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Pharmacognostical and phytochemical analysis of Pippali (*Piper longum* Linn.)

Sadhana Singh, Apurva Priyadarshi, Brijesh Singh and Poonam Sharma

Abstract
Ayurveda is the science and philosophy of life. *Ayurveda* reveals that we had a rich knowledge of the application of medicinal plants from very ancient times. Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served human as well as valuable components of medicines, seasoning, beverages, cosmetics and drugs. In this study Botanical description, chemical Constituents, Ayurvedic Properties of Pippali, medicinal properties, formulation, dose, and pharmacological activity of *Piper longum* Linn. are described. The present work deals with the pharmacognostical and preliminary phytochemical studies on Fruit of *Piper longum* Linn. Macroscopical and Microscopical Characters, physico-chemical constants, quantitative microscopy parameters, extractive values, TLC and HPTLC were studied. Preliminary phytochemical screening on Fruit of *Piper longum* Linn. were studied. The determination of these study will help future researchers in their Phytochemical as well as Pharmacological evaluation of this plant.

Keywords: *Piper longum* Linn. preliminary phytochemical, pharmacognostical study

Introduction

Botanical Description

Habitat: It occurs in the hotter parts of India, from the central Himalayas to Assam, the khasi & the mikir hills, the lower hills of Bengal [1] & the evergreen forests of the Western Ghats from konkan to Travancore

Habit: A slender, aromatic climber with perennial woody roots.

Root: Root stock erect, thick, jointed, and branched.

Stem: Stems numerous, 0.6 -0.9m, ascending or prostrate (not climbing) much branched, stout, cylindrical, thickened above nodes, finely pubescent.

Leaves: Ovate, cordate, subacute, entire, glabrous.

Flowers: Flowers are unisexual, minute, sessile bracteate without perianth very densely packed in spikate inflorescences. The male & female spikes are on separate plants.

Fruit: Ovoid, yellowish orange, sunk in fleshy spike [2].

Pippali of Pippali

Dry fruit

Chemical Constituents

Two alkaloids piperlongumine & piperlonguminine, n-hexadecane, n- heptadecane, n octadecane, n- nonadecane n-eicosane, n-heneicosane, - thujene, terpinolene, zingiberene, p-cymene, p-methoxy acetophenone, traces of dihydrocarveol, phenyl ethyl alcohol & two sesquiterpenes; piperine, piplartin, triacontane, dihydrostigmasterol, an unidentified setroid, reducing sugar, glycosides, sesamin & methyl- 3.4.5-trimethoxycinnamate (root) ; major alkaloid piperine & sesamin (stem & fruit); sesquiterpene hydrocarbon, caryophyllene, a sesquiterpene alcohol, carbonyl compound (essential oil), N-isobutyldeca- trans- 2-trans- 4- dienamide, piperine, piplartine & a lignan d- sesamin, two piperidine alkaloids pipernonaline & piperundecalidine (fruit), sylvatin sesamin & diaeudesmin (seed) [2].

Ayurvedic Properties of Pippali [3]

Rasa : Katu, Tikta, Madhura.
Guna : Laghu, Snigdha.
Virya : Anushna.
Vipaka : Madhura.
Doshakarma : Kapha vata shamaka

Rogaghnata

Kasa, Shvasa, Hikka, Aruci, Agnimandya, Ajirna, Vibandha, Gulma, Udara vikara, Arsha, Yakridvikara, Pliha vriddhi, Krimiroga, Hriddourbalya, Pandu, Raktavikara, Amvata, Vatarakta, Kshaya, Yakshma, Shukradourbalya, Rajorodha, Kashtaprasava, Kushtha, Jirna jvara, Vishamajvara, Dourbalya[5,6,7,8].

Karma

Kasahara, Shvasahara, Hikkanigrahana, Rasayana Medhya, Vatahara Mutrala, Dipana, Vatanulomana, Shulaprashamana, Mridurecana, Krimighna, Raktotkleshaka, Jantughna, Shrovirecana, Plihavriddhihara, Yakriduttejaka, Vishamajvara, Raktotkleshaka, Vrishya, Balya, Garbhashyasamkocaka [8].
Prayojyanga : Phala, mula [10]
Dose : Curna 500mg-1gm [10]

Pharmacological activities

Antibacterial, anti-inflammatory, insecticidal, antimalarial, CNS stimulant, ntitubercular, anthelmintic, hypoglycemic, antispasmodic, cough suppressor, antigiardial, immunostimulatory, hepatoprotective, analeptic, antinarcotic, antiulcerogenic.

Formulations (Yoga)

Gudha Pippali, Pippalyasava, Pippali khandha, Pippalyadi leha, Pippalyadi curna, Pippali rasayanam, Vardhamana Pippali, Pippali ghritam.

Propagation and cultivation

Piper longum is cultivated on a large scale in limestone soil, 450-600m below the Cherrapunji region which receives very heavy rains from the end of March to the middle of September and where the relative humidity is high. Long pepper is cultivated mainly by layering of mature branches or by suckers planted at the beginning of the rainy season. The vines are well manured with cowdung cake and start bearing 3-4 years after planting. The spikes are harvested in January, while still green and unripe, as they are most pungent at this stage. They are dried in the sun when they turn grey.

Plant regeneration from callus cultures of *Piper longum* Linn. was achieved through organogenesis. *In vitro* grown shoots were used as explants for callus injunction. Competent callus was initiated around the nodal ring of tissue using Murashige and Skoog's medium supplemented with 1.0 mg/1œ-naphthaleneacetic acid and 0.2 mg/1, N-benzyladenine. Optimum growth regulator concentration for shoot induction and shoot elongation were found to be 0.5mg/1 indole -3-acetic acid with 1.5mg/1benzyladenine and 0.1mg/1 indole-3-acetic acid with 0.2 mg/1 benzyladenine respectively. Elongated shoots were rooted on half strength Murashige and Skoog's medium having 0.1mg/1 indole-3-acetic acid. The rooted plantlets were successfully established in soil.

Morphogenetic potential of root, leaf, node and internode explants of P. longum has been reported. The highest number of shoot buds was produced on root explants followed by node, internode and leaf explants. Benzyladenine was suitable for shoot induction and its optimum concentration is 1-2 mg /l.

Material and Method

Microscopical Characteristics of Powdered Pippali (Friut)

Fragments of thin walled cell and starch grains were seen from the powder of PippalÍ fruit. Coarse powder of dull dark brown to black in colour. In microscopic powder study it shows Lignified sclerenchymatous cells of pink to purple colour and calcium oxalate crystals of grey in colour. Aleurone grains of green colour and oil globules of orangish pink stain

S. No.	Reagents	Observations	Characteristics
1.	Phloroglucinol+Conc. Hcl	Pink	Lignified sclerenchymatous cells
2.	Dil. Iodine solution	Blue	Starch present in perisperm
3.	Dil. Sulpuric Acid	White	Calcium oxalate crystals
4.	Alcoholic Picric Acid	Yellow	Starch grains
5.	Sudan Red III	Red	Oil globules and cuticles.

Table 1: Certificate of Analysis of Pippali

S. No.	Parameters	Observation
I	**Physical tests**	
	Nature	Coarse powder
	Colour	Dull dark brown to black
	Odour	Characteristic
	Taste	Bitter
II	Foreign matter	Nil
III	Moisture content (w/w%)	9.34
IV	Ash value (% w/w)	
	Total ash	4.4
	Acid insoluble ash	0.05
	Water soluble ash	2.077

Table 2: Percentage yield of Extracts of Pippali

S. No.	Extracts	Nature of Extract	Weight (gm)	% Yield w/w
I	Hydro-alcohol	Viscous	18.117	18.117

Preliminary phytochemical testing of Pippali (*Piper longum* Linn.)
Genuine sample of Pippali gave the presence of following Phytochemicals:

Phytoconstituents	
Alkaloids	+
Glycosides	-
Flavonoids	-
Steroid	-
Phenolic & tannins	-
Terpenoid	-
Sterol	+
Carbohydrates	-
Proteins	+
Amino Acids	-

(+) indicate present, (-) indicate absent

Thin Layer Chromatography (TLC) of Extracts
TLC or Thin Layer Chromatography is a type of planar chromatography. TLC is routinely used by researcher in the field of phyto-chemicals, biochemistry etc. to identify the components in a compound mixture like alkaloids, phospholipids, amino acids etc. It is a semi quantitative method of analysis and its sophisticated version or highly precise quantitative version is High performance thin layer chromatography (HPTLC). Similar to other chromatographic methods TLC is also based on the principle of separation. The separation depends on the relative affinity of compounds towards stationary and mobile phase. The compounds that under the influence of mobile phase (driven by capillary action) travel over the surface of stationary phase. During this movement the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus separation of components in the mixture is achieved. Once separation occurs individual components are visualized as spots at respective level of travel on the plate. Their nature or characters are identified by means of suitable detection techniques. TLC System consists of a TLC plates preferably readymade with stationary phase: These are stable and chemically inert plates on to whose surface a thin layer of stationary phase is applied. The stationary phase on the plates is of uniform thickness and consists of fine particle size.
R_f value= a/b
= Distance travelled by the solute/ Distance travelled by the solvent

a= Distance travelled by the solute
b= Distance travelled by the solvent

TLC of Hydroalcoholic extract of Pippali
A mixture of 6 ml of Toluene, 6 ml of Ethyl acetate, 1.8 formic acid, 0.25 methanol.

Heat: Heat at 110 °C for 10 minutes and examines the plate under day light

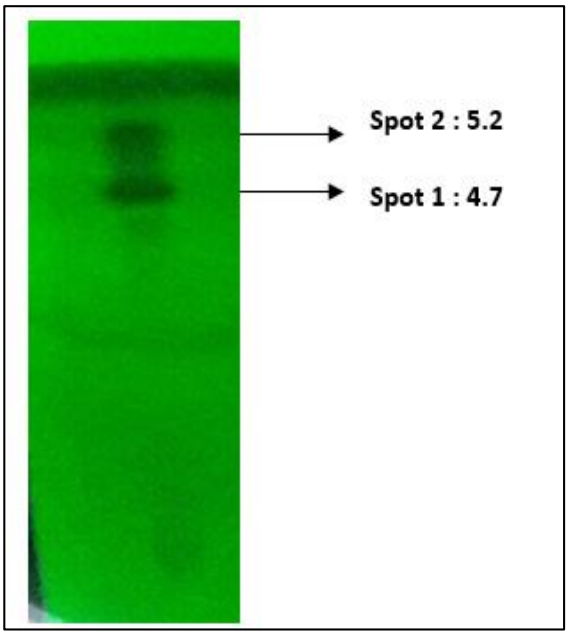

Fig 1: Tlc Fringer Print of Hydroalcoholic Extract of Pippali

For Spot 1

$$\text{Rf Value} = \frac{4.7}{5.5} = 0.8$$

For Spot 2

$$\text{Rf Value} = \frac{5.2}{5.5} = 0.94$$

Fig 2: HPTLC (High Performance Thin Layer Chromatography)

Methodology
- 0.3g of extract was dissolved with 1 ml of water and 1ml of ethyle alcohol and 3, 6 and 9µl of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator.
- The plate was developed in Toluene: Ethyl-acetate: formic acid: methanol (6:6:1.8:0.25). The developed plates were visualized in UV 254 and 366 and scanned under UV 254 and 366 nm. R_f, of the spots and densitometric scan were recorded.

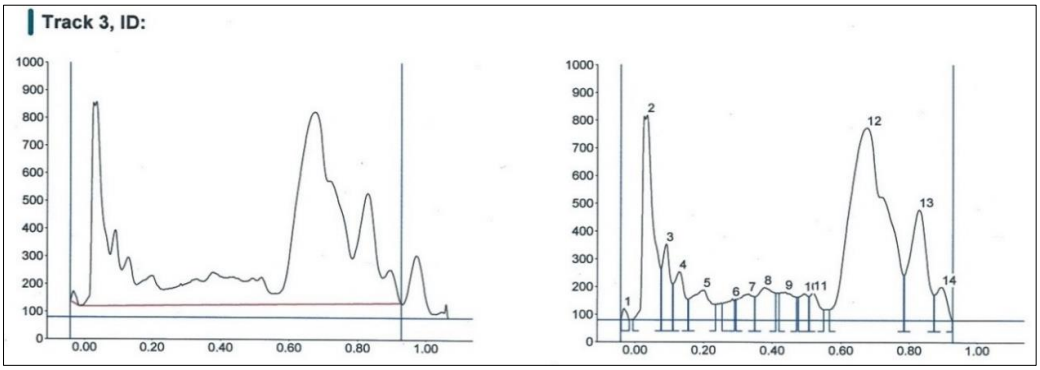

Fig 3: HPTLC chromatogram of hydroalcoholic extract of Pippali (Fruit)

Table 3: HPTLC chromatogram *of* Hydroalcoholic extract of Pippali

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.04	6.1	-0.03	40.7	1.30	-0.02	0.5	427.4	0.32
2	-0.01	1.5	0.04	738.3	23.61	0.08	184.3	19224.6	14.49
3	0.08	186.0	0.09	273.6	8.75	0.11	128.5	5149.0	3.88
4	0.11	129.5	0.13	174.6	5.56	0.16	74.5	4207.4	3.17
5	0.16	74.5	0.20	107.1	3.44	0.24	56.1	4856.6	3.66
6	0.26	60.1	0.28	76.1	2.43	0.29	71.5	1805.7	1.36
7	0.30	75.1	0.33	93.8	3.00	0.35	84.1	3425.3	2.56
8	0.35	84.2	0.38	117.3	3.75	0.41	97.7	4566.8	3.44
9	0.42	96.7	0.44	100.7	3.22	0.57	83.6	3571.5	2.69
10	0.48	84.0	0.49	95.3	3.05	0.51	86.6	2059.5	1.55
11	0.51	86.9	0.52	98.5	3.15	0.55	39.1	2217.6	1.67
12	0.57	38.8	0.68	693.4	22.17	0.79	163.5	61664.4	46.48
13	0.79	164.0	0.83	397.8	12.72	0.88	93.1	16281.7	12.27
14	0.88	93.3	0.90	120.1	3.84	0.93	1.5	3217.1	2.42

Discussion and Conclusion

Pharmacognosy may be defined as a branch of bioscience which treats in detail medicinal and related product of crude or primary type obtained from plant, animal and mineral origins. In short it is an objective study of crude drugs from natural sources treated scientifically and it encompasses the knowledge of the history, distribution, cultivation, collection, processing for market and preservation, the study of sensory, physical, chemical, and structural characters and the uses of crude drugs [10].

The macroscopic and microscopic identifying characters of useful part of Pippali was included from the Ayurvedic Pharmacopoeia of India monograph considering the legal document of the Government of India. Powder microscopy of Fruit showed the presence of Fragments of thin walled cell, starch grains, Lignified sclerenchymatous cells, calcium oxalate crystals, Aleurone grains and oil globules. On physiochemical analysis, the moisture content was found in-Pippali 9.34%. The Total ash was found 4.4%; Acid insoluble ash was 0.05%, and; water soluble ash was 2.077% in fruit of Pippali. The phytochemical investigation shows the presence of alkaloid, sterol and protein in fruit of Pippali. The TLC and HPTLC was performed and the developed plates were visualized in UV 254nm, 366nm, which are presented in previous picture as TLC finger print as well as HPTLC chromatogram of hydro- alcoholic extract of Pippali. This study will help both physicians and researchers in their respective fields, either for the treatment of diseases or in the field of research.

References

1. The Ayurvedic Pharmacopoeia of India, First edition, New Delhi; Govt. of India, 'Ministry of Health and Family welfare' Department of Indian Systems of Medicine and Homeopathy, 1999, 133-134.
2. Database on Medicinal Plants Used in Ayurveda, by PC Sharma, MB Yelne and TJ Dennis, Central Council For Reseach In Ayurveda & Siddha, Reprint Janakpuri, New Delhi, Part I, 2005; II(3):472
3. Bhavaprakasha Nighantu of Bhavamishra, Commentary by Krishnachandra Chunekar, Edited by GangÁsahÁya Pandey, Chaukambha Bharati Academy, Varanasi, Reprint, 1999, 15.
4. Caraka Samhita. of Agnivesa, elaborated by Caraka and Dridabala, Edited with 'Caraka-Candrika' Hindi commentary along with special deliberation by Dr. Brahmanand Tripathi, Chaukambha Surbharati Prakashan, Varanasi, 3rd Edition, 1994.
5. Sushruta Samhita. Edited with Ayurveda Tattva-Sandipika by Kaviraja Ambika Dutta Shastri; Chaukambha Sanskrit Sansthan, Varanasi, 5th edition, 1982
6. Ashanga. Samgraha of Vagbhata with Indu Vyakhya by Prof. Jyotir Mitra, Chaukambha Sanskrit Series, Varanasi, 13th Edition, 2008.
7. Ashtanga Hridaya of Vagbhata, Translated by Prof. K.R. Srikantha Murthy, Krishnadas Academy, Varanasi, 3rd Edition, 2000.
8. Dravyaguna Vigyana by Dr. Gyanendra Pandey, Chaukhambha Krishanadas Academy, Varanasi, Reprint 2004; 3:116-122.
9. Dravya Guna Vigyana by P.V. Sharma Chaukhambha Bharati Academy, Varanasi, Reprint 2003; II:278.
10. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, Fiftieth Edition, Nirali Prakashan, Shivaji Nagar, Pune, 2014, 1.3