Research Article

Pharmacognostic and Phytochemical investigation of stems of *Pergularia daemia*

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Abstract

Objective: The main objective of the present work is to study the pharmacognostic and phytochemical characteristics of *Pergularia daemia* stems belonging to the family *Asclepiadaceae*. **Materials and Methods:** Ethanolic and aqueous extracts of *Pergularia daemia* stems were prepared by using Soxhlet and cold maceration technique respectively. The extracts were concentrated using rotary evaporator. Morphological evaluation, microscopical characterization, powder analysis, physicochemical evaluation, fluorescence analysis, preliminary phytochemical screening and TLC profiling of *Pergularia daemia* was performed. **Results:** Pharmacognostic and phytochemical studies of stem of *Pergularia daemia*. Microscopical characters were determined by performing transverse section of stem and powder microscopy. Standardization of whole plant was done with the help of ash value (total ash, acid insoluble ash and water soluble ash), water soluble extractive and alcohol soluble extractive value. Fluorescence analysis was also carried out ultraviolet chamber. Alcoholic and aqueous extracts were prepared and preliminary phytochemical analysis was carried out. Alcoholic extract shows the presence of carbohydrates, alkaloids, flavonoids whereas aqueous extract shows presence of steroids and tannins. Thin layer chromatography of alcoholic extract shows the eight numbers of spot having Rf value 0.12, 0.8, 0.38, 0.45, 0.58, 0.74, 0.87 and 0.93 respectively. **Conclusion:** Pharmacognostic and phytochemical investigation of powdered stem of *Pergularia daemia* describing its morphological evaluation, microscopical characterization, powder analysis, physicochemical evaluation, fluorescence analysis, preliminary phytochemical screening and TLC profiling has been studied in detail so as to develop a reference for academic and commercial purpose. Further, it can be used for the standardization and pharmacopoeial parameters development.

Keywords: *Pegularia daemia,* extraction, morphology, microscopy, phytochemical analysis

Introduction

India is popular for its rich natural resources. In India, there are about 6,000 plants which are used in herbal medicine. Over 1500 plants identified by Indian system of medicines of which 500 plants are commonly used for different ailments (Muley et al., 2009). Medicinal plants contains primary compounds like chlorophyll, sugars, proteins etc. and secondary compounds like alkaloids, glycosides, steroids, and phenolic compounds. These compounds contain many active components that take parts in the different activity called as phytochemicals (Wadood et al., 2013).

Pergularia daemia (Asclepiadaceae) is commonly known as "Veliparuthi" in Tamil, "Uttaravaruni" in Sanskrit and "Utranajutuka" in Hindi (Khare, 2007). It shows anthelmintic, laxative, antipyretic, and expectorant properties and it is also used to treat malarial fevers (Hase and Nasreen, 2016). The preliminary phytochemical analysis of various plant extract of *Pergularia daemia* showed the presence of various phytochemicals. In order to evaluate the quality of crude plant material, it is necessary to determine pharmacognostic parameters like ash value, extractive value, fluorescent analysis and microscopy. Phytochemical analysis of crude drug can be very useful for developing the quality parameters. There are no reports of pharmacognostic studies and phytochemical analysis of *Pergularia daemia*

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stem so it was envisaged that above mentioned studies will be worth and will be useful for the scientific community as well as commercial herbal market.

Materials and Methods

Plant materials

The plants specimen for the proposed study was collected from Aurangabad district, Maharashtra. It was identified and authenticated by Department of Botany, Dr. Babasaheb Ambedkar Marathwada University Aurangabad (accession No. 0631).

Chemicals

Analytical grade chemicals were used for the proposed work. Hydrochloric acid, ethanol and methanol were purchased from Merck, Mumbai. Sodium hydroxide, sulphuric acid, ferric acid, iodine was purchased from Avra synthsis Pvt. Ltd. Hyderabad.

Morphological evaluation

Fresh stems of *Pegularia daemia* were collected and different organoleptic features *viz.* shape, size, color, type, odour, taste were observed for morphology. These parameters were evaluated as per standard WHO guidelines.

Microscopical characterization

Microscopical studies were carried out using simple microscope (DMWB1-223ASC). Transverse sections of stem were studied for different microscopic characters (Sass, 1998).

Powder analysis

The shade dried stems of the plant were powdered and passed through 100 # sieve. A small quantity of dried powder was placed on the glass slide. It was cleared from chlorophyll by heating with chloral hydrate solution and was suspended in 50% v/v glycerol in water. Powder was observed under microscope to study different cell content *viz.* trichomes, xylem, phloem, chlorenchyma, cortex, epidermis, and pith and recorded with help of digital camera (Akbar et al., 2014).

Physicochemical evaluation

Physicochemical parameters of *Pegularia daemia stem* powder such as ash values, extractive values and loss on drying were determined according to the official methods prescribed in Indian pharmacopeia and the WHO guidelines on quality control methods for medicinal plants materials **(**WHO, 1998**).**

Fluorescence analysis

The fluorescence analysis of dried powder of *Pegularia daemia* stem was carried out using Kokoski standard method (Kokoski et al., 1958). It was examined by treating the stem powder with acidic, basic, organic and inorganic solvents *viz.* aqueous Sodium hydroxide, HCL, 50 % sulphuric acid, methanol, iodine, 5 % ferric chloride and NaOH. After the treatment, samples were observed under the visible light, short ultraviolet light and log ultraviolet light (Kumar et al, 2018).

Preparation of extract

The collected stems of *Pegularia daemia* was made free from any foreign organic matter, dried under shade and powdered. The 95% ethanolic extract was prepared using Soxhlet extraction apparatus whereas aqueous extract was prepared by cold maceration technique (for 24 hours). The extracts were dried under vacuum using rotary evaporator (Laborota, Heidolph 4001 efficient). Dried extracts were used for phytochemical screening and establishment of Thin Layer Chromatography (TLC) profile.

Preliminary phytochemical screening of extracts

Ethanolic and aqueous extracts of *Pergularia daemia* were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, glycosides, tannins and phenolic compounds, flavonoids, steroids, saponins, proteins, amino acids, carbohydrates and triterpenoids. This examination was done using standard procedure (Wagner and Bladt, 1996).

Thin layer chromatography profiling of extracts

Ethanolic and aqueous extracts of *Pergularia daemia* were diluted with chloroform, methanol and n-hexane in the ratio of (9: 0.5: 0.5 v/v) and then applied (1-10µl) to the origins of a TLC plate 2 cm above its bottom with the help of capillary tubes. After application, TLC plate was kept in saturated solvent TLC glass chamber and solvent was allowed to move through adsorbent phase up to $3/4^{th}$ of the plate. TLC plate was observed for the various spots and their respective Rf values.

Results

Morphological evaluation

Morphological and organoleptic parameters of the *Pergularia daemia* stem were study. The stem was pentagonal, pale yellow, smooth, hollow, about 2-4 mm thick and slightly bitter in taste (Figure 1).

Figure 1. *Pergularia daemia*

Microscopical characterization

From the microscopical study, it was observed that, the stem of *Pergularia daemia* contains single layered

epidermis with arranged parenchyma cells. Below the epidermis, multilayered cortex section was observed which forms hypodermis with arranged parenchyma cells with vascular bundle. It also showed presence of trichomes, xylem and pith (Figure 2).

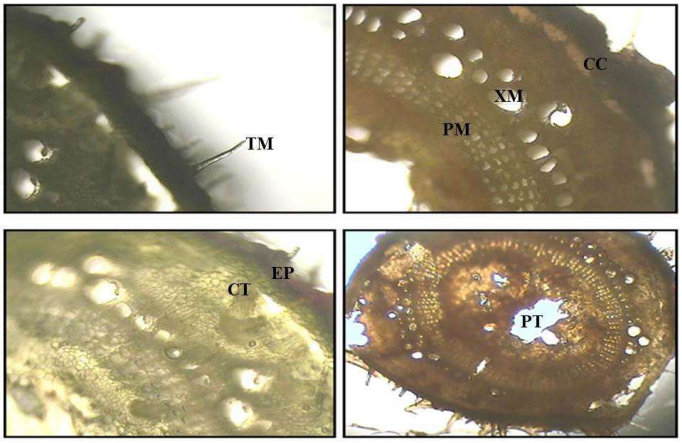

Figure 2. Transverse section of stems of *Pergularia daemia*: **TM**- Trichomes; **XM**- Xylem; **PM**- Phloem; **CC**- Chlorenchyma; **CT**- Cortex; **EP**- Epidermis; **PT**- Pith.

Powder analysis

Powder microscopy of dried stem powder of *Pergularia daemia* showed presence of fragments of parenchymatous cells, lignified pitted vessel, calcium oxalate crystals, mucilaginous cells, epidermal fragments, fiber and lignified fiber (Figure 3).

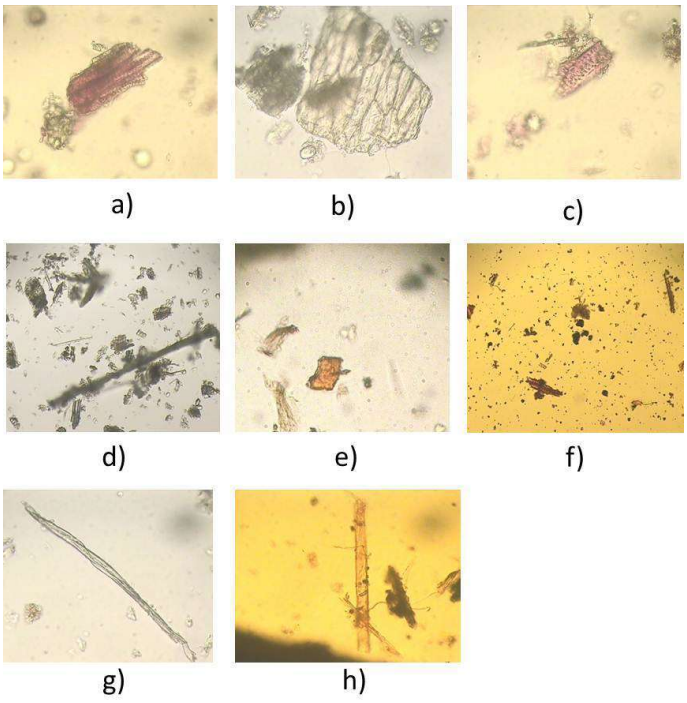

Figure 3. Powder microscopy: (a) Xylem fibers ; (b) Fragments of parenchymatous cells; (c) Lignified pitted vessel (d) calcium oxalate crystals; (e) mucilaginous cells; (f) Epidermal fragments (g) fiber; (h) Lignified fiber.

Physicochemical evaluation

Results of various physicochemical parameters *viz.* ash, extractive values and loss on drying are summarized in table 1.

Table 1. Physicochemical analysis of the stems of *Pergularia daemia*

Sr. No.	Parameters	Mean ± SD Values (% W/W)
1	**Ash Value**	
	Total ash	10.1± 0.24
	Water soluble ash	7.2± 0.43
	Acid insoluble ash	2.5±0.28
2	**Extractive value**	
	Water soluble extractives	12.8±0.45
	Alcohol soluble extractives	3.6± 0.22
	Ethyl acetate soluble extractives	1.5±0.40
3	**Loss on drying**	4.2±0.34

Fluorescence analysis

The fluorescence analysis results of *Pergularia daemia* stem powder are depicted in table 2.

Table 2. Fluorescence analysis of powder of the stems of the *Pergularia daemia*

Reagents	Color observed in Short UV	Color observed in Long UV	Color observed in Visible light
Powder	Green	Dark green	Pale green
Powder + aqueous Sodium hydroxide	Green	Yellowish green	
Powder + hydrochloric acid	Green	Blackish green	Yellow
Powder + 50% sulphuric Acid	Green	Black	Yellow
Powder + methanol	Fluorescent green	Green	Yellowish green
Powder + Iodine	Brown	Black	Yellowish green
Powder + 5 % ferric chloride	Pale green	Black	Yellow
Powder + NaOH + methanol	Dark green	Yellowish green	Yellowish green

Table 3. Phytochemical analysis of *Pegularia daemia*

Sr. No	Test of constituents	Ethanol extract	Aqueous extract
1	Carbohydrates	+	-
2	Steroids	+	+
3	Glycosides	+	-
4	Flavonoids	+	-
5	Alkaloids	+	-
6	Phenols	-	-
7	Tannins	-	+
8	Terpenoids	-	-
9	Saponins	-	-
10	Volatile oils	-	-
11	Gums	-	-
12	Reducing sugar	-	+

Preliminary phytochemical screening of extracts

Ethanolic extract of *Pergularia daemia* aerial parts showed presence of alkaloids, flavonoids, glycoside, steroids, and carbohydrates while its aqueous extract showed presence of steroid, tannins and reducing sugar. Results are depicted in table 3.

Thin layer chromatography profiling of extracts

TLC pattern of ethanolic extract showed eight spots with Rf value 0.12, 0.18, 0.38, 0.45, 0.58, 0.74, 0.87 and 0.93 using the solvent system Chloroform: methanol: n-Hexane (9:0.5:0.5) (Figure 4).

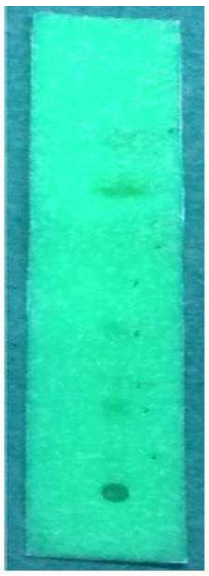

Figure 4. Thin Layer Chromatography of alcoholic extract

Discussion

Across the globe, herbal medicines are gaining attention and trust of people as a reliable and safe way of treatment. Thousands of plants and or their parts are used worldwide for the therapeutic purposes. The therapeutic efficacy of any plant material largely depends on its source, geographical location, authenticity, purity and the chemical composition. Availability of such data can be very useful in predicting the therapeutic effects in precise manner. Present work is an attempt to establish such data. In India, *Pergularia daemia* is found to be one of the therapeutically important plants. Since long, it has been used for treatment of variety of ailments but the details of its quality attributes like pharmacognostic, phytochemical and physicochemical characteristics were not available. In order to fulfill said gap, present work was designed. Firstly, ash values of the powdered stem of *Pergularia daemia* were calculated. Ash values are the criteria to judge the identity or purity of crude herbal material. Ash values of powdered stem of *Pergularia daemia* were found to be less than 10.2% which indicates that crude drug is clean and free from dirt and sandy material.

Extractive values of powdered stem of *Pergularia daemia* were calculated to find out the nature of chemical constituents present. As compare to alcohol and ethyl acetate, high water soluble extractive value of powdered stem of *Pergularia daemia* indicates presence of hydrophilic constituents.

In order to find out the moisture and volatile matter in powdered stem of *Pergularia daemia*, test for loss on drying was carried out. It was observed that moisture and volatile matter content of powdered stem was less than 5%. Lower moisture content of the stem powder signifies the extended stability of the said crude material.

Some of the phytochemicals shows fluorescence after bombardment of visible or ultraviolet light. Presence of such phytochemicals in crude drugs can be qualitatively evaluated using fluorescence analysis. In acidic and alcoholic environment, powdered stem of *Pergularia daemia* showed fluorescence which in good agreement with its phytochemical analysis. Phytochemical analysis of ethanolic extract revealed presence of alkaloids, glycosides, flavonoids, steroids and carbohydrates.

TLC profiling of the ethanolic extract showed eight distinct spots with good Rf difference which again confirms presence of at least eight different phytochemicals.

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