

PHARMACOGNOSTICAL STUDY ON *PIPER TRIOICUM* ROXB

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Received: 21 Feb 2011, Revised and Accepted: 21 March 2011

ABSTRACT

This paper deals with the detailed pharmacognostical evaluation of the *Piper trioicum* Roxb (Piparacae). Morphology of the entire plant have been studied with the aim to aid pharmacognostic and taxonomic species identification using light and confocal microscopy, WHO recommended physico-chemical, morphological and histological parameters presented in this paper may be proposed as parameters to establishes the authenticity of *Piper trioicum* Roxb and can possibly help to differentiate the drug from its other species /varieties.

Keywords: *Piper trioicum* Roxb, Microscopy, Nutritional value, Microscopy, Preliminary chemical tests

INTRODUCTION

Piper trioicum Roxb belongs to Piparacae family, distributed in south asian countries. The whole plant is used as rubefacient, diuretic, hepatoprotective and used for diabetes, muscular pains, headache, toothache and internal remedy for cholera in folk medicine; the root is used as diuretic¹. *Piper trioicum* Roxb is herbs, shrubs, or climbers, rarely trees, usually aromatic. Vascular bundles are scattered in transverse section in a monocotyledon like manner. Tip of stem sometimes enclosed within a stipule like sheath, the prophyll, sometimes adnate to petiole, absent in Peperomia. Leaves alternate, often opposite or whorled in Peperomia, simple, base often asymmetric, palmately or pinnately veined. Inflorescence a pedunculate spike, rarely grouped into an umbel, rarely a raceme (in Zippelia), leaf-opposed or axillary, rarely terminal. Flowers are small, bisexual, hermaphroditic, polygamous or dioecious, nearly always sessile; bracts small, usually peltate or cupular, usually without perianth. Fruits are a small drupe or nutlet; pericarp fleshy, thin or dry, sometimes with sticky papillae (in Peperomia) or glochidiate spines (in Zippelia). Seeds are with copious starchy perisperm and a minute embryo embedded in small endosperm. To ensure reproducible quality of herbal medicines, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication followed by creating numerical values of standards for comparison. Pharmacognostical parameters for easy identification like leaf constants, microscopy & physico chemical analyses are few of the basic protocol for standardization of herbals. Hence, in the present work the pharmacognostical standardization has been performed for the leaf of the plant.

MATERIALS AND METHODS**Collection and authentication**

Plant material of *Piper trioicum* (PT) was collected from local areas of Talakona, Andhra Pradesh and plant was authenticated by Mr. Madhavachetty, Botanist, S.V.University, Thirupati, Andhra Pradesh. Plant was dried in the shade and ground into uniform powder using milling machine.

For the microscopic studies, transverse sections were prepared and stained^{2,3}. The leaves were boiled separately with saturated chloral hydrate solution for surface studies and quantitative microscopical observation of leaf. The selected whole plant of PT was subjected to organoleptic (colour, odour and taste were recorded), microscopic (essential for powdered crude drugs consist of the fragments of cells in the form of recognizable tissues and the study of surface constants like fibres, lignified vessel, epidermal cells, calcium oxalate crystals, starch grains, etc.)^{4,5,6} and physico-chemical values such as the percentage of total ash, acid-insoluble ash, water-soluble ash, sulphated ash, water and alcohol soluble extractives, crude fiber content and foreign matter were calculated as per the Indian

Pharmacopoeia^{7,8} and fluorescence analysis of crude powder were estimated using various chemical and organic reagents.

Determination of stomatal index

Leaf fragments were observed under microscope for the presence and quantification of epidermal cells, stomata (type and distribution), palisade cells, vein islet number and veinlet termination number. Stomatal index was calculated as the percentage of number of stomata present per number of epidermal cells and each stoma was counted as one cell.

Preparation of extracts

The extracts of leaves of PT were prepared by successive soxhlation with various solvents. The shade dried leaf powder was packed in thimble kept in the soxhlet apparatus and extraction was allowed to run successively using the solvents, petroleum ether (60-80°C), chloroform and ethanol. Finally, the marc was dried and macerated with chloroform-water for 24 hours to obtain the aqueous extract. Each extract was concentrated by evaporating the solvent on the water-bath and the obtained extracts were weighed. The physical characteristics and percentage yield of various extracts were tabulated.

Phyto chemical screening

All the extracts were subjected to preliminary phytochemical screening for the detection of various chemical constituents. The presence or absence of different phytoconstituents viz. triterpenoids, steroids, alkaloids, sugar, tannins, glycosides and flavanoids, etc. were detected by usual prescribed methods⁹.

RESULTS AND DISCUSSION**Macroscopic studies**

The macroscopical studies revealed that leaves are ovate corate 2 to 4.5 inches long and 1.5 to 3 inches in width, abruptly acuminate with entire margin, green veins, round or perinath shaped base. Organoleptic properties are green color, spicy taste and spicy odour.

Transverse section of leaf

The transverse section of the leaf showed dorsiventral nature. Upper epidermis was double layered with rectangular epidermal cells with distinct cuticle, abundant were observed. Mesophyll was differentiated into palisade and parenchyma. Palisade cells were elongated arranged compactly in single layer and were discontinued over midrib. Parenchyma consists of loosely arranged parenchymatous cells of 4-5 layers. Lower epidermis was single layered with rectangular cells. Vascular elements were arranged in 2-3 layers. The dorsal surface was strongly convex and epidermal layer of lamina continued along midrib below the upper epidermis and above the lower epidermis. Vascular bundles were arch shaped,

collateral in nature. 4 to 5 layers of collenchyma cells are present below upper epidermis and above lower epidermis (Figure 1).

Powder microscopy

In the powdered preparation, fibres were mostly narrow, long and cylindrical with thick walls and narrow lumen. Pits were not evident up to 101.28µm long and 4.585µm wide. Epidermal, parenchymatic, trichomes and elements were observed. The adaxial epidermis in surface view has highly lobed cells; their anticlinal walls are thick and much undulate. Long, narrow, cylindrical vascular elements are thick and much undulate (figure 2, 3). The results of quantitative microscopy of leaf / leaf powder of PT were present in table 1.

Physico chemical properties

The physico-chemical characters of powdered drug of whole plant of PT such as the percentage of total ash, acid-insoluble ash, water-soluble ash, sulphated ash, alcohol soluble extractives, moisture content and foreign matter were presented in Table 2. The fluorescence analysis of the powdered drug of PT in various solvents and chemical reagents was performed under normal and Ultra Violet (UV) light (Table 3).

Preliminary phytochemical screening

After extraction with different solvents, the residues were dried and measured. The residue obtained was 0.2%, 4.2%, 1.8% and 3.8%w/w for petroleum ether, chloroform, alcohol and water extract of PT respectively. The dark green, green, brown and brown residues were for petroleum ether, chloroform, alcohol and water extract of PT respectively. The preliminary phytochemical investigation of the aqueous, ethanol, chloroform and petroleum ether extracts of PT showed the presence of alkaloids, steroids,

flavonoids, phenolic compounds, carbohydrates, tannins and glycosides (Table 4).

Table 1: Quantitative microscopy of leaf / leaf powder of PT

| Parameter | Value |
|-----------------------------------|----------------------|
| Phloem fibers (width) | 26.08 – 51.5µ |
| Phloem fibers (length) | 133 – 558.6 µ |
| Starch grains (diameter) | 13.3– 66.5µ |
| Calcium oxalate crystals | 1-2/mm ² |
| Stomatal number (lower epidermis) | 7% |
| Stomatal index (lower epidermis) | 12.96% |
| Stomatal number (upper epidermis) | 11% |
| Stomatal index (upper epidermis) | 22.42% |
| Vein islet number | 14/ mm ² |
| Veinlet termination number | 10 / mm ² |
| Palisade ratio | 23 / mm ² |
| Length of Trichomes | 81-369.6 µ |
| width of xylem vessels | 133- 359.1µ |

Table 2: Physicochemical properties of PT

| Quantitative Parameter | Values obtained | %w/w |
|-------------------------------|-----------------|------|
| Total ash value | 12.5% | |
| Acid insoluble ash | 3% | |
| Water soluble ash | 6.5% | |
| Sulphated ash | 6.6% | |
| Moisture content | 8.96% | |
| Foreign matter | 4% | |
| Alcohol soluble extract value | 0.64% | |
| Crude fiber content | 47% | |

Table 3: Fluorescence analysis

| Solvents used | Daylight | UV light (254nm) | UV light (366nm) |
|--------------------------------------|---------------|------------------|-------------------|
| 50%H ₂ SO ₄ | Yellow green | Light green | BrownishYellow |
| 50%HNO ₃ | Green | Green | Brown |
| 5%NaOH | Green | Green | Pale Yellow |
| 1NMethanolic NaOH | Green | Bright green | Yellow |
| 1N KOH | Yellow | Bright green | Yellow |
| 5%KOH | Yellow | Green | Green |
| 5% FeCl ₃ | Brown | Green | Yellow |
| Methanol | Light green | Bottle green | Yellow |
| Conc. HCl | Yellow | Dark green | Green |
| Conc. H ₂ SO ₄ | Brown | Bottle green | Florescent yellow |
| Ammonia | Yellow | Green | Greenish yellow |
| Conc HNO ₃ | Reddish brown | Green | Green |

Table 4: Qualitative preliminary phytochemical studies

| Extracts | Pet. ether | Chloroform | Alcohol | Water |
|----------------------|------------|------------|---------|-------|
| Carbohydrates | - | - | + | + |
| Flavonoids | - | - | - | + |
| Alkaloids | - | - | - | + |
| Proteins& Aminoacids | - | - | - | - |
| Phenolic compounds | + | + | + | - |
| Steroids | - | - | - | - |
| Tannins | + | + | + | - |
| Terpenoids | - | - | - | - |
| Saponins | + | + | + | + |
| Glycosides | - | - | - | - |

+ present, - absent

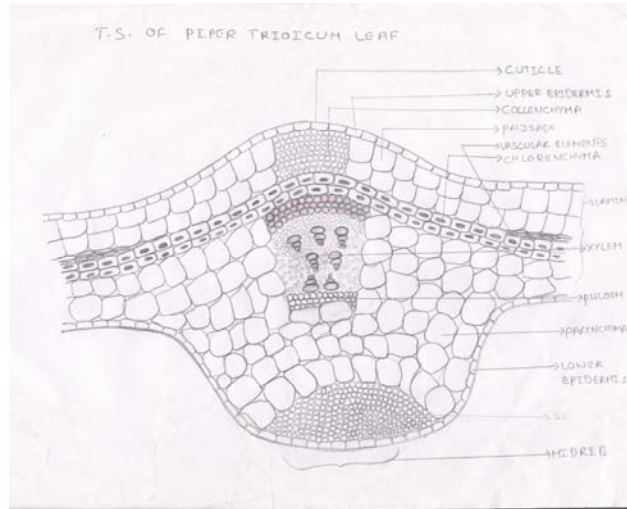


Fig. 1: Leaf T.S of *Piper trioicum* Roxb.



Fig. 2: Powder microscopy studies



Fig. 3: Powder microscopy studies

DISCUSSION

As a part of standardization study, the macroscopical examination of PT was studied. Macroscopical evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of drugs. The macroscopical characters of the PT can serve as diagnostic parameters.

The extractive value, ash value, loss on drying and fluorescent analysis of whole plant extracts have been carried out. The results showed greater extractive values in hot extraction, indicating the effect of elevated temperature on extraction. Percentages of the extractive values were calculated with reference to air-dried drug. The percent extractives in different solvents indicate the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent. The fluorescence analysis of the powdered drug from the PT in various solvents was performed under normal and UV light. All the whole plant extracts are examined in short UV (254nm) and long UV (366 nm) to detect the fluorescent compounds.

CONCLUSION

The pharmacognostic parameters, which are being reported for the first time, could be useful in the identification and standardization of a crude drug. The data produced in the present investigation is also helpful in the preparation of the crude drug's monograph and inclusion in various pharmacopoeias.

ACKNOWLEDGEMENT

The authors sincerely thank Management, Nalanda College of Pharmacy for providing necessary facilities to carry out study.

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