

Pharmacobotanical Studies on *Piper longum* L.

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Abstract

Piper longum L. is used as drug in Unani, Ayurvedic and Siddha System of medicine. The drug (dried fruit) is aromatic, stomachic, carminative, expectorant, digestive and antiseptic and also used as spice in commerce. Very few, clinical studies have been conducted on this drug. The present studies deal with detailed pharmacognostic studies and reviews related information on this drug.

Key Words – *Piper longum* L, Drug Standardization, Quality Specifications

Introduction

Piper longum L. (Family- Piperaceae) is commonly known as Pippali, it is widely used as a pungent spice. The fruit of the pippali plant is a common ingredient in many recipes. It rehabilitates vitiated vata and kapha, the dried spikes are acrid, mildly thermogenic, stomachic, carminative, expectorant, tonic, laxative, digestive and antiseptic. It native of the Indo Malya region. Pippali is a slender aromatic climber; it grows in mountain valleys and coastal areas of tropical as well as sub-tropical regions. The plants are found as creepers or root climbers with fastigate branches and are considered as indigenous to the hotter parts of India. It is found growing wild in the west coast as undergrowth in the evergreen forests of the Western Ghats. It is also occasionally cultivated in large scale especially in West Bengal and Southern states (Shah and Qudry, 1990-91)

Pippali is one of the ingredients of the Ayurvedic drug “Trikatu” .There is a mention of 4 types of Pippali in Rajnighantu, namely pippali, vanapippali, saimhali and gajapippali. Sharma (1983) has equated the former 3 with *Piper longum*, *Piper sylvaticum* Roxb. and *Piper retrofractum* vahl. respectively of this *Piper sylvaticum* is a Himalayan species and *Piper retrofractum* is an indeterminable taxon as mentioned by Hooker (1872).

Methodology

Drug samples were collected from different places with a view to find out any significant difference present within the same species. For studying powder, Jackson and Snowdon (1992) was followed. To determine physico-chemical constants, Indian Pharmacopoeia (Anonymous, 1966) was consulted and for fluorescence study schedules mentioned by Trease and Evans (1972)

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were followed. Colours were named by consulting Rayner (1970). Standard prescribed procedures for histochemical studies (Johanson, 1940; Youngken, 1951; Cromwell, 1955, Trease and Evans, 1978), organic group detection (Robinson, 1963); U.V. Spectrophotometry (Willard *et al.*, 1965) and Chromatography (Shellard, 1968; Stahl, 1969; Smith and Feinberg, 1972) were adopted. The informatics is complied by reviewing the available literature.

Systematics

Piper longum L. (Family: Piperaceae)

Plant is a slender aromatic climber, rooting at the nodes, the branches erect subscentent, swollen at the nodes; leaves alternate, lower ones broadly ovate, cordate, upper ones oblong oval all entire, smooth, thin with reticulate venation, flowers in solitary spikes, fruits berries small, red when ripe completely sunk in solid fleshy spike. (Fig 1 A). The plant is widely distributed in India. This plant grows in moist deciduous to evergreen forests. It is found Ceylon, Malay Peninsula, Malay Islands, and hotter provinces of India. In India *Piper longum* cultivated in Bihar, Assam, Khasia hills.

Drug Specification

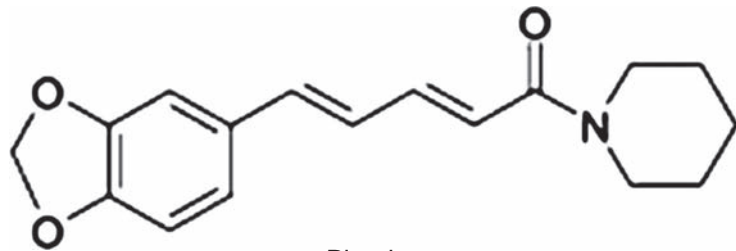
The drug consists of dried, greenish-black to black, immature, catkin-like long fruits with bracts.

Nomenclature

The plant is known by different vernacular names e.g. Lindi peeper, Pipali (Gujarati), Pipar (Hindi), Pippali (Malayalam), Pimpali, Lendi Pimpali (Marathi), Pipali, Pippali (Oriya), Arisi Tippali, Thippili (Tamil), Pippalu (Telugu) and Filfil Daraz (Urdu) etc.

Chemical Constituents

Piperine is the major and active constituent of long pepper (*Piper longum*). Pippali fruit contain volatile oil, resin, isobutylamides (retrofractamide, brachystamide longamide), lignans (sesamin, pulviatiol, fargesin), esters and alkaloids (Piperine, Piperlongumine, Piperlonguminine etc), a waxy alkaloid N-isobutyl, deca trans-2-trans-4-dienamide and a turpenoid substance. Other substances such as calcium, phosphorus and iron also present in pippali powder (Shah and Qudry *et al.*, 1990-91).



Piperine

Pharmacology

It is rejuvenative for the lungs, encourages vasodilatation and increase circulation, specifically to the lungs. It has the anthelmintic qualities, used to kill worms, amoebas and parasites and hepatoprotective effect that benefit in fibrosis. It shows anti-bacterial activity against *B. cereus*, *B. subtilis*, *M. tuberculosis*, *Staph. Albus*, *Esch. Coli* etc. It also has anathematic, anti-inflammatory, hepatoprotective, hypoglycemic, antispasmodic and insecticidal activity (Satyavati *et al.*, 1987).

Therapeutic and non-therapeutic Uses

Charak has described the medicinal properties of the plant as an appetizer, stimulant, antipolice, antitussive, and inducing resistance to infections. *Piper longum* is in widespread use for various ailments for many centuries. The dried fruits are acrid, mildly thermogenic, stomachic, aphrodisiac, carminative, expectorant, febrifuge, tonic, laxative, digestive, emollient and antiseptic. They are useful in anorexia, dyspepsia, flatulent colic, asthma, bronchitis, gastropathy, epilepsy, fevers, gonorrhoea, hemorrhoids, vitiated conditions of Vata, Gout and Lumbago.

Pippali is widely used remedy in Ayurvedic medicine and it's also used as a simple home remedy in treatment of disorders such as dyspepsia, coryza, thirst, fever, abdominal disease, bronchitis, diabetes and worms etc. Powdered of pippali, administered with honey, to relieve cough, asthma, hoarseness, hiccup and sleeplessness (Georgeking *et al.*, 1989).

Classical Formulations

Ayurvedic- Gudapippali, Amrtarista, Ayasakrti, Asvaganshadyarista, Kumaryasava, Candanasava, Cayavanaprasa avaleha, Siva Gutika, Kaisora Guggulu (API)

Siddha - Attatic Curanam, Civanar Amirtam, Kakkuvan Ilakam, Kunmak Kutorri Meluku, Palacancivi Mattirai, Tippili Iracayanam (SPI).

Regulatory Status

Piper longum L. is an official drug in following pharmacopoeias and formularies of India:-

- i. Ayurvedic Pharmacopoeia of India, Part I, Vol. IV.
- ii. Ayurvedic Formulary of India, Part I & II.
- iii. Indian Pharmacopoeia, 2007, 2010.
- iv. National Formulary of Unani Medicine Part I-V.
- v. Siddha Pharmacopoeia of India, Part I, Vol. I.
- vi. Siddha Formulary of India, Part-I.

Observations

I. Organoleptic Characteristics

- Entire Drug-Fruit elongated cylindrical, catkin-like spikes of immature individual fruit; fused together in spirals. Spike is ovoid-oblong erect, 2.5-5 cm, blunt, greenish black to black in colour, and shining.
- Powdered Drug-The Powder colour is greyish-black; odour aromatic and taste, pungent, (Fig. 1 B,C)

II. Micro-Morphological Characteristics

Powdered Drug The epicarp is polygonal-shaped cells, sclereids from mesocarp with adhering epicarp containing pigment and calcium oxalate crystals in surface. A hypodermis with elongated stone cell; sclereids from the mesocarp. An endocarp of elongated, porous, sclerechymatous cells; cells of the endocarp in surface view with underlying pigment layer and hyaline layer of testa. Perisperm cells densely packed with mass of starch granules. Endosperm and fibro-vascular tissue are also present (Fig. 2).

III. Histochemistry

Micro – Chemical Tests and Behaviour of specific reagents towards Plant/Drug Tissues – Observations and results pertaining to micro-chemical tests and behaviour of specific reagent towards plant tissues are presented in Table-1.

Table 1 : Micro-chemical Tests and behaviour of specific reagents towards plant tissues and cells contents.

Sl. No.	Reagent	Test for	Inference	Histological zone/ cell contents responded.
1.	Dragendorff's reagent	Alkaloid	+	Perisperm cells
2.	Marme's reagent	Alkaloid	+	Perisperm cells
3.	Wagner's reagent	Alkaloid	+	Perisperm cells
4.	Potassium hydroxide solution (5% w/v)	Anthocynin	-	Not Responded
5.	Sulphuric acid (66% v/v)	Anthocynin	-	Not Responded
6.	Acetic acid	Calcium oxalate	+	Prismatic calcium oxalate in mesocarp cells
7.	Potassium hydroxide solution (5% v/v) + Hydrochloric acid	Calcium oxalate	+	Same as above
8.	Sulphuric acid	Calcium oxalate	+	Same as above
9.	Kedde reagent	Cardiac glycoside	-	Not Responded
10.	Iodine Solution followed by Sulphuric acid	Cellulose	-	Not Responded
11.	Sudan III	Fixed oil and fats	+	Perisperm cells
12.	Chlor-zinc-Iodine Solution	Latex	-	Not Responded
13.	Aniline sulphate Solution followed by Sulphuric acid	Lignin	+	Sclereids from mesocarp
14.	Phloroglucinol HCl	Lignin	+	Same as above
15.	Lugol's solution	Protein	-	Endosperm cells
16.	Millon's reagent	Protein	-	Same as above
17.	Picric acid	Protein	-	Same as above
18.	Heating with KOH (5% w/v) + H ₂ SO ₄	Suberin	-	Not Responded
19.	Sudan III	Suberin	-	Not Responded
20.	Weak Iodine solution	Starch	+	Starch grains in perisperm cells
21.	Potassium hydroxide solution (5% w/v)	Starch	+	Starch grains in perisperm cells
22.	Sulphuric acid	Starch	+	Starch grains in perisperm cells

Indications: '-' Absence and '+' presence of constituent.

Organic Groups of Chemical Constituents – The extracts of the drug were tested for presence of different organic groups and results are presented in Table – 2.

Table 2 : Major Group of Organic Chemical Constituents of Drug.

Sl. No.	Organic Groups of Chemical Constituents	Reagents/Tests	Inference
1.	Alkaloid	Dragendorff's and Mayer's reagents	+
2.	Anthraquinone	Borntrager reaction	+
3.	Coumarin	Alcoholic potassium hydroxide	+
4.	Flavonoid	Shinoda reaction	-
5.	Glycoside	Mollisch's test	-
6.	Protein	Xanthoprotein test	+
7.	Resin	Ferric chloride reagent	+
8.	Saponin	Liebermann-Burchard reaction	-
9.	Steroid	Salkowski reaction	-
10.	Tannin	Gelation test	+

IV. Identity, Purity & Strength

Physico-Chemical Constants – The analytical values in respect of physico-chemical constant of drug were established and results are reported in Table-3.

Table 3 : Analytical Values of Physico-chemical Constants

Sl. No.	Physico-Chemical Constants	Analytical values
	Moisture content, % w/w	11.0
	Total Ash, % w/w	5.0
	Acid insoluble ash, % w/w	0.50
	Alcohol soluble extractive % w/w	10.5
	Water soluble extractive % w/w	23.50
	Essential oil, %, v/w	—

V. Fluorescence & Spectroscopy

Fluorescence Characteristic of Powdered drug under Ultra-Violet Light – Powdered drug was screened for fluorescence characteristic with or without chemical treatment. The observations pertaining to their colour in daylight and under ultra-violet light were noticed and are presented in Table-4.

Table 4 : Fluorescence Characteristic of Powdered Drug under Ultra-Violet Light.

Sl. No.	Treatments	Coriandrum sativum	
		Colour in day light	Nature of colour in fluorescence
1.	Powder as such	Yellowish brown	Brown
2.	Powder with		
	Carbon tetra chloride	Brown	Brown
	Ethyl acetate	Light brown	Brown
	Hydrochloric acid	Greenish brown	Greenish brown
	Nitric acid + water	Light brown	Brownish
	Sodium hydroxide + methanol	Yellowish brown	Brown
	Sodium hydroxide + water	Greenish brown	Brown
	Sulphuric acid + water	Light brown	Greenish brown
	Buffer- pH 5	Light brown	Brownish
	Buffer- pH 7	Light brown	Brownish
	Buffer- pH 9	Light brown	Brownish

Ultra-Violet Spectroscopy – The data related to Ultra-Violet Spectrophotometric characteristics as computed in Table-5.

Table 5 : Ultra-Violet Spectrophotometer characteristic of drugs.

Sl. No.	Specifications	Data
1.	Tincture dilution ml/ml	1
2.	Maximum absorption peak	0.911 1.525 1.493
3.	I Maxima at, nm	331.35 259.50 215.45

VI. Chromatographic Profile

Thin-Layer Chromatography – Best separation for TLC fingerprinting were obtained by using different layers and solvent systems. Inferences are shown in Table-6.

Table 6 : TLC fingerprinting data

S. No.	Drug	Mobile Phase/ Solvent System	Derivatizing Reagents	Visualizations	No. of Spots	R _f Values of bands
1.	<i>Piper longum</i> L.	Toluene: Ethyl acetate (9:1) v/v	Anisaldehyde-Sulphuric Acid	Under UV 254 nm	4	0.10, 0.19, 0.26 (all dark grey and 0.63 (grey)
				Under UV 366 nm	4	0.10 (blue), 0.20 (blue), 0.34 (light blue) and 0.63 (blue)
				After derivatization	6	0.10, 0.19 (both brown), 0.26 (violet), 0.37 (brown), 0.63 (blue) and 0.85 (grey)

Table 7 : Regulatory Specifications for fruits of *P. longum* L. in different regulatory compendium.

Sl. No.	Quality Specification	Ayurvedica Pharmacopoeia of India (API)	Siddha Pharmacopoeia of India (SPI)	India Pharmacopoeia IP 2007, 2010
1.	Official Title	Pippali	Tippili	Pippali, Large
2.	Botanical Species	<i>Piper longum</i> L.	<i>Piper longum</i> L.	<i>Piper retrofractum</i> Vahl.
3.	Morphological part/ Official part	Dried immature fruits	Dried immature fruits	Fruit Spike
4.	Description	I. Macroscopic II. Microscopic III. Powder	I. Macroscopic II. Microscopic III. Powder	I. Macroscopical II. Microscopical

5.	Identity, Purity & Strength			
	Foreign Matter	2.0%, Not more than	2.0 %, Not more than	2.0%, Not more than
	Total Ash	7.0% Not more than	7.0% Not more than	8.0%, Not less than
	Acid insoluble ash	0.5%, Not more than	0.5%, Not more than	3.0%, Not more than
	Alcohol soluble extractive	5.0%, Not less than	5.0%, Not less than	8.0%, Not less than
	Water soluble Extractive	7.0%, Not less than	7.0%, Not less than	10.0%, Not less than
	Loss on Drying	---	---	12.0% , Not more than
6.	Assay	---	---	1.0% piperine, not less than
7.	Heavy metals	---	---	Compliance with prescribed limit
8.	Microbial contamination	---	---	Compliance with prescribed limit
9.	Thin layer chromatography	TLC profile	TLC profile	TLC profile



B. Fruits



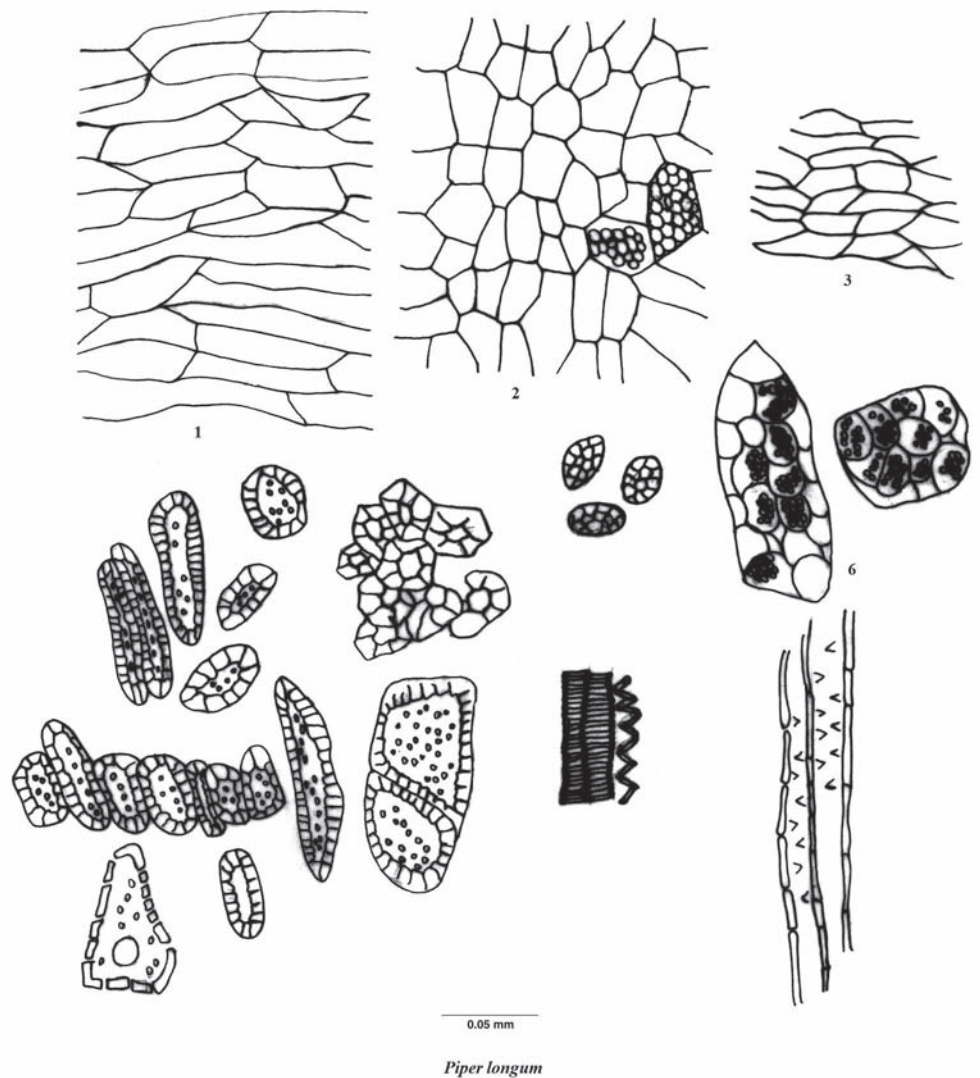
A. Fruiting Plant



C. Fruits (Magnified)



D. Fruit Powdered



1. Outer layer of seed-coat, 2. 3. Epicarp in surface view showing starch granules in some of the cells, 4. Stone cells, 5. Endosperm cells containing starch granules, 6. Xylem vessels, 8. Tracheids.

Figure-1

Discussion

The fruits of *Piper longum* L. are used in a number of classical and patent and proprietary formulations of Unani, Ayurveda and Siddha preparation. It is also most commonly used as a spice. Pharmacopoeia provides its specification in respect of macro-morphology, micro-morphology, physico-chemical constants (total ash value, alcohol insoluble, water soluble extractive and alcohol soluble extractive), assay (essential oil limits) and Thin layer chromatography. In the present study pharmacognostic standardization of ripe fruit of *Piper longum* L. is carried out which can be employed in quality control of *Piper longum* L. used either as drug or spice or as other commodity in commerce. It also provides review on different aspects of drug.

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