Morpho-Anatomical, Preliminary Phytochemical and Hptlc Profile of Extra-Pharmacopoeial Herb Passiflora foetida Linn. Leaf

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ABSTRACT

Background: Medicine has evolved after a lot of trial and error practices. Ayurveda is a life science which is meant for well-being of all living creature. The medicinal importance of wide range of plants has been recorded in Ayurveda, but some plants which are found later or exotic are not mentioned in Ayurveda, they are named as Anukta which means untold. Ethnobotany is the branch of botany which deals with the study of relationship between people and plants. Passiflora foetida Linn. is an extra pharmacopoeial drug in Ayurveda, which is used by folklore and traditional healers for various ailments like hysteria, skin disease, asthma, headache, for poisonous bites, digestive complaints etc.

Methodology: Pharmacognostic, physico-chemical, preliminary phytochemical study and HPTLC studies were carried out following standard procedures mentioned in Pharmacopoeia. Results: Pharmacognostic study revealed macroscopic features of the plant. Physicochemical studies gave information about the moisture content, total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive and water soluble extractive. Preliminary phytochemical evaluation of alcoholic extract revealed the presence of phenols, alkaloids and resins. HPTLC study revealed the fingerprint of extract of the plant sample. Conclusion: The results obtained from the studies will help in the standardisation of the plant material.

Keywords: Anukta, Ayurveda, Ethnobotany, Extra-pharmacopoeial, Quality control, Standardisation.

INTRODUCTION

There are some traditional healers, who practice rare treatments and have some secret therapy. Most of them may never reveal this secret to others; as a result, certain techniques and great knowledge tend to vanish from earth after their death. Some plants which are very common are considered as weed until its medicinal value is known to human kind. Potency of certain plants remains in shadow till scientific exploration is done. Presently Indian systems of medicine use around 1100 medicinal plants, which are mostly collected from their natural habitat. Many of plants found in common are not included in these records; sometimes they may be widely used by tribal people as folklore medicines or used in codified systems of medicine. Classical literatures of Ayurveda such as Samhitás, Nighántus and related text books have recorded certain medicinal plants for therapeutic uses and advised the physicians to learn uses of new plants from the cowherds/ traditional practitioners, shepherds for improving ones knowledge.

Passiflora foetida Linn. (Passifloraceae) is a herbaceous climber, more or less viscous, densely hirsute; tendrils axillary, simple, leaves up to 10 x 9 cm, usually 3-lobed, silky hairy, with glandular hairs on margins; petioles glandular-ciliate but without true petiolar glands; stipules pectinate; corolla white; corona violet and white or purple and white; berries globose, 2 cm across, orange when ripe; flowers occur during October to March [1].

The plant is known by different names in different languages such as Assamese - Koth-bel, junka phool, mewa, lota bel, jumka lata; Bengali - Jumka lata; English - Stinking passion flower, love in a mist; Hindi - Jumka lata, raaki phul, krishna kamal; Iruka - Varingodi; Kannada - Kukkiballi; Malayalam - Poochappazham, amoornapazham; Manipuri - Lam radhikanachom; Marathi - Vel ghani; Tamil - Mossukattan, siruppannaikalli; Telugu - Thealajumuki; Sanskrit - Mupparisavalli, mukkoepra, lomaphala, swaduphala [2].

P. foetida occur around the globe as a weed and it is used in the treatment of hysteria, asthma, giddiness, skin diseases [3]. diarrhoea, digestive problems, for poisonous bites [4], etc. Though the plant is useful medicinal there are no pharmacognostical studies in report.
Proper identification of this plant by macro-microscopy and chemical techniques are reported in this study.

![Figure 1: Habit of Passiflora foetida Linn](image)

**MATERIALS AND METHODS**

**Collection**

Fresh plants of *P. foetida* were collected from its natural habitat at Kambar, Mogral Puthur, Kasaragod, Kerala in the month of December. Morphological features were noted referring to regional flora [5]. The material was authenticated by Dr. Krishnakumar G, Professor Department of Botany Mangalore University. The Pharmacognostical and Phytochemical studies were carried out in the Department of Pharmacognosy & Phytochemistry, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka.

**Macroscopy**

The sample is by observed with naked eye and later with a magnifying lens. Description of specimen’s general characteristics, such as size, shape, outer surface and inner surface etc were recorded. Leaf of *P. foetida* was placed on a white sheet with scale and a clear photograph is taken. Measurements of the whole sample are recorded with a scale. Morphological characters such as color, odour, texture and taste were noted [6].

**Microscopy**

Sample was preserved in fixative solution FAA (Formalin – 5 ml + Acetic acid – 5 ml + 70% Ethyl alcohol – 90 ml). The materials preserved in FAA for more than 48 h were cut into thin transverse section. Transverse sections were stained with safranin and mounted with glycerine before photographing using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars [7].

**Physico-chemical analysis**

Leaves were plucked carefully, shade dried completely and made in to powder with the help of a mixer grinder. Coarse powder was stored in air tight neat containers and stored in dry clean places for further studies [8]. Physicochemical parameters like loss on drying, total ash, acid insoluble ash, water soluble extractive were done according to the standard protocol [9].

**Preliminary phytochemical analysis**

Test for alkaloids, phenols, flavonoids, saponins, tanins, and carbohydrates are done as per standard methods in the alcoholic extract of the leaf, which is obtained by the maceration method [10].

**HPTLC Profile**

One gram of dried leaf powder of *P. foetida* was extracted with 10 ml of methanol by maceration method. Three, 6 and 9 µl of the above extract were applied on a pre-coated silica gel F254 on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Ethyl acetate: methanol: water: formic acid (5:0.2: 0.3: 0.6) in a double trough chamber. The developed plates were visualized in short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under UV 254 nm, 366 nm and 620 nm. Rs colour of the spots and densitometric scan were recorded [11].

**RESULTS**

**Macroscopy**

Fresh leaf is simple, petiolate, ovate, trilobed, base cordate and tip acute, pubescent on both surfaces, including the serrate margin of the leaf; thin and light to dark green in colour; 6 to 11 x 4 to 5 cm; odour foul when crushed, taste astringent, bitter.

![Figure 2: Macroscopy of Passiflora foetida leaf](image)

**Microscopy**

TS of petiole is convex shaped with many unicellular covering trichomes. A single layeres epidermis with a thin cuticle is present as an outermost layer with 3 to 4 layers of collenchyma underneath. The ground tissue is parenchymatous, formed of thin walled parenchyma without prominent intercellular spaces, a few of them loaded with rosette crystals of calcium oxalate; there are 3 major vascular bundles, the one at the middle upper side is oval shaped and the 2 lateral ones triangular shaped; there are 3 minor vascular bundles intermittent to the aforesaid 3 major bundles, all the 6 bundles being suspended in the ground tissue are xylem towards the centre and phloem towards the periphery; the parenchyma surrounding the phloem shows crowding of rosette crystals (Fig 3a,b).
Detailed TS of leaf passing through midrib are broadly convex at the lower side and slightly convex on upper side, single layered upper and lower epidermii covered with thin cuticle bears uni and bi-cellular covering and a few multi-head glandular trichomes; epidermis is followed by an arch of angular collenchymatous mechanical tissue, upper epidermis followed by trace bundle and centrally located well developed half-moon shaped vascular bundle capped by an arch of pericyclic tissue at the lower side; vascular bundle consists of xylem and phloem with usual elements; ground tissue having thin-walled parenchymatous cells embedded with brownish content, prismatic and...
rosette crystals of calcium oxalate. Lamina shows single layer of upper and lower epidermal cells covered with thin cuticle with uni and bicellular covering and a few multi-head glandular trichomes; mesophyll tissue consists single layer of palisade parenchyma cells and 3 to 4 cells rows of spongy parenchyma cells contain chlorophyll contents; vascular strands are embedded in centre of mesophyll region with usual elements (Fig 3c).

Physicochemical analysis

The physicochemical analysis of *P. foetida* leaf powder showed loss on drying to be 14.57 ± 0.01% w/w, total ash value was 12.745 ± 0.14% w/w, acid insoluble ash was 0.39 ± 0.01% w/w, water soluble ash was 6.67 ± 0.01% w/w, alcohol soluble extractive was found to be 1.71 ± 0.01% w/w and water soluble extractive value was 22.11 ± 0.01% w/w.

Preliminary Phytochemical Analysis

The preliminary phytochemical analysis of alcoholic extract of *P. foetida* showed the presence of alkaloid, phenols and resin.

HPTLC

HPTLC developed plates when observed under short UV (254 nm) showed 2 bands at Rf 0.06, 0.37 (all green). Under long UV (366 nm) at Rf 0.06, 0.17 three bands were found in fluorescent blue colour, and at Rf 0.38 one band was found in fluorescent red colour. After the derivatization (620 nm) of plate with vanillin sulphuric acid reagent, no bands were found (Table 1).

| Table 1: RF values of *Passiflora foetida* L. leaf ethanolic extract |
|---------------------------------|
| Short UV | Long UV | After derivatisation |
| 0.06 (Green) | 0.06 (F. blue) | - |
| - | 0.09 (F. blue) | - |
| - | 0.17 (F. blue) | - |
| 0.37 (Green) | 0.38 (F. red) | - |

Densitometric scan of the plate at 254 nm showed 5 peaks having maximum absorption at Rf 0.08 (42.40%) and at 366 nm it showed 6 peaks having maximum absorption at Rf 0.11 (12.67%) and at Rf 0.22 (35.09%). After derivatization with VSA reagent it showed only 2 peaks scanned under deuterium lamp the absorption was found only at Rf 0.05 (80.2%) was evident (Fig 4,5).

Track 1 – 3 µl; 2 – 6 µl; 3 – 9 µl Solvent system – Ethyl acetate: Methanol: Water: Formic acid (5:0:2:0:3:0.6).

**DISCUSSION**

*Passiflora foetida* is a climber which is found as a weed. The plant has wide range of medicinal properties [12]. The process of derivation of quality standards of herbal drugs need information from basic disciplines of plants science such as taxonomy, morphology, anatomy etc, for identifying plant drugs. At the same time, to evolve standards on quality specifications of a herb in terms of its chemical composition,
analytical and phytochemical expertise is also required. From above study we found there are a lot of vernacular names for this plant in India, most of them were coined on the basis of its morphological features. Pharmacognostical study helped in evaluating the macroscopic and microscopic characters of the plant. These features evaluated for herbal drugs help in confirmation of its botanical source even when it is in dried form. Though plants can be easily identified in its fresh form, the same is difficult when it is dry. Here in this sample the macroscopic study revealed it as a simple trilobed leaf which is entire green and pubescent in fresh form which was matching with the morphological characters of *P. foetida* explained by Patil et al [13]. The organoleptic characters were also able to find the colour, odour, texture and taste of the plant leaf powder. The physicochemical analysis determined the loss on drying, total ash, acid insoluble ash, water soluble ash, alcohol soluble and water soluble extractive value. The ash value helped in determining the amount of impurities and the inorganic matter present in the leaf powder. The constants obtained in the present study will serve as an indication of chemical quality of *P. foetida* for quality control and standardisation of this herbal drug in future researches. A preliminary examination for different phytochemical entities by colour tests indicated preliminary chemistry of the plant. The preliminary phytochemical study from alcoholic extract of *P. foetida* leaf showed the presence of alkaloid, phenols and resins, which was matching with the result of phytochemical screening mentioned by Asadujjaman et al [14]. This plant is used in hysteria and sleep disorder, this action can be due to the presence of different alkaloids present in the plant [15,16]. These phytoconstituents supports the ethnomedical claims of the plant. HPTLC also helped to know that there is wide range of chemical moieties present in the alcoholic extract of *P. foetida* leaf. From the results of HPTLC densitometric scan, five chemical components at 254 nm, six chemical components at 366 nm and two chemical components at 620 nm were found in leaves of *P. foetida*.

**CONCLUSION**

The results obtained from the pharmacognostical, physico chemical tests, preliminary phytochemical analysis and HPTLC studies help in the standardisation of the drug. The preliminary phytochemical analysis of *P. foetida* helped knowing the presence of phyto-constituents. As there are a number of phyto-constituents, the plant possess wide range of medicinal properties also. Further studies might be carried out to explore more about the plant. Studies could be carried out following Ayurvedic protocols, to find, *rasa, guna, veerya, vipaka* etc for adding this extra-pharmacopeial plant *P. foetida* in Ayurvedic Pharmacopeia.

**Conflict of Interest**

None declared.

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None declared.

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