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A Critical Review on the Pharmacognosy and Chemistry of the Fruiting Spikes of Piper Longum L.

KEYWORDS: Fruiting spike, Piper longum, pharmacognosy, aliphatic hydrocarbons

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ABSTRACT *The present study deals with the pharmacognostic studies on the fruiting spikes of Piper longum L. and an assessment on the chemical constituents of the volatile oil reported by earlier workers. A clear morphological description of the fruiting spike is given. A number of unambiguous pharmacognostic characters such as rectangular or square oil-containing cells, elongated parenchyma with large irregular chloroplasts, oval or isodiametric cells containing both chloroplasts and starch grains and oval or isodiametric colorless cells with clusters of starch grains as well as absence of sclerieds and sclerenchyma distinguishes this material. The absence of seed is another marker here. The conflicting reports on the constituents of the volatile oil also is put to rest because it seems one of those studies probably on some material adulterated by some other species of Piper.*

Introduction
The fruits of Piper longum L., commercially known as Long pepper (English) or Pipal (Hindi) and Pipali (Sanskrit), is an important medhya rasayana drug which is capable of improving intellect and memory power as also regain health by dispelling diseases (Sivarajan and Balachandran, 1994). Dried fruits and roots are the official parts. It is considered digestive, appetizer, aphrodisiac and tonic and cures among many diseases, cough, piles, anaemia, fever and leprosy. The pharmacological properties which are validated to some extent are curative action against bronchial asthma, as digestive stimulant, antiulcer, amoebicidal, hypoglycemic, anti-inflammatory, anticancer and immunomodulatory activities (Sukh Dev, 2006). The plant is indigenous to hotter parts of India and is cultivated at many places.

Root and stem (available under the name "Piparimool") also are used as stomachic, laxative, anthelmintic, carminative; improving appetite, useful in bronchitis, abdominal pains, tumours and diseases of the spleen.

The plant is a dioecious, glabrous, scandant shrub with lenticels in older portions, leafy stipules, ovate or elliptic leaves having a cordate base and undulate margin. Male spike slender, cylindrical, 5-7 cm long, and female spike cylindrical, 0.5 – 1.5 cm long, 4 mm across. Bracts peltate-orbicular, single bract covers partly four adjacent flowers. Fruiting spike cylindrical, upto 2.5 cm long, black when ripe, pungent.

Pharmacognostic parameters of this important drug are poorly studied and much confusion exists on the morphology and histochemistry of the fruit. At this age, in which the authentic material being scarce, a number of similar looking substitutes are flooding the market. It is commented that in Indian market fruiting spikes of P. longum are usually not available and instead under the name of 'chotipeepal', spikes of P. peepuloides and under the name of 'Baripeepal', spikes of P. chaba are supplied (Mehra, 1970). Due to the confusion existing in the chemistry especially on the constituents of volatile oils and the large number of diverse compounds reported from various parts of this plant, chemical characterization of the drug is becoming more and more difficult. At this stage only pharmacognosy can provide reliable biomarkers to test the drug at raw material stage, both entire fruit or in powder form. In one of the very first pioneering studies published, detailed description of the plant and histology of the root (Piplamool) are given (Aiyer and Kolammal, 1966). In a recent work, an attempt is done to provide the pharmacognostic characters of the fruit (Gupta et al., 2007). But a number of inadvertent and or conceptual errors have crept inside and a student of plant sciences will find such data far from satisfactory.

The plant provides a plethora of compounds from various parts. The major compounds are alkaloids, lignans, steroids, fatty oil (in fruits) and volatile oils (mainly in fruits). Alkaloids include piperidines, alkamides, aristolactams and dioxoaporphines. About 16 alkamides are extracted together with 10 aporphines, five dioxoaporphines and five aristolactams from the plant. From the leaves two isobutyl amides, guineensine and retrofractamide A are isolated (Parmar et al., 1998); whereas from the stem one more isobutyl amide, pellitorine (Das et al., 1998) coupled with two piperidine amides, piperine and piperlonguimine are isolated. Aristolactams and dioxoaporphines are concentrated more in the roots; amides and piperidine type alkaloids are confined to fruits. The piperidine amides isolated from the roots are piperine, piperlongumine and dehydropipernonaline (Chatterjee and Dutta, 1967; Tabuneng et al., 1983) but the only isobutylamide from the roots is piperlonguminine. Aristolactams like aristolactam A 11, cepharanone B, piperolactam A, B and C and dioxoaporphines like cepharadione A and B, norcepharadione B, piperadione and 2 – hydroxy – 1 – methoxy – 4H – dibenzo quinoline 4, 5 (6H) dione (Desai et al., 1989) are also extracted from the roots. Nine isobutyl amides detected in the fruits are pellitorine, sylvatin, piperlonguminine, (2E, 4E) – N – isobutyl eicosadienamide, (2E, 4E, 8Z) – N – isobutyleicosatrienamide, (2E, 4E) – N – isobutyl octadecadienamide, guineensine (Tabuneng et al., 1983) and brachystamide (Das et al., 1998) Further, the piperidine amides same as those from the roots together with pipernonaline and piperundecalidine (Dutta et al., 1977; Tabuneng et al., 1983; and Oizumi et al., 1987) are also found in the fruits. Piperine and Piplartine are found in the stems of P.longum (Atal and Banga,1963) commercially available as 'piplamool'.

Five lignans also have also been isolated from P. longum. Sesamin is confined to stem, root and fruits (Das et al., 1998) whereas (+) – asarinin is isolated from stems and leaves (Miyakado et al., 1983). Lignans (+) – diaeudesmin, fargesin (Miyakado 1983) and pluviatilol are reported from the fruits only. The yield of fatty oil from fruit is 8.5%. The fatty acids present are palmitic, hexadecenoic, stearic, oleic, linoleic, linolenic, arachidic and behenic acids (Bedi and Atal, 1971). Sitosterol is isolated from roots and fruits whereas stigmasterol and daucosterol (Zhang et al., 1996) are isolated from the stem

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and fruit of the plant respectively.

The volatile oil which is produced maximum in fruits of P. longum is dextrorotatory and the yield is 1% (Govindarajan, 1977). There exists conflicting reports on the constituents of this oil. In one report, oxygenated compounds constituted 8-10% of the volatile oil, but the straight chain hydrocarbons (i.e. alkanes and alkenes) are present to the extent of 35-40%. The major constituents of the oil are sesquiterpenes such as β–caryophyllene (17%), α – zingiberene (15%) and β – bisabolene (11.2%) and saturated aliphatic hydrocarbons such as pentadecane (17.8%) and tridecane (6.8%). Other sesquiterpenoids present are germacrene-D (4.9%), cis-β-farnesene (3.7%), spathulenol (3%) and globulol (2.6%), δ-elemene, α-cubebene, α-γalangene, α-copaene, β-bourbonene, β-elemene, α-gurjunene, α-humulene, γ-muurolene, germacrene B, β-selinene, calamenene, γ-elemene, cubebol and δ-cadinol. Other aliphatic components are heptadecane (5.7%), n-hexadecane, n-octadecane, undecane, n-nonadecane, n-eicosane, n-heneicosane and phenyl ethyl alcohol. The monoterpenoids occur in traces and they are α-thujene, terpinolene, p-cymene, dihydrocarveol, , α-pinene, β-pinene, myrcene, α-phellandrene, 1, 8 – cineole, limonene, γ-terpinene, linalool, camphor, terpinen – 4 – ol and α-terpineol. p-Methoxyacetophenone also is found in traces (Anonymous, 1969, Zaveri et al., 2011).

In another significant study on the volatile oil in the fruits of P. longum, 45 components were identified (Liu et al., 2007) , and the highest-content component was also β –caryophyllene (33.44%). Other sesquiterpenids located are β -elemene (2.33%), germacrene- D (3.41%), zingiberene (6.68%), cadina-1,4-diene, β -eudesmol (2.30%) and cubenol (3.64%). Surprisingly the monoterpenes in the oil amounted to about 24%, a surprisingly higher amount and consisted of eugenol (7.39%), Δ^3-carene (7.58%), D-limonene (6.70%) and β -pinene (2.26%). Only one aliphatic hydrocarbon, nonadecane was located in this oil, that too in negligible amounts (0.23%)

Though flavonoid spot pattern is studied no flavonoid or phenolics acids were identified from any part of this plant. Therefore the present work is taken up to evaluate the fruiting spike in terms of its morphology and histochemical characters which will provide the diagnostic markers of this drug. In addition, the phenolic chemistry of the fruits also is studied and a possible reasoning of the observed variations in volatile oil chemistry is attempted.

Materials and Methods

The authentic fruiting spikes of P. longum were procured from the markets of Wayanad, Kerala. The identity of this drug was confirmed by comparing the same with the authentic material preserved in Indian Institute of Spices Research Centre, Calicut. Pharmacognosy studies were conducted on the fruiting spikes by recommended procedures (Wallis, 1957). Standard procedures (Ibrahim and Towers, 1960; Mabry et al., 1970) were followed for identification of flavonoids and phenolics acids in the drug.

Results

The fruits of Piper are one seeded berries. In plants like P. longum, the bracts and bracteoles as well as the peduncle become fleshy and coalesce to form one unified structure in which only the tips of the peltate bracts and the remnants of style are seen jutting out. This is called the "fruiting spike" and is similar to the fruit "sorosis" of jack fruit in morphology. This fruiting spike is a brownish black long cylindrical structure in which individual fruits are arranged in spirals and each turn of spiral contains about 10 fruits. These spikes have an average length of 2.5 cm and diameter of 5mm. The fruit tastes slightly pungent with a slight bitter aftertaste. The tips of bracts are peltate-orbicular having a diameter of 1mm, and single bract covers four adjacent flowers partly. The stalk of bract is fused with the adjacent fruits. The outlines of fruits are not clearly visible but the remnant of the style appeared spherical or bilobed. From the number of bracts and remnant of styles there appear to be 100 to 125 fruits in a fruiting spike (Fig. 1). In T.S., the spikes are found to contain about 8 fruits. The seeds are found to be aborted and only a crushed band of cells are seen within the endocarp. Thus the fruits are seedless in nature.

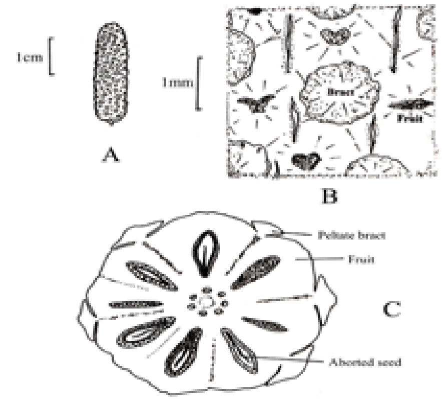

Fig 1: Fruiting spike of P. longum (A); a portion of the spike enlarged (B); Transverse section of fruiting spike (C).

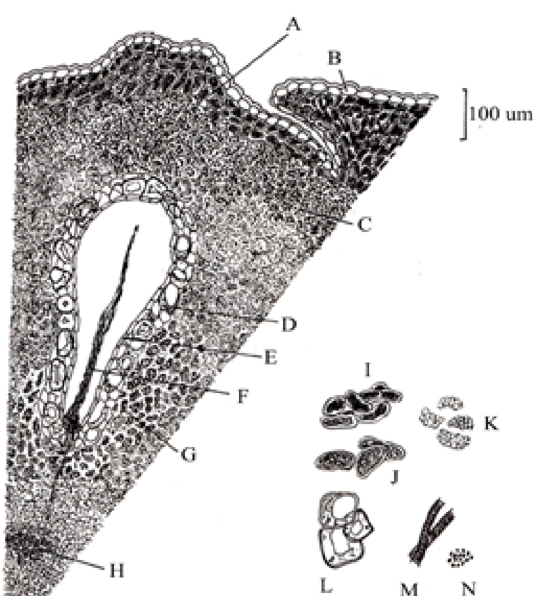

Fig 2: Transverse section of fruiting spike of P. longum showing (A) Hypodermal parenchyma with large chloroplasts; (B) Bract; (C) Parenchyma with large chloroplasts and starch grains; (D) Oil cell; (E) Endocarp; (F) Aborted seed; (G) Parenchyma containing clusters of starch grains; (H) Vascular bundle

Powder of P. longum showing (I) Hypodermal parenchyma with large chloroplasts; (J) Outer mesocarp cells with chloroplasts and starch cells; (K) Inner mesocarp cells with clusters of starch cells; (L) Oil cells; (M) Tracheids; (N) Starch grains.

In the T.S of spike (Fig. 2) the cross sections of fruits, bracts and the axis are seen. The stylar region appears as a bilobed crest. The epidermis consists of barrel shaped cells covered by a thick cuticle. The hypodermis (outer region of mesocarp) contains thin, radially elongated parenchyma each containing a very large elongated irregular chloroplast. These cells have an average length of 50µ and a breadth of 15µ. The middle portion of mesocarp consists of large oval parenchyma having about 40µ length and 25µ in breadth or isodiametric cells of 35- 40µ in diameter. These cells contain both small chloroplasts and starch grains and appear green in colour. Inner to this is a white region consisting a large number of oval cells having about 40µ length and 25µ in breadth or spherical cells (35 to 42µ in diameter) containing closely packed starch grains only. The innermost region of mesocarp adjoining the endocarp is a continuous layer of radially arranged rectangular (70µ x 35µ) or squire (40µ x 40µ) oil cells. Endocarp consists of a single layer of rectangular (35µ x 12µ) thin walled cells. The seed is represented by a dry crushed radially elongated mass of undifferentiated cells. In the centre of the axis of spike there is a ring of 6-8 vascular bundles surrounding the parenchymatous pith. In some samples, the pith is hollow due to the disintegration of parenchymatous cells.

The powder (Fig. 2) of the fruiting spike of P. longum is distinguished by elongated parenchyma with large irregular chloroplasts of hypodermis, oval or isodiametric cells containing both chloroplasts and starch grains (from outer mesocarp region), oval or isodiametric colorless cells with clusters of starch grains (middle mesocarp), rectangular or square oil cells containing oil, tracheids and small starch grains. Sclerenchyma and sclerieds are conspicuously absent.

The fruits are found to contain a flavone, apigenin and syringic, melilotic and gentisic acids. Coumarins, simple phenols, tannins and iridoids were found to be absent in the plant. As there are abundant data on the volatile oil and alkaloids of fruits, they are not analyzed here.

Discussion

The presence of elongated parenchyma with large irregular chloroplasts, oval or isodiametric cells containing both chloroplasts and starch grains, oval or isodiametric colorless cells with clusters of starch grains, rectangular or square oil cells containing oil and small angular starch grains as well as absence of sclerenchyma and sclerieds are important characters distinguishing the fruiting spikes of Piper longum from related species of Piper (having similar fruiting spikes) such as P. hapnium, P.peepaloides, P. chaba, P. jekkoanum and P. sylvaticum. Piper hapnium and P. chaba are different from P. longum in having large rhomboidal starch crystals in perisperm; P.peepaloides in containing mucilage cells and spherical oil cells, P. jekkoanum in containing raphides and P. sylvaticum in having endocarp of beaker cells (Mariamma, 2002). The terms used by some earlier workers such as "aggregate fruits", "fruitlets", "kernel", "perisperm" etc. are erroneous and thus unwarranted.

It is possible to explain the differences reported in constituents of volatile oil from two different schools. In one report aliphatic hydrocarbons form a major portion of oil and monoterpenes are virtually absent (Zaveri et al.,, 2011). This report corroborates earlier reports on the chemistry of this oil (Handa et al., 1963; Anonymous, 1969; Shankaracharya et al., 1997). But in the second report (Liu et al., 2007), the oil of fruiting spikes of monoterpenes in the oil amounted to about 24%, while aliphatic hydrocarbon, (only one located, nonadecane) was in negligible amounts. In both cases sesquiterpenes formed the major components. In both cases the results i.e., predominance of monoterpenes in one sample and of aliphatic hydrocarbons in another, are baffling and send wrong signals to the workers trying to explain the chemical markers as well as the pharamacological actions of long pepper.

During our survey on the chemical constituents especially on the volatile oils of the genus Piper (Mariamma, 2002) we happened to screen a number of species of this genus all collected and authenticated from TBGRI, Palode and Indian Institute of Spices Research Centre, Calicut. On examining those data in terms of the earlier comments (Mehra 1970) that in Indian market fruiting spikes of P. longum are usually not available and instead under the name of 'chotipeepal', spikes of P. peepuloides and under the name of 'Baripeepal', spikes of P. chaba are supplied, we could get some leads which we are presenting here. The volatile oil of P. chaba consisted of 53 components of which only 26 could be identified with the help of three libraries, WILEY 139.LIB, NIST 62.LIB and NIST 12.LIB, present in GCMS-5000 at Southern Petrochemical and Industrial Corporation (SPIC), Science Foundation, Chennai. The principal components were sesquiterpenes like bisabolene, caryophyllene humulene etc. while monoterpenes like eugenol and isoeugenol also were present in this oil. The volatile oil of P. peepuloides also contained 36 compounds of which only 12 could be identified with the help of spectral libraries. All the compounds identified were sesquiterpenes and eugenol or isoeugenol were not seen in this oil even in traces. Incidentally only another species of Piper i.e. P. jekkoanum (out of the total of 13 species screened) was found to possess eugenol and isoeugenol.

The comments mentioned above can probably put the confusion on the chemistry of the volatile oil of P. longum at rest. Most probably the plant material used in one of the study conducted by Liu and co-workers (Liu et al., 2007) was adulterated with that of P. chaba. If that is the case, the volatile oil of P. longum can be seen consisting of sesquiterpenes and aliphatic hydrocarbons.

Conclusions

The present study provides a number of unambiguous pharmacognostic characters such as rectangular or square oil cells, elongated parenchyma with large chloroplasts, oval or isodiametric cells containing both chloroplasts and starch grains and oval or isodiametric colorless cells with clusters of starch grains as well as absence of sclerieds and sclerenchyma. The absence of seed is another marker here. The conflicting reports on the constituents of the volatile oil also is put to rest because it seems one of those studies probably on material adulterated by some other species of Piper.

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