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A COMPARATIVE PHYTOCHEMICAL SCREENING OF ROOT AND STEM OF *PIPER LONGUM* LINN.

Ujjaliya Nitin B.L*, P. Vivek, R. Remadevi
Dept. of Dravyaguna Vijnan, V.P.S.V. Ayurveda College, Kottakkal, Kerala, India

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***Corresponding author**
Dr. Nitin Ujjaliya B.L., MD Scholar, Dept. of Dravyaguna Vijnan, V.P.S.V. Ayurveda College, Kottakkal, Kerala, India
Email: drnujjaliya@gmail.com

ABSTRACT
Piper longum Linn. is a well known plant being used in home remedies as well as in Ayurveda since long. Nowadays Pippalī mūla (root of *P. longum*) is not easily available in the market. Stem cutting of *P. longum* is being marketed as substitute. Manufacturing companies have started using stem cuttings instead of root. This alteration may affect quality of Ayurvedic preparations. The demand of Ayurvedic medicine has increased globally as well as the concern regarding quality and authenticity of product. The standard parameters of root of *Piper longum* have not been prepared yet. This work was an attempt to establish preliminary physicochemical and phytochemical standards of root as well as comparison with stem cuttings according to the parameters of Ayurvedic pharmacopoeia of India (API). Thin Layer Chromatography profile was prepared to compare their chemical constituents. The profile suggested similarity between root and stem cuttings but the percentage of chemical constituents was found more in stem. Additional researches will substantiate stem cuttings efficiency as a good quality alternative for root.
Keywords: *Piper longum,* physicochemical analysis, phytochemical analysis, Thin Layer Chromatography, Rf value.

INTRODUCTION

Piper longum Linn. belongs to Piperaceae family, is a renowned plant in the field of Ayurveda. It is one of the famous spices of Kerala. Literary review reveals that it is an effective medicine for the diseases of digestive system like loss of appetite, indigestion, flatulence and the ailments of respiratory system such as cough, asthma, allergic rhinitis.[1] Its root (mūla) is ingredient of different formulations in Ayurvedic classics. Quality of formulations depends on availability of genuine raw material. Popularity and demand increases the chance of adulteration and substitution. Like many other Ayurvedic herbs even Pippalī mūla is facing problem of non availability in market.[2] Standardization of Ayurvedic medicine is essential for better therapeutic results and for global acceptance. In Ayurveda, uses of root are mentioned in various contexts but not about stem cuttings. It may be because of therapeutic variation between root and stem of same plant. Similarly therapeutic utility of root of Pippalī is given, but not about stem. Nowadays herbal industries are using stem cuttings as a substitute of root of Pippalī. Quality standard and chemo-fingerprint profile of root has not prepared by any authorities. Ayurvedic Pharmacopoeia of India (API) also has given the standards of stem cuttings instead of root by the name Pippalī mūla.[3] So, an attempt has been made to standardize and compare root and stem cuttings according to the parameters of API.

MATERIAL AND METHODS

The samples were collected from the Botanical garden of V.P.S.V. Ayurveda College, Kottakkal, Kerala and authentically identified in the Pharmacognosy section of the Dravyaguna department using external morphological and histological characters.[4] After identification, samples were subjected for physicochemical standardization.[5] TLC profile of both the samples was done to compare the phyto-constituents.

Physico-Chemical Constants

The moisture content, pH of 10% solution, total ash, water and acid insoluble ash were determined using standard operating procedure.[6] Water soluble, cold alcohol soluble and hot alcohol soluble extractives were obtained and measured. Water soluble extracts of the drug mainly represents the percentage of organic constituents such as tannins, sugars, plant acids, mucilage and glycosides. Alcohol soluble extracts mainly represents the percentage of organic constituents such as alkaloids, phenols, flavanoids, steroids etc. Water and alcohol is the base for most of the Ayurvedic preparations. So these were used as solvent in this study. (Table 1 and 2)

Phytochemical Screening

The extracts obtained were subjected to qualitative tests for the identification of various plant constituents. Tests for alkaloids, steroids, flavonoids, phenols and for the presence of tannin were done as per the standard methods. The extracts were indicative of approximate measure of their chemical constituents. (Table 3).

Table 1: **Physicochemical analysis of stem and root of *P. longum***

S.N	Experiments	Percentage in Stem	Percentage in Root
1.	pH of 10% solution	6.3	6.5
2.	Loss on drying	9.0 %	7.0 %
3.	Total ash	10.5 %	9.2 %
4.	Water insoluble ash	4.66 %	3.2 %
5.	Acid insoluble ash	0.8 %	0.56 %

Table 2: **Percentage of water soluble and alcohol soluble extractives**

S.N	Extractives	Percentage in stem	Percentage in root	Color/Consistency for Both samples
1.	Water soluble	6.05 %	2.8 %	Brown / Dry
2.	Cold alcohol soluble	4.65 %	2.8 %	Leafy green / Oily
3.	Hot alcohol soluble	9.0 %	6.2 %	Leafy green / Oily

Table 3: **Qualitative Phyto-chemical analysis of the extractives**

Solvent	Steroids	Alkaloid-by Mayer's reagent	Alkaloid-by Dragendroff's	Phenol	Flavonoid	Tannin
Water	+	+	+	–	+	+
Cold alcohol	+	+	+	+	+	+
Hot alcohol	+	+	+	+	+	+

TLC Profile

TLC profile was done to compare the phytochemical constituents of both the samples. A pre-coated silica gel 60 F_{254} TLC plate (E. Merck) of 0.2 mm thickness was used for thin layer chromatography. Visualization was done under 366 nm and 254 nm. Rf values were recorded at 366 nm (Table 4). Chemical profile of the extractives gave an initiative regarding pharmacological action and can be used further as standard parameter for examination the adulteration. Rf values were found similar for both the samples but the band width was not equal for each chemical component. (Figure 1)

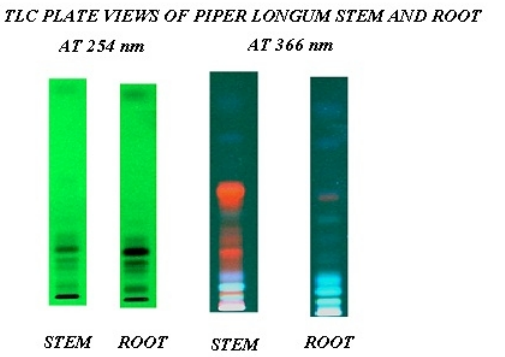

Figure 1: TLC profile of Methanolic extract of Pippalī stem and root

Table 4: TLC details of Methanolic extract of Stem and Root of Pippalī at 366 nm

Rf value	Color of the band in extract of Stem	Color of the band in extract of Root
0.08	Sky blue	Fluorescent blue
0.10	Queen red	Sky blue
0.21	Fluorescent blue	Sky blue
0.28	Blue	Sky blue
0.36	Dark red	Light blue
0.50	Dark red	Light blue
0.58	Dark blue	Blue
0.64	Red	Blue
0.72	Dark blue	Blue
0.80	Dark blue	Blue

RESULT AND DISCUSSION

The study of Pharmacognostical feature of medicinal plants in general is an imperative process to know their quality, purity and to check for the adulterants and substitutes. This study was specially carried out to standardize the root of *P. longum* Linn. Root of Pippalī is not available in the market. Stem cuttings are being used for preparation of medicines. So, in the second part of the study a comparative TLC profile was prepared to assess the pharmacological properties of stem through phytochemical screening. In chromatography it was found that, Rf values were similar but stem cuttings of long piper showed high percentage of chemical constituents than root. At 254 nm stem and root extracts showed very similar constituents (Rf values) and band widths were thick in root extract TLC plate. At 366 nm Rf values are quit similar but the width and color of bands were not similar. In the present study, as water is the universal solvent, the highest percentage of extracts obtained in it. Qualitative analysis suggested that steroids, alkaloids, flavonoids and tannins were present in all solvents. Phenols were present in all solvent except water. The evolved physicochemical parameters and chromatographic finger print profiles were found to be complement to each other and are efficient for establishing the standards.

CONCLUSION

Root of *Piper longum* is an important drug used in Ayurveda and its non-availability has increased the chance of adulteration. The above physicochemical and phytochemical studies including TLC profile have significance in this regard. TLC showed presence of similar chemical constituents in both the samples. On the basis of phytochemical screening it can be suggested that stem cuttings may be a good alternative for roots of Pippalī. Further detailed analysis of percentage of chemical constituents and clinical studies on both the parts of long pepper should be done to evaluate comparative therapeutic efficacy.

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