

Pharmacognostic Evaluation of *Piper longum* Linn. Fruit

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Abstract – The present study includes macro and microscopical details, powder study, physico-chemical study and HPTLC fingerprinting of the *Piper longum* fruits. Microscopic studies revealed the presence of stone cells, starch grains and thin walled fragments of parenchymatous cells. Physico-chemical studies showed alcohol and ether soluble extract 24.53 and 6.7, sugar 0.35, starch 21.33 and tannin 0.83% respectively. Successive soxhlet extract showed maximum percentage of hexane soluble fraction i.e. 22.52. The HPTLC profile has also been performed against the reference marker piperine, which was identified at R_f 0.42. In the present paper a detailed pharmacognostical evaluation of fruit has been undertaken.

Keywords – *Piper longum*, Pharmacognosy, HPTLC.

Introduction

Piper longum (Piperaceae) is a slender aromatic climber with perennial woody roots occurring in the hotter parts of India. It is an important medicinal plant, used in traditional medicine by many people in Asia and Pacific islands especially in Indian Systems Medicine (Guido *et al.*, 1998). The fruits commonly known as 'Pippali' are used as a spice, a preservative in pickles, and cattle feed. In traditional Indian Medicinal system, *Piper longum* fruits have been advocated to be beneficial in diseases of respiratory tract infections such as cough, bronchitis, asthma, malarial fever, diarrhea and jaundice (Kirtikar and Basu, 1933; Chopra *et al.*, 1958; Shusurut Samhita, 1963; Krishnamurthi, 1969). *Piper longum* is a component of medicines reported as good remedy for treating gonorrhoea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, chronic gut related pain, and arthritic conditions (Singh, 1992). Other reported beneficial effects of *Piper longum* include analgesic and diuretic effects, relaxation of muscle tension, and alleviation of anxiety (Singh and Blue Mental, 1997). Piperine was the first amide isolated from *Piper* species and was reported to display central nervous system depression, antipyretic, and anti-inflammatory activity (Virinder *et al.*, 1997), some rare alkaloids has also been reported (Das *et al.* 1998).

Piperine is a potent inhibitor of the mixed function oxygenase system and non-specific inhibition of P₄₅₀ isoenzymes (Atal *et al.*, 1985). Constituents of *Piper* species have inhibitory activity on prostaglandin and leukotriene biosynthesis *in vitro* (Stohr *et al.*, 2001). It is also reported to be a good antiviral agent (Ratner *et al.*, 1991; Van den Brock *et al.* 1996; Couragot *et al.*, 2000; Durantal *et al.*, 2001).

Due to its vast diversified medicinal values, the present study has been done to document the pharmacognostic standards of this species which will be not only helpful to the industries for the quality control of the raw material but will also ensure batch to batch consistency of the drug.

Experimental

The plant material was collected from the Banthara field station, Lucknow of UttarPradesh, India [LWG 221874, 2005] and the fruits were preserved in 70% ethyl alcohol for histological studies. Microtome sections were cut and stained with safranin and fast green and photographed with Nikon F70X camera (Johansen, 1940). Physico-chemical and phytochemical studies like, total ash, acid insoluble ash, and tannins were calculated from the shade dried powdered material according to the recommended procedures (Peach and Tracy, 1955; Anonymous, 1965; Anonymous 1984). The behavior of the powdered drug with different chemical reagents was also studied as per

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methods described by Chase and Pratt (1949) and Kokoski *et al.* (1958).

Results

A Brief taxonomic description – A slender aromatic climber with perennial woody roots, stems creeping, round, smooth, woody with articulations, swelling near the joints and branched; young shoots downy; leaves 5 - 9 cm long, 3 - 5 cm wide with seven nerves, ovate, cordate with broad rounded lobes at base, subacute, entire, glabrous; Flowers small, white, sessile, covering a tubular spadix; spikes cylindrical pedunculate, male larger and slender, female 1.3 - 2.5 cm long and 4 - 5 mm diam.; fruits ovoid, yellowish orange, sunk in fleshy spike and surface coarsely wrinkled.

Macroscopic – Fruit very small, ovoid, completely sunken embedded in solid fleshy spike, 2.5 - 4.0 cm long. Colour of fruit is light green to olive green when immature, after ripening colour changed to shining blackish green.

Microscopic – Transverse section (T.S.) of the aggregate fruit shows 5 - 7 rows of fruitlets, which are spiral or vertical on a central axis. Outer epidermal cells of the fruitlets are containing brown contents; the cells of which are irregular in shape. Mesocarp consists of large parenchymatous cells, filled with starch grains, traversed with a number of stone cells either solitary or in groups. Endocarp and seed coat fused to form a deep zone, round to oval measuring 3 to 8 m in diameter. The central axis is parenchymatous, 5 to 8 collateral vascular bundles arranged in a ring. Second layer contains tangential cells with orange red pigment. The outer most layer of kernel consisting of hexagonal or irregular cells filled with starch grains.

Powder Study – The powder of fruit is dark brown in colour pungent and heating in taste. The microscopical study of powder shows large polygonal perisperm cells, isolated or in group of 2 or 3, packed with simple and compound starch grains measuring 2 to 5 m in diameter; stone cells measuring 130 to 190 m in diameter with broad lumen in groups of 2 to 8, stone cells, thin walled fragments of parenchymatous cells from mesocarp.

Phytochemical studies – Physico- chemical parameters (moisture, total ash, acid insoluble ash, water soluble extractive, alcohol and ether soluble extractive, protein, sugar/starch and tannins) were determined by using standard methods and techniques, results are tabulated in Fig. 1.

Determination of different solvent extractive of *Pippali* fruit were done through successive soxhlet extraction and result percentage are presented in Fig. 2.

HPTLC studies – For proper and meaningful utilization

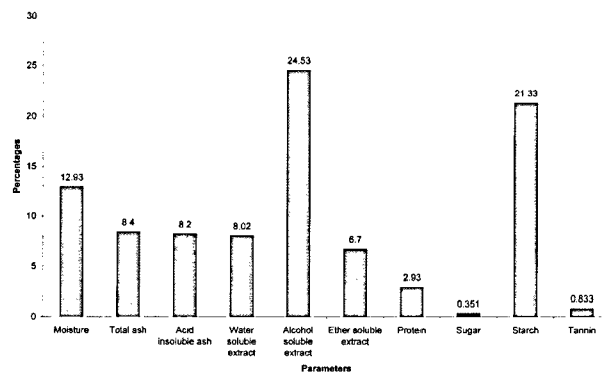


Fig. 1. Physicochemical values of *Piper longum* fruits.

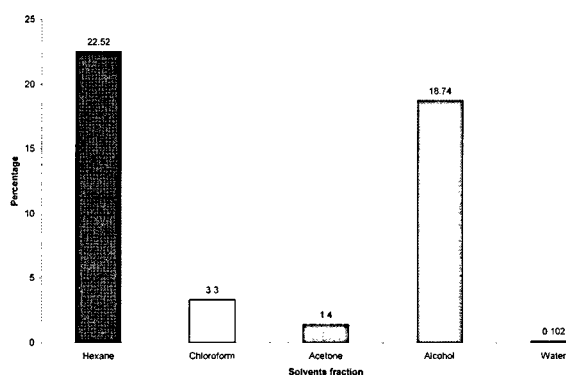


Fig. 2. Successive soxhlet values *Piper longum* fruits.

it is important to have quality standards of material and for this quality standardization HPTLC finger print profile was developed against the marker compound piperine. The HPTLC analysis was carried out on percoated Silica gel 60-F₂₅₄ Merck TLC glass plate of 5 × 10 cm with the help of Camag Linomat -IV applicator. The plate was visualized in UV 366 nm in solvent system Hexane: Chloroform (3 : 2). The band in the sample were obtained at R_f 0.07, 0.14, 0.21, 0.23, 0.30, 0.42, 0.48, 0.55, 0.63, 0.66, 0.85 and 0.90 which can be used as identifying marker. The Piperine was identified at R_f 0.42.

Discussion

From the above studies fruit of *Pippali* can be easily differentiated on the basis of organoleptic and microscopical characters. Physico-chemical values viz. moisture content, total ash, acid insoluble ash, water, alcohol, ether soluble extractives, protein, sugar/starch, and tannin were determined by using standard method and techniques. The total ash and acid insoluble ash which are important parameter for detecting the presence of inorganic substances was found 8.40 and 8.20% respectively. Similarly the

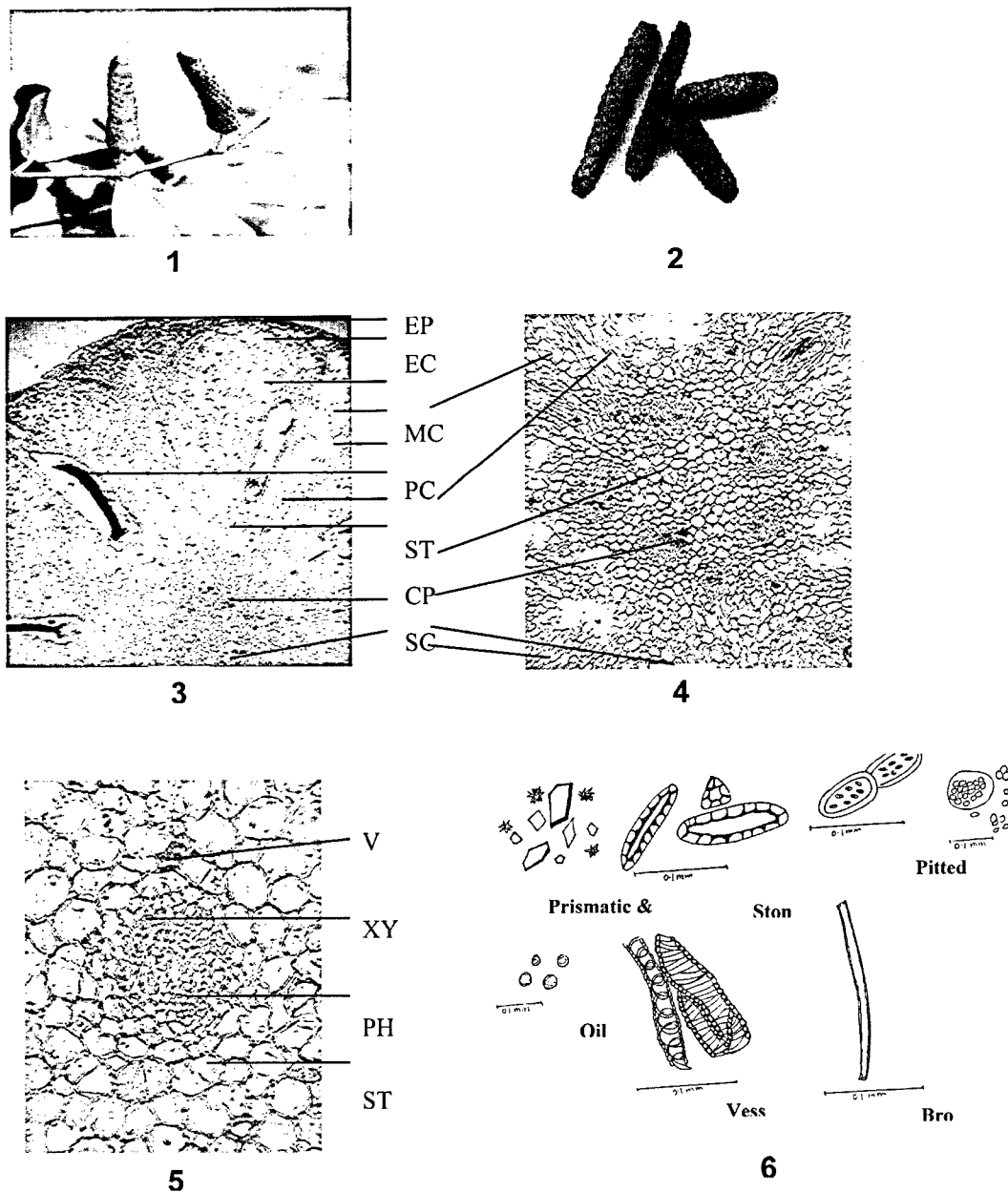


Plate 1. Macroscopic & Microscopic characters of *Piper longum* Fruit

1. A flowering twig
2. Dried Fruits of *Piper longum*
3. T. S. Diagrammatic representation of Fruit (x25)
4. T.S. Cellular of Fruit showing cortical zone (x40)
5. T.S. Cellular of Fruit showing vascular elements (x40)
6. Powder study of *Piper longum* fruit

CP, Carpel; EP, Epidermis; EdC, Endocarp; EC, Exocarp; MC, Mesocarp; OC, Oil cells; PC, Parenchymatous cells; PH, Phloem; SC, Sclereids; V, Vessels; XY, Xylem; ST, Starch grains

water, alcohol and ether soluble extractives, which are indicator of total solvent soluble component, are 8.02,

24.53 and 6.70% respectively. Determination of different solvent extractive of *Pippali* fruit was completed through

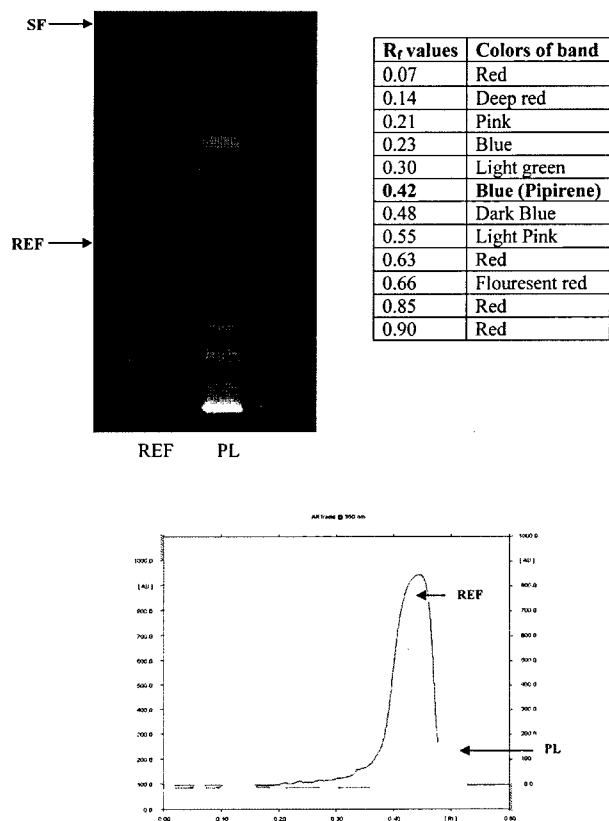


PLATE 2. HPTLC profile and densitometric chromatogram of *Piper longum* fruit and reference sample (under UV-366).

Ref, Reference; PL, *Piper longum*.

successive soxhlet extraction from nonpolar to polar solvent viz. hexane, chloroform, acetone, alcohol and water were also carried out to estimate the successive extractive values.

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