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Pharmacognostical aspects of *Pergularia daemia* Leaves

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Abstract

Plant and plant products are being used as a source of medicine since long. In fact the many of currently available drugs were derived either directly or indirectly from them. The *Pergularia daemia* has been traditionally used as anthelmintic, laxative, antipyretic, expectorant and also used to treat malarial intermittent fevers. In the past decade, research has been focused on scientific evaluation of traditional drugs of plant origin for the treatment. *Pergularia daemia* is a slender, hispid, fetid-smelling perennial climber, which is used in several traditional medicines to cure various diseases. Phytochemically the plant has been investigated for cardenolides, alkaloids, saponins, tannins and flavonoids. This review is a sincere attempt to summarize the information concerning pharmacognostical features of *Pergularia daemia*.

Keywords: *Pergularia daemia*, drugs, phytochemicals, pharmacognostical

Introduction

The herbal drug industry is considered to be a high growth industry of the late 90s and seeing the demand, it is all set to flourish in the next century. The trend for the increasing of medicinal herbs in countries like America, Australia and Germany is well supported by statistical data. In ayurveda, the ancient Indian system of medicine, strongly believe in polyherbal formulations and scientists of modern era often ask for scientific validation of herbal remedies (Soni *et al.*, 2008) [24]. The efficacy of some herbal products is beyond doubt, the most recent examples being *Artemisia annua* (artemisinin), *Taxus brevifolia* (taxols) and *Silybum marianum* (Silymarin). (hypericin and hyperforin), *Allium sativum* (allicin and allin), *Ginkgo biloba* (Ginkgolic acid) are popularly used herbal remedies among people. All these herbals are standardized for active constituent. Standardization means adjusting the herbal drug preparation to a defined content of the active constituent. Extract refers to a concentrated preparation of active constituent of a medicinal herb. The concept of standardized extracts definitely provides a solid platform for scientific validation of herbal products. Medicinal plant materials are characterized according sensory microscopic characteristics (Joshi, 1947) [17]. Owing to the global trend towards improved 'quality of life', there is considerable evidence of an increase in demand from medicinal plant (Kotnis *et al.*, 2004) [18]. Organoleptic evaluation can be done by means of organs of sense. This evaluation provides the simplest and quickest means to establish the identity and purity and thereby ensure quality of a particular sample. A number of different bases are for morphological studies and a natural variation in these characteristics plays an important role for preliminary evaluation of crude drugs. The basis of an analysis by evaluation of microscopic characters is that there are always sufficient differences in the same type or different types of plants as for as the cell characteristics are concerned. Standardization profiles of herbal drugs are not available for most drugs.

This study is an attempt to establish the standardization parameters for pharmacognostic evaluation of *Pergularia daemia*. It includes microscopic and macroscopic characters.

Materials and Methods

The fresh leaves of *Pergularia daemia* were collected in the month of April from Sangamner, Dist. Ahmednagar (MS), India. These were identified, confirmed and authenticated with the use of local floras (Cooke, 1901-1908; Pradhan and Singh, 1999) [8, 21], at post graduate Department of Botany, Sangamner College Sangamner.

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A voucher specimen of the leaf is deposited in department for future reference. Collected fresh leaves were washed and use for study of macroscopic and microscopic characteristics.

Morphology and Medicinal uses

Pergularia daemia s.) Choiv.

Family: Asclepiadaceae Local name: Uтарan, Uтарanajutuka (Hindi).

Plant perennial twining herb, foetid when brushed and much milky juice. Stem clothed with spreading hairs. Leaves thin 2-4 by 1/2- 3/2 inch, broadly ovate or suborbicular, acuminate, glabrous or more or less shortly pubescent above usually valvately pubescent beneath, the margins ciliate base deeply, cordate the basal lobes semi orbicular. Petioles 3/4 or 3/2 inch long, pubescent. Flowers greenish- yellow or dull white in lateral cyme which is at first corymbose, alter words racemose, peduncles pubescent, coming off from between the petioles, through not quite mid way between them 3-6 inches long or longer pedicels capillary 3/4- 1/3 inches long pubescent bracts minute, lanceolate divided to the base, sepals 1/8 inches long, lobes spreading, 1/4 inches long, ovate oblong, acute; ciliate outer corona membranous, subquadrate, truncate, inner corona lobes 1/5-1/3 inch in long including the subulate horns which are curved high over the stamina column, spur acute. Follicles reflexed, 2-3 by 1/2 inches lanceolate, attenuated, into a long beak echinate with soft spines seeds 1/3 by 1/6 inches, ovate, truncate at the apex, densely valvately pubescent on both sides, narrowly margined, crenate at the rounded base, corona 1-3/2 inch long. (Cooke T, 1901-1908) [8].

Flower and fruits: March – December

Medicinal uses: The leaf juice is used as an expectorant and catarrhal disease; also the decoction of leaves is given in cough as an emetic and anthelmintic for removing intestinal worms. A mixture of the leaf juice and staked lime is applied to rheumatic swellings, hard tumours and cysts (Dastur, 1962) [9]. Leaf juice is used to treat infantile diarrhea, asthma and catarrhal affection, rheumatism and amenorrhoea. It is also useful in curing gynecological conditions like excessive bleeding. Squeezed fresh leaves are applied as poultice in carbuncle (Bhattacharya, 1998) [4]. Decoction of the leaves is given to children as an anthelmintic, in doses not exceeding three table-spoonfuls, "in one to two once doses it is good expectorant", decoction or juice or leaves is useful also in asthma and snake bite." Powered leaves in doses of 5 to 10 grains are also good expectorant " (Chopra, 1956) [7], externally the juice combined with lime is applied to rheumatic swellings. A mixture of the juice of these leaves and of the leaves of *Ocimum sanctum* obtained by squeezing them between the palms of the hands is a stimulating emetic; " Honey is also added to the decoction of the leaves to help the expectorant effect " (Chopra), combined with ginger, the juice of the leaves is given in rheumatism. Fresh leaves made in to a pulp are used as a stimulating poultice in carbuncle with benefit. Juice of the leaves is employed in the preparation of medicinal oil used in rheumatism amenorrhoea and dysmenorrhoea and an the root-bark is used as a purgative in rheumatic cases in doses of 1 to 2 drachms mixed with cow's milk (Nadkarni, 1976) [20].



Fig 1: A flowering twig: *Pergularia daemia*

Microscopic study

Fresh leaves of *Pergularia daemia* were selected for the microscopical studies.

For anatomical work, collected plant part was fixed in FAA (95% Ethyl alcohol 50 parts+ Glacial acetic acid 5 parts + Formalian 10 parts + distilled water 35 parts) in the field itself. Some plant materials were also preserved in 70% alcohol.

Microscopic sections were cut on by free hand sectioning. Numerous temporary and permanent mounts of the microscopical sections of the leaf specimen were made and examined microscopically. Leaf epidermal studies were carried out on fresh specimens. Peels were obtained using sharp pointed forceps and sometimes by using some chemicals. They were stained in 1% safranin mounted in glycerin and made semi-permanent by ringing with DPX solution. Stomatal index (SI) was calculated as defined by Salisbury (1927, 1932) viz.

$$SI = \frac{S}{E \times S} \times 100$$

Where,

S= number of stomata per unit area,

E = number of epidermal cells in the same area.

Stomatal frequency and stomatal index have been calculated out of an average of 10 readings. Also, the measurements of different cells and cell contents were done with the help of calibrated ocular micrometer.

Transections of leaf and petiole were taken by free hand for that fresh and preserved material was used. Sections were stained in safranin (1%), light green (1%) and mounted in DPX after the customary dehydration. Some hand sections were also examined in glycerine. Microphotographs of leaf were taken by using Camera. The histochemical tests were performed for identifying different constituents.

Histochemical Tests:

Histochemical tests were performed on fresh plant materials according to the methods of Johansen (1940) [16] and Gurr (1965) [14]. The descriptions of the tests done are as follows

Lignin

Sections were treated with aniline sulphate solution (sat. aniline sulphate and sulphuric acid) which gives yellow color.

Starch

0.3 gm of iodine and 1.5 gm of potassium iodide were dissolved in 100 ml of distilled water. A drop of this solution was added on the section, washed with water and observed under microscope indicating presence of blue violet starch grains.

Fats

0.5 gm of Sudan IV dye was dissolved in 100 ml 70% alcohol. Sections were kept in this stain for 20 minutes, rinsed quickly with 50% alcohol and mounted in glycerine for observations. Blue, red or pink precipitate indicated the presence of fat.

Alkaloids

Transverse sections of the different plant were treated with the Dragendoeff's reagent. The formation of precipitate or development of turbidity in the sections clearly indicated the presence of alkaloids.

Saponins

Sections were placed in saturated Barium hydroxide solution for 24 hours. Then they were washed with Calcium chloride solution followed by Potassium dichromate solution wash. Yellow colour indicated the presence of saponins.

Tannins

Reagent used was 10% aqueous ferric chloride made alkaline with sodium carbonate, which produced blue green color with tannin.

Flavonoids

To determine the localization of the flavonoid, sections were put in a drop of solution of vanillin sulphuric acid for two minutes, yellow colour in the tissues indicates presence of flavonoids.

Calcium oxalate

The sections were treated with 2N acetic acid for about 15 minutes then removed and transferred to a solution prepared at 1% silver nitrate in 15% hydrogen peroxide for about 15 minutes. Afterwards, the above section was washed with distilled water. Counterstained the section with 2% safranin for 1-3 minutes. Mounted the section with usual technique in glycerine water and observed under microscope. The calcium oxalate crystal appears black against red background.

Result and Discussion

Morphologically, the leaves are simple, Leaves thin 2-4 by 1/2- 3/2 inch, broadly ovate or suborbicular, acuminate, glabrous or more or less shortly pubescent above usually valvately pubescent beneath, the margins ciliate base deeply, cordate the basal lobes semi orbicular. The petioles 3/4 or 3/2 inch long, pubescent.

Epidermal features

Cells of upper epidermis are larger having 72.96 μ length and 67.26 μ width, thick, cells usually penta or hexagonal cells wall thick and straight walled. Lower epidermis is smaller having 61.52 μ length and 40.38 μ breadth, cells with cell wall thin slightly wavy stomata are present on both upper and lower epidermis, the upper surface having 44.46 μ length and 24.04 μ breadth, while the lower stomata having 46.74 μ length and 22.5 μ breadths. They are paracytic, but with cells parallel to the pore sometimes secondarily divided (Fig. A and B). The trichome is multicellular, uniseriate and thick walled unbranched having 165.12 μ and 34.2 μ breadth (Table 1).

T.S. of Petiole

In sectional view, the petiole appears more or less circular in outline with concave adaxial side. The epidermis consist of closely packed thick walled cells with thick cuticle. It is followed by many layered collenchymas occurs on adaxial and abaxial side. The ground tissue is parenchymatous vascular tissues comprises of and are which is centrally located above which two cortical bundle. The system is surrounded by phloem at outer and inner side (fig. C).

T.S of Leaf

The leaf is dorsiventral and amphistomatic. The upper epidermis has basal shaped closely enlarged thick walled cells. The cuticle is thick. The lower epidermis is of small cells and thin walled. The stomata are present on both the surfaces. The trichomes are multicellular uniseriate and thick walled. The mesophyll is differentiated in to palisade and spongy tissue. Palisade cells are double layered elongated and compactly arranged. While spongy parenchyma which is composed of polygonal cells irregularly arranged and filter entire space of leaf lamina. The vascular tissue has bicollateral vascular bundle which extends through the mesophyll. The midrib has typical an arch shaped strand. The ground is parenchymatous. (Fig. D).

Table 1: Quantitative Microscopy

Sr. No.	Parameters	Leaf Surface	Range (μ)	Mean(μ)	
1	Epidermal Cell Measurements	Length	Upper	68.4-85.5	72.96
			Lower	52-72.1	61.52
		Breadth	Upper	51.3-79.8	67.26
			Lower	26.5-55	40.38
2	Stomatal Measurements	Length	Upper	39.9-51.3	44.46
			Lower	39.9-51.3	46.74
		Breadth	Upper	17.1-28.7	24.04
			Lower	18.7-27.6	22.5
3	Stomatal Number	Upper	5-6	5.2	
		Lower	19-23	20.66	
4	Stomatal index	Upper	4-5.32	4.49	
		Lower	7.10-13.29	10.70	
5	Trichome Measurements	Length	102--285	165.12	
		Breadth	22.8-57	34.2	

The histochemical color reactions were carried out on transverse section of the fresh leaf (Table II). The results

indicated that presence of lignin, starch, fats, alkaloids, saponins, tannins, flavonoids and calcium oxalate crystals.

Table 2: Histochemical color reactions

Reagents	Constituents	Color	Histological Zone	Degree of Intensity
Aniline SO ₄ + H ₂ SO ₄	Lignin	Yellow	Xy.	++
Weak Iodine Solution	Starch	Blue	Mesophyll	+
SudanIII/IV	Fats	Bink /Red	Mesophyll	+
Dragendroff's Reagent	Alkaloid	Turbidly Brown	Mesophyll, M. cor.	++
Ba(OH) ₂ +K ₂ Cr ₂ O ₇ +CaCl ₂	Saponins	Yellow	Mesophyll.M.cor.	++
FeCl ₃	Tanins	Blue green	M.cor.	+
Vanilline+HCl	Flavonoids	Yellow	M.col., Mesophyll, M. cor.	+++
AgNO ₃ +H ₂ O ₂	Crystals	Black	Mesophyll, M. cor.	++

Abbreviations = Xy. - Xylem; M. cor. - Midrib cortex; M. col.- Midrib collenchyma.

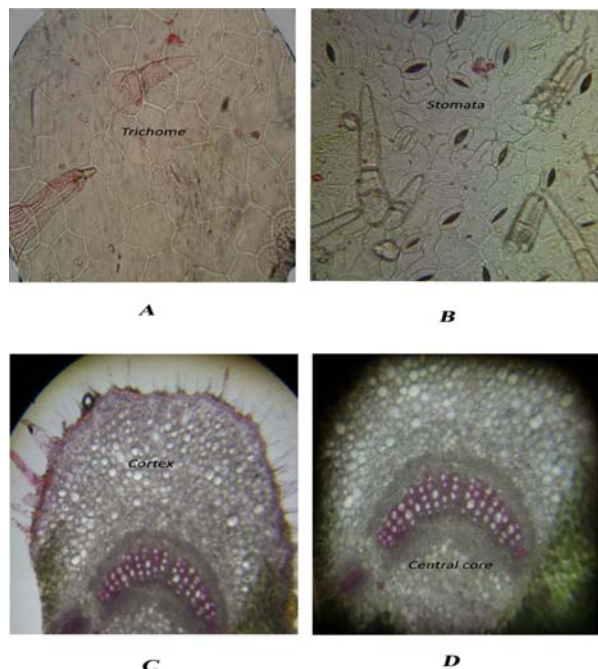


Fig 2: *Pergularia daemia*
A: Upper epidermis B: Lower epidermis
C: T.S. of left D: Central zone

Conclusion

The scientists are sincerely trying to evaluate many plant drugs used in traditional system of medicinal. Empirical knowledge about medicinal plants plays a vital role in primary health care and has great potential for the discovery of new herbal drugs. The pharmacognostical studies including macroscopic and microscopic evaluation of leaves of *Pergularia daemia* has considerable use in the identification of this drug.

Anatomical structures are most likely to provide concerning the interrelationship of larger groups such as families, or in helping to establish the real affinities of genera of uncertain taxonomic status. These findings will be useful to supplement existing information with regard to the identification and standardization of *Pergularia daemia* to distinguish it from substitutes and adulterants. In conclusion, the present manuscript will be useful to supplement information with regard to its identification and in carrying out further research for the use in the treatment of various diseases. The present study of pharmacognostic characteristics of *Pergularia daemia* leaf will provide useful information for its correct identity.

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