Abstract
A large number of plants are claimed to possess the medicinal properties in the traditional system and are also used extensively by the people of Assam. Pippali is an important ingredient of many Ayurvedic formulations. It is also used by the common people of Assam for multi-dimensional purpose such as medicine, recipe, spice, etc. Such traditionally used herbs are needed to be standardized for the proper use by the people and also for the establishment of a unique identification data among the common species. Present study was carried out to get a standardized data of Piper longum Linn which is found in Assam. Even though this plant fruit has gained scientific importance, there is a need of standardized data. Hence, in the present work the fruit part of the plant was subjected to various pharmacognostical, physicochemical and phytochemical evaluations. In the microscopical studies, the different cell structures and arrangements were studied and in physical evaluation, the ash values, extractive values, etc were studied. The various pharmacognostical constants were obtained which can help in the development of a suitable monograph for the plant. These studies are important in the way of acceptability of herbal drugs in present scenario of lacking regulatory laws to control quality of herbal drugs.

Keywords
Pippali, Piper longum Linn, pharmacognostic study, physicochemical study, thin layer chromatography

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INTRODUCTION
North east India is the richest reservoir of plant diversity in India and one of the richest biodiversity areas of the world. Assam is a home to a good number of plants having medicinal as well as traditional uses. Altogether, 952 plants species have been identified which have uses in medical practices in some form or other. Pippali (Piper longum L.) is one of them\textsuperscript{1}. Piper longum is a native of North-East India. It occurs in the hotter parts of India, from Central Himalayas to Assam, Khasi and the Mikir hills, the lower hills of West Bengal, and the evergreen forests of the Western Ghats from Konkan to Travancore\textsuperscript{2}. Pippali, belonging to the family Piperaceae is a highly valuable drug and is one of the essential ingredients in most of the compound preparations included in Ayurvedic literature\textsuperscript{3}. Due to the multi-dimensional action of Pippali, it is a very popular medicine since Samhita period of Ayurveda. References of both medicinal and dietary uses of Pippali are found in ancient classics. In Charak Samhita, the drug has been mentioned in several Mahakasaya, Yavagu, different formulations like Vardhamana Pippali Rasayana, Sitopaladi churna, Navayas churna, etc\textsuperscript{4}. Acharya Susruta has described Pippali in Saka varga\textsuperscript{5}. Since ages, Pippali is used as spice, ingredient and medicine traditionally. Pippali has been used as a crude drug for the treatment of gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infection, chronic gut-related pain and arthritic conditions\textsuperscript{6}. A use of combination of fruits of Pippali, seeds of Vidanga and borax powder has already been cited in Ayurveda as contraceptive\textsuperscript{7}. Common people of Assam also use Pippali for such multi dimensional purposes. It is also added in soup, pickle, sauce, stew and curry as a spice\textsuperscript{8}. Hence, there is an utmost need to standardize such traditionally used herbs for the proper use by the people and also for the establishment of a unique identification data among the common species. Keeping all these in views, the present study was carried out to get a standardized data of Piper longum Linn found in Assam.

MATERIALS AND METHODS:
COLLECTION OF PLANT MATERIAL:
Fresh Pippali fruits were collected from the village Mandakata of Kamrup district, Assam and identified in the pharmacognosy section of Govt. Ayurvedic College,
Guwahati-Assam. The collected fruits were then shade dried, powdered after keeping some fruits in fixatives for microscopic studies and the dried fruit material was stored in an air tight container for future use.

**PLACE OF WORK:**
Pharmocognostic, physicochemical and phytochemical studies were carried out in State Drug Testing Laboratory, AYUSH, Govt. Ayurvedic College and Hospital, Ghy-14.

**PHARMACOGNOSTIC STUDIES:**
Fresh fruits were taken for morphological and histological studies. Coarse powder (60 #) was used to study microscopical characters, physicochemical parameters and phytochemical investigation. For the microscopical studies, transverse sections of fruits were prepared and stained as per standard procedure. The powder microscopy was performed according to the method of Khandelwal.

**Macroscopic study:**
It refers to evaluation through organs of sense and includes the macroscopic appearance, color, odour, taste etc. of the drugs.

**Microscopy study:**
A transverse section of fresh pippali fruit was taken and cleaned. The sample was treated with chloral hydrate solution and different staining reagents and chemicals were used to detect the lignified cells in the cross sections and in the powder drugs. The section was mounted on slides and studied under Trinocular Research Microscope.

**PHYTOCHEMICAL SCREENING:**
The aqueous and methanolic extracts along with other solvent extracts of plant fruit materials were studied for various phytochemicals like alkaloids, carbohydrates, flavonoids, glycosides, gums and mucilages, phenols, tannins, reducing sugars, saponins, steroids and terpenoids by using precipitation and coloration reactions.

**EXTRACTION:**
Powdered Pippali, 300gm, was extracted successively with solvents like petroleum ether, benzene, chloroform, acetone and methanol respectively in a Soxhlet apparatus. Each solvent extract was then concentrated by distilling off the solvent under reduced pressure.

**THIN LAYER CHROMATOGRAPHY:**
Thin layer chromatography was carried out with the methanolic extract and maximum spots been separated on precoated silicagel G TLC plate with trial and error methods.
**PHYSICOCHEMICAL PROPERTIES:**
Physicochemical parameters were determined as per guidelines of WHO\(^5\). Total ash value, loss on drying, water soluble ash, acid insoluble ash, solubility of the extract in different solvents, pH, heavy metal analysis, petroleum ether soluble extractive, alcohol soluble extractive and water soluble extractive values were determined.

**RESULTS AND OBSERVATION**
The Pippali fruit was investigated in a systematic way covering pharmacognostical, physicochemical and phytochemical aspects to rationalize its use as a drug of therapeutic importance.

**MACROSCOPIC CHARACTERISTICS:**
The various macroscopic characters like colour, odour, taste, size, shape, etc of both fresh and dried Pippali fruit have been listed in Table 1.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Pippali fruit (fresh)</th>
<th>Pippali fruit (dried)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Greenish black</td>
<td>Greyish black</td>
</tr>
<tr>
<td>Odour</td>
<td>Aromatic</td>
<td>Aromatic</td>
</tr>
<tr>
<td>Taste</td>
<td>Less pungent</td>
<td>Pungent</td>
</tr>
<tr>
<td>Touch</td>
<td>Slightly rough</td>
<td>Rough</td>
</tr>
<tr>
<td>Size</td>
<td>2.5-3 cm, 4.5 mm diameter</td>
<td>2.2-5 cm, 3-4 mm diameter</td>
</tr>
<tr>
<td>Shape</td>
<td>Cylindrical, straight</td>
<td>Cylindrical, slightly curved</td>
</tr>
<tr>
<td>Surface</td>
<td>Rough and beady</td>
<td>Rough</td>
</tr>
</tbody>
</table>

**MICROSCOPICAL CHARACTERISTICS**

*Fruit microscopy*
Mesocarp shows broad zone of parenchyma cells with plenty oil globules. Oil zones composed of 2-5 rows of angular embedded oleoresin cells which are oval to circular in shape. Vascular bundle is well developed, clearly differentiated with protoxylem and metaxylem.
Determination of Quantitative Standards:

The various physicochemical parameters like Ash value, total ash, pH, LOD, etc of Pippali fruit have been given in Table 2.

Table 2: Physicochemical parameters of Pippali fruit

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ash value</td>
<td>4.88%</td>
</tr>
<tr>
<td></td>
<td>Total ash</td>
<td>0.09%</td>
</tr>
<tr>
<td></td>
<td>Acid insoluble ash</td>
<td>0.09%</td>
</tr>
<tr>
<td>2</td>
<td>Extractives</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water soluble extractive</td>
<td>9.92%</td>
</tr>
<tr>
<td></td>
<td>Alcohol soluble extractive</td>
<td>17.00%</td>
</tr>
<tr>
<td>3</td>
<td>Loss on drying</td>
<td>10.88%</td>
</tr>
<tr>
<td>4</td>
<td>pH</td>
<td>5.0</td>
</tr>
<tr>
<td>5</td>
<td>Foreign matter</td>
<td>0%</td>
</tr>
</tbody>
</table>

Chromatographic Profile of Crude Extract of *Piper longum* Linn

In the chromatographic profile of the methanolic extract of Pippali fruit, different major spots were observed with Rf value of 0.6, 0.5, 0.3, 0.1, which signifies presence of several active constituents. The details of solvent system and the Rf values have been listed in Table 3, Figure 4.

Table 3: The details of solvent system and the Rf values

<table>
<thead>
<tr>
<th>Extract</th>
<th>Solvent system</th>
<th>No.of spots</th>
<th>Rf values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>Dichloromethane : nHexane</td>
<td>4</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.10</td>
</tr>
</tbody>
</table>

Phytochemical Study:

The detailed phytochemical study of Pippali fruit reveals significant data of its presence of alkaloids, glycosides, tannins, phenols, flavonoids, sterols, etc as shown in Table 4.

Table 4: Phytochemical Analysis Of Pippali Fruit Extract

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Constants</th>
<th>Present/Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+ve</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+ve</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>+ve</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>-ve</td>
</tr>
<tr>
<td>5</td>
<td>Tannins and phenols</td>
<td>+ve</td>
</tr>
<tr>
<td>6</td>
<td>Sterols</td>
<td>+ve</td>
</tr>
<tr>
<td>7</td>
<td>Quinines</td>
<td>-ve</td>
</tr>
<tr>
<td>8</td>
<td>Lignins</td>
<td>-ve</td>
</tr>
</tbody>
</table>

Discussion

The various pharmacognostic parameters and standards of a crude drug must be established before its inclusion in a herbal
pharmacopoeia. Hence, a drug should be standardized to establish its correct identity. Microscopic method is one of the easiest and cheapest methods to start with for establishing the correct identity of the source materials. The physical constant evaluation of a drug is an important parameter in detecting adulteration or improper handling of drugs. The macroscopical characters of the fruit can serve as diagnostic parameters. Ash values and extractive values are important in evaluating the purity of drugs i.e. the presence or absence of foreign inorganic matter. Extractive values help in estimation of specific constituents soluble in particular solvents and in evaluation of the chemical constituents present in the drug. Physicochemical parameters of the Pippali fruit showed that alcohol soluble extractive value is more than water soluble extractive value, which indicates the presence of more alcohol soluble contents in the drug. pH of the drug determines acidity or alkalinity of drug. The test drug has pH 5.0 indicating its acidic nature. Phytochemical analysis of the drug showed presence of alkaloids, flavonoids, glycosides, sterols, tannins and phenols.

**CONCLUSION**

It is an essential part of discovery, development and manufacture of Pharmaceuticals to provide timely, accurate and reliable data. Here an attempt was made to get a standardized data of Pippali available in Assam. The pharmacognostical, phytochemical and physicochemical characters of Pippali fruit are useful to generate standards to assess the quality and purity of the drug. The information provided by this study may be useful to carry out further study of Ayurvedic drugs of traditional medicinal practice of present era.
REFERENCES: