Research Article

PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF *PIPER LONGUM* L. SPIKE OBTAINED FROM TISSUE CULTURE AND NATURAL HABITAT: A COMPARATIVE STUDYM.S Heera¹*, T.S Mahesh², Babu Thushar³, S. Ravi Rao⁴¹PG Scholar, Department of PG studies in Dravyaguna Vijnana, Alva's Ayurveda Medical College, Moodbidri, Karnataka, India²Professor, Department of PG studies in Dravyaguna Vijnana, Alva's Ayurveda Medical College, Moodbidri, Karnataka, India³PG Scholar, Department of PG studies in Shalyatantra, Alva's Ayurveda Medical College, Moodbidri, Karnataka, India⁴Professor, Department of PG studies in Dravyaguna Vijnana, Alva's Ayurveda Medical College, Moodbidri, Karnataka, India

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ABSTRACT

Piper longum L. is a slender perennial climber used in Ayurveda particularly for respiratory tract disorders. Both spikes and roots of this plant are said to have medicinal value. Over the years, industrial demand of Pippali is continuously increasing which is largely met by collection from its natural habitat, endangering its natural resource. Red list status of *P. longum* L. has been assessed and it is included in the short supply list of medicinal plants. As conventional propagation shows delayed rooting and crop losses, there is a need for alternative propagation methods to conserve and sustain it. Micro propagation is an emerging method to propagate plants which allows rapid multiplication of disease free plants in limited time and space. Thus the purpose of the study is to evaluate whether the tissue cultured plant differs from the natural variety. Pippali spikes were collected from tissue culture and natural variety *P. longum* L. plants. Pharmacognostical study, comparative TLC and HPTLC were conducted and the results revealed that inspite of some differences; there are similarities between both the samples. Since the major phyto-constituents are present, the tissue culture Pippali will perform all the major pharmacological actions which are attributed to the natural variety. Thus, micro propagation of *P. longum* L. can be adopted for mass multiplication of healthy and disease free plants thereby arriving at a solution for conservation of its species. Hence it can be used as an alternate method to overcome the scarcity of natural Pippali.

Keywords: *Piper longum* L.; Tissue culture; Pippali, Pharmacognostic study, Phytochemical study**INTRODUCTION**

Plant tissue culture is the technique of growing plant cells, tissues and organs in an artificial nutrient medium static or liquid, under aseptic conditions.¹ This technology is widely accepted as a tool of biotechnology for vegetative propagation of plants of agricultural, horticultural and forestry importance. It has now been commercialized globally and has contributed significantly towards the enhanced production of high quality planting material. Most of the medicinal plants either do not produce seeds or seeds are too small and do not germinate in soils. Thus, tissue culture methods can be useful for rapid multiplication of plants which are difficult to regenerate by conventional methods.² Also, mass multiplication of disease free planting material is a general problem. In this regard, micro propagation holds significant promise for production of true to type, rapid and mass multiplication of plants under disease free conditions. The drug, Pippali (*Piper longum* Linn., Piperaceae family) is a prominent drug of Indian medicine. Both spikes and roots (Pippalimoola) of this plant are used as medicine³. It is used particularly for respiratory tract disorders and as digestive, appetizer, carminative, hematinic, general tonic etc. and is one of the essential ingredients in most of the Ayurvedic formulations. Over the years, industrial demand of Pippali is continuously increasing which is largely met by collection from its natural habitat, endangering the natural resource of this species. Hence it has been included in the short supply list of medicinal plants. Red list status of *Piper longum* Linn. has been assessed as Endangered for Tamil Nadu, India and lower risk for Kerala, India⁴. Conventional propagation is beset with problems of scanty and delayed rooting of vegetative cuttings along with

crop losses due to pests and diseases.⁵ As tissue culture methods helps to produce true to type plants, production of *P. longum* L. by tissue culture method will be helpful in propagating and conserving its species. There are various research studies available regarding micro propagation of *P. longum* L.⁶ but no studies have been done based on the characters of their medicinally useful parts of tissue culture *P. longum* L. Thus the study has been undertaken to assess and compare the quality of *Piper longum* Linn. obtained from natural habitat and tissue culture variety.

MATERIALS AND METHODS**Pharmacognostical study****Collection of the samples**

Botanically identified authentic samples of *Piper longum* L. spikes were collected from the natural variety plants. Mature spikes from tissue culture raised *Piper longum* L. plants were also collected. The samples were subjected to macroscopic and microscopic studies.

Place of Work

Pharmacognostical study was carried out in Department of Pharmacognosy, S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka, India.

Procedure of Microscopy

Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5 ml + Acetic acid-5 ml + 70 % Ethyl alcohol-90 ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. Transverse sections were

photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axiocam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

Phytochemical study

The samples obtained were dried in shade and was made into fine powder. Comparative TLC and HPTLC profiles of *P. longum* L. spike samples were evaluated.

Procedure

One gram of powder was extracted in 10 ml of methanol and filtered. 5 µl of the filtrate of both natural and tissue culture

Pippali extract along with piperine marker were applied on aluminium plate pre-coated with silica gel 60 F 254 of 0.2 mm thickness using LINOMAT 5 applicator. The plate was developed in developing chamber using Toluene: Ethyl acetate: Formic acid (7: 2: 0.1) as mobile phase. The developed plates were visualized in visualizing chamber and scanned in scanner 4 under UV 254, 366 and under white light at 540 nm. The plate was dipped in Vanillin- Sulphuric acid and heated at 105°C till the spots appeared. The plate was also visualized by dipping in Dragendroff's reagent. R_f values and densitometric scan were recorded.

Table 1: Macroscopy of *P. longum* L. - fresh spike

	Tissue culture Spike	Natural variety Spike
Colour	Greenish black	Greenish black
Odour	Aromatic	Aromatic
Taste	Less Pungent, Slightly bitter	Less Pungent, Slightly bitter
Touch	Slightly Rough	Slightly Rough
Size	2-2.5cm in length, 4-5mm diameter	2-2.5 cm in length, 4-5 mm diameter
Shape	Cylindrical, straight, blunt	Cylindrical, straight, blunt
Surface	Rough and beady	Rough and beady

Table 2: Macroscopy of *P. longum* L. - dried spike

	Tissue culture Spike	Natural variety Spike
Colour	Greyish black to brown	Greyish black to brown
Odour	Aromatic	Aromatic
Taste	Pungent, producing numbness	Pungent, producing numbness
Touch	Rough	Rough
Size	1-2 cm in length, 2-3 mm diameter	1-2 cm in length, 2-3 mm diameter
Shape	Cylindrical, slightly curved, blunt	Cylindrical, slightly curved, blunt
Surface	Rough, broken surface shows a central axis around which 5-7 fruitlets are arranged.	Rough, broken surface shows a central axis around which 5-7 fruitlets are arranged.

Table 3: T. S. of spike of *P. longum* L.

	Tissue culture spike	Natural variety spike
Epicarp	Outer epidermal layer of irregular cells filled with deep brown content, covered externally by cuticle.	Outer epidermal layer of irregular cells filled with deep brown content, covered externally by cuticle.
Mesocarp	Wide zone, tangentially elongated parenchymatous tissue	Wide zone, tangentially elongated parenchymatous tissue
Endocarp	Outer layer composed of thin walled cells, colourless, inner layer of tangentially elongated cells, with reddish brown content.	Outer layer composed of thin walled cells, colourless, inner layer of tangentially elongated cells, with reddish brown content.
Vascular bundles	Showing xylem and phloem.	Showing xylem and phloem.

Figure 1: Macroscopy of spikes of *Piper longum* L. - Tissue culture (fresh and dried)

Figure 2: Macroscopy of spikes of *Piper longum* L. - Natural variety (fresh and dried)

Figure 3: Transverse section of spikes of tissue culture and natural variety

Figure 4: Microscopy of tissue culture fruit and natural variety fruit
(EC, epicarp; End, endocarp; Me, mesocarp; Ph, phloem; VB, vascular bundle; Xy, xylem)

Figure 5: TLC photo documentation of Methanolic extract of Pippali from natural and tissue culture along with piperine standard
[Track 1- Pippali natural variety (5 μ l), Track 2- Piperine standard (5 μ l), Track 3- Pippali tissue culture (5 μ l),
Solvent system- Toluene: Ethyl acetate: Formic acid (7:2:0.1)]

Figure 6: HPTLC photo documentation of Pippali tissue culture (5 μ l), piperine standard (5 μ l), Pippali natural (5 μ l) at 254 nm

RESULTS

Pharmacognostical study

Macroscopic study of the fruit samples

While comparing the macroscopic features of fresh and dried spikes (Table 1, 2) (Figure 1, 2), it was found that there was no difference between the spikes of tissue culture and natural variety. Even the number of fruitlets and size of the spike was also similar.

Microscopic study of the fruit samples

While comparing the microscopic features of dried spikes (Table 3) (Figure 3, 4), it was found that there was no difference between the spikes of tissue culture and natural variety.

Thus, both the varieties had similar pattern macroscopically and microscopically suggesting that the spikes cannot be differentiated even though the plant is tissue cultured.

Phytochemical study

Comparative TLC and HPTLC profiles of *P. longum* L. spike samples

TLC photo documentation of Methanolic extract of Pippali from natural and tissue culture (Figure 5) revealed that the bands obtained were almost similar except that few bands were missing and few were seen in addition. It was also observed that piperine was present in both the samples at R_f 0.23.

- At 254 nm, 9 bands in natural variety and 6 bands in tissue culture Pippali.
- At 366 nm, 12 bands in natural variety and 10 bands in tissue culture Pippali.
- After derivatisation with Vanillin sulphuric acid, 10 bands in natural variety and 12 bands in tissue culture Pippali.
- After derivatisation with Dragendorff's reagent, 7 bands in natural variety and 8 bands in tissue culture Pippali.

HPTLC documentation of *P. longum* L. spikes of natural and tissue culture variety (Figure 6) were done in comparison with standard piperine.

- At 254nm, 13 peaks were obtained in natural variety whereas 14 peaks were obtained in tissue culture Pippali.
- The peak of standard piperine was obtained at 0.23 R_f . The same peak was obtained in both the samples indicating the presence of piperine in both the spikes.

DISCUSSION

Thus, the present study to evaluate the difference between tissue culture and natural variety Pippali revealed both similarities and dissimilarities between the two samples. Pharmacognostical study of the spike samples had similar pattern macroscopically and microscopically. While comparing TLC and HPTLC documentation of spike samples, a similarity was noticed in the presence of phyto-constituents, though there were some changes in the pattern of distribution. The differences were noted as;

Presence of additional bands/peaks

This may be due to the reason that even though both the plant varieties were maintained in the same habitat, tissue culture plants started their life cycle in an artificially prepared medium. Further scope of the study involves identification of these compounds.

Absence of some bands/peaks

This may be either due to difference in maturity or due to the fact that tissue culture plants started photosynthesis lately i.e. after hardening and acclimatization. Thus some of the secondary metabolites may be absent.

The differences which were noted should be studied thoroughly especially in terms of maturity, identification of compounds and their pharmacological properties. As the study duration was less, first fruits of tissue culture plants were taken for the present study. The later mature fruits should also be studied to exclude any possible changes in the distribution of chemical constituents. But inspite of these differences, they still share the phyto-constituent piperine in common, which is the major alkaloid present in *P. longum* L. Thus based on the present study, it can be concluded that, inspite of showing some differences, there is similarity between Tissue culture and Natural variety Pippali. To know about the extent of similarity and therapeutic value, detailed chromatographic studies, experimental and clinical studies on various pharmacological actions should be conducted.

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