PHYSICOCHEMICAL AND HPTLC ANALYSIS OF PIPPALIMULA (ROOT OF Piper longum Linn.)

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ABSTRACT
Pippalimula (root of Piper longum Linn.) is the spice of Indian cuisine having immense therapeutic properties. The study was conducted to evaluate Physicochemical, phytochemical analysis and HPTLC analysis using specific solvent system for Pippalimula. Material and Methods: Fine powder of Grade I Pippalimula (root of Piper longum Linn.) and alchoholic extract were obtained and subjected to phytochemical analysis and chromatographic study. Discussion: Physicochemical analysis of the root was carried out. HPTLC analysis of the methanolic extract of the root powder was performed with Toluene: Ethyl acetate 9:1. (v/v). Conclusion: 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. The study will provide referential information for the correct identification of the crude drugs.

Introduction
Pippalimula is an essential flavour of Indian cuisine which is also having vast therapeutic value. Ayurveda has valued its utilization by including it in various formulations i.e. Panchakol churna, Dashmulah-shatpal ghrita. Acharya charaka has included it in Ayurveda ascribed to adding it as Dipaniya-Pachanay-Ahara prashamanamun. He has enlisted the drug in Dipaniya and Shulapramahana dashemani. It is used in approximate 30 formulations by Acharya charaka. [1]The drug possesses Katu Rasa (pungent taste); Laghu (Light), Ruksha (rough) and Ushna (hot) guna (properties); Madhura vipaka (specific digestion) and used in various disease conditions i.e. Krimi (parasitic disease), shwasa (dyspnoea), Kshaya (Pulmonary tuberculosis), Pila roga (spleen disorders), Vishama jvara (intermittent fever), Arsha (piles), Urustambha (stiffness of thigh), Vatayadh (Nervous diseases), Nidranasha (Insomnia), Grahan (Dysentery) etc. [2]Vangasena, a medieval compendium identified sedative property of Pippalimula and suggested it with jaggery as anupana (vehicle). It is excerpted as origin of health – Arogyamoolam by authors of Siddhabheshaja manimala. Pippali mula have been used as stomachic, thermogenic, aphrodisiac, carminative, expectorant, laxative, digestive and emollient, anti-giardias, anti-moebic, anti-asthmatic, antiseptic and also active against bacterial diseases. The root is reported to have weak opioid but potent NSAID type of analgesic activity, anti oxidant activity, antimicrobial activity. [3] In Indian market Pippali (fruit of Piper longum Linn.) and Pippalimula (root of Piper longum Linn.) is possessing high demand due to its therapeutic properties. As reported by NMPB it is one of the highly traded medicinal plants procured from cultivation.[4] The plant is reported as endangered for Tamilnadu and at lower risk for Kerala.[5] 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 phytochemicals or compounds in the test drug. It is commonly used in clinical, chemical 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 [6], Vyaghr haritaki Avaleha [7], Kanakabindvarishta [8], Mahasudarshan churna [9] Balachaturbhadra churna [10] etc. The chromatographic method with modification of sample preparation stage can also contribute in the identification of...
rasa based traditional classification method of drugs.[11] Keeping this in view, attempt has been made to evaluate the physicochemical, phytochemical and HPTLC analysis of root of *Piper longum* Linn. The drug subjected to physico-chemical and HPTLC analysis by following the standard procedures mentioned in Ayurvedic Pharmacopeia of India.[12]

### Material and methods

#### Procurement of raw materials

The healthy, dried Grade – I *Piper longum* root[13] was collected from Paderu district, Andhra Pradesh where it is cultivated for medicinal purpose in the month of January. After proper authantification of the drug, it was made into powder form using 60 no.mesh. Sample of the drug was extracted successively with methanol using a maceration extraction 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.

#### Phase I (Chromatographic analytical study of *Pippalimula*)

HPTLC study of sample of test drugs [20]

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 H.P.T.L.C.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

#### Physicochemical parameters

**Table 1: Physico-chemical parameters of powder of *Piper longum* Linn. Root (average of 3 samples)**

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Test</th>
<th>As per API</th>
<th>Res</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loss on Drying</td>
<td>-</td>
<td>4.62 % w/w</td>
</tr>
<tr>
<td>2</td>
<td>Ash Value</td>
<td>Not &gt;5.5%</td>
<td>5.68 % w/w</td>
</tr>
<tr>
<td>3</td>
<td>Acid Insoluble Ash</td>
<td>Not &gt;0.2%</td>
<td>0.263 % w/w</td>
</tr>
<tr>
<td>4</td>
<td>Water Soluble Extract</td>
<td>Not &lt;12%</td>
<td>18.30 % w/w</td>
</tr>
<tr>
<td>5</td>
<td>Methanol Soluble Extract</td>
<td>Not &lt;4%</td>
<td>11 % w/w</td>
</tr>
<tr>
<td>6</td>
<td>pH</td>
<td>-</td>
<td>5.5</td>
</tr>
</tbody>
</table>

**Table 2 Qualitative tests of powder of *Piper longum* Linn. Root**

<table>
<thead>
<tr>
<th>Application mode</th>
<th>Camag Linomat V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plates Chamber</td>
<td>Camag Twin trough Chamber</td>
</tr>
<tr>
<td>Plates</td>
<td>Precoated Silica Gel GF254</td>
</tr>
<tr>
<td>Chamber Saturation</td>
<td>30 min.</td>
</tr>
<tr>
<td>Development Time</td>
<td>30 min.</td>
</tr>
<tr>
<td>Development distance</td>
<td>7 cm.</td>
</tr>
<tr>
<td>Scanner</td>
<td>Camag Scanner III.</td>
</tr>
<tr>
<td>Detection</td>
<td>Deuterium lamp, Tungsten lamp</td>
</tr>
<tr>
<td>Data System</td>
<td>Win cats software</td>
</tr>
<tr>
<td>Visualization</td>
<td>Long and short UV</td>
</tr>
<tr>
<td>Spray reagent</td>
<td>Anisaldehyde – Sulphuric acid spray reagent.</td>
</tr>
</tbody>
</table>

**Results**

**Plant material**

Powder of *Piper longum* Linn root: Brownish cream colour.
Sr. No. | Qualitative tests | Pippalimula *(piper longum Linn.)*
--- | --- | ---
1. | Alkaloids | -
2. | Steroids | +
3. | Amino acids | -
4. | Carbohydrates | +
5. | Glycosides | -
6. | Tannins | +
7. | Proteins | -
8. | Volatile oils | +

**HPTLC of the raw drugs**
Alcohol extract of *Piper longum* Linn. *(Pippalimula)* root powder was subjected to HPTLC and visualized in short U.V. (254 nm) and Long U.V. (366 nm) as well as the spray detection. Densitometry analysis (Image 3.1) on CAMAG Scanner 3 of spots generated *in situ* comparison.

**Table 3 Spots of Pippalimula(Piper longum Linn. Root) methanolic extract**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvent system</th>
<th>Spots</th>
<th>Visualization of the derivatization with anisaldehyde Sulfuric acid <em>(Rf values)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pippalimula Piper longum Linn. Root</td>
<td>Toluene: Ethyl acetate 9:1</td>
<td>11</td>
<td>@254 nm @366 nm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.04, 0.20, 0.40, 0.51, 0.62, 0.73</td>
</tr>
</tbody>
</table>

**Discussion**

The physical constant evaluation of drugs is an important parameter in detecting adulteration or improper handling of drugs. Physico chemical analysis of the root was carried out and found similar to reported API standard limits. (Table 1) Quantitative estimation has been carried out on certain constituents like alkaloids, flavonoids, phenols and tannins and preliminary analytical studies were done for authentication of the drug. Qualitative tests of the drug showed presence of steroids, carbohydrates, tannin and phenolic compounds. (Table 2)Polyphenols such as tannins and flavonoids are reported numerous health protective benefits like lowering of blood lipids. [21] Tannins are known to possess general antimicrobial and antioxidant activities[22]. Phenolic phytochemicals have antioxidantive, antidiabetic, anticaarcinogenic, antimicrobial, anti-allergic, antimutagenic and anti-inflammatory activities[23],[24]. These secondary metabolites are the essential part of the drug which make the plant useful for treating different ailments and having the potential of providing useful drugs for the management of various conditions.

The phytochemicals constituents identified from different extracts of *Pipar longum* Linn. root are piperine, piplartine, triocontane, 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) [25].

HPTLC fingerprinting is proved to be a reliable, accurate and precised method for herbal identification and authentication. Thus the developed chromatogram and *Rf* value will be specific with selected solvent system, and serve the better tool for standardization of the test drug. [26] HPTLC analysis of the methanolic extract of the root powder was performed using Toluene: Ethyl acetate 9:1 as solvent. 11 spots were observed at 254 nm with *Rf* values 0.04, 0.20, 0.40, 0.51, 0.62, 0.73 and at 366 nm with *Rf* values 0.04, 0.22, 0.42, 0.52, 0.75 in mobile phase respectively.(Table 3)

**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 *Rf* values reflect the phytoconstituents 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.).
Image 1: Graphic chart of Pippalimula at 254 nm

Image 2: Graphic chart of Pippalimula at 366 nm
Conflict of interest: We declare that we have no conflict of interest.

References

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