

ORIGINAL ARTICLE

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.

Address for Correspondence:

Dr. P. Padma Rao

Drug Standardisation Unit

O.U.B.32, Road No. 4, Habsiguda, Hyderabad

Email: padmarao.pogaku@yahoo.com

Received on 04.07.12

Approved on 26.10.12

Indian Journal of Research in Homoeopathy
Vol. 6, No. 3, July - September 2012

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 pre-coated 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 panicle 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 tanniniferous 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 R_f 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

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

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

Alcohol	65 % v/v
Drug strength	1/10
Preparation:	
<i>Pimenta officinalis</i> 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

S. No.	Parameters	Observations
1.	Organoleptic profile	
	a. Appearance	Clear, non viscous, foam on shaking
	b. Colour	Blackish yellow
	c. Odour	Strong aromatic tobacco like smell
2.	Sediments	Absent
3.	Weight per ml	Not more than 0.88 g
4.	Total solids	Not less than 1.13% v/w
5.	Alcohol content	61-64 % v/v
6.	pH	5.0– 6.0
7.	λ max	341, 278, 226 nm

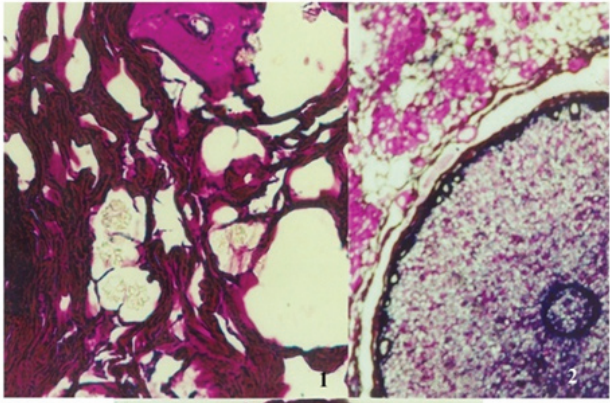


Figure 1

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

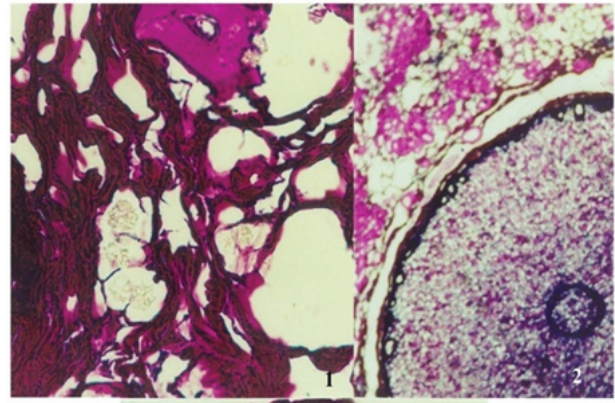


Figure 2

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

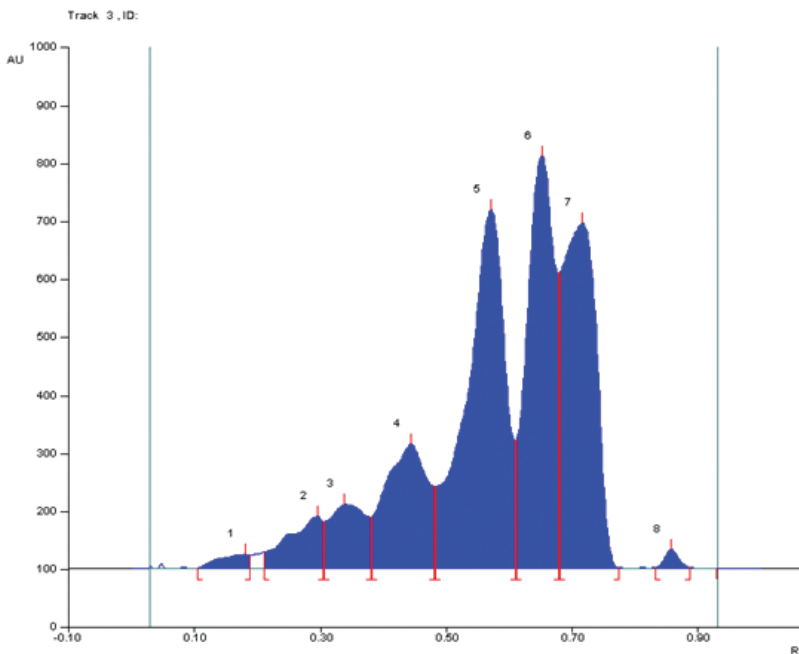


Figure 3: HPTLC finger printing of *Pimenta officinalis* mother tincture scanned at 366

at R_f 0.71, 0.65 and 0.57 respectively. At 254 nm, nine spots appear at R_f 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 R_f 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 μ m (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 μ m (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 λ_{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.

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पिमेन्टा ओफिसनालिस एल. एक झाड़ीनुमा पेड होता है जिसे सामान्यत 'सभी मसाले' के रूप में जाना जाता है, इसका संबंध माइर्टेसी परिवार से है। यह होम्योपैथी में एक संभावित औषधि है जिसका प्रयोग न्यूरालगियस और गर्मी एवं सर्दी के प्रति अनियमित उत्तेजना में किया जाता है। सूखा अपरिपक्व फल औषधि के रूप में उपयोग किया जाता है। फल का आकार एक छोटे मटर के दाने जितना होता है, जो कि बेर जैसा भूरे काले से लाल भूरे रंग जैसा होता है। बाह्यफलभित्ति असंख्य बड़ी स्रावी ग्रंथियों सहित गुहिकाओं के कारण विशिष्ट होती है। फलभित्ति लगभग 2 मि.मी. होती है। मिसोकार्पिक कोशिकाओं में राल, टेनिनस् और स्फेरोक्रिस्टल होते हैं। स्टोन कोशिकाएं भी मिसोकार्प में मौजूद होती है। बीज की सबसे बाहरी परत अत्यधिक नज़दीक टेस्टा के साथ स्टोन कोशिकाओं की बनी होती है। एंडोस्पर्म स्ट्राज अनाज कोशिकाओं के बने होते हैं। पाउडर की अति सूक्ष्म और इंद्रिय ग्राही विशेषताएं प्रदर्शित की गई हैं। कच्ची औषधियों के भौतिक रासायनिक मापदण्ड जैसे की अर्कमूल्य राख मूल्य और मदर टिंचर का सूत्रीकरण और मानकीकरण जैसे प्रति मि.ग्रा. भार, कुल ठोस, अल्कोहल की मात्रा पीएच सहित एचपीटीएलसी और यूवी अध्ययन शुरू किये गये।

