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Krutika Joshi

Ph. D. Scholar, Dravaguna
Department, IPGT and RA,
GAU, Jamnagar.

Dr. VJ Shukla

Head, Pharmaceutical
Laboratory, IPGT and RA,
GAU, Jamnagar.

K Nishteswar

Head, Dravaguna Department,
IPGT and RA, GAU, Jamnagar.

Dr. Mandip Goyale

Assistant Professor,
Kayachikitsa Department, IPGT
and RA, GAU, Jamnagar.

Dr. Rahul Shingadiya

Ph.D. Scholar, RSBK
department, IPGT and RA,
GAU, Jamnagar.

Dr. Suhas Chaudhary

Lecturer, Shri Gulabkunvarba
Ayurveda mahavidyalaya, GAU,
Jamnagar.

Correspondence:

Krutika Joshi

Ph. D. Scholar, Dravaguna
Department, IPGT and RA,
GAU, Jamnagar.

Physicochemical and HPTLC analysis of Pippalimula (Root of *Piper longum* Linn.)

Krutika Joshi, VJ Shukla, K Nishteswar, Mandip Goyale, Rahul Shingadiya, Suhas Chaudhary

Abstract

Background: Pippalimula (root of *Piper longum* Linn.) is a well-known herb used as single drug or in compound formulations for various disorders.

Aim: The study was planned to carry out the Physicochemical and HPTLC analysis of Pippalimula. **Material and Methods:** Fine powder of Pippalimula and alcoholic extract were obtained and subjected to phytochemical analysis and chromatographic study.

Result: Physico chemical analysis of the root was carried out and found similar to reported API standard limits. HPTLC analysis was performed with Toluene: Ethyle acetate: acetic acid (7:2:1) v/v. Which showed 3 spots on 254 nm. HPTLC analysis of root of *Piper longum* Linn. Can provide standard analysis with selected solvent system and can be used as a reference for the authentication and quality control of the drug.

Keywords: Physicochemical, Hptlc Analysis, *Piper longum* L., alcoholic extract

1. Introduction

Phytochemicals are compounds present in plants that are used as food and medicine to protect against illness and to maintain human health. Phytochemicals have antioxidant or hormone-like effect which helps in fighting against many diseases including cancer, heart disease, diabetes, high blood pressure and preventing the formation of carcinogens on their target tissues.

The genus *Piper* (L.) contains more than 700 species grown in tropical and subtropical rain forest. Plants belonging to the genus *Piper* are reputed in the Indian Ayurvedic system of medicine for their medicinal properties. Previous phytochemical studies of this genus have led to the isolation of a lots of interesting chemical constituents which include lignins, amides, alkaloids and flavonoids [1].

In Indian market *Pippali* (fruit of *Piper longum* Linn.) and Pippalimula (root of *Piper longum* Linn.) are possessing high demand due to their therapeutic properties. As reported by NMPB it is one of the highly traded medicinal plants procured from cultivation. Approximate annual consumption of *Piper longum* Linn. (fruits and roots) is 1737 Metric Ton, but maximum amount of those consumptions are fulfilled through import. In the year 2004-2005; about 9,067,191 Kg Pippali was imported which includes its fruit and root [2]. The plant is reported as endangered for Tamil Nadu and at lower risk for Kerala [3]. Moreover, the import of the plant is additionally expensive. Popularity and demand increase the chance of adulteration and substitution. To prevent this, standardization of the medicine is vital for better results.

Analytical procedure helps in determination of the presence of the materials in terms of elements or compounds in the test drug. It is commonly used in chemical, clinical and pharmaceutical research laboratories as a part of quality control measures. It is used for the standardizations of various Ayurvedic formulations i.e., Vasa Avaleha [1], Kanakabindvarishta, Balachaturbhadrha churna [2] etc. The chromatographic method with modification of sample preparation stage can also contribute in the identification of *rasa* based traditional classification method of drugs. Keeping this in view, attempt has been made to qualify the drug material by the physicochemical and phytochemical analysis. HPTLC analysis of the root of *Piper longum* Linn. was carried out to observe the performance of methanolic extract in presence of acetic acid.

The test drug used for the current research work was subjected to physico-chemical and HPTLC analysis in the Pharmaceutical Chemistry Laboratory of I.P.G.T & R.A, Jamnagar. All the experiments were done by following the standard procedures mentioned in Ayur

Pharmacopeia of India. Hence, the study was conducted with the aim to carry out the Physicochemical and HPTLC analysis of Pippalimula (root of *Piper longum* Linn.).

Material and Methods

Procurement of raw materials

The healthy, dried Pippalimula (root of *Piper longum* Linn.) was collected from Paderu district, Andhra Pradesh where it is cultivated for medicinal purpose in the month of January. After proper authentication of the drug, it was made into powder form by using 60 no. mess.

Preparation of Solvent extracts

Sample of the drug was extracted successively with methanol using a maceration extraction method. Extracts, thus obtained, were subjected to phytochemical analysis and chromatographic study.

Phase 1 Organoleptic parameters

Colour: Creamish brown

Smell: Strong pungent

Taste: Pungent

Form: powder

Touch: Fine

Physicochemical analysis

Physicochemical study of sample was carried out by using various physicochemical parameters as mentioned in *Ayurvedic Pharmacopoeia of India*.

- Determination of loss on drying
- Determination of total ash
- Determination of acid insoluble ash
- Determination of Extractive values
- Determination of water soluble extractive value
- Determination of alcohol soluble extractive value
- Qualitative chemical test

Qualitative tests for various functional groups like alkaloids, glycosides etc., were carried out by using the methanol soluble extracts of the samples and by following standard procedures.

Table 1: Qualitative tests of methanolic extract of *Piper longum* root powder

Sr. No.	Phyto-constituents	Performed test
1.	Alkaloids	Dragondroff's test
2.	Steroids	Solkowski reaction
3.	Amino acids	Ninhydrin test
4.	Carbohydrates	Banediets reagent test
5.	Glycosides	Keller killiani test
6.	Tannins	Lead acetate test
7.	Proteins	Biurate test
8.	Volatile oils	Volatile oil test

Phase 2: (Chromatographic analytical study of *Pippalimula*)

HPTLC study of sample of test drugs

Principle remains the same as of TLC i.e. adsorption. One or more compounds were spotted in a thin layer of adsorbent

coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action (against gravitational force). The component with more affinity towards stationary phase travels faster. Thus the components were separated on a thin layer chromatographic plate based on the affinity of the components towards the stationary phase.

Steps involved in HPTLC were as followed

1. Selection of chromatographic layer
2. Sample and standard preparation
3. Layer pre-washing
4. Layer pre-conditioning
5. Application of sample and standard
6. Chromatographic development
7. Detection of spots
8. Scanning and Documentation

Chromatographic conditions

Stationary phase : Silica gel GF 254

Mobile phase : Toluene: Ethyl acetate: acetic acid (7:2:1) v/v

Application mode : Camag Linomat V

Development Chamber: Camag Twin trough Chamber.

Plates : Precoated Silica Gel GF254 Plates.

Chamber Saturation : 30 min.

Development Time : 30 min.

Development distance: 7 cm.

Scanner : Camag Scanner III.

Detection : Deuterium lamp, Tungsten lamp

Data System : Win cats software

Visualization : Long and short UV

Spray reagent : (Anisaldehyde-Sulphuric acid) spray reagent.

Results

Plant material

Powder of *Piper longum* Linn root: Brownish cream colour.

Physicochemical parameters

Table 2: Physico-chemical parameters of powder of *piper longum* Linn. Root

Sr. No.	Test	As per API	Result (Average of 3 samples)
1	Loss on Drying	-	4.62 % w/w
2	Ash Value	Not >5.5%	5.68 % w/w
3	Acid Insoluble Ash	Not >0.2%	0.263 % w/w
4	Water Soluble Extract	Not <12%	18.30 % w/w
5	Methanol Soluble Extract	Not <4%	11 % w/w
6	pH (5% aqueous solution)	-	5.5

Qualitative tests

Table 3: Qualitative tests of powder of *piper longum* Linn. Root

Sr. No.	Qualitative tests	result
1.	Alkaloids	-
2.	Steroids	+
3.	Amino acids	-
4.	Carbohydrates	+
5.	Glycosides	-
6.	Tannins	+
7.	Proteins	-
8.	Volatile oils	+

HPTLC of the raw drugs

Alcoholic extract of *Piper longum* Linn. (*Pippalimula*) root powder was subjected to HPTLC and visualized in short U.V. (254nm) and Long U.V. (366 nm) as well as the spray detection. Densitometry analysis (Image 1) shows 3 peaks @254 nm after spray with anisaldehyde Sulfuric acid.

Table 4: Spots of *Pippalimula* methanolic extract

Sample	Solvent system	Visualization of the derivatization	No of spots	(R_f values)
Pippalimula		@254 nm	3	0.07, 0.70 and 0.86
		@366 nm	0	-

Discussion

Most of the people think that spices like Pippalimula, used in their kitchen are only intended to flavour to their food, but they also play a vital role in keeping us healthy and fit. The phytochemicals are either the product of plant metabolism or synthesized for defence purposes which are known to have bioactivity. The bioactive phytochemical of *Piper longum* root can be used for medicinal purpose. Numbers of studies were found having reported phytochemical screening of the root of *Piper longum* Linn. Extracted in various solvents. Constituents identified from different extracts of *Pipar longum* Linn. root are piperine, pipartine, triacontane, dihydro-stigmasterol, an unidentified steroid, reducing sugars, glycosides, sesamin and methyl-3,4,5-trimethoxycinnamate (roots); two alkaloids piperlongumine and piperlonguminine characterised as N- (3, 4, 5- trimethoxy cinnamoyl)- Δ - piperidin- 2- one and isobutylamide of piperic acid respectively (stem and roots).

Quantitative estimation has been carried out on certain constituents like alkaloids, flavonoids, phenols and tannins and very preliminary analytical studies were done with the aim of authentication of the drug.

Physico chemical analysis of the root was carried out and found similar to reported API standard limits. The physical constant evaluation of drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity of the drugs i.e. the presence or absence of foreign inorganic matter.

The moisture content of the drug is not too high thus it could discourage the multiplication of bacteria, fungi and yeast.

Qualitative tests of the drug showed presence of steroids, carbohydrates, tannin and phenolic compounds. Polyphenols such as tannins and flavanoides have been shown to have numerous health protective benefits, which include lowering of blood lipids. Tannins are known to possess general antimicrobial and antioxidant activities. Recent reports show that tannins may have potential value as cytotoxic and antineoplastic agents. Phenolic phytochemicals have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities. These secondary metabolites could make the plant useful for treating different ailments and having the potential of providing useful drugs for the management of various conditions.

HPTLC fingerprinting is proved to be a reliable, accurate and precised method for herbal identification and authentication. Thus the developed chromatogram and R_f value will be specific with selected solvent system, and serve the better tool for standardization of the test drug. HPTLC analysis of the methanolic extract of the root powder was performed. Using Toluene: Ethyle acetate: acitic acid (7:2:1) as solvent. Three spots were observed at 254 nm with R_f values 0.07, 0.70, and 0.86 in mobile phase.

API mentioned chromatographic separation using Toluene ethyle.

Conclusion

The results obtained from qualitative evaluation of HPTLC fingerprint images will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy. The R_f values reflect the phyto constituents of the plant which may establish the identification of the genuine source. Thus the present study will provide sufficient information about the identification, standardization and quality control of Pippalimula (Root of *Piper longum* Linn.).

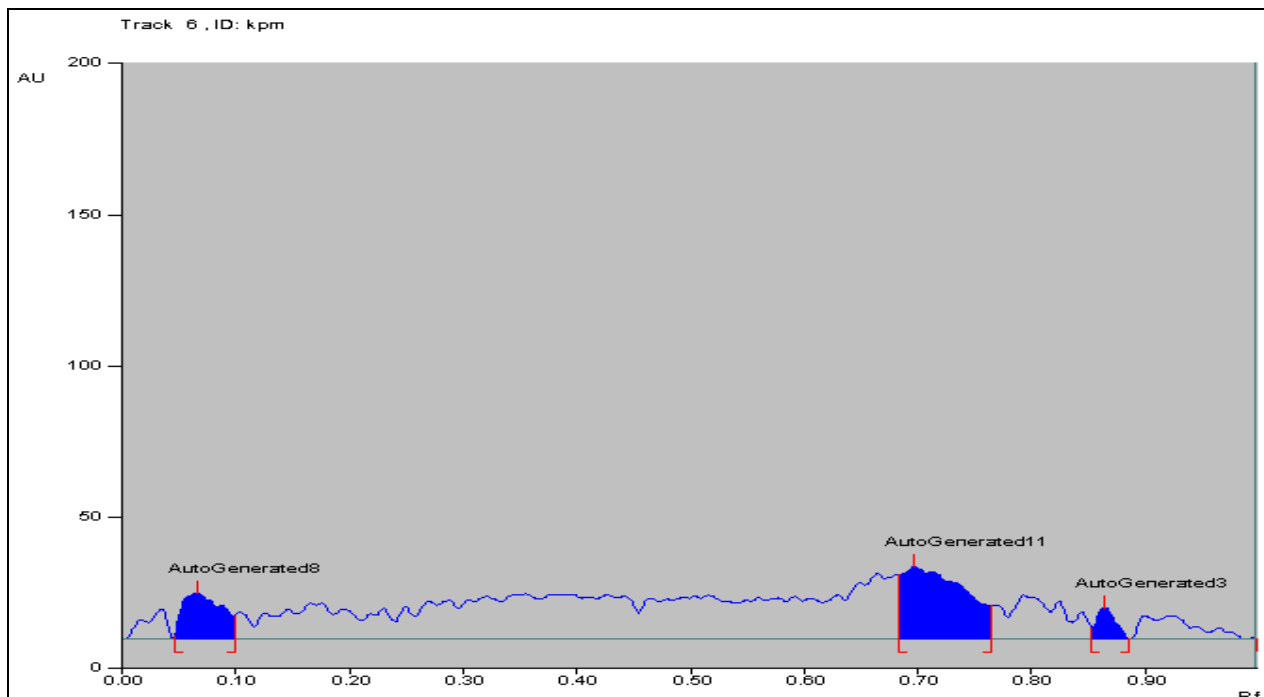


Fig 3.1: Densitogram of *Pippalimula* methanolic extract in mobile phase @254 nm

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