INTRODUCTION
Pippali is one of the oldest medicinal plant known to Indians that dates back to 2000-3000 years. Medicinal properties of Pippali are well known and well documented since vedic period. In Atharva veda, Pippali is mentioned as rasayana, Kshipra bhashaja, Atividdha bhashaja, and Vatakrit Bhashaja. Acharya Sayana has quoted Pipplai in the treatment of Vatavyadhis like Dhanurvata and Akshepaka. In Kaushika Dharmasutra, Pippali and Sarshapa khanda along with other herbs are advocated as medhya rasayana for neonates. According to book called Keshava paddhati (26/33-40), it is indicated for vata vikaras. Pippali one of the best drugs in Ayurveda that nourishes all dhatus of

MICROSCOPICAL IDENTIFICATION OF TWO PIPER SPP.,
PIPER LONGUM AND PIPER RETROFRACTUM

C.S. RANA,1* G.P KIMOTHI,2 V. SASIBHUSHAN,3 R.K. RAI,4 S.B. NARAYAN,5 J.L.N. SASTRY6**

Dabur Research and Development Centre (DRDC)1-6, Dabur India Ltd. Sahibabad, Ghaziabad, Uttar Pradesh - 201010 (India)

Abstract: Background: The genera Piper belong to the family Piperaceae. About 2000 species of Piper are extended all over the world and 52 species are found in different part of India. Piper longum Linn, P. retrofractum Vahl, P. betel, P. nigrum, and P. cubeba are some important species which are utilized for the medicinal property in traditional as well as modern system. Fruits stem and root parts are generally used both of the species in spices. Materials and Methods: Plant species (Piper longum Linn. and Piper retrofractum Vahl) were collected from wild as well as cultivated sources. Plant specimens made into Herbarium following Jain and Rao, (1977), given accession numbers and have been identified by the Botanical Survey of India, Dehradun (BSD). Microscopical studies were conducted following standard methods of Pharmacognosy. Results: In the T.S of stem and roots of Piper longum and Piper retrofractum some key features have been noticed and which will be key identification of the individual species too. The stellar region are mostly appears in penta and hexagonal wedged shape. In root the vascular system appears in single layer while as in the stem parts of both species it would be appearing in multi layered. Single layer of vascular system in root parts and double layer of vasculum in stem parts must be key identification of the individual species. Discussion: An adulteration, either intentional or unintentional reduces potency of herbal drugs. Keeping the above facts in mind the present study was designed and to carry out revamp gap of misidentification and adulteration of the species. Conclusion: Detailed taxonomical history along with etymology of the concern species has been discussed and emphasized to mitigate the gaps of misidentification of plant parts.

Keywords: Ayurveda, Chavica longa, Chavica officinarum, Histology, Pippali, Taxonomy.
body and maintains health. Pippali is one such drug which is commonly used as spice and also having great therapeutic importance [1-3] (Ravindran et al., 1992; Chauhan et al., 2011; Sharma, PV 2013). Pipplamool possesses bioavailability enhancing properties. Piperine was shown to enhance the bioavailability of antitubercular drugs rifampicin, pyrazinamide, isoniazid and ethambutol and also the antileprotic drug[4] (Bhardwaj et al., 2002). The fruits of Pippali is an important rasayana drug which is capable of improving intellect and memory power as also regains health by dispelling diseases. It is considered digestive, appetizer, aphrodisiac and tonic and also it cures many other diseases like cough, piles, anemia, fever and leprosy [5] (Kaushik et al., 2012). The pharmacological properties of this plant was validated to some extend of its curative properties as antiasthmatic, as digestive stimulant, antiulcer, amoebicidal, hypoglycemic, anti-inflammatory, anticancer and as an immune-modulator [6, 7] (Madhavi et al., 2012; Nabi et al., 2013).

The genus *Piper* is largest genus in the family Piperaceae, which is found throughout the tropical and subtropical regions. More than 2000 species were recorded in the genus *Piper*, of which 52 species were found in India [8, 9] (Gamble, JS 1925; Hooker, JD 1886). Several species of *Piper* are used as medicine. *Piper betle* L., *P. longum* Linn, *P. cubeba* L.f. *P. retrofractum* Vahl, *P. nigrum*L., etc, are some of the species used in indigenous medicinal system and herbal industries. The distribution of *Piper* spp ranges from sea level to the high ranges of Andes and Sub Himalayas [8-10] (Gamble, JS 1925; Hooker, JD 1886; Tavan et al., 2002). Trans-Gangetic region and the South Deccan are considered to be the two independent centers of origin of the genus *Piper* in India [8-9] (Gamble, JS 1925; Hooker, JD 1886). The sub mountainous tracts of the Western Ghats are believed to be the centre of origin of *Piper* spp.[1](Ravindran et al., 1992).

According to Ayurvedic classics two types of Pippali are mentioned. One is Gajapippali (*Piper chaba*). Another is Long Pippali (*Piper longum*). Bhavmishra has mentioned the fruit of Chhya/chavika as Gajapippali. Bhavamishra has mentioned the fruit of Chavya/Chavika as Gajapippalai. Raja nighantu mentioned four types of pippali i.e. 1.Pippali, 2.Gajapippali, 3. Simhala Pippali 4.Vana Pippali [3] (Sharma, PV 2003). Simhala and Vana Pippali appear to be Ceylon and wild varieties respectively. Simhala and Vana Pippali appear to be Ceylon and Wild varieties respectively. In market two more types of Pippali (*Piper sylvacticum / P. Peepuloides*) are also available. On physical appearance we concluded that they are mostly used as adulterants to each other. A quality formulation depends on availability of genuine raw materials. Popularity and demand increases the chance of adulteration and substitution of the species [11] (Nitin et al., 2012). Like many other Ayurvedic herbs even Pipalimula is facing problem of non-availability in market. Quality standardization of Ayurvedic medicine is an essential for better therapeutic results [11, 12] (Nitin et al., 2012; Vinay et al., 2012).Uses of pippalimula (roots of piper) was mentioned in various Ayurvedic contexts instead of stem.. The present study was undertaken to investigate grouping of the species on the basis of taxonomy and histology, to describe the external and internal key characteristics features of the individual species for their accurate identification. The present study has been carried out with the following aim and objectives; to find out Histology of stem and root parts of *Piper* species, to validate species of Pipalamool and Chabya through Taxonomy and Histology.

**MATERIALS AND METHODS**

Plant species (*Piper longum* Linn.and *Piper retrofractum* Vahl) were collected from wild as well as cultivated sources. Plant specimens made herbarium and accession numbers were given and plant species were identified in Botanical Survey of India, Dehradun (BSD). Thin sections were prepared by adopting standard procedures and sections were mounted in slides and observed under microscope.
RESULTS

The stem and root of Piper longum L. and Piper retrofractum Vahl were carried out and the obtained results were presented and used these data for scientific validation of the above mentioned species.

TAXONOMICAL DESCRIPTION


Vern. Pippali; H. Pippla; Sans. Pipplamool; Eng. Long pepper

Trailing or climbing herb. Stem terete, angular and grooved with nodes and internodes. Leaves 2.5-10 X 1-6 cm, ovate, cordate, acuminate, membranous, glabrous, 5-7 nerves; petiole 5 x 2.2 cm long; lower basal leaves are generally long petioled, ovate, cordate shaped, upper caule leaves are narrower oblong-cordate, sessile, amplexicaule, elliptic-lanceolate. The female spike is up to 2.5 cm long and 4-5 mm in diameter but the male spikes is larger, slender and narrow, yellow. Flowers dioecious; bracts stalked, peltate. Ovary sunk. Fruiting spike 1.8-2.5 X .6-.75 cm and fleshy.

Distribution: Hotter parts of India, Sri Lanka, Malacca.

Piper chaba Hunter in Asiat. Res. 9. 391; Roxb. Fl. India. 1: 156. P. officinarum Cas DC. in Prodr. 16. 356. P. peepuloides Wall. Cat. 6650 E.F. Chavica officinarum Miq. Syst. Pip. 256. II. Pip. 39. T. 34. and Fl. Ind. Bat. I.2.444.(Fig.2)


Climbers, glabrous, stem stout climbing and rooting. Stems brownish when dry, ca. 2 mm thick, terete, striated. Petiole 5-11 mm, sheathed at base only; leaves very short petioled rather coriaceous oblong-ovate or lanceolate acuminate 3-5 nerves at the very obliquely cordate auricled base pinnienerved above it, fruiting spike stoutly peduncled, suberect, conico cylindric; leaf blade narrowly elliptic, ovate-oblong, or elliptic, 8.5-16 × 3.2-7.5 cm, papery, glaucous when dry, densely glandular, base with both sides rounded or 1 side slightly tapered and short, tapered and short side sometimes concave to semicordate, ± symmetric to oblique, bilateral difference 0-5 mm, apex shortly acuminate to acute; veins 9-11, pinnate, usually 4 or 5 on each side of midvein. Plant is dioecious. Male spikes 5-6.5 cm; peduncle slightly longer than petioles; bracts orbicular, 1-1.2 mm wide, peltate, sessile. Stamens 2 or 3; filaments nearly absent; anthers broadly ellipsoid. Ovary immersed in rachis; stigmas 3, ovate-acute, recurved. Female spikes 3-4 cm × 7 mm; peduncle and bracts as in male spikes. Fl. May-Jul.; Fr. Jun.-Sept.

ANATOMICAL FEATURES

Piper longum root: Roots are long cylindrical 0.4-0.6 cm thick, reddish brown to muddy; odour- aromatic; taste- pungent to acrid.

Microscopic

The epidermis appears in double layers. Parenchymatous cells of cortex are arranged in concentric circle. Cortical cells containing starch grains and crystals. Cortex is differentiated as an exodermis. The innermost layer of the cortex is usually differentiated as an endodermis. The pericycle is usually a single layer cells lying within endodermis and peripheral to the vascular bundles. Xylem cells are surrounded by phloem cells and the xylem is exarch because the protoxylem is lies towards periphery and metaxylem towards the centre. The vascular system consists of hexagonal rays of thick
Macroscopic Identification of Pippali and Chavya: C S. Rana, et al.

walled, lignified tracheary elements, alternating with arcs of thin walled pith cells. Vascular bundle is hexarch (Fig. 5).

*Piper longum*: Stem in cut piece usually 0.5-2.0 cm in width, cylindrical and somewhat twisted, greyish-brown, surface smooth with a few longitudinal wrinkles, nodes and internodes are distinct, fractures are short; odour - peppery; taste - acrid.

**Microscopic**

Stem shows a single or multi layered epidermis followed by a continuous ring of collenchymatous and round to oval thin-walled, parenchymatous cells; vascular bundles show peripheral and medullary arrangement, separated from each other by a wavy strip of sclerenchyma forming a ring, enclosing pith; bundles collateral and arranged in rings, having sclerenchymatous sheath of pericyclic cap over phloem; xylem usually six wedge-shaped; starch grains simple and compound having 2-7 components, round to oval (Fig. 7).

*Piper retrofractum* root: Roots are cylindrical to oval 0.2-0.4 cm thick, reddish brown to muddy; odour - aromatic; taste – pungent to acrid.

**Microscopic**

The epidermis is appeared in two layered. Parenchymatous cortex cells which are arranged regularly in concentric ring. Cortical cells often contain starch grains and sometimes many crystals. The outermost layers of the cortex, just beneath the epidermis differentiated as an exodermis, a kind of hypodermis. The innermost layer of the cortex is usually differentiated as an endodermis. The pericycle is usually single to multiple layers of parenchymatous cells lying just beneath the endodermis and peripheral to the vascular arch, protoxylem is lies towards periphery and metaxylem towards the centre. Xylem vessels are surrounded by thread like phloem. The vascular system consists of pentagonal rays of thick walled, lignified tracheary elements, alternating central core of pith. (Fig. 6).

*Piper retrofractum* stem: Cut pieces, having distinct internodes and nodes; stout, cylindrical, 0.2-0.6 cm thick, reddish brown to grey; odour - aromatic; taste- pungent.

**Microscopic**

Stem shows a thin cork consisting of 2-4 layers of rectangular, brownish cells; secondary cortex a wide zone, consisting of round, oval to rectangular, thin-walled, parenchymatous cells with prominent intercellular spaces; plenty of simple round to oval starch granules are present; endodermis single layered; stelar region composed of five wedge-shaped vascular bundles alternating with wide medullary rays. A ring of conjoint collateral perimedullary bundles lie around the centrally located parenchymatous cells; phloem lies towards outer side and composed of sieve elements, parenchyma and phloem fibres occurring in groups or as a capped; xylem lies towards centre and composed of vessels, tracheid and fibres; isolated vessels barrel-shaped with pitted and reticulate thickenings; fibres spindle-shaped, medullary rays are multi seriate (Fig. 8).
DISCUSSION

Plant species have been the source of medicines since thousands of years. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries using medicinal plants for primary health care [13] (Mishra, P 2010).
Macroscopic Identification of Pippali and Chavya: C S. Rana, et al.

Most of the drugs which derived from traditional medicine are now in high demands due to commercialization impact. Floristic inventory has done by many workers. Some study also focused on genera *Piper* and others emphasized on their habit and habitats [1] (Ravindran et al., 1992). Pharmacognostical and anatomical study has been carried out by [15] (Saraswathy et al., 2013) and they concluded that the drugs sold in the market under the name of unauthentic Chavya (*Piper retrofractum* Vahl.) are mixture of *Piper* spp.

Botanical and taxonomical history of the *Piper longum* (Pipalimula) observed that *P. longum* has the synonyms and basionyms like *Chavica longa* (L.) Karst. *C. roxburghii* Miq., *C. sarmentosa* Miq., *Piper latifolium* Hunter. Meanwhile, *Piper retrofractum* Vahl., *Chavica officinarum* Miq., *C. parviflora* Hassk, *Piper chaba* Hunter, *P. officinarum* (Miq.) C. DC. Collectors might be mixed up unintentionally with *Chavica* and *Longa* at the time of collection of root, stem and fruit parts of the individual species. While comparing the herbarium specimen (Fig. 1-2) of both species are very
similar because *Piper longum* and *Piper retrofractum* both are having basal and cauline leaves (reticulate venation). During the collection for herbarium specimens could be challenging to differentiate them. Basal leaves of the *Piper longum* is cordate shaped, while as cauline leaves of the *P. longum* and *P. retrofractum* are elliptic instead of the central nerves venation or arrangement of the veins. Young chaps may be puzzled with the basal as well as cauline leaves in earlier and on maturation of the plants during collection of fruits, stem and root parts respectively (Fig. 3-4). Generally when plants parts like root and stem are difficult to identify and separate each other diagnostic characteristics. Meanwhile the name *Piper longum* represented as long fruits and leaves of the species. In case of *Piper retrofractum* the vernacular name of Gaja Pippali also conflict the collection situation. Definitely, an adulterated form of fruits, stem and roots has come generally in this case. Cavya sometimes Chabya consists of dried root of *Piper retrofractum* Vahl. Syn. *P. chaba* Hunter non Blume. *P. officinicum* DC. Meanwhile, Piplamool consist of dried stem of *Piper longum* L. seems as collection of the root part of the Pipali. But which species have high constituents that is important concern for the validation and standardization.

In the T.S of stem and roots of *Piper longum* and *Piper retrofractum*, some of the key features have been noticed and which will be key identification of the individual species too. The stelar region are mostly appears in penta and hexagonal wedged shape. In root, the vascular system appears in single layer while as in the stem parts of both species it would be appearing in multi layered. Single layer of vascular system in root parts and double layer of vasculum in stem parts must be key identification of the individual species (Fig. 5-8).

**CONCLUSION**

Root and stem parts of *Piper longum* and *P. retrofractum* are important drugs used in Ayurvedic formulations. Its non-availability in the market generally leads probabilities of inter and intra species adulteration and substitution. Roots of *Piper longum* and *P. retrofractum* are generally not available in the market due to small yields. Stem (aerial ground stem parts) cuttings are being used for preparation of medicines. Presence of penta to hexagonal vascular system in single and double layer in the stem and root parts of the individual specie are the unique characteristic features for accurate identification.

**RECOMMENDATIONS**

As per API both species have stem parts used in Ayurvedic formulation, we recommend separate use (root and ground stem) for the particular complaints. Because biological active marker compounds of individual species may affectively differ for the individual disease and complaints.

**ACKNOWLEDGMENTS**

We are thankful to Dr Padmanabha Rugvedi MD Ayurveda at Dabur Research and Development Centre (DRDC) for critical review. We appreciate Officials of Herbarium division, CNH, Howrah for sharing the original image of the *Piper retrofractum* and *P. longum* herbarium specimens. We acknowledge Anonymous reviewers for fruitful suggestions and reviewing the paper properly.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.
Macroscopic Identification of Pippali and Chavya: C S. Rana, et al.

References


*Corresponding Author: Dr. Charan S Rana, PhD, Principal Scientist, Dabur Research & Development Centre (DRDC), Dabur India Ltd. Sahibabad, Ghaziabad, (UP). e-mail: charan.rana@dabur.com Contact No: 9953399279*