Research Article

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COMPARATIVE PHARMACOGNOSTICAL, PHYTOCHEMICAL AND HPTLC STUDY OF SOME COMMON MEDICINAL PIPER SPECIES

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ABSTRACT

Piper species are reported to have great medicinal values in Indian system medicines. The present study was therefore carried out to provide the requisite pharmacognostic, phytochemical and HPTLC study of the four piper species namely *Piper longum, Piper nigrum, Piper cubeba and Piper retrofractum*. The marker compound present in all these four species is Piperine, an alkaloid found naturally in plants belonging to the pyridine group of Piperaceae family. This study will be the ready reference for the correct identification of the four crude drugs.

Keywords: *Piper longum, piper nigrum, Piper cubeba and Piper retrofractum,* piperine, HPTLC.

INTRODUCTION

Plants have been the source of medicines for thousands of years. Species of the genus piper are among the important medicinal plants used in Traditional systems of medicine. Piper Species are widely distributed in the tropical and subtropical regions of the world and have high commercial and economic potential. A country like India has got a variety of geo-climatic conditions and seasons favourable for the growth of many plant species.[1] The family Piperaceae comprises of 12 genera and about 1400 species mainly found in the tropical region. The genus Piper contains more than700 species, grow in the southern part of India are economically important and used in various systems of medicine. Several species of Piper are used in the Indigenous system of medicine in India. [2] Piperine ($C_{17}H_{19}NO_3$) is the main alkaloid present in the fruits and roots of these species. Piperine has been reported from 19 Piper species and more than 600 secondary metabolites are isolated from the genus Piper.[3] Piperine has diverse biological activities and responsible factor for the pungency of the useful parts. Piperine has a broad spectrum of activities. It increases the bioavailability of other drugs by enhancing their absorption from the gut. It also displays analgesic- antipyretic, anti-inflammatory, growth stimulatory, anti-thyroid, chemopreventive, insecticidal, immune-modulator, antitumor, anti-depressant and anti-apoptotic activities.[4]

The present study reveals relevant pharmacognostic, phytochemical and HPTLC study of four piper species namely *Piper longum, Piper nigrum, Piper cubeba and Piper retrofractum* commonly used in Traditional systems of medicine.

Piper longum Linn or Pippali consists of dried fruits, a slender, aromatic, perennial herb, native of the hotter parts of the country and found wild as well as cultivated extensively in Assam, lower hills of Bengal, evergreen forests of western ghats, along the west coast and southern states of India. Fruits are harvested in January. The fruits are useful in cough, asthma, bronchitis and other respiratory disorders.[5]

Black pepper contains fully mature dried fruits of *Piper nigrum* L., a climber, cultivated from Konkan southwards, especially in North Konkan, Kerala and also in Assam of India. Fruits ripen from December-March depending upon climate conditions, Fruits harvested from December to April. Pepper is useful for digestive and respiratory disorders.[6] *Piper cubeba* (cubeb) or tailed pepper, a plant in genus Piper, cultivated for its fruit and essential oil. It is mostly grown in Java and Sumatra, hence sometimes called Java pepper. It is a perennial plant, with a climbing stem, round branches, about as thick as a goose-quill, ash-colored and rooting at the joints. Fruits are useful for halitosis, dental and gum disorders.[7]

Chavya is named as *Piper Chaba or Piper retrofractum* belongs to the family Piperaceae. Its root is considered as Chavya or Gaja Pippali. It is useful for digestive disorders and hemorrhoids. In all these four species, the major chemical constituent is alkaloid piperine.[8]

Chemical Constituents

The fruit of *P.longum* contains a large number of alkaloids in which the major is piperine. The piperine content is 3-5% (on dry weight basis) in *P.longum*. The other alkaloids and gvamides are methylpiperine, iperonaline, piperettine, asarimine, pellitorine, piperundecalidine, piperlongumine, piperlonguminine, refractomide, pregumidiene, brachystamide, brachystamide-A, brachystine, pipercide, piperderidine, longamide and tetrahydropiperine, tetrahydropiperlongumine, dehydropipernonaline, piperidine and tri-methoxycinnamoyl-piperidine. Some lignans like sesamin, pulvuatilol, forgesin and others have been isolated from the fruit of *P.longum*. The fruit of *P.longum* contains some esters like tridecyl-dihydro-p-coumarate, eicosanyl-(E)-p-coumarate. and Z-12-octandecenoic–glycerol-monoester.[9] The essential oil of the fruit *P. longum* is a complex mixture, the three major components of which are (excluding the volatile piperine) caryophylleneandpentadecane (both about 17.8%) and bisaboline (11%).Others include thujine, terpinoline,

zingiberine, pcymene, p-methoxyacetophenone, and dihydrocarveol.[10]

The fruit of *P.nigrum* also contains a large number of alkaloids like *P. longum*in which the major alkaloid is Piperine (3.15%-4.82%) and another is piperitone. Piperine is the Trans stereoisomer of 1-Piperoyl piperidine. This alkaloid is responsible for the pungency of black pepper. The other chemical constituents are betapinene, limonene. Furthermore terpinene, alpha-pinene, myrcene, delta3-carene and monoterpene derivatives (borneol, carvone, carvacrol, 1,8-cineol, linalool). Black pepper contains a volatile oil (including beta-bisabolene, camphene, beta-caryophyllene, and many other terpenes and sesquiterpenes), up to 9% alkaloids (especially piperine, largely responsible for the herb's acrid taste), about 11 % proteins, and small amounts of Minerals.[7] Black pepper is an excellent source of Manganese, a very good source of iron and vitamin K and a good source of dietary fiber.[11] Through GC-MS, different components isolated from the oil of hot petroleum ether extract of *P.nigrum*(Fruit) are methyl benzene,δ–elemene, α–copane, caryophyllene, α–caryophyllene, β–bisabolene and δ–cadinene.[12]

The dried cubeb fruits mainly contain the alkaloids, lignans, and essential oil. The major alkaloid is piperine and essential oil consisting monoterpenes (sabinene 50%, α-thujene, and carene)and sesquiterpenes (caryophyllene, copaene, α- and β-cubebene, δ-cadinene, germacrene), the oxides 1,4- and 1,8-cineole and the alcohol cubebol.

The root of Chavya (*Piper retrofractum)* contains piperine 6.55-0.18%, pippalotin (0.13-0.20%), piperceaguminin, sterol, and glycoside.[8]

MATERIALS AND METHODS

Plant collection

The raw materials of all these four species were procured from local market of Kolkata, West Bengal and authenticated by Botany department of the Institute as per usual norms.[13] Dried materials of these species were used for pharmacognostic evaluation and some preliminary phytochemical tests. [14]

Extraction of plant material

Extraction was carried out at room temperature under normal condition. About 15 g shade dried powder of fruits of piper species were successively extracted with petroleum ether, chloroform, and ethanol.[15] The Extracts obtained were filtered and concentrated by evaporating on a water bath.

Phytochemical analysis

The extract was used for preliminary screening of phytochemicals such as alkaloids, tannins, flavonoids, terpenoid/steroid. The screening was done as per the standard method. [16]

HPTLC Study

A simple and convenient HPTLC method was developed for standardization of four piper species along with standard marker compound piperine.[17, 18] A CAMAG HPTLC system (Switzerland) comprising CAMAG Linomat5 applicator, CAMAG TLC scanner3, CAMAG wincats software, version 1.44, Hamilton Syringe (100µl), CAMAG Reprospar3, CAMAG TLC plate heater, CAMAG UV cabinet were used for the study. Silica gel ^{60} F_{254}Aluminium plates (Merck) was used as stationary phase. Toluene: Diethyl ether: 1-4 dioxane (14:5:4v/v) was used as mobile phase. The sample solution was prepared by taking 2 g of fine dust of each piper species separately and subjected to cold extraction with methanol solvent for 3days and extracts were filtered using filter paper (Whatman no. 40). Whole extracts were concentrated and used for HPTLC profile.

Standard solution of piperine was prepared by taking 1mg of piperine dissolved in 10ml of methanol in a volumetric flask. This solution was used for HPTLC study.

RESULTS

Pharmacognostical study
Piper longum L (Pippali)
Macroscopic

Fruits of Pippali i.e. *Piper longum*L. (Piperaceae) are very small, ovoid, completely sunken embedded in solid fresh spike, 2.5-4.0 cm long. The color of fruit in light green to olive green when immature. After ripening colour changed to shining blackish green with aromatic odour and pungent taste producing numbness on the tongue. Broken surface shows a central axis around which 5-7 fruit-lets are arranged. [Figure 1(a)]

Figure 1(a): Fruits of *Piper longum* L. (Pippali)

Powder Microscopy

Deep grayish brown to dark brown with a pungent odour and bitter acrid taste, shows abundant polyhedral, elongated parenchymatous cells from perisperm, oval to slightly elongated stone cells interspersed with thin walled polygonal hypodermal cells, oil globules and round to oval starch grains, measuring 3 to 9µmin diameter. [Figure 1(b)]

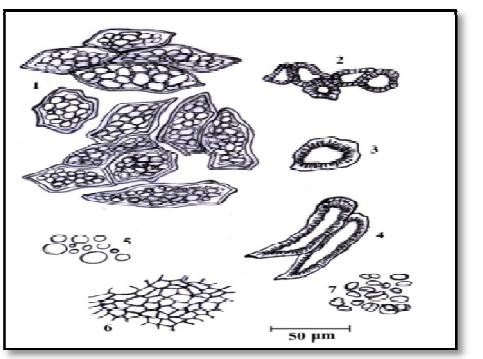


Figure 1(b): Powder microscopy of fruit of *Piper longum* L. (Pippali)

***Piper nigrum* L. (Maricha)**

Macroscopic

Fruits of Maricha i.e. *Piper nigrum*L. (Piperaceae) are cylindrical, matured, hard, wrinkled, grayish-black to black, 0.3-0.5 cm in diameter with specific pungent aromatic odour and pungent taste. [Figure 2(a)]

Figure 2(a): Fruits of *Piper nigrum* (Maricha)

Powder Microscopy

Colour blackish-grey with characteristic more or less isodiametric stone cells single and in groups, few groups of thick walled sclerenchymatous cells, thin-walled polygonal hypodermal cells, beaker-shaped stone cells from endocarp and abundant polyhedral elongated cells from perisperm, packed tightly with masses of starch grains,aleurone grains and oil globules. Starch grains are minute oval to round single or compound having 2-3 components. [Figure 2(b)]

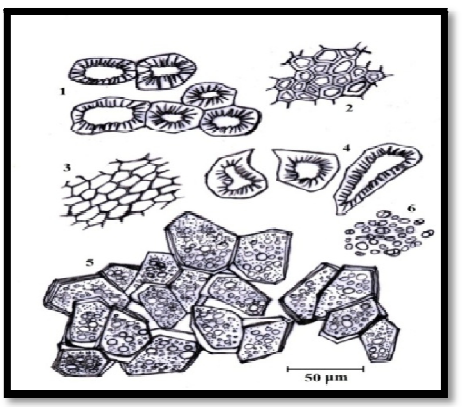


Figure 2(b): Powder microscopy of fruit of *Piper nigrum* L. (Maricha)

***Piper cubeba* Linn. F (Kankola)**

Macroscopic

Fruits of Kankola i.e. *Piper cubeba*Linn. F.(family Piperaceae) are hard wrinkled, cylindrical or rounded, 5-7 mm in diameter, grayish brown to dark brown to brownish black in color, attached with5-8 mm long stalk; pericarp light reddish brown fused with testa; albumen stony creams and oily; odour, characteristic, aromatic and pleasant with slightly bitter, pungent taste. [Figure 3(a)]

Figure 3(a): Fruits of *Piper cubeba* Linn. F (Kankola)

Powder Microscopy

Deep grayish brown to dark brown with characteristic, pleasant aromatic smell and slightly bitter pungent taste, shows small, brown and thick-walled cells, spiral xylem vessels, fibre, large, thin-walled parenchymatous cells in group, oil cells, heavily lignified sclereids with narrow lumen, few prisms of Ca-oxalate crystals, elongated hyaline cells of tegmen, group of greyish kernel cells with packed cell content, oval starch grains. [Figure 3(b)]

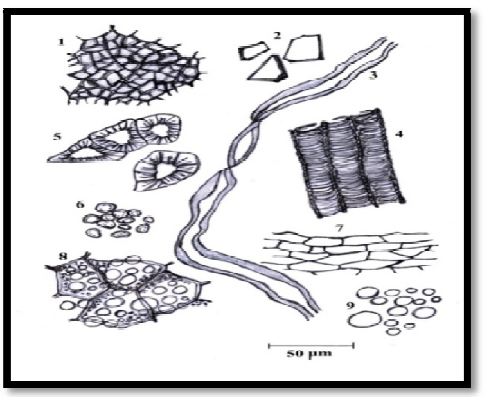


Figure 3(b): Powder microscopy of the fruit of *Piper cubeba* Linn. F (Kankola)

***Piper retrofractum* Vahl. (Chavya)**

Macroscopic

Dried cut pieces of the stem of Cavya or Cavika i.e. *Piper retrofractum* Vahl. (family Piperaceae) are of variable length and 0.5-2.0 cm in width, cylindrical and somewhat twisted and pressed, with distinct nodes and internodes, greyish-brown, surface smooth with a few longitudinal wrinkles, fracture, short; odour peppery with an acrid taste. [Figure 4(a)]

Figure 4(a): Fragmented stem of *Piper retrofractum* Vahl (Chavya)

Powder Microscopy

Greyish-brown shows fragments of pitted and reticulate vessels, tracheids, needle and spindle-shaped fibers and simple, oval to rectangular, thin-walled, parenchymatous cells with plenty of round to oval starch grains, measuring 3-14µ in diameter. [Figure 4(b)]

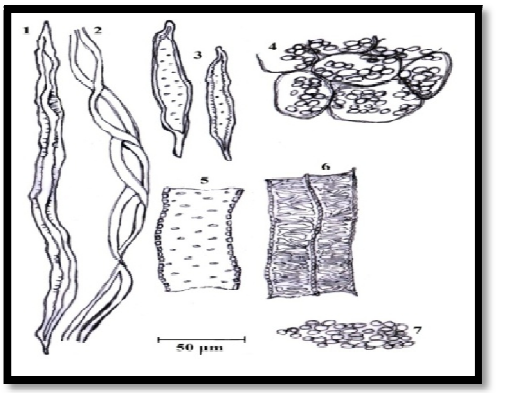


Figure 4(b): Powder microscopy of stem of *Piper retrofractum* Vahl (Chavya)

Phytochemical Screening

Phytochemical analysis of four piper species revealed the presence of alkaloids, tannins, flavonoids, terpenoid are shown in Table1-4.

Table 1: Preliminary Phytochemical screening of *P. longum*

Name of the test	Petroleum ether extract	Chloroform extract	Ethanol extract
Test of Alkaloid (Dragendorff's test)	+	+	+
Test of Flavonoids ($FeCl_3$ test)	+	+	+
Test for Tannins	+	+	+
Test for Terpenoid/Steroid (L.B test)	+ (for terpenoid) + (for steroid)	+	+

Table 2: Preliminary Phytochemical screening of *P.nigrum*

Name of the test	Petroleum ether extract	Chloroform extract	Ethanol extract
Test of Alkaloid (Dragendorff's test)	+	+	+
Test of Flavonoids ($FeCl_3$ test)	+	+	+
Test for Tannins	+	+	+
Test for Terpenoid/Steroid (L.B test)	+ (for terpenoid)	+	+

Table 3: Preliminary Phytochemical screening of *P.cubeba*

Name of the test	Petroleum ether extract	Chloroform extract	Ethanol extract
Test of Alkaloid (Dragendorff's test)	+	+	+
Test of Flavonoids ($FeCl_3$ test)	+	+	+
Test for Tannins	+	+	+
Test for Terpenoid/Steroid (L.B test)	+ (for terpenoid)	+	+

Table 4: Preliminary Phytochemical screening of *P.retrofractum*

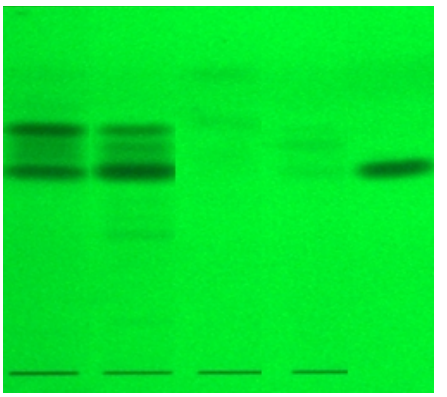
Name of the test	Petroleum ether extract	Chloroform extract	Ethanol extract
Test of Alkaloid (Dragendorff's test)	+	+	+
Test of Flavonoids ($FeCl_3$ test)	+	+	+
Test for Tannins	+	+	+
Test for Terpenoid/Steroid (L.B test)	+ (for terpenoid)	-	-

(+) : Present , (-) : Absent

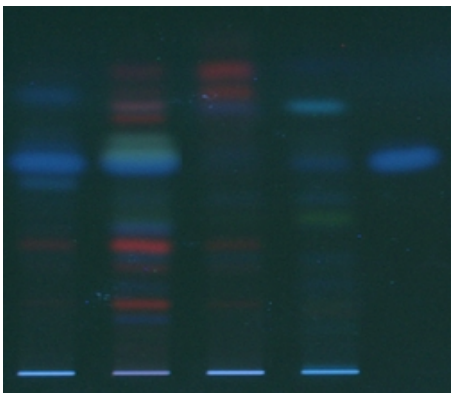
HPTLC Profile of four Piper species along with Piperine (marker compound)

Chromatography experiments

i) Stationary Phase :-
Aluminum plate precoated with silica gel $^{60}F_{254}$
ii) Mobile Phase :-
Toluene:Diethyl ether:1,4 Di- oxane (14:5:4)
iii) Sample application :-
Applied volume 5µl, band length 8mm and applied at 10mm from the base of the plate. Plate size was 10x10 cm.
iv) Development :-
Development up to 85mm in CAMAG Twin trough chamber. Plate preconditioning (temp 30^0C and relative humidity were 60%)
v) Photography:-



Observed at 254nm



Observed at 366nm

***P. longum, P.nigrum, P.cubeba, Piper retrofractum,* Piperine (marker compound)**

Table 5: HPTLC analysis of Piperine and methanolic extract of four piper species

Conditions	Standard Piperine	Methanolic extract of *P. longum*	Methanolic extract of *P.nigrum*	Methanolic extract of *P.cubeba*	Methanolic extract of *Piper retrofractum*
	R_f value	R_f value	R_f value	R_f value	R_f value
UV254 nm	0.57	0.57,0.62,0.65 0.70	0.39,0.44,0.57, 0.61,0.64,0.70	0.57,0.62,0.71,0.85	0.57,0.65,0.70
UV366 nm	0.57	0.20,0.29,0.36,0.52,0.57, 0.63,0.77	0.15,0.19,0.29,0.32,0.36,0.40 ,0.45,0.49,0.57,0.60,0.64,0.7 1,0.74,0.82	0.19,0.36,0.41,0.49,0.57,0.6 2,0.70,0.73,0.77,0.83	0.18,0.32,0.43,0.48,0.5 7,0.73

DISCUSSION

The current study will serve to become a ready reference for identification, phytochemical analysis as well as HPTLC of four piper species on the basis of microscopy and chemical analysis. The preliminary phytochemical analysis indicated the presence of alkaloids, tannins, flavonoids, terpenoid and steroid in the crude petroleum ether, chloroform and ethanolic extracts of four piper species. The presence of alkaloids in all species of Piper indicates Piperine which is an alkaloid may be present. In future, this investigation will further help in isolation of important compounds from these four piper species. Moreover, from comparative HPTLC study, we can get a preliminary idea about the content of Piperine present in these four species. The trend of occurrence of piperine in these species are P.nigrum>*P. longum, Piper retrofractum>P.cubeba.*

CONCLUSION

The current study will be the ready reference for the correct identification of the four crude drugs as well as gives a preliminary idea about the phytochemicals present and qualitative level of piperine content in these common piper species used in traditional medicine, however, a comparative quantitative study is suggested further.

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