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Research Article

Pharmacognostic and phytochemical studies on *Parthenium hysterophorus* L.

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ABSTRACT

From last few decades there is an increased use of herbal medicines all over the world and now there is need to study herbal drugs on scientific basis to develop their monographs. The present research work was an attempt to establish parameters for identification of *Parthenium hysterophorus* L. (Fam. Asteraceae) according to guidelines of WHO, by studying its morphological and organoleptic characters, detailed microscopic evaluation, histochemical studies, fluorescence analysis, loss on drying, extractive values, swelling index, foaming index and preliminary phytochemical screening of flower heads, leaves, stem and root. Although conventional, but it is simple, easy and quick way for identification and standardization of herbal substances affordable even in developing countries. This is the first report on pharmacognostical and physicochemical studies on this plant which can be helpful in establishing pharmacopoeial monograph of this plant.

Key words: *Parthenium hysterophorus*, carrot grass, pharmacognostic, Asteraceae.

INTRODUCTION

Since the birth of humans, plants have been a good source of medicines [1]. At the end of 19th century, after synthesis of aspirin, research on herbal products was halted and researchers started focusing on synthetic and semi-synthetic drugs. But from last few decades, there is an upsurge in use and research on botanical drugs. About 25% of the prescribed drugs are derived from higher plants and this value is increased to 50% if animal and microbial products are also included [2-3].

Parthenium hysterophorus L. is commonly known as carrot grass, congress grass, white head and white top [4]. It belongs to family Asteraceae also called Compositae [5]. In folklore medicines, it is used to treat allergies, sores, anemia, fever, facial neuralgia, skin diseases and as tonic, blood purifier, vermifuge, ammenagague, abortive and insecticide [4, 6]. This plant is native to Gulf of Mexico and USA, from where it was spread all over the world, except Europe [7-10]. Sesquiterpene lactones are the most abundant phytoconstituents of this plant which are responsible for harmful effects e.g. strong allelopathic effect [11-13], dermatitis [14] and live stock toxicity [15-17]. This plant has many beneficial effects e.g. antibacterial activity [18-20], larvicidal activity against *Aedes aegypti* L. [21-22], acaricidal activity [23], nematicidal activity [24], antioxidant activity [25-26], cytotoxic activity [27-29], skeletal muscle relaxant activity [30], act as hypoglycemic agent [31], as herbicidal agent [32], as pesticidal agent [33], as a source of anti-HIV agent [34], as a source of food for animals [35], used for the production of oxalic acid [36], for the production of xylanase [37], for production of bioethanol [38-39], for removal of *p*-cresol, dyes and heavy metal ions from industrial wastewater [40-46], as an additive in biogas production [47], as reducing agent in formation of silver nanoparticles [48], in production of zinc nanoparticles [49] and as organic manure [50-52]. The present study was conducted to establish parameters for the identifiacation of this plant according to the guidelines of WHO [53].

MATERIALS AND METHODS

All chemicals, reagents and solvents used were of analytical grade. Reagents and solutions were prepared according to British Pharmacopoeia and United States Pharmacopoeia.

Plant collection and preparation:

The plant was collected from Bio-Park, Bahauddin Zakariya University Multan, Pakistan in the month of August 2015 and was identified as *Parthenium hysterophorus* L. (Fam. Asteraceae) by Dr. Zafar Ullah Zafar, taxonomist of B.Z. University. Capitula,

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leaves, roots and stems were separated and dried under shade for about 40 days. These parts were then ground separately. For microscopic analysis fresh plant was used [54].

Macroscopic characters and preliminary tests:

Macroscopic characters of fresh and shade dried capitulum, leaf, stem and root were studied and preliminary tests on shade dried powders were performed e.g. taste, odor and color were noted. Small quantity of powder was pressed between filter paper to check oily stain, small quantity was mixed with water and shaken vigorously to check persistent froth indicative of saponins and some powder was allowed to stand in water to observe gummy substances [1, 53, 55].

Microscopic and histochemical studies:

Microscopic and histochemical studies were performed according to the methods of WHO, Johansen and Khandelwal [54, 56].

Fluorescence analysis:

Fluorescence analysis was performed by treating the powders with different reagents [57-58].

Physico-chemical parameters:

Physicochemical parameters were studied according to the guidelines of WHO [53].

Phytochemical studies:

Preliminary phytochemical screening was done according to the prescribed procedures [59].

RESULTS

MACROSCOPIC CHARACTERS

Flower head (capitulum):

In fresh plant, numerous creamy white colored capitula having light indistinct odor were seen at the tips of branches. Each capitulum was 3.5-4 mm in diameter and pentangular in shape having five ray florets and about 40-47 disk florets. Shade dried capitulum was odorless, brown in color and 3-3.5 mm in diameter. External row of the involucre was formed by five phyllaries which were non-hairy, thin, nearly translucent, green in color, small in size, ovate in shape and flexible in texture. In shade dried capitulum, these phyllaries were inflexible and light green to light brown colored. Color of these phyllaries changed to brown on maturity of capitulum before seed dispersion. Internal row of the involucre was formed by five papery, whitish green in fresh state and light green in shade dried phyllaries which were transformed into palea (Fig. 1A, 1B). Green colored pedicel was 2-7 mm in length and covered with very small hairs and its color changed to light brown when dried under the shade. Very light weight achene (cypsela) was dark brown to black in color, obovate in shape and 1.5 mm in length (Fig. 1C, 1D). Five cypselae were produced per capitulum. Each achene had two dead tubular florets appearing as papery structures, a pappus consisting of three parts and a palea.

Leaf:

Fresh leaves were soft and flexible in texture, bipinnatfid in shape and alternate in arrangement possessing light indistinct odor. Width and length of lamina was 12-15 cm and 15-22 cm respectively with 2 cm long petiole (Fig. 1E, 1F). Adaxial side was green while abaxial side was yellowish green. Petiole and lamina were covered with fine soft hairs, abaxial side being more pubescent. Leaves towards the apex were less divided and smaller. In shade dried condition, leaves were odorless, light green colored, crumpled, inflexible and pubescence was less prominent.

Stem:

In fresh plant, stem was erected, herbaceous, green colored, highly branched towards the apex, octangular in shape, pubescent and longitudinally grooved possessing light indistinct odor (Fig. 1G, 1H). Fracture of stem was fibrous and tough in fresh state and hard in shade dried form with outer covering being peeled off during fracture. Pith was soft, continuous at apex, chambered at base, white colored in fresh stem and light brown to dark brown in shade dried condition. Grooves became more prominent in shade dried condition.

Root:

Light brown colored tap root system with numerous root hairs and possessing light indistinct odor was observed in fresh plant. Length was about 15-18 cm and fracture was fibrous and tough (Fig. 1I, 1J). Shade dried form was almost odorless, dark brown colored with very hard fracture.

PRELIMINARY TESTS OF POWDERED PLANT PARTS

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Results of preliminary tests performed on powdered plant parts are described in table 1.

MICROSCOPIC EVALUATION

Flower head (capitulum):

Wavy walled rectangular shaped epidermal cells were seen on both surfaces of phyllaries and palea, walls of palea cells being wavier than those of phyllaries (Fig. 2A, 2B). Uniseriate multicellular oblong non-glandular hairs were found on pedicel and on both sides of phyllaries, being relatively more abundant on peripheries (Fig. 2D). Capitate glandular trichomes with multicellular stalk were prominent on plea and disk florets (Fig. 2C). Capitate multicellular trichomes were also seen on pappus and achene (Fig. 2E). Round shaped pollen grains were abundant (Fig. 2F). In powdered form, epidermal cells, pollen grains, broken trichomes similar to those of fresh plant were seen.

Leaf:

Polygonal and irregular shaped epidermal cells with anomocytic type of stomata and uniseriate multicellular non-glandular trichomes with pointed ends were found on both surfaces of leaf (Fig. 2J). Abaxial side trichomes were relatively longer (Fig. 2G, 2H). In transverse section, outline of midrib had irregular ridges and was not smooth. Outermost layer was epidermis, covered externally by cuticle and possessing trichomes. Variable layers of collenchyma cells were found inside the ridges. Oval shaped vascular tissues surrounded by collenchyma cells were prominent forming an arc (Fig. 3A). Xylem was toward adaxial side and phloem toward abaxial side (Fig. 3B). Remaining space of midrib was packed with parenchyma cells. In powder, anomocytic stomata, irregular shaped epidermal cells, intact and broken trichomes, pitted and spiral vessel elements were seen (Fig. 2I).

Stem:

In transverse section, stem was octangular in outline and eight ridges were visible even with naked eye (Fig. 3C). Epidermis consisting of round cells and forming the outer most layer was covered by cuticle and trichomes were prominent (Fig. 3I). Epidermis layer contained two raised stomata in each trough, inside each raised stoma there was air space and two to three layers of chlorenchyma cells (Fig. 3E). Ridges were packed with angular collenchyma cells (Fig. 3F). Cortex consisted of four to five layers of parenchyma cells. Oval shaped vascular bundles were seen in circular form, xylem being inside in the form of radial rows and phloem outside (Fig. 3D). Over the phloem, there was sclerenchyma fiber cap consisting of round to somewhat elliptical shaped thick walled cells. Vascular bundles inside the ridges were relatively larger, having more fibers inside the xylem. Pith was connected to the cortex by three to four layers of parenchyma cells. Pith consisted of thin walled cells which were small and round near vascular bundles and became elongated in the center of stem. In powder form, intact and broken trichomes, epidermal and pith cells, spiral vessel elements, vessel segments containing round bordered pits, few small starch granules were seen (Fig. 3G, 3H).

Root:

In transverse section, there was broken periderm, thin cortex, 27-30 thin walled medullary rays and vascular tissue. Xylem occupied the most of the part (Fig. 3J).

HISTOCHEMICAL STUDIES

Results of histochemical analysis are described in table 2-5.

FLUORESCENCE ANALYSIS

Results of fluorescence analysis are described in table 6-9.

PHYSICO-CHEMICAL PARAMETERS

Physico-chemical parameters are listed in table 10.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Results of preliminary phytochemical screening are presented in table 11.

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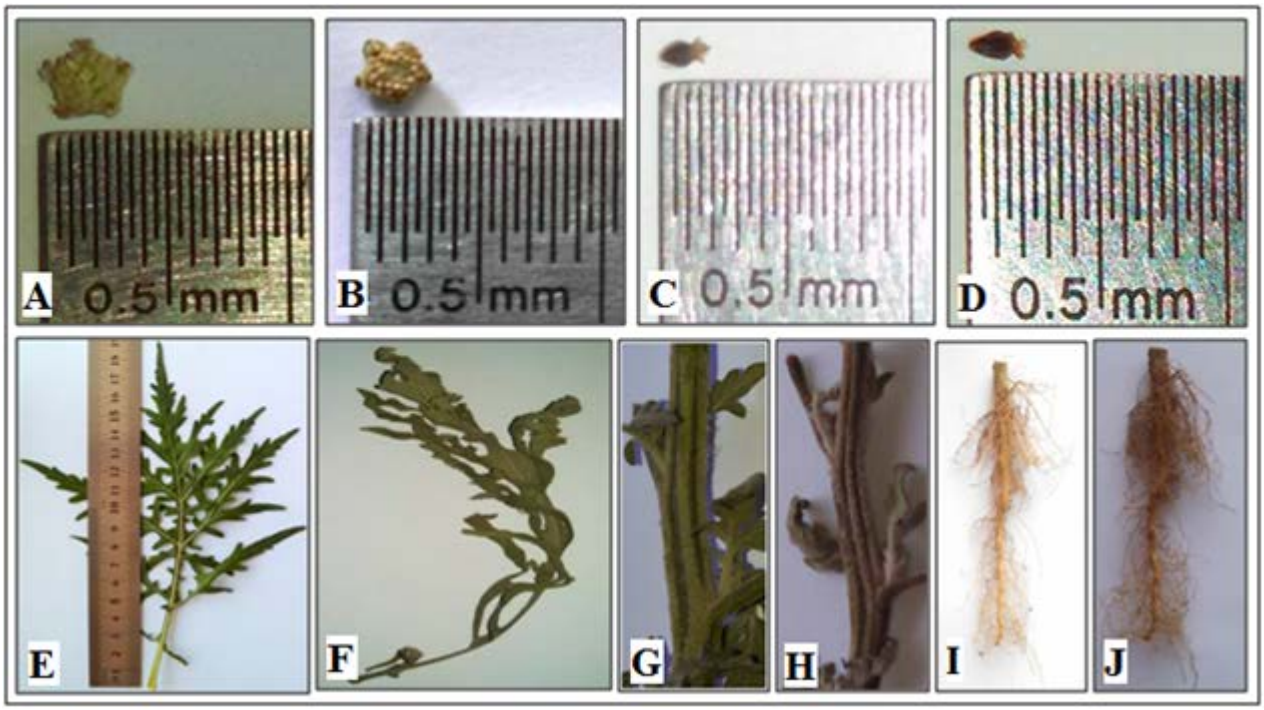

Figure 1: A: capitulum (fresh); B: capitulum (shade dried); C: cypsela (fresh); D: cypsela (shade dried); E: leaf (fresh); F: leaf (shade dried); G: stem (fresh); H: stem (shade dried); I: root (fresh); J: root (shade dried).

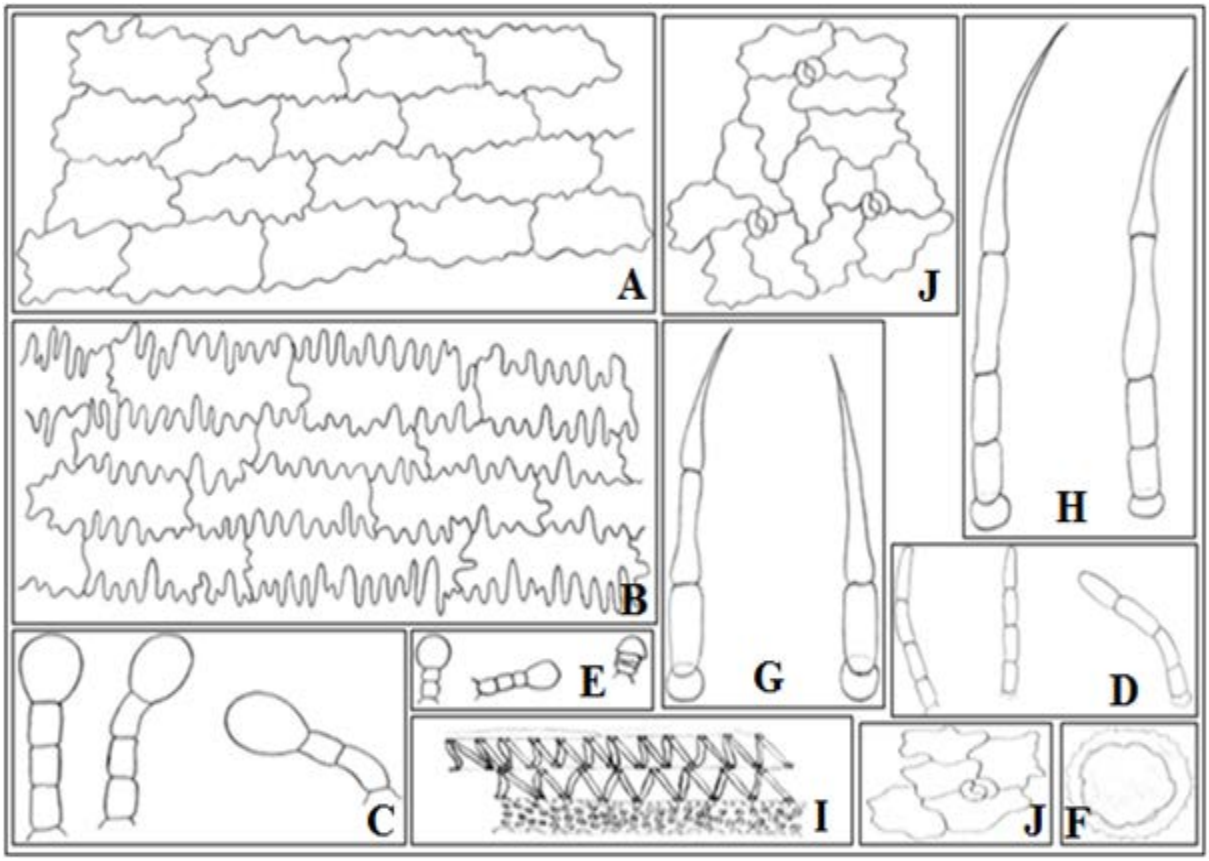

Figure 2: A: Epidermal cells of phyllaries in surface view; B: Epidermal cells of palea in surface view; C: Glandular trichomes of disk florets and palea; D: Non-glandular trichomes of phyllaries; E: Trichomes of achene; F: Pollen grain; G: Trichomes on adaxial side of leaf; H: Trichomes on abaxial side of leaf; I: Spiral and pitted vessel elements seen in leaf powder; J: Leaf epidermis in surface view.

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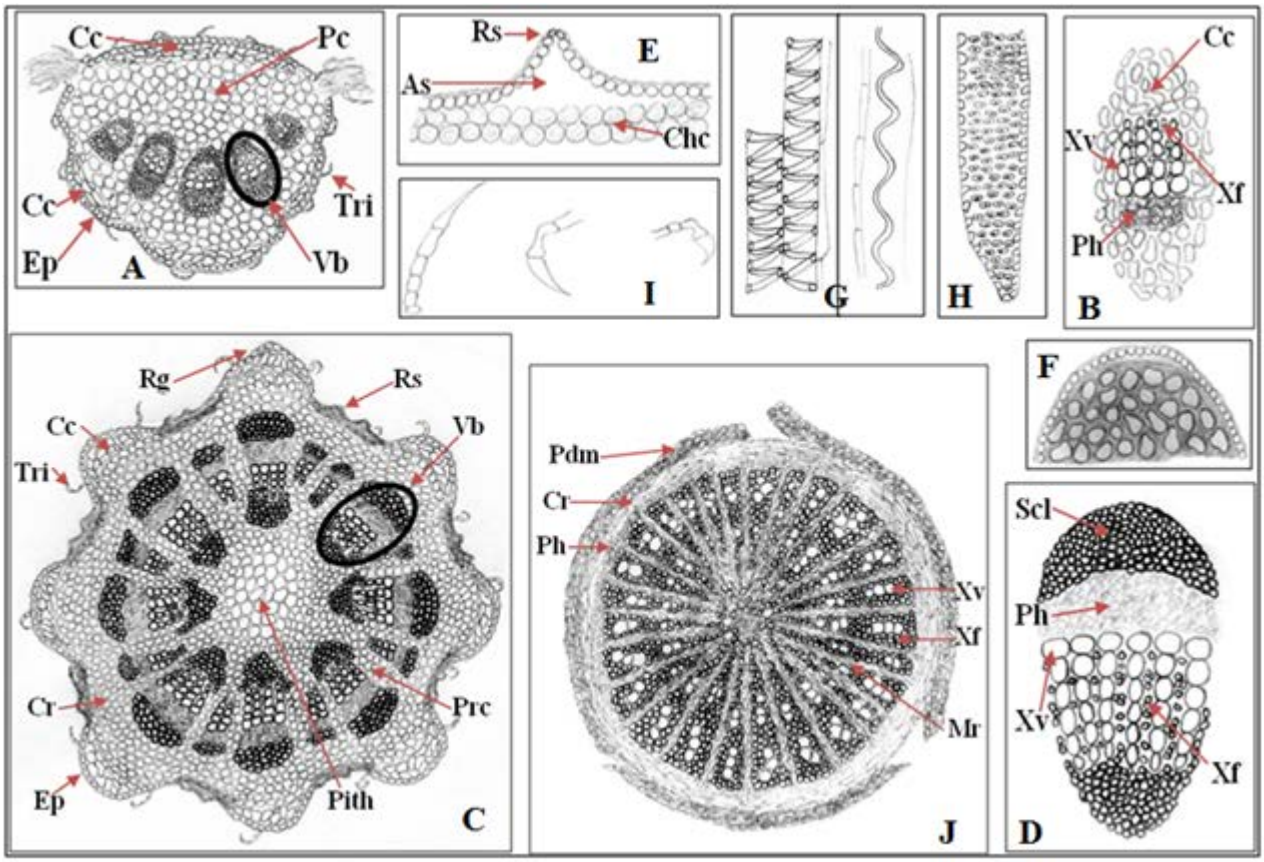

Figure 3: A: Midrib of leaf in transverse section; B: Vascular bundle of leaf at 400X; C: TS of stem; D: Vascular bundle of stem at 400X; E: Raised stoma at 400X; F: Ridge of stem filled by angular collenchyma at 400X; G: Spiral vessel elements of stem; H: Vessel elements of stem having round bordered pits; I: Trichomes of stem at 400X; J: TS of root.

Abbreviations: As: Air space; Cc: Collenchyma cells; Chc: Chlorenchyma cells; Cr: Cortex; Ep: Epidermis; Mr: Medullary ray; Pc: Parenchyma cells; Pdm: Periderm; Ph: Phloem; Prc: Parenchyma cells connecting pith to cortex; Rg: Ridge; Rs: Raised stomata; Scl: Sclerenchyma fiber cap; Tri: Trichomes; Xf: Xylem fibers; Xv: Xylem vessels.

Table 1: Preliminary tests of powdered plant parts

Tests performed	Capitulum	Leaves	Stem	Root
Color	Light green	Green	Yellowish green	Yellowish brown
Odor	Odorless	Like green tea	Light indistinct	Odorless
Taste	Bitter	Bitter	Light indistinct	Light indistinct
Oily stain on filter paper	Absent	Absent	Absent	Absent
Gummy material	Absent	Absent	Absent	Absent
Persistent froth	Absent	Absent	Absent	Absent

Table 2: Histochemical studies of capitulum

Test for	Reagent used	Histochemical zone	Color	Result
Lignin	Phloroglucinol soln.	Trichomes	No change	Absent
Lignin	Safranin solution	Trichomes	No change	Absent
Lignin	Phloroglucinol soln.	Vascular bundles	Cherry red Pink	Present
Lignin	Safranin solution	Vascular bundles	No change	Present
Starch granules	I_2 solution	Powder of capitulum	Blue	Absent
Cellulose	I_2 solution+H_2SO_4	Disk florets	No change	Present
Tannins	$FeCl_3$ solution	Powder of capitulum	No change	Absent
Volatile oil, fat	Sudan Red solution	Powder of capitulum		Absent

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Table 3: Histochemical studies of leaf

Test for	Reagent used	Histochemical zone	Color	Result
Lignin	Phloroglucinol soln.	Trichomes	No change	Absent
Lignin	Safranin solution	Trichomes	No change	Absent
Lignin	Phloroglucinol soln.	Xylem vessels and fibers	Cherry red	Present
Lignin	Safranin solution	Xylem vessels and fibers	Pink	Present
Starch granules	Iodine solution	Powder of leaf	No change	Absent
Cellulose	I_2 solution+H_2SO_4	Collenchyma cells	Blue	Present
Tannin	$FeCl_3$ solution	Whole TS and powder	No change	Absent
Volatile oil, fat	Sudan Red solution	Whole TS and powder	No change	Absent

Table 4: Histochemical studies of stem

Test for	Reagent used	Histochemical zone	Color	Result
Lignin	Phloroglucinol soln.	Trichomes	No change	Absent
Lignin	Safranin solution	Trichomes	No change	Absent
Lignin	Phloroglucinol soln.	Xylem vessels and fibers	Cherry red	Present
Lignin	Safranin solution	Xylem vessels and fibers	Pink	Present
Starch granules	Iodine solution	Powder of stem	Blue	Present
Cellulose	I_2 solution+H_2SO_4	Collenchyma cells	Blue	Present
Tannin	$FeCl_3$ solution	Whole TS and powder	No change	Absent
Volatile oil, fat	Sudan Red solution	Whole TS and powder	No change	Absent

Table 5: Histochemical studies of root

Test for	Reagent used	Histochemical zone	Color	Result
Lignin	Phloroglucinol soln.	Xylem vessels and fibers	Cherry red	Present
Lignin	Safranin solution	Xylem vessels and fibers	Pink	Present
Starch granules	Iodine solution	Powder of root	Blue	Present
Cellulose	I_2 solution+H_2SO_4	Cork cells	Blue	Present
Tannin	$FeCl_3$ solution	Whole TS and powder	No change	Absent
Volatile oil, fat	Sudan Red solution	Whole TS and powder	No change	Absent

Table 6: Fluorescence analysis of capitulum

Treatment	Visible (Day light)	Ultra violet	
		Short (254 nm)	Long (366 nm)
P (powder)	Light green	Yellowish brown	Yellowish brown
P+water	Brownish green	Light green	Light green
P+95% ethanol	Dark brown	Light green	Light green
Aqueous extract	Dark brown	Greenish	Yellowish green
P+conc.H_2SO_4	Yellowish black	Yellowish green	Yellowish green
P+conc.HCL	Blackish brown	Light brown	Dark brown
P+glacial acetic acid	Dark brown	Yellowish green	Yellowish brown
P+chloroform	Greenish brown	Blackish	Blackish
P+dil.ammonia solution	Brownish green	Brownish	Blackish
P+I_2 solution	Black	Dark brown	Black
P+$FeCl_3$ solution	Blackish green	Blackish	Blackish

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Table 7: Fluorescence analysis of leaf

Treatment	Visible (Day light)	Ultra violet	
		Short (254 nm)	Long (366 nm)
P (powder)	Green	Brown	Dark brown
P+water	Green	Light green	Green
P+95% ethanol	Dark green	Dark green	Blackish
Aqueous extract	Blackish brown	Blackish green	Yellowish black
P+conc.H_2SO_4	Greenish black	Yellowish brown	Dark brown
P+conc.HCL	Blackish green	Dark brown	Blackish brown
P+glacial acetic acid	Blackish green	Yellowish brown	Dark brown
P+chloroform	Dark green	Greenish black	Black
P+dil.ammonia solution	Dark green	Blackish	Blackish
P+I_2 solution	Greenish black	Dark brown	Black
P+$FeCl_3$ solution	Green	Blackish	Blackish

Table 8: Fluorescence analysis of stem

Treatment	Visible (Day light)	Ultra violet	
		Short (254 nm)	Long (366 nm)
P (powder)	Yellowish green	Light brown	Yellow
P+water	Yellowish green	Light green	Light green
P+95% ethanol	Brown	Brown	Light green
Aqueous extract	Light brown	Yellowish green	Greenish yellow
P+conc.H_2SO_4	Brownish black	Black	Dark brown
P+conc.HCL	Light brown	Yellowish green	Blackish green
P+glacial Acetic acid	Greenish brown	Yellowish green	Yellowish green
P+chloroform	Brownish green	Light green	Light green
P+dil.ammonia solution	Light green	Light green	Blackish green
P+I_2 solution	Black	Dark brown	Black
P+$FeCl_3$ solution	Yellowish brown	Brownish black	Blackish

Table 9: Fluorescence analysis of root

Treatment	Visible (Day light)	Ultra violet	
		Short (254 nm)	Long (366 nm)
P (powder)	Yellowish brown	Dark brown	Brown
P+water	Yellowish brown	Brown	Brown
P+95% ethanol	Dark brown	Brown	Brown
Aqueous extract	Reddish brown	Yellowish brown	Yellowish brown
P+conc.H_2SO_4	Brownish black	Black	Dark brown
P+conc.HCL	Grey	Blackish brown	Blackish brown
P+glacial acetic acid	Light brown	Yellowish green	Yellowish green
P+chloroform	Light brown	Brownish	Brown
P+dil.ammonia solution	Light brown	Light brown	Blackish brown
P+I_2 solution	Black	Dark brown	Blackish
P+$FeCl_3$ solution	Dark brown	Brownish black	Black

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Table 10: Physico-chemical parameters

Parameters	Capitulum	Leaf	Stem	Root
Foaming index	Less than 100	Less than 100	Less than 100	Less than 100
Swelling index	8.5 mL	8 mL	7.5 mL	7 mL
Loss on drying	9.73% w/w	9.50% w/w	5.71% w/w	7.3% w/w
Water soluble extractive value (cold)	6.25% w/w	8.35% w/w	4.5% w/w	4.5% w/w
Water soluble extractive value (hot)	7.67% w/w	9.7% w/w	5.25% w/w	4.62%w/w

Table 11: Preliminary phytochemical analysis

Phytoconstituents	Flower heads	Leaves	Stem	Root
Alkaloids	–	–	–	–
Cardiac glycosides	+ +	+	+	+ +
Anthraquinone glycosides	–	–	–	–
Free anthraquinones	–	–	–	–
Tannins	–	–	–	–
Saponins	–	–	–	–

–: Absent +: Present

DISCUSSION AND CONCLUSION

Some important characters about the authenticity of *P. hysterophorus* are explored in this research work which might be helpful in identification of this plant even if it is in dried form, powdered form or adulterated. Wavy walls of rectangular shaped epidermal cells of palea and phyllaries, oblong non-glandular trichomes of pedicel and phyllaries, glandular trichomes of achene and pappus, typical shaped pollen grains, anomocytic stomata, pointed trichomes of leaf, oval shaped vascular bundles in midrib forming an arc, octangular stem with trichomes and raised stomata, presence of angular collenchyma cells in ridges of stem, sclerenchyma fiber cap over vascular bundles of stem, periderm and medullary rays of root are characteristic features. All the trichomes are non-lignified but lignin and cellulose is found abundantly in vascular bundles and collenchyma cells respectively which were observed in histochemical studies. This lignin is the main barrier in production of biogas from this plant [60]. Plant has bitter taste characteristic of sesquiterpene lactones. Tannins and saponins are absent while cardiac glycosides are present in all parts of the plant. Many aspects of this plant still need to be studied e.g. quantitative microscopic evaluation, DNA based authentication, exploration of more therapeutic effects and useful constituents.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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