PRELIMINARY PHYTOCHEMICAL SCREENING AND PHYSICOCHEMICAL ANALYSIS OF LEAVES OF *PERGULARIA DAEMIA* (FORSSK.) CHIOV.

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ABSTRACT

Pergularia daemia (Forssk.) Chiov. of family Asclepidaceae commonly known as utaran which is used to cure cough, asthma and treating various diseases in traditional system of medicine. The present investigation is based on the screening of various phytochemicals and to analyze physicochemical characteristics from *Pergularia daemia* leaves. The pharmacognostical characteristics like macroscopic, microscopic features and leaf constants were determined. The physicochemical analysis was determined (Kokate, (2006) to check ash values, total ash, acid soluble and acid insoluble ash value extractive values (alcohol and water soluble) and moisture content of powdered leaf drug. Preliminary phytochemical screening shows the presence of alkaloids, flavonoids, tannins, terpenoids, saponins, carbohydrates and amino acid.

KEYWORDS: *Pergularia daemia*, physicochemical analysis, pharmacognostic study and phytochemical screening.

INTRODUCTION

Traditional medicine is the major and cheaper method for treating various diseases among marginalized peoples in developing countries. As there are the tremendous advances in the synthetic drugs and dependence on the modern medicine even though most of the world population still prefers the herbal drugs. Man has been using herbal drugs for combating various harmful (killing) diseases. Herbal medicines are essential because they are having the ability to produce the non nutritive plant phytochemicals which have the disease preventive properties. The wild medicinal plants contains so many phytochemicals and each

phytochemical having different effect. Such plants and their products can be used as a source of herbal medicine from years ago. According to WHO 80% of the world population using herbal medicines in the form of decoctions, teas, extracts with water, milk or alcohol (Fransworth, 1990).[1] Plant contains a potential source of novel drug components which are still unexplored.

Pergularia daemia (Forssk.) Chiov. Commonly called as Utaran. It is a foetid smelling laticiferous twinner herb widely distributed worldwide in the tropical and subtropical regions (Pankaj, 2003)[2], in India it is found mostly along roadsides growing on another trees. Leaves are simple, opposite, cordate, velvety and pubescent with milky latex, 4-5 cm long. Flowers are greenish yellow or dull white, tinged with purple found in corymbose clusters. Flowering may occur many times in year between Augusts to January. Fruits are paired follicles (6-8 cm) with long beak facing each other covered with soft spines. Seeds are many velvety with long white hairs on both sides.

The whole plant exudates the milky latex which is used as medicine i.e. used against toothache (Patrik, 1992 and Hebbar *et al*., 2004).[3 and 4] The Ayurvedic system of medicine recorded various uses of *Pergularia daemia* such as the leaf juice is given in asthma, cough, applied on rheumatic swelling with lime and ginger, amenorrhea, dysmenorrhea, bronchitis. The leaves are used against stomachache and tetanus (Irvine, 1952).[5] The whole plant can be used against malaria, fever (Bruce, 1998)[6], rheumatism, menstrual disorder facilitating parturition and gastric ulcers (Singh *et al*., 2002).[7]

The present study is an attempt to evaluate the pharmacognonstical features, physicochemical and phytochemical analysis of leaves of *Pergularia daemia*.

MATERIALS AND METHODS

Plant material

The fresh leaves of plant *Pergularia daemia* were collected from the Ahmedpur Tahsil of Latur district (Maharashtra) and authenticated by Botanical Survey of India, Pune. The collected leaves were washed, shade dried and grind with mechanical grinder. The fine powder was collected and used for powder microscopy. The fresh plant material was used for the microscopic study.

Pharmacognonstical studies

Macroscopic

Morphological studies of leaves like the colour, odour and taste were done as per standard methods.

Microscopic

Microscopic studies were done by free hand transverse sectioning of midrib and the lamina of leaves of *Pergularia daemia.* The sections were stained and mounted in glycerin and observed under microscope.

The histochemical tests were carried out by using conc. HCl, iodine solution for identification of starch grains, sulphuric acid for calcium oxalate crystals and ferric chloride for phenolic compounds in the powder of leaf. The leaf constants like stomatal number, stomatal index, palisade ratio, vein iselet number and vein termination number were also determined by using fresh plant leaves (Kokate, 2006).[8]

Physicochemical evaluation

The determination of moisture content, ash value i.e. total ash, acid soluble, acid insoluble, water soluble ash, extractive values (alcohol and water soluble) were determined as per standard procedures.

Phytochemical screening

The extracts were prepared by using the dried powder of leaf and subjected for the Soxhelet extraction method with different solvents like petroleum ether, ethanol, methanol, acetone and distilled water as per polarity. The extracts were filtered and concentrated in water bath. The various extracts were subjected for phytochemical screening by dissolving them in respective solvents. The extract shows the presence of alkaloids, flavonoids, tannins, terpenoids, saponins, carbohydrates, amino acids. (Harbone, 1973)[9] and (Trease, 1989).[10]

RESULTS AND DISCUSSION

Microscopic character

Transverse section of leaf of *Pergularia daemia* showed the single layered upper epidermis with unicellular-multicellular trichomes in laminar region. The epidermis followed by compactly arranged columnar palisade parenchyma cells forming 1-2 layers. The spongy parenchyma arises from palisade tissue with intercellular spaces and forms the spongy mesophyll just above the lower epidermis which is single layered. The midrib region shows

curved structure on abaxial side and contains ground tissue. The adaxial surface followed by collenchyma cells which contains closed collateral vascular bundles (Fig. No.1) contains endarch, lignified xylem and exarch phloem which is non-lignified. Stomata are found on both the surfaces and are of anisocytic type surrounded by three subsidiary cells. The trichomes are also present on both surfaces and are multi cellular, unbranched, uniseriate, multiseriate type.

Powder microscopy

The leaf powder of *Pergularia daemia* was characterized by observing its morphological features like green colour, presence of characteristic odour and astringent taste. The leaf powder was used for micro chemical tests. For the detection of calcium oxalate crystals, the powder was added with acetic acid and dil. HCl when added with conc. sulphuric acid it shows green colour which indicates the presence of stone cells. Presence of starch was detected by dil. Iodine solution while Hemicellulose detected with dil. Iodine and Conc. H_2SO_4. The results were tabulated in table No. 1.

Quantitative analysis

The quantitative microscopy of fresh leaves sample of *Pergularia daemia* were shows various leaf constants like stomatal number, stomatal index, vein iselet number, vein let termination number and palisade ratio. The results are tabulated in table No. 2.

Physicochemical studies

The moisture content of leaf was 8.79%. The total ash value was 24.1% w/w, acid insoluble ash was 11.1% and the water soluble ash was 12.5% w/w. such characters helps to evaluate the crude drug. The alcohol and water soluble extractive values are 23.2%, 35.2% respectively. which can determine the quality of plant material (Table No. 3).

Phytochemical screening

The preliminary phytochemical screening of leaf extract of *Pergularia daemia* shows the presence of alkaloids in all extracts but absent in ethanol extract while the flavonoids and Saponins are found in petroleum ether, ethanol and distilled water extract but not detected in methanol and acetone extract. Tannins are present in all the extracts except petroleum ether extract while the carbohydrates, terpenoids and amino acids are found in all the leaf extracts (Table No. 4).

Herbal based drugs have many advantages than the synthetic drugs but one should have to know about the proper knowledge about the use of herbal drugs as in the traditional system or modern system. Pharmacognostic study is an important meant for the identification, authentification and detection of adulteration for crude drugs (Kokate and Purohit, 2006).[8]

The anatomy of the leaf laminar region reveals the presence of trichomes at upper and lower epidermis, compact palisade parenchyma and spongy mesophyll. The midrib part has collenchyma cells with collateral vascular bundles.

The micro chemical tests of powder showed the presence of stone cells and calcium oxalate crystals. The physicochemical analysis showed that the total ash two times greater than the water soluble ash. The water soluble extractives were more than the alcohol soluble extractives. The leaf contains various types of secondary metabolites such as Alkaloids, Flavonoids, Tannins, Terpenoids, Saponins, Carbohydrates, Amino acids.

Adulteration and misidentification of medicinal plants can be harmful to the consumers and also create major problems for the pharmaceutical industries. The observation of microscopic characters is the major aid for authentification of drug, such characters are important for identification of powdered drug because most of the morphological features lost (Kumar *et al*., 2011).[11]

The microscopical examination of leaf powder shows the presence of trichomes, epidermal cells, stomata etc. The moisture content of the drug reveals that the storage conditions of crude drug. Study of organoleptic characters of a crude drug is used for the qualitative evaluation to develop morphological profile of a medicinal plant (Agrawal *et al*., 2004).[12]

The extractive values plays an important role in the determination of adulterated drug/ in the detection of adulteration (Thomas *et al.*, 2008).[13] Phytochemical screening plays significant role in the detection of phytochemicals such as, alkaloids, flavonoids, tannins, terpenoids, saponins, carbohydrates, amino acids etc. (Karthikeyan *et al*., 2014).[14]

Table No. 1- Micro-chemical tests of *Pergularia daemia* leaves.

Sr.No.	Reagent	Observation	Characteristic
1	Powder + Acetic acid	Soluble	Calcium oxalate crystal
2	Powder + dil. HCl	Soluble	Calcium oxalate crystal
3	Powder + conc. Sulphuric acid	Green colour	Stone cells present
4	Powder + dil. Iodine solution	No blue colour	Starch is absent
5	Powder + dil. Iodine solution+ H_2so_4	Black colour	Hemicellulose absent

Table No. 2 - quantitative characteristics of *Pergularia daemia* leaves.

S. No.	Parameter	Surface	Range
1	Stomatal frequency	Upper	20-25
		Lower	320-331
2	Stomatal index	Upper	1.88-2.33
		Lower	17.33-18.03
3	Palisade ratio	-	6-7
4	Vein iselet number	-	9-11
5	Vein-let termination number	-	13-15

Table No. 3– physicochemical analysis of *P. daemia* leaves.

Plant part	Parameter	Observation
Pergularia daemia leaf	Moisture content	8.79%
	Total ash	24.1%
	Acid insoluble ash	11.1%
	Water soluble ash	12.5%
	Alcohol soluble extractives	23.2%
	Water soluble extractives	35.2%

Table No. 4 – Phyto chemical screening of *Pergularia daemia* leaves.

Sr. No.	Plant part	Extract	Alkaloids	Flavonoids	Saponins	Tanins	Carbo-hydrates	Terpenoids	Amino Acids
1	**LEAF**	P. Ether	+	+	+	-	+	+	+
		Ethanol	-	+	+	+	+	+	+
		Methanol	+	+	-	+	+	+	+
		Acetone	+	+	-	+	+	+	+
		Dist.water	+	+	+	+	+	+	+

+: Present,   - : Absent
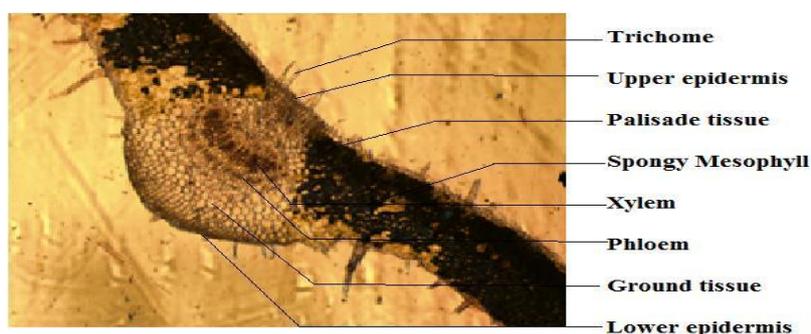
Fig. 1 – Transverse section of *P. daemia* leaf.

CONCLUSION

The pharmacognonstical studies like microscopic characters, quantitative analysis, physicochemical studies of *Pergularia daemia* are useful for the standardization of crude drug and to know the purity of a drug.

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