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Analysis for piperine in leaves, roots and spikes in *Piper longum* L

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Abstract
Piper longum L (Pipali) is an important medicinal plant used as an analgesic, antipyretic, CNS depressant, anti-inflammatory, anti-tumor, sedative, hypnotic, tranquilizer and muscle relaxant, hepatoprotective both in digestive and respiratory systems. The fruit of *Piper longum* contains a large number of alkaloids and related compounds, among them the most important is piperine. Attempts were made to detect piperine in different parts of the *Piper longum* plant. Leaf and root of both male and female plants and spike from only female plant were used. Methanol extracts of above mentioned parts were prepared and subjected to thin layer chromatography (TLC) along with piperine as a standard, using toluene-ethyl acetate (70:30) mobile phase. Upon treatment with vanillin-sulphuric acid reagent and subsequent heating the silica gels and viewed under UV light. Appearance of yellow fluorescence was observed in all the samples except the male leaf. This confirmed the presence of piperine in all parts except male leaf of *Piper longum* plant.

Keywords: *Piper longum* L., Piperine, TLC, leaf, spike

Introduction
Piper longum (Piperaceae) is used in traditional medicine and also as a spice. It is commonly known as long pepper (pipali) which is native to the Indo-Malaya region. It is found both in the wild and cultivated forms in the hot and humid parts of India, from Central Himalayas to Assam, Khasi and the Mikir hill, the lower hills of West Bengal, and the evergreen forests of the Western Ghats from Konkan to Travancore and has also been recorded in the Car Nicobar Islands (Manoj *et al.*, 2004) [10]. The plant is a slender, aromatic, perennial climber, with large woody roots and numerous creeping, joint stems with thick nodes containing cordate leaves (Mishra *et al.*, 2010) [11]. Spike (inflorescence) is the most important and useful part of the plant. The inflorescence is cylindrical, pedunculate and flowers grow on solitary spike, the female flower is up to 2.5 cm long and 4-5 mm in diameter but the male spikes are larger, slender with narrow bracts. The fruits are small, ovoid berries, shiny blackish green, embedded in fleshy spikes (Zaveri *et al.*, 2010) [23].

The extract of fruits of *P. longum* "Piperis Longi Fructus" (crude drug) is frequently used in folk medicine to treat bronchial trouble. Further, it is used as a carminative and analgesic (Jung *et al.*, 1989; Parmar *et al.*, 1997) [7, 14]. Piperine is an important and abundant alkaloid extensively used for enhancing the bio-availability and bio-efficacy of various drugs (Atal and Bedi, 2010) [1]. Further its derivatives have various traditional uses as an analgesic, antipyretic, CNS depressant, anti-inflammatory (Chauhan *et al.*, 2011) [3], anti-tumor, sedative, hypnotic, tranquilizer, muscle-relaxant, hepatoprotective both in digestive and respiratory systems (Sharma, 1996) [18].

The fruit of *P. longum* contains a large number of alkaloids and related compounds, the most abundant of which is piperine, together with methyl Piperine, Piperonaline, piperettine, asinine, pellitorine, piperundecalidine, piperlongumine, piperlonguminine, refractometer A, pregna diene, brachystamide, brachystamide-A, brachystine, pipercide, piperderidine, longamide and tetrahydropiperine, tetrahydro piperlongumine, dihydro pipernonaline piperidine, Piperine, tetrahydro piperlongumine, trimethoxycinnamoyl-piperidine and piperlongumine have been found in the root of *P. longum* (Zaveri *et al.*, 2010) [23].

Spike and root of *P. longum* are used for pharmaceutical purpose and for drug preparation. There is no report available on the distribution of piperine in different parts of male and female plants for which the present study has been designed. The presence of piperine in different parts of plant was determined using Thin Layer Chromatography (TLC) method.

Material and Methods

Collection of plant sample

Leaves and roots of both female and male plant but spike only from female *P. longum* were collected from the botanical garden, Department of Botany, Dayalbagh Educational Institute, Agra, for the experimental work. Leaves at third position from top were collected.

Sample preparation

The freshly collected plant samples were washed thoroughly with distilled water and air dried under shade at room temperature for at least 10 days. After drying samples were grounds into powder in grinder or mortar pestle. All the samples were sieved through a specific pore (1 mm) size and then made into a thimble for reflux. In methanol (Merck) all the samples were refluxed for 24 h at 60ºC and filtered through Whatman no.1 filter paper. Extracts were concentrated in a rotary evaporator.

Tests for alkaloids

1. Dragendorff's test: This test is specific for alkaloids. Solution A- 1.7 g of bismuth nitrate (Hi-media) was dissolved in 100 ml water: glacial acetic acid mixture (80:20). Solution B- 40 g of KI (Potassium iodide; Merck) was dissolved in 100 ml distilled water. The final reagent was prepared by mixing 5 ml of solution A, 5 ml of solution B, 20 ml glacial acetic acid and 70 ml distilled water. Reagent was filled in spraying bottle.

2. Wagner's test: This test is also specific for alkaloids. Dissolved 2 g of Iodine and 6 g of potassium iodide in 100 ml distilled water forming Wagner's reagent (Iodo-potassium iodide). To presence of 2-3 drops of reagent were added to the sample.

Mobile phase preparation

Toluene (Merck) and ethyl acetate (Merck) were used as a mobile phase of chromatographic method. Specific ratios of 70:30 toluene-ethyl acetate were used for separation of alkaloids.

Preparation of standard solution

For preparation of standard solution of 0.1 mg piperine (Sigma-Aldrich) was dissolved in 1 ml of methanol for the formation of 0.1 mg/ml concentrate solution.

Preparation of sample solution

The concentrated sample solutions were evaporated and converted to dried form. A specific amount of dried samples were taken and dissolved in methanol for further use in chromatography.

Plate formation for thin layer chromatography

For TLC plate formation silica gel 60 F_{254} (Merck) were mixed with distilled water in a specific ratio. The paste of silica gel was homogeneous and smooth. With the help of spreader the paste was spread uniformly on a glass plate. Glass plates ready with silica gel were kept in oven overnight to dry.

Spray reagent formation

The spray reagent was prepared by adding 1 g vanillin (Hi-media) to 100 ml absolute ethanol with 1.5 ml concentrated sulphuric acid and was taken in a reagent bottle (Spangenberg *et al*., 2011) [19].

Thin layer chromatography method for piperine detection

On pre-coated silica gel plates 2 µl of sample solution was placed with the help of micro pipet. Then plates were partially dipped in mobile phase (toluene- ethyl acetate solution) till flow of solution became saturated. Plates were taken out of the solution, dried and sprayed with the reagent (vanillin-sulphuric acid reagent). After spraying, the plates were heated for 10 minutes at 100°C in oven. Plates were observed in UV light for presence of piperine.

Results and Discussion

The phytochemical screening of different parts of *P. longum* plant was done to identify many bioactive compounds. Alkaloids are known to be present in different parts of the plant producing it. In *P. nigrum* and *P. longum* piperine has been reported from both root and spike (Shaila *et al*., 2005; Kanaki *et al*., 2008, Upadhyay *et al*., 2013; Chitlange *et al*., 2016) [16, 8, 22, 4]. In case of *Couroupita guianesis* a comparative analysis of different biochemical compounds were studied in different parts of plant like leaf, flower and fruits (Pandurangan *et al*., 2018) [13]. Tylophorine is also distributed throughout *Tylophora asthmatica* (Gupta *et al*., 2012) [6]. In the present study main emphasis was given on the distribution of alkaloid piperine in *P. longum*.

Phytochemical tests are used to screen presence of alkaloids in plant parts (Kodangala *et al*., 2010; Rami *et al*., 2013, Sasikala and Sundaraganapathy, 2017) [9, 15, 17]. Results of the two alkaloids specific tests, Dragendorff's test and Wagner's reagent test for different parts *P. longum* is presented in Table 1. All the plant parts as leaf, root and spike from female plants and only roots from male plants showed presence of alkaloids.

Thin layer chromatography (TLC) is a versatile technique used to separate a non-volatile mixture. TLC is extensively used to separate chemical constituents as specific band patterns in different plant parts (Ujjaliya *et al*., 2012; Biradar *et al*., 2013) [21, 2]. In the present study, investigations were made to detect piperine in different parts of male and female piper plants using TLC. Piperine is an important alkaloid present in *Piper*, but distribution of piperine in this plant is not well described in the literature. Extracts from samples *viz.* leaf, root of male and female plants and spike from female plants only were applied on silica gel plate and separated, using solvent toluene-ethyl acetate (70:30) as mobile phase (Nica-badea *et al*., 2015; Elhag *et al*., 2015) [12, 5]. The silica gel plates were removed from mobile phase, dried and treated with vanillin-sulphuric acid reagent, and spots were visualized by projecting ultraviolet light. Piperine bands present fluoresced yellow. Along with test samples piperine standard was also run to further confirm the result. Significant signals were detected in all parts of sample except leaf of male part (Fig. 1). Piperine standard and the yellow bands appearing from the test sample gave an R_f value of 0.51±0.04.

Table 1: Analysis of Alkaloid in different plant parts.

Plant parts	Alkaloid test by using Dragendorff's reagent	Alkaloid test by using Wagner's reagent
Leaves from female plant	+	+
Root from female plant	+	+
Spike from female plant	+	+
Leaves from Male plant	-	-
Root from Male plant	+	+

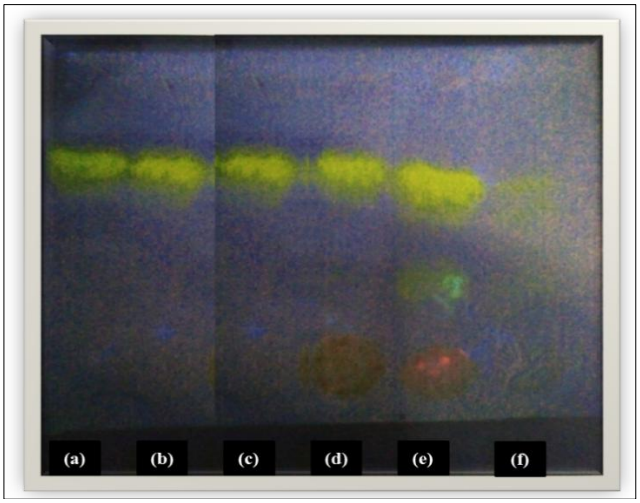

Fig 1: (a) standard Piperine, (b) female spike, (c) female roots extract (d) female leaves extract, (e) male roots extract and (f) male leaves extract

Conclusion

In the present study distribution of piperine in different parts of male and female *P. longum* parts was determined. The two alkaloid specific tests of Dragendorff and Wagner indicated presence of an alkaloid in roots, leaves and spikes of female plant but only in roots of male plants. Piperine standard gave yellow bands on the TLC plate when sprayed with vanillin-sulphuric acid reagent and viewed under UV. Extracts from different parts except leaves of male plant of *P. longum* also gave yellow band and had the same R_f value as Piperine standard.

$$\mathbf{R_f\ value}=\frac{Distance of the spot on the TLC-plate}{Distance of the solvent front}$$

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