



**STUDIES ON QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL
ANALYSIS OF *PIPER LONGUM* LINN.**

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ABSTRACT

The present study aimed to carried out a qualitative and quantitative analysis of *Piper longum*. Various extract of different plant parts of *piper longum* revealed the presence of various important bioactive compounds viz. alkaloid, flavonoids, glycosides, tannin, phenol and sterol. Quantitative analysis showed highest amount of phenol content (24.27 mg GAE/ gm) in leaf and total flavonoids content (3.27 mg quercetin/gm) content in root in Methanolic extract.

KEY WORDS: *Piper longum*, Phytochemical analysis, phenol, flavonoid



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INTRODUCTION

Piper longum L. is popularly known as “pippali” or “long pepper”, belonging to family piperaceae. It is an important medicinal plant in Indian traditional medicine. It is distributed throughout the Indian subcontinent; this herb also grows wild in Malaysia, Singapore, Bhutan and Myanmar¹. Flowers are yellow, in elongate spikes and the fruits are small, ovoid berries, shiny blackish green, embedded in fleshy spikes². They have a pungent taste and cause salivation and numbness of the mouth³. Long pepper is very effective in the treatment of bronchial asthma in children^{4,5}. It has been reported that the fruits extract of the plant had antidepressant, antinociceptive, anti-inflammatory, antioxidant, anticancer, antidiabetic, antibacterial, antifungal, antitumor, anti allergic, antiasthmatic, antifertility, antiulcer, antihypertensive, antiplatelet, antithyroid, immunomodulatory, antiameobic, hepatoprotective, vasodilating, insecticidal and mosquito larvicidal activities^{6,7,8}. Roots and fruits are also used as anti-dote in snake biting and scorpion sting⁹. *P. longum* L. has been used in traditional remedies as well as in the Ayurvedic system of medicine against various disorders^{10,11}. This plant possesses alkaloid viz. Piperine which is involved to enhance the bioavailability of many drugs¹⁹.

There is very limited work on qualitative and quantitative analysis of *Piper longum* L. In the present study an attempt was made to screen different extract prepared from different plant parts of *Piper longum* L. for phytochemical screening and also determine total phenol and flavonoids contents of different plant parts of *Piper longum* L.

MATERIALS AND METHODS

Collection of plant samples

The leaves, fruits and root of *Piper longum* (L.) Vent. were collected from the botanical garden of Hemchandracharya North Gujarat University (HNGU), Patan, in the month of Aug-sept. 2012. The plant material was authenticated

and identified in the Department of Botany, HNG University, Patan, Gujarat, India.

Preparation of plant materials

The freshly collected samples were washed thoroughly with distilled water and air-dried under shade at room temperature for 7-10 days. After drying, the samples were reduced to small piece; material was grounded in to fine powder using mortal-pestle then sieved using a muslin cloth. Powdered samples were then stored in air tight containers for further use.

Preparation of extract

The air-dried finely powdered plant samples (2.0 g each) were soaked in 20 ml of methanol, aqueous and hexane for 24hrs. at room temperature. The extracts were filtered through what man No.1 filter paper. The supernatants were collected, covered, labeled and used for the screening of various phytochemicals.

Qualitative phytochemical analysis

Qualitative analysis of methanol, aqueous extract and hexane extract of *Piper longum* L. were carried out to determine the presence of various bioactive compounds using the standard qualitative procedures^{12,13,14}.

Quantitative phytochemical analysis

The phytochemicals which are present in the methanol extract of *Piper longum* L. was quantified by standard procedures.

Tests performed for the presence of qualitative phytochemical analysis

a) Tests for alkaloids

1) Dragendorff's test: To 1 ml of each of the sample solution taken in a test tube few drops of Dragendorff's reagent (potassium bismuth iodide solution) was added. A reddish brown precipitate was observed indicating the presence of alkaloids.

2) Meyer's test: To 1ml of each of the sample solution few drops of Meyer's reagent (potassium mercuric chloride solution) was

added. A creamish white precipitate was formed indicating the presence of alkaloids.

3) Wagner's test: To few ml of each of the sample solution, Wagner's reagent (Iodine in potassium iodide) was added, which resulted in the formation of reddish brown precipitate indicating the presence of alkaloids.

b) Tests for Flavonoids

1) Lead acetate test: When aqueous basic lead acetate was added to test sample produces a reddish brown precipitate.

2) Ferric chloride test: To few ml of test samples taken separately, few drops of ferric chloride were added which resulted in the formation of blackish red precipitate.

3) Shinoda test: (Magnesium hydrochloride reduction test): To the test solution few fragments of magnesium ribbon and concentrated hydrochloric acid were added drop wise and reddish to pink colour was resulted.

4) Alkaline reagent test: When sodium hydroxide solution was added to the test samples formation of intense yellow color, which turns to color less on addition of few drops of dilute acid indicates the presence of Flavonoids.

c) Test for Tannins and Phenol

1. Ferric chloride test: When a few drops of ferric chloride were added to sample solution a blackish precipitate appears.

2. Gelatin test: When gelatin and water were added to test samples formation of white precipitate was resulted.

3. Lead acetate: Few ml of test samples were taken in different test tubes followed by the addition of aqueous basic lead acetate. It results in the formation of a reddish brown bulky precipitate.

4. Ellagic acid test: Equivalent amount (5%) of glacial acetic acid and sodium nitrite were added to plant extract a muddy niger brown colour appears, which is a positive result for phenols.

d) Tests for Glycosides

1. Legal's test: When the test samples were treated with pyridine and sodium nitroprusside solution blood red colour appears.

2. Kellar Kiliani test: 1ml of concentrated sulphuric acid was taken in a test tube then 5ml of extract and 2ml of glacial acetic acid with one drop of ferric chloride were added, formation of a blue colour.

3. Concentrated Sulphuric acid test: Conc.H₂SO₄ was added to test sample which resulted in appearance of reddish colour.

e) Tests for Sterols

1. Libermann-Buchard test: When samples were treated with few drops of acetic anhydride, boiled and few drops of concentrated sulphuric acid from the sides of the test tube were added, shows a brown ring at the junction of two layers and the upper layer turns green which shows the presence of sterols.

2. Salkowski test: Few drops of concentrated sulphuric acid were added to the test samples in chloroform, a red colour appears at the lower layer indicates the presence of sterols.

f) Tests for Quinones

1. Alcoholic KOH test: When alcoholic KOH was added to the test samples red to blue colour appears reacting positively for quinines.

g) Tests for Saponins

1. Foam test: 5ml of extract was shaken vigorously to obtain a stable persistent froth. The froth was then mixed with three drops of olive oil and observed for the formation of an emulsion, which indicated the presence of saponins.

Tests performed for the presence of quantitative phytochemical analysis

Total flavonoids determination

The total flavonoid content (TFC) of different parts such as leaf, root and fruits of *Piper longum* Linn. was determined using the aluminium chloride assay through colorimetry¹⁵. Each plant extracts (0.5 ml of 1:10 g ml⁻¹) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible

spectrophotometer (USA). The concentrations of flavonoids in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample.

Total phenols determination

Total phenols were determined by Folin Ciocalteu reagent¹⁶. For the estimation of Phenol, gallic acid used as a standard compound. A diluted plant extract and Gallic acid (0.5 ml of 1:10 g ml⁻¹) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na₂CO₃ (4 ml, 1 M). The mixture were incubated for 15 min and the total phenols were determined by colorimetry at 765 nm. All determination was performed in triplicate. A standard calibration plot was generated at 650nm using known concentrations of gallic acid. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg gallic acid equivalent of phenol/g of sample.

RESULTS AND DISCUSSION

The preliminary phytochemicals screening of different parts of *Piper longum* were done to identify various bioactive compound. In these screening process alkaloids, tannins, phenols, saponins, Flavonoids, glycosides, sterol, quinine and lignin shows different types of results in different solvents. The results of the phytochemical screening of leaf, root and fruits of *Piper longum* (L.) are as presented in table-1, 2, 3.

1. QUALITATIVE PHYTOCHEMICAL ANALYSIS

Leaf extract

The leaves extract gives positive results for alkaloids and sterol in all the three organic solvents. While saponin, and quinone were shows negative results in all the extract. Flavonoids, tannin and phenol were absent in methanol extract while positive in hexane and aqueous extract. Lignin present in aqueous extract and methanol extract while it was absent in hexane extract. Glycoside was present only in hexane extract. Similar report was reported In *Piper nigrum* Linn.¹⁷.

Root extract

The screening for the phytochemicals present in the root extract of *Piper longum* L. revealed the presence of alkaloids, tannin, phenol, saponin and sterol in all the organic solvent while glycosides and quinone was completely absent in all the extract. Lignin were present in methanol extract while absent in aqueous and hexane extract.

Fruits extract

All the solvent extract gives positive result for alkaloids, tannin, phenol and sterol. flavonoids present in methanol extract while not present in Aqueous and hexane extract. Glycosides gives positive result in aqueous and methanol while negative in hexane extract. Quinone present in aqueous extract while absent in hexane and methanol. Lignin were completely absent in aqueous, methanol while positive in hexane. Saponin was completely absent in all the three organic solvent. Similar result was reported¹⁸.

Table-1
Phytochemicals Analysis of on leaf extract of Piper longum.

Phytocompound	Test for phytocompound	Solvent system		
		methanol	Hexane	Aqueous
Alkaloids	Mayer	+	+	+
	Wagner	-	-	+
	Dragendroff	-	-	+
Tannin & Phenol	Gelatin	-	+	+
	FeCl ₃	-	+	+
	Lead acetate	-	+	+
Saponins	Foam test	-	-	-
Flavonoids	Alkaline reagent test	-	+	-
	Mg ribbon test	-	-	+
	Lead acetate	-	-	-
Glycosides	Legal's test	-	+	-
	Kellar kiliani test	-	-	-
Sterol	Libermann Burchard test	+	+	+
	Salkowski test	-	-	-
	Alcoholic KOH Test	-	-	-
Quinone	Alcoholic KOH Test	-	-	-
Lignin	Labat test	+	-	+

+ = Present, - = Absence

Table-2
Phytochemicals Analysis of on root extract of Piper longum.

Phytocompound	Test for phytocompound	Solvent system		
		methanol	Hexane	Aqueous
Alkaloids	Mayer	+	-	+
	Wagner	-	-	+
	Dragendroff	+	+	+
Tannin & Phenol	Gelatin	+	+	-
	FeCl ₃	+	+	+
	Lead acetate	-	-	+
Saponins	Foam test	+	+	+
Flavonoids	Alkaline reagent test	-	-	+
	Mg ribbon test	-	+	-
	Lead acetate	+	-	-
Glycosides	Legal's test	-	-	-
	Kellar kiliani test	-	-	-
Sterol	Libermann Burchard test	+	-	-
	Salkowski test	+	+	+
	Alcoholic KOH Test	-	-	-
Quinone	Alcoholic KOH Test	-	-	-
Lignin	Labat test	+	-	-

+ = Present, - = Absence

Table-3
Phytochemicals Analysis of on fruits extract of *Piper longum*

Phytocompound	Test for Phytocompound	Solvent system		
		Methanol	Hexane	Aqueous
Alkaloids	Mayer	+	+	+
	Wagner	+	-	+
	Dragendroff	-	-	+
Tannin & Phenol	Gelatin	-	-	-
	FeCl ₃	-	+	+
	Lead acetate	+	-	+
Saponins	Foam test	-	-	-
Flavonoids	Alkaline reagent test	-	-	-
	Mg ribbon test	-	-	-
	Lead acetate	+	-	-
Glycosides	Legal's test	-	-	+
	Kellar kiliani test	+	-	-
Sterol	Libermann Burchard test	+	+	+
	Salkowski test	+	+	+
	Alcoholic KOH Test	-	-	+
Lignin	Labat test	-	+	-

+ = Present, - = Absence

2. QUANTITATIVE PHYTOCHEMICALS ANALYSIS

Methanolic extract of *Piper longum* L. was analyzed for their phytoconstituents. Total phenol and flavonoids present in methanolic extract of leaves, root and fruits are shown in Table-4. Total phenol is usually determined in powder drug and extracted by using the Folin-Ciocalteus method. For the total phenol quantification Gallic acid was used as a standard compound and it was expressed as mg/g gallic acid equivalent using the standard curve equation: $y = 0.004x + 0.017$, $R^2 = 0.997$. Where y is absorbance at 760 nm and x is total phenolic

content in the different plant parts of *Piper longum* expressed in mg/gm. The leaf contains the maximum and the fruit contains the minimum amount of phenolic compounds. Flavonoids are polyphenolic compound, which play significant role in plant. Total flavonoids is determined by colorimetric method using aluminium chloride. Total flavonoids content of Methanolic extract of *Piper longum* were between 0.39 ± 0.014 mg/gm and 3.27 ± 0.020 mg/gm The flavonoid contents of the extracts in terms of quercetin equivalent (the standard curve equation: $y = 0.028x - 0.046$, $R^2 = 0.995$). Total flavonoid content of root is high compare to leaf and fruits.

Figure-1
Standard curve of Quercetin for flavonoids determination.

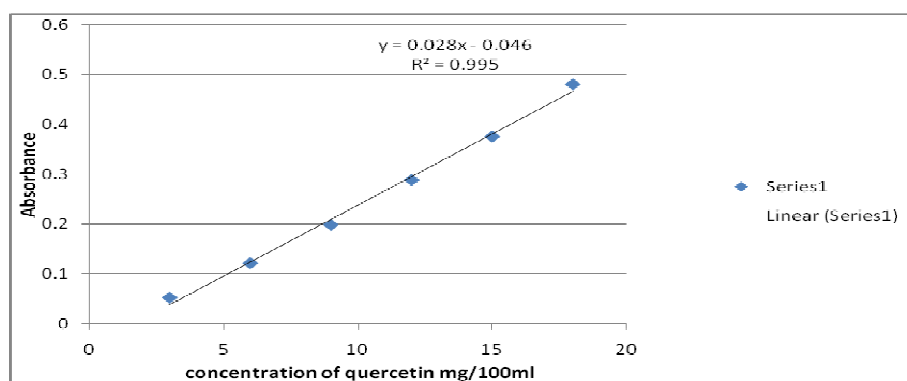


Figure-2
Standard curve of Gallic acid for phenolics determination.

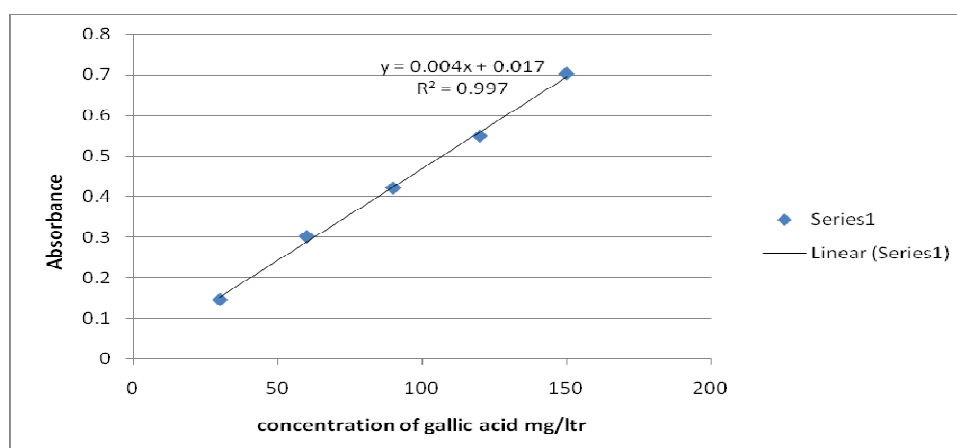


Table-4
Quantitative analysis of phenol and flavonoids

samples	Phenol mg/gm	Favonoids mg/gm
Leaf	24.27±0.008	0.39±0.014
root	1.26±0.013	3.27 ±0.020
fruit	0.04±0.010	0.45±0.011

All values are expressed as mean ± STD (n=3)

CONCLUSION

The present investigation revealed that methanol, hexane and aqueous extract of leaves, root and fruits of *Piper longum* contain different bioactive compound. Quantitative analysis showed a significant amount of phenols and flavonoids in leaf and root. However, further research is required to isolate bioactive compound which is present in *Piper longum* for its potent antioxidant properties.

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