



## PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDY OF *PIPER LONGUM* L. AND *PIPER RETROFRACTUM* VAHL.

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### ABSTRACT

Piperine, an alkaloid with diverse biological activity commonly occurring in fruits of *Piper* sp., has high commercial, economical, and medicinal value. In this study densitometric estimation of piperine from fruits of *Piper longum* L and *Piper retrofractum* Vahl. It can be stated from this study that at industrial level *P. longum* Lin. is more suitable because of its preservation less and high percentage of Piperine (3.26µg/ml) than *P. retrofractum* Vahl. (0.49µg/ml) pharmacognostic and phytochemical characters of both species is discussed in present study.

**KEY WORDS:** Piperine, *Piper longum*, *Piper retrofractum*, HPTLC.

### INTRODUCTION

The country like India has got variety of climatic conditions and seasons favorable for growth of many species of plants. *Piper longum* L. popularly known as Pippali belonging to the family Piperaceae, an important medicinal plant is used in traditional medicine in Asia and Pacific islands especially in Indian medicine. The plant is a native of Indo-Malaya region. It was very early introduced to Europe and was highly regarded as a flavour ingredient by the Romans. The Greek name "Peperi", the Latin "Piper" and the English "Pepper" were derived from the Sanskrit name "Pippali". It grows wild in the tropical rain forests of India, Nepal, Indonesia, Malaysia, Srilanka, Rhio.

*P. longum* is a highly valuable drug and is one of the essential ingredients in the most of the compound preparations included in Ayurvedic literature. Piper are among the important medicinal plants used in various systems of medicine<sup>1,2</sup> Ayurvedic Formulary of India Pippali is being used in 324 formulations where as Sunthi is being used in 260 formulations as an ingredient. It is also one of the ingredient of a frequently used formulations i.e. Trikatu churna, Chatuh-Shashti-Prahari Pippali churna in Ayurvedic practice<sup>3</sup>.

*P. longum* is a component of medicines which is reported as good remedy for treating gonorrhoea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, chronic gut-related pain and arthritic conditions. Other reported beneficial effects of *P. longum* include analgesic and diuretic effects, relaxation of muscle tension and alleviation of anxiety. Since a long time *P. longum* has been used to possess immunomodulatory and antitumor activity.<sup>4</sup> Pharmacological and clinical studies have revealed that piperine has also been shown to enhance the bioavailability of several drugs, for example sulfadiazine, tetracycline, streptomycin, rifampicin, pyrazinamide, ionized, ethambutol, and phenytoin<sup>5</sup>.

In general at commercial level there is another variety *P. retrofractum* Vahl. also used as the name of *Badi Pippali*. It is also have therapeutic importance but at commercial point of view it has more price than *P. longum* and it is hard to store

in pharmacy and also it has low percentage of piperine content<sup>6</sup>.

*P. retrofractum* or long *Cavya* in Sanskrit pepper is originally from South East Asia and is mostly cultivated in Indonesia and Thailand. The used plant part is the fruit, the tiny berries, which merge to a single, rod-like structure which bears some resemblance to catkins (flowers of trees like hazelnut or willow). The sensory quality of *P. retrofractum* is hot and warm, with sweet overtones. It has been used in traditional medicine as a stimulant, carminative, tonic and for postpartum women (fruit); antihypertensive and relaxes muscles (plant); antifungal (stem); and colic (root). The main constituent is Piperine and content is slightly higher than in black pepper (about 6%). This active compound derived from the fruit of *P. retrofractum* also showed insecticidal activity against larvae of plants insects<sup>7</sup> and anti microbial activity. The extract of *P. retrofractum* has been known to have insecticidal activity to *P. xylostella* and *C. pavonana*.<sup>8</sup>

### MATERIAL AND METHOD

Three samples were taken two samples of *P. longum* Lin. Fig. no.1. and *P. retrofractum* vahl. (Fig.no.1) collected in Pharmacy G.A.U Jamnagar and Young fruits were collected from Plakkad Kerala, India fig. No.3.

### Physicochemical Evaluation

In physical evaluation, Loss on Drying, Ash Value, Water soluble Extractive, Methanol Soluble Extractive, Dichloromethane Soluble Extractive Refractive, pH Value are studied as per standards<sup>9</sup>. Extracts obtained by exhausting crude drugs are indicative of approximate measures of certain chemical compounds they contain, the diversity in chemical nature and properties of contents of drug. The determinations were performed by preparing three batches (Table no.3).

### Preparation of standard Piperine solution

Piperine standard (2mg) was accurately weighed and transferred to 2ml of volumetric flask. Crystals were dissolved in methanol and the volume was adjusted up to 10 ml with methanol (0.2mg/ml). It was prepared in different three concentrations respectively 5, 10, 15µg.

**Calibration curve for Piperine**

In Calibration graph was found to be linear over the concentration range 2- 9µg/spots. Linearity was evaluated by determining seven standard working solutions in duplicate. The peak area and concentration was subjected to least square linear regression analysis to calculate the calibration equation  $Y=841.16+1086.46X$  and regression coefficient (r<sup>2</sup>) was 0.997(Fig. no. 3).

**For HPTLC investigation**

A Camag HPTLC equipped with a sample applicator Linomat V, twin trough Fig. development chamber, TLC Scanner III, Reprostar and Wincats 4.02, integration software (Switzerland). and stationary phase precoated silica gel 60F 254 were used.

**Chromatographic Conditions:**

Application mode: Camag Linomat V, Hamilton syringe  
 Development chamber: Camag Twin Through Chamber (20x10cm<sup>2</sup>)  
 Fig.s: Precoated silica Fig.  
 Chamber saturation: 30 min  
 Development distance: 8.5 cm  
 Development time: 30 min  
 Scanner: Camag Scanner II (Ver. 3.14)  
 Detection : Tungsten lamp.  
 Photo Documentation : Camag Reprostar  
 Data System : Cats soft ware (Ver. 3.17)

**RESULT AND DISCUSSION:**

After observing under microscope stalked mushroom shaped bracts were observed with clearly differentiation between epicarp, mesocarp, oil zone Clearly developed vascular bundle differentiated with meta and proto xylem was also observed. Some comparative characteristics are shown in Table no.2, Fig. no.1.

By table no. 4 it observed that sensitivity of component is more in short UV as compared to long UV because more spot was found in all samples in short UV. More no. of

compound are traced out in matured fruits of *P.longum* as compared to Fresh *P.longum* and *P.retrofractum*. (table no.4) By this profile it is observed that Piperine is present in all samples and separated spot compared with Piperine fraction at R<sub>f</sub> value 0.64 in short UV (254 nm) found similar chemical properties (Suprime possible UV spectrum).

**Quantitative Estimation**

In the chromatogram of the drugs extracted from the Piper samples , many well resolved spots were observed, out of these spots one spot matched with the R<sub>f</sub> value shown by standard Piperine and having the same λ<sub>max</sub> 254 nm. Piperine amount was carried out by mathematical formula and it is shown in table no.5. Where amount of piperine is traced out more in matured fruits of *P.longum* as compared to Fresh *P.longum* and *P.retrofractum*.(Table no.5.)

As this study gives an practical verification behind the statement of *Acharya Sharnagdhara* where it was suggested to use *Pippali* (*Pipper longum*), *Marich* (*P.nigrum*) *Vidanga* (*Embelia ribes*),*Shada*(Honey),*Ghee*, *Dhanya* (Cereal) better to be use older<sup>10</sup> , Behind this methodology it might be possible that therapeutic activity and percentage of some chemical constituents increases. And in this study Piperine content found more in old Pippali than younger fruits. Table no.5.

**CONCLUSION**

In present study the pharmacognostical study reveals that the difference between morphology and microscopic whereas in phytochemical study reveals that When samples subjected for the quantitative estimation of piperine in different samples it vary the percentage of Piperine. On the basis of this study it can be stated that at commercial level Old matured fruits of *P. longum* should be used and it should not be adulterated with other verity of Pippali (*P.retrofractum*) which has lower amount of its main chemical constituent and also hard to store.

**Table no. 1**  
**Organoleptic characters:**

Sample	Test	Colour	Odour	Touch
<i>P. longum</i> (Fresh)	Less pungent	Greenish brown	Aromatic	Fine
<i>P. longum</i> (Old)	Strong Pungent	Brown	Aromatic	Fine
<i>P. retrofractum</i>	Moderate Pungent	Green	Strong Aromatic	Corse

**Table no. 2**  
Microscopic characters of spike of *Piper longum* L. is tabulated below

Characters	<i>Piper longum</i> (Fresh)	<i>Piper longum</i> (Old)	<i>Piper retrofractum</i>
<b>Bracts</b>	Sessile, well differentiated, containing parenchymateous tissue and oil globules	Sessile, present near the margin of peduncle, Convex shape, Composed of parenchymateous tissue and volatile oil.	Not well differentiated, merge with Epicarp, Irregular shaped Parenchymateous tissue and volatile oil
<b>1. Epicarp</b>	Outer most layer with very thin layer of cuticle with polygonal shaped cells	The outermost single layer covered with cuticle, Polygonal shaped cells	The outermost single layer covered with cuticle. Polygonal shaped cells
<b>3.Hypoderm</b>	2-6 tangentially elongated parenchymatous cell with stone cells	2-4 tangentially elongated parenchymatous cell with large number of stone cells.	2-4 tangentially elongated parenchymatous cell, stone cells absent
<b>3. Mesocarp</b>	Broad zone of parenchyma cells with plenty oil globules	Consist broad zone of parenchyma cells with oil globules and alurone grains.	Consist broad zone of parenchyma cells with oil globules
<b>4. Oil zone</b>	Composed of 2-5 rows of angular embedded oleoresin cells which are oval to circular in shape	Composed of 2-5 rows of angular embedded oleoresin cells which are oval to circular in shape.	Poorly developed 1-2 layers
<b>5. Innermost mesocarp</b>	With very less no. of starch grains	Mesocarp cells filled with starch grains and are smaller in size.	Mesocarp cells filled with starch grains and are smaller in size.
<b>6. Endocarp</b>	2-3 layer light yellow to brown colour cells	Single layer narrow elongated dark brown content and thickened.	Single layer narrow elongated dark brown content with pointed end
<b>7.Spermoderm</b>	Well developed ovule attached with endocarp	Ovule is attached to the inner wall of the endocarp. Ovule is brown in colour.	Poorly developed ovule
<b>8. Vascular bundle</b>	Well developed, clearly differentiated with Proto-xylem and meta-xylem	Well developed, clearly differentiated with Proto-xylem and meta-xylem	Showing meta and proto xylem with phloem

**Table no. 3**  
Physicochemical results

Parameters	<i>P. longum</i> (Fresh)	<i>P. longum</i> (Old)	<i>P. retrofractum</i>
Loss on Drying	12.20	9.20	8.90
Ash Value	2.50	5.50	1.23
Water soluble Extractive	5.00	12.00	21.34
Methanol Soluble Extractive	7.10	9.10	18.28
pH Value	5.89	5.43	5.23

**Table no. 4**  
HPTLC of Standard Piperine and different samples of Pippali

Track	Under 254nm (Short UV)		Under 366nm (LongUV)	
	No. of spots	Rf Value	No. of spots	Rf Value
<b>1. <i>P.longum</i> (old)</b>	2	0.03,0.63	3	0.03,0.23,0.62
<b>2. <i>P.longum</i> (fresh)</b>	3	0.03,0.62,0.83	3	0.12,0.81,0.63
<b>3. <i>P. retrofractum</i></b>	9	0.03,0.13,0.21,0.29, 0.37,0.45,0.53,0.60,0.84	8	0.03,0.13,0.21,0.29, 0.37,0.45,0.53,0.60
<b>4.Piperine (2.5%)</b>	3	0.13,0.46,0.59	4	0.03,0.13, 0.45,0.59
<b>5.Piperine (5%)</b>	3	0.13,0.46,0.61	4	0.03,0.14,0.46,0.59
<b>6. Piperine (7.5%)</b>	4	0.46,0.60,0.86	4	0.04,0.014,0.046,0.60

**Table no. 5**  
Amount of Piperine

S.no	Sample	Amount of piperine (µg/ml)
<b>1.</b>	<i>P. longum</i> (fresh)	0.76
<b>2.</b>	<i>P. longum</i> (old)	3.26
<b>3.</b>	<i>P. retrofractum</i>	0.49



Fig. no. 1  
Microscopy of different samples of pippali

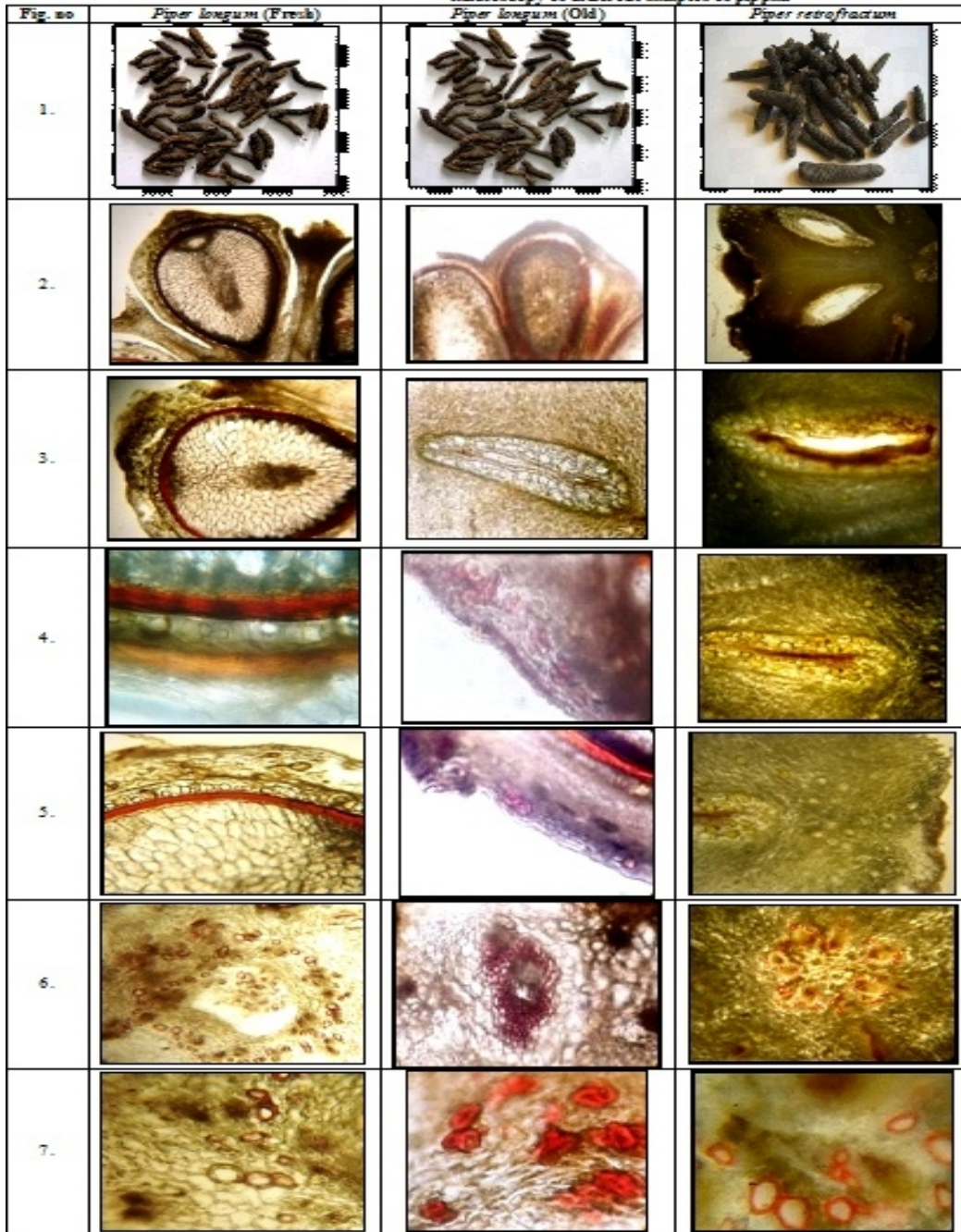


Fig. no. 2  
HPTLC Fig. 2 in different conditions

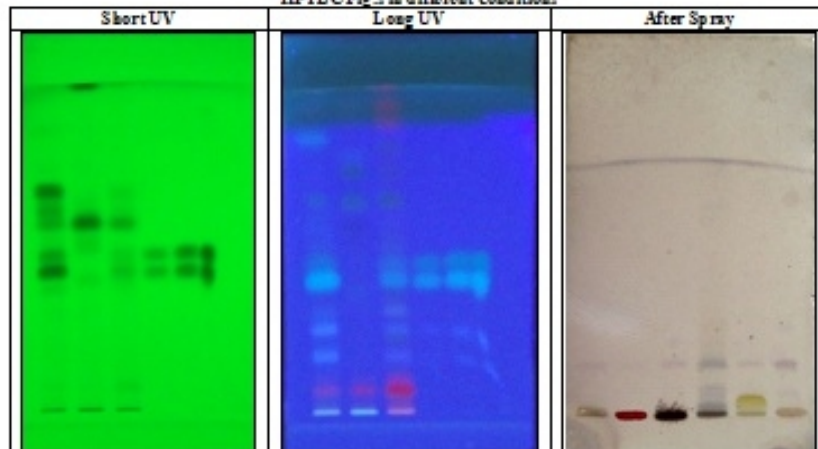
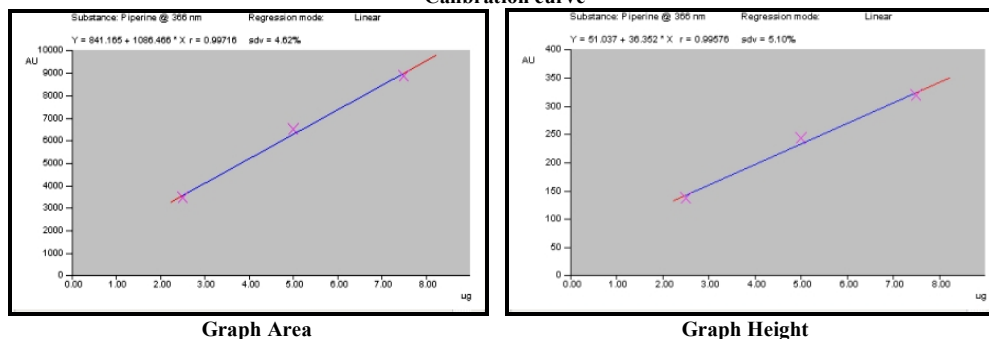


Fig. no. 3  
Calibration curve



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