Standardisation and Quality Control of Homoeopathic Drug *Pimenta officinalis* Lindl.

P. Padma Rao*1, P. Subramanian1, P. Sudhakar1, T. Sheshashena Reddy1, P.R. Reddy1 and H.C. Gupta2

1Drug Standardisation Unit, Hyderabad
2Central Council for Research in Homoeopathy, New Delhi

*Pimenta officinalis* Lindl., a bushy tree commonly known as ‘all spice’ belongs to the family Myrtaceae. It is a potential drug in homoeopathy used in neuralgias and disturbed sensations of heat and cold. The dried unripe fruits are used as medicine. The fruit is a small pea-sized berry, blackish to reddish brown. The epicarp is conspicuous with numerous large glandular secretory cavities. The pericarp is about 2 mm thick. The mesocarpic cells contain resin, tannins and sphaerocrystals. Stone cells are also present in mesocarp. The outermost layer of seed consists of closely packed testa with attached stone cells. The endosperm consists of cells containing starch grains. The powder microscopical and organoleptic characters of powder are presented.

Physico-chemical parameters of raw drug viz., extractive values, ash values, formulation and standardisation of mother tincture like wt. per ml, total solids, alcohol content, pH along with HPTLC and UV studies have been undertaken.

**INTRODUCTION**

*Pimenta officinalis* Lindl., commonly known as “All spice” is a bushy evergreen tree belongs to the family Myrtaceae. It is indigenous to the West Indies and tropical America and also grown in gardens in India.1 In homoeopathy, the dried unripe fruits are used in the preparation of medicine. It is used in neuralgias and disturbed sensations of heat and cold.2,3 Besides, it is an aromatic stimulant used in digestive troubles and flatulence, dyspepsia and diarrhoea.4 Chemically the fruit is found to contain eugenol as a principal constituent, besides vanillin,1,8 cineole, caryophellene, quercetin glycosides, catechins, phenyl propanoid and 3-(4- hydroxy- 3- methoxyphenyl) propane – 1,2-diol.5,6 Earlier studies pertaining to pharmacognosy and physico- chemical parameters of fruit are rather scanty.4,7,8 The drug is also not included in HPI. Hence, at present, more detailed morpho-anatomical and physico-chemical studies of dried unripe fruits have been undertaken. Besides HPTLC, finger printing and UV absorbance of the drug are also recorded.

**MATERIAL AND METHODS**

**Pharmacognosy**

The authentic material, i.e., dried unripe fruits of *Pimenta officinalis* was obtained from Survey of Medicinal Plants and Collection Unit, Emerald, Nilgiri Dist. Tamil Nadu (India). The material was preserved in F.A.A and used for anatomical studies following the method of Johansen.9 The epidermis of fruit wall was obtained by scraping with a razor blade. Microtome sections were taken, stained and mounted as per standard method.9 Photomicrographs were taken with Olympus CH- 2 trinocular microscope.

**Physico-chemical**

The air dried fruits were coarsely powdered to 10/44 (sieve size) and were subjected to determination of moisture content (loss on drying at 105°C), total ash, water soluble ash, acid insoluble ash, extractability in different solvents, physico-chemical constants, HPTLC and UV aspects of mother tincture following official methods.10,11,12 Mother tincture was prepared as per HPI by the percolation method.13

**HPTLC analysis**

Evaporate 25 ml mother tincture on water bath to remove alcohol. Extract the residue with 3x25 ml
chloroform. Concentrated chloroform extract is used for the HPTLC study. The concentrated chloroform extract was spotted in the form of band of width 4 mm with a Camag microlitre syringe on precoated silica gel aluminium plate 60F-254, (5 x 10 cm with 0.25 mm thickness; Merck, Darmstadt, Germany) using a Linomat IV sample applicator (Camag, Muttenz, Switzerland, supplied by Anchrom technologists, Mumbai). A constant application rate of 6 ml/sec was employed. The slit dimension was kept at 4 x 0.45 mm and 20 mm/sec scanning speed was employed. The mobile phase consisted of chloroform: methanol (9:1 v/v) and 10 ml of mobile phase was used for chromatography. Linear ascending development was carried out in a 10 x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase at room temperature (25±2°C). The length of the chromatogram run was 8 cm and subsequent to the development, the TLC plates were dried in a current of air with the help of hot air dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed (Camag TLC scanner III) at 254 nm and 366 nm by reflectance scanning and operated by CATS 4 software (v 4.05, Camag) resident in the system.14,15,16

OBSERVATIONS AND RESULTS

Morphology: A bushy evergreen tree 6-9 m in height. Its leaves oblong to oblong lanceolate, leathery; flowers white, in terminal and axillary trichotomous paniculate cymes; fruit a globose berry with the size of a pea, black to purple, two seeded; seeds reniform, dark brown.17

Macroscopy: The fruit is a globose berry, small pea sized, up to 0.5 cm in diameter, apex with four small calyx teeth, surrounding a short style; externally rough, with undulated surface.

Microscopy: The pericarp is up to 2 mm thick, made up of outer epicarp, middle mesocarp and inner most endocarp. In surface, the epicarp possesses large rounded glandular secretory cavities, 170 – 255 μm (204) in diameter with a thick ridge and a large central cavities often with yellowish oily contents. The intermittent areas are hyaline.

In transection, the epicarp consists of 1- layered epidermis of elongated, oblong to elliptic and few oval to spherical cells as a narrow chain often densely filled and covered by a thick cuticle. The hypodermis is 4 – 6 layered, collenchymatous, consists of elongated cells often with tanniferous contents. Beneath the collenchyma, secretory oil cavities are present. The secretory cavities are large, spherical and covered with 2 – 3 layered epithelium. The cortex or mesocarp is prominent, consisting of spherical to polygonal and elongated cells often containing resin, tannins and sphaerocrystals.

The mesocarp often possesses polygonal to spherical, lignified stone cells, either solitary or in groups. Vascular bundles are dispersed in the mesocarp. The endocarp is inconspicuous and made up of 2 – 3 layers of small closely arranged cells with dense contents.

In transection, the seed possesses outermost thick layered testa consists of thick walled cells. The hypodermal region is made up of layer of isolated stone cells. Internally, the endosperm consists of polygonal to spherical cells with dense starch grains.

Powder microscopy
1. Oil glandular secretory canals, either single or in groups with dark contents.
2. Stone cells numerous, isolated or in groups.
3. Many starch grains.
4. Sphaerocrystals, isolated a few.
5. Cortical cells with sphaerocrystals.
6. Endosperm tissue with starch grains.
7. Masses of brownish yellow resins.

Organoleptic characters
Colour - reddish brown
Touch - coarse
Odour - strong aromatic, spicy
Taste - astringent

Physico-chemical studies

The data under the physico-chemical study for the raw drug is summarized in Table 1 and that of mother tincture preparation and its standardization in Tables 2 and 3 respectively.

Qualitative phytochemical tests
Preliminary phytochemical study indicates the presence of alkaloids, essential oil, terpenoids, tannins and volatile organic matter, besides traces of volatile oils.

HPTLC fingerprinting

The profile of chromatographic separation scanned at 254 nm, reveals nine spots. Among them 8, 7 and 6 spots possess maximum composition with Rf at 0.72, 0.65 and 0.57 respectively. While, chromatogram scanned at 366 nm shown in Figure 3, reveal 8 spots with 7, 6 and 5 spots showing maximum composition.
Table 1: Standardisation of Raw Drug

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Quantitative values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moisture content (Loss on drying at 105° C)</td>
<td>Not more than 9.50 % w/w</td>
</tr>
<tr>
<td>2</td>
<td>Total ash</td>
<td>Not more than 5.74 % w/w</td>
</tr>
<tr>
<td>3</td>
<td>Acid insoluble ash</td>
<td>Not more than 0.63 % w/w</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble ash</td>
<td>Not more than 1.89 % w/w</td>
</tr>
<tr>
<td>5</td>
<td>Alcohol soluble extractive</td>
<td>Not less than 6.52 % w/w</td>
</tr>
<tr>
<td>6</td>
<td>Water soluble extractive</td>
<td>Not less than 11.32 % w/w</td>
</tr>
<tr>
<td>7</td>
<td>Extractive values in</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Hexane</td>
<td>Not less than 0.40 % w/w</td>
</tr>
<tr>
<td></td>
<td>b. Chloroform</td>
<td>Not less than 1.92 % w/w</td>
</tr>
<tr>
<td></td>
<td>c. Methanol</td>
<td>Not less than 8.9 % w/w</td>
</tr>
</tbody>
</table>

Table 2: Formulation of mother tincture  (Percolation technique was used13.)

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>65 % v/v</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug strength</td>
<td>1/10</td>
</tr>
</tbody>
</table>

Preparation:

Pimenta officinalis in coarse powder 100 g
Strong alcohol 678 ml
Purified water 350 ml
To make one thousand millilitres of the mother tincture

Table 3: Standardization of Mother Tincture

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Organoleptic profile</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Appearance</td>
<td>Clear, non viscous, foam on shaking</td>
</tr>
<tr>
<td></td>
<td>b. Colour</td>
<td>Blackish yellow</td>
</tr>
<tr>
<td></td>
<td>c. Odour</td>
<td>Strong aromatic tobacco like smell</td>
</tr>
<tr>
<td>2.</td>
<td>Sediments</td>
<td>Absent</td>
</tr>
<tr>
<td>3.</td>
<td>Weight per ml</td>
<td>Not more than 0.88 g</td>
</tr>
<tr>
<td>4.</td>
<td>Total solids</td>
<td>Not less than 1.13% v/w</td>
</tr>
<tr>
<td>5.</td>
<td>Alcohol content</td>
<td>61-64 % v/v</td>
</tr>
<tr>
<td>6.</td>
<td>pH</td>
<td>5.0– 6.0</td>
</tr>
<tr>
<td>7.</td>
<td>λ max</td>
<td>341, 278, 226 nm</td>
</tr>
</tbody>
</table>
Standardisation and Quality Control of Homoeopathic Drug Pimenta officinalis Lindl.

P. Padma Rao et al

Figure 1

1. Epicarp in surface x 59
2. T.S. of pericarp x 57
3. T.S. of pericarp (enlarged) x 106

Abbreviations:
- c – cortex or mesocarp;
- h – hyaline;
- e – epidermis;
- oc – oil cavities;
- st – stone cells;
- v – vascular bundles

Figure 2

1. T.S. pericarp (enlarged) showing sphaeraphides x 570
2. T.S. of fruit pericarp and seed x 62
3. T.S. of seed with endosperm x 139

Abbreviations:
- ec – endocarp;
- end – endosperm;
- sph – sphaeraphides;
- st – stone cells;
- sg – starch grains;
- t – testa

Figure 3: HPTLC finger printing of Pimenta officinalis mother tincture scanned at 366
at Rf 0.71, 0.65 and 0.57 respectively. At 254 nm, nine spots appear at Rf 0.25, 0.31, 0.34, 0.39, 0.42, 0.57, 0.65, 0.72 and 0.86 with various concentrations while at 366 nm, eight spots at Rf 0.18, 0.29, 0.34, 0.44, 0.57, 0.65, 0.71 and 0.86 (Fig.3).

**DISCUSSION AND CONCLUSION**

*Pimenta officinalis* is bushy evergreen tree belongs to the family Myrtaceae. The berries are nearly globose up to 0.5 cm in diameter, apex with four small calyx teeth surrounding a short style, externally rough and undulated, reddish brown to black as also reported.4,8 The pericarp is upto 2 mm thick, reddish brown with numerous oil glands and confirms earlier studies. The epicarp in transection possess large secretory oil glands 170-255 μm (204) in diameter with a 2-3 layered thick ridge containing yellowish oil contents. The mesocarp possesses polygonal to spherical lignified stone cells, either solitary or in groups. Sphaerocrystals 5.5-25 μm (13) in diameter occur in cortex. The seed in transection has a thick layered testa. The endosperm consists polygonal to spherical cells with dense starch grains. The powder microscopic features and organoleptic characters of the powder of fruit are also presented. The salient macroscopic and microscopic characters along with microscopic features of powder and organoleptic features are diagnostic and can be considered as standard for the drug.

The values of total ash, water soluble ash and acid insoluble ash, loss on drying, extractability in water and alcohol and different solvents falls in the acceptable range. The methodology for preparation of the mother tincture and its standardization are presented in Tables 2 & 3, which are specific to homoeopathic pharmacopoeial standards and will supplement substantially. The results of HPTLC studies reveals eight distinct spots and UV spectrophotometric study exhibits $\lambda_{\text{max}}$ (maximum absorption) with three prominent peaks, which can be taken as the characteristic standard for the drug.

The physico-chemical data, macro and microscopical characters of the drug and methodology employed in the study will help in identification, authentication and to ensure quality, purity and efficacy of the drug.

**ACKNOWLEDGEMENT**

Dr. Alok Kumar, Director General I/C, Central Council for Research in Homoeopathy, New Delhi, for the facilities and encouragement; Dr. Uday Kumar, Deputy Director, Pathology Division, National Institute of Nutrition, Hyderabad for the microtome facility and Director, Indian Institute of Chemical Technology, Hyderabad for the HPTLC facility.

**REFERENCES**

पिमेंटा ओफिसिनालिस एल. एक झाड़ीनुमा पेड़ होता है जिसे सामान्यतः ‘सभी मसाले’ के रूप में जाना जाता है, इसका संबंध माइरसी परिवार से है। यह होम्योपेथी में एक संभावित औषधि है जिसका प्रयोग न्यूरालिगिक्स और गर्मी एवं सर्दी के प्रति अनियमित उत्सर्जना में किया जाता है। सूखा अपरिपक्व फल औषधि के रूप में उपयोग किया जाता है। फल का आकार एक छोटे मटर के दाने जिलना होता है, जो कि बेर जैसा भूरे काले से लाल भूरे रंग जेसा होता है। बाह्यफलभिंति असंख्य बड़ी सादी ग्रंथियों सहित गुहिकाओं के कारण विशाल होती है। फलभिंति लगभग 2 मिमी. होती है। मिसोकार्औषधि कोशिकाओं में राल, तेनिनस् और स्क्रीनिस्तिल होते हैं। स्टॉन कोशिकाएं भी मिसोकार्औषधि में मौजूद होती हैं। बीज की सबसे बाहरी परत अत्यधिक जन्तुवादी टेस्ट से साथ स्टॉन कोशिकाओं की बनी होती है। एंडोस्पर्म स्ट्राज अनाज कोशिकाओं के बने होते हैं। पाउडर की अति सूखर्त और इंदिरा गाँधी विशेषताएं प्रदर्शित की गई हैं। कच्चे औषधियों के भौतिक रासायनिक मापदंड जैसे की अर्कमूल्य राख मूल्य और मदर टिंवर का सूत्रीकरण और मानकीकरण जैसे प्रति म्या. भार, कुल टोस, अल्कोहल की मात्रा पीएच सहित एचपीटीएल्सी और यूवी अभ्ययन शुरू किये गये।