leaf with cuneate base and reticulate venation. Leaf colour is typically green, being dark green in the adaxial and pale green in the abaxial sides. Phyllotaxy is alternate or sub opposite. A reduction in the leaf size is observed in the successive pairs of leaves. This rather gives an alternate appearance for the leaf.

T.S of leaf showed the presence of upper and lower epidermis with barrel shaped or rectangular shaped cells, typical of most leaves. Presence of cystolithis evident in the epidermis. Ground tissue is predominantly made of collenchyma. Patches of parenchyma with calcium oxalate crystals embedded in it has also been noted. Horseshoe shaped vascular bundle with xylem and phloem in the midrib is a key characteristic of the species. Presence of large sclereids (200 – 400 µm) is typical to the species. Perhaps, the roughness of the leaves is due to the presence of sclereids. Stomata in the species is helicocytic characterised by a helix of four or more subsidiary cells surrounding the guard cells. This is the first report of the presence of helicocytic stomata in the species. Payne stressed that helicocytic stomata is an unrecognised stomatal pattern in dicotyledonae and he observed this type of stomata in many species including its close ally, *Pellionia daveauana*.[11] The present observation of helicocytic stomata in *P. heyneana* is in broad consensus with Payne (1970), whereas Ushakumari *et al.*(2004) reported the presence of cyclocyticstomatata in *P. heyneana*.[12] Total absence of stomata in the adaxial surface is a feature of importance in the identification of the drug. Absence of palisade tissue in the lamina is yet another feature specifically confronting the species. These features might offer authenticity in the identification of the species which would be largely helpful in distinguishing it from adulterants.

Fluorescence analysis is important in distinguishing the drug from its adulterants in its powdered form.[13,14] Comparative account of the fluorescence analysis treated with different chemical reagents and observed at 254 and 366 nm UV are depicted in Table 3.

Evaluation of physicochemical parameters aids in the formulation of pharmacopoeial standards. Among the various physicochemical characteristics evaluated in the present study (Table 4), ash value is one of the common features used to determine the identity and purity of the drug.[15] The extract left behind after exhausting the crude drug is indicative of the chemical constituents of the drug in question. Total ash usually contains carbonates, phosphates, silicates and silica.[3,16] High total ash value of *P. heyneana* (18.61% w/w) noted in the study may be due to high quantity of calcium crystals of the plant. This could be well corroborated with the results of T.S. of lamina in which are seen patches of parenchyma with calcium oxalate crystals. The water soluble ash gives an estimate of the amount of inorganic elements in the sample. Acid insoluble ash in *P. heyneana* amounts to 11.63% w/w, which indicates that almost half of the total ash is soluble in acid. The extractive values are useful to evaluate the nature of chemical constituents present in crude drug and also to help in estimation of specific constituents soluble in a particular solvent.[17] The extractive value of the three serial fractions revealed that the quantity of extract is maximum in ethanolic fractions which means the plant contains more polar compounds.

CONCLUSION

Setting up standards for the correct identity and quality of a crude drug is a fundamental process of drug development. In the present study, a great bulk of information on identity, purity and quality of plant material is gainedwhile evaluating the macroscopy, microscopy, powder characters and physicochemical parameters. Thus the present investigation put forward important standardization parameters for a meagrely studied medicinal herb *P. heyneana* which has a new entry as medicinal aid and also as a tool for identifying authenticity of plant material.

The pharmacognostical evaluation of *P. heyneana* leaf revealed the presence of characteristic microscopic features of the crude drug like cystoliths in upper epidermis, helicocytic stomata in lower epidermis, large number of peculiar shaped, huge foliar sclereids, absence of palisade tissue in the lamina *etc*. Powder analysis, fluorescence analysis and physicochemical parameters of the leaf powder conducted in the present study will be extremely beneficial to identify the authenticity of the plant leaf even from the crushed or powdered form.

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CONFLICT OF INTEREST

Declared None.

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