

## Evaluation of Piperine Content from Roots of *Piper Longum* Linn., Originated from Different Sources with Comparison of Zonal Variation in Odisha, India

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### ABSTRACT

Plant phytochemicals are found to be responsible for various pharmacological effects on human health care system. Piperine, the principle bio-molecular active compound of *Piper longum*, elicits diverse pharmacological activities like analgesic, anti-pyretic bioavailability enhancer, antioxidant, immune-stimulant, hepatoprotective and many more. It is generally extracted or traditionally obtained from fruits (pippali) and roots (pippalimula) of wild *Piper longum*, a RET medicinal plant of the family Piperaceae. In this study, piperine content was assessed through Spectrophotometric and HPLC method from roots of *Piper longum*, collected from various agro-climatic zones of Odisha. Precisely the roots taken for experiment were originated from three types of cuttings used for propagation viz. nodal, Petiolar and apical. Through Spectrophotometric method of analysis piperine content in *P. longum* roots extracted through Methanol ranges in between 0.0176-1.424% dry wt. and ethanol extracted samples in between 0.0094-0.267% dry wt. Similarly in case of HPLC analysis, piperine content, extracted through Methanol ranges in between 0.104-0.74% dry wt. and ethanol extracted samples in between 0.042-0.363% dry wt. From the experiment Piperine content was appraised to be highest in roots of plants grown through nodal cuttings followed by Petiolar and apical leaf cuttings. Similarly amongst the selected agro-climatic zones, Keonjhar region showed highest yield of piperine in root samples followed by G. Udayagiri and finally by Khurda. Moreover among the selected solvent systems, methanol showed promising result in extraction process of piperine than ethanol.

**Keywords:** Apical, HPLC, piper longum, piperine, petiolar, roots

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## 1. INTRODUCTION

*Piper longum* Linn., commonly known as Long Pepper, is an endangered medicinal plant belonging to the family Piperaceae. The native of plant is considered to be South Asia and is found both wild as well as cultivated, throughout the hotter parts of India from central to the north-eastern Himalayas. The herb also grows wild in Malaysia, Singapore, Bhutan and Myanmar [1]. In Odisha it is found in Koraput, Ganjam, Phulbani, Kalahandi, Khurda, Keonjhar, Mayurbhanj, Puri, Angul [2]. The principle bioactive compound found in *P. longum* is an alkaloid, 5-9% of piperine [3-5]. Systemic pharmacological studies on piperine have revealed that this compound elicited diverse pharmacological activities

like analgesic [6], bioenhancer [7], anti-pyretic, anti-inflammatory, antioxidant, hepatoprotective, antithyroid, antihypertensive, antitumor, antiasthmatic & CNS-depressant activities [8, 9].

From ancient era the main source of piperine from *P. longum* are the Spikes and Roots. Several literatures are available regarding piperine content of *P. longum* spikes, but only few literatures are available regarding the piperine content from the root parts [10-12]. Moreover no such reports are available regarding the piperine content in the roots of *P. longum*, collected from various agro-climatic zones of Odisha. Previously the whole plantlet was being successfully developed from the leaf

cuttings (Apical and Basal Petiolar leaf cuttings) of *P. longum* plants [13]. Now the variation in the piperine content present in the root parts, developed from various source types, is to be compared to know the superior root type as the source of piperine content. In this experiment we have focused our concern on the quantitative assessment of piperine content from various root parts of *P. longum* plant viz. roots from nodal vine cuttings, petiolar leaf cuttings and apical leaf cuttings, collected from various agro-climatic zones of Odisha through spectrophotometric and HPLC method of analysis.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Root samples were collected from different plant parts of *P. longum* plants (Nodal, Apical and Petiolar) of three agro-climatic zones of Odisha viz. Keonjhar, Khurda and Phulbani (G. Udayagiri). The samples were compared with herbarium specimens present in Regional Plant Resource Centre herbarium store and also identified through the reference book The Flora of Odisha (Fig. 1, 2 & 3).

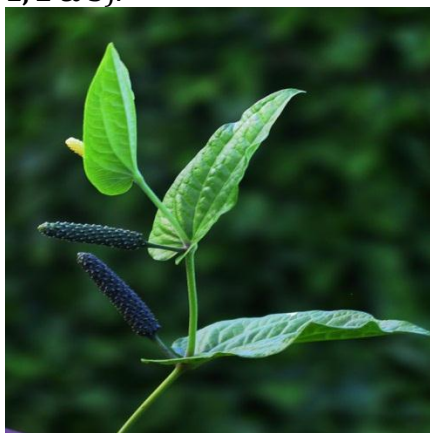


Figure 1: *Piper longum*



Figure 2: Vine Roots



Figure 3: Apical & petiolar leaf Root

### 2.2. Methods

#### 2.2.1. Standard Preparation

Standard stock solution of 1 mg/ml was prepared taking standard piperine (SIGMA Aldrich, Germany) in Methanol and kept at 4°C for further use.

#### 2.2.2. Extraction of Piperine

Ten gm of fine powdered root samples of *P. longum* was extracted through Soxhlet apparatus for 8-10 hrs with HPLC grade Methanol and Ethanol solvent systems separately. The total extract was condensed in dry bath and kept as piperine stock sample [12, 14].

#### 2.2.3. Quantitative Estimation of Piperine through Spectrophotometric Method

Amount of piperine present in the different root part is estimated through Spectrophotometric analysis. To the piperine sample, 0.1 ml of Gallic acid was added. To this solution 5 ml of Conc. H<sub>2</sub>SO<sub>4</sub> was added and the mixture was incubated in water bath at 47 °c for 3-4 mins. The absorbance of this mixture was measured at 656 nm wavelength. The piperine content of the extracted samples was then calculated from the standard curve of pure piperine made by taking different concentrations of it in gradual increasing order [15].

#### 2.2.4. Quantitative Estimation of Piperine through HPLC Method

##### 2.2.4.1. Identification & Isolation through Thin Layer Chromatography (TLC) Procedure

Crude piperine extract (10 µl each) solution was identified and isolated through thin layer chromatography (TLC) process. For this purpose the mobile phase selected was Toluene: Ethyl Acetate in a ratio of 90: 10. The detection reagent used for detecting the pure piperine bands was 1% solution of Vanillin in methanolic Sulphuric acid and viewed under ultraviolet light at 365 nm wavelength for the identification of the separated compounds. The Rf value of sample was determined to check the presence of piperine in the extracted sample; against the standard [14].

#### 2.2.4.2. Assessment of Piperine through HPLC

HPLC analysis was performed in HPLC system (Make-Waters) equipped with binary pump (Model-1525) and porous

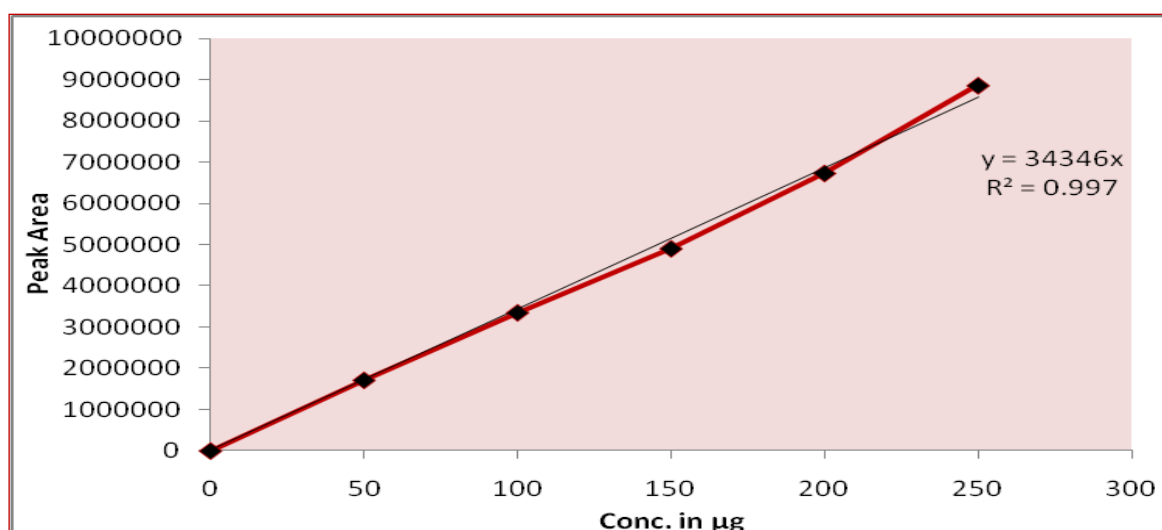
Silica with 5 µm diameter C<sub>18</sub> 4.6 × 150 mm column. The mobile phase selected was consisted of a mixture of HPLC grade Acetonitrile and water, in a ratio of 70: 30, at a flow rate of 1 ml/min. The peaks were detected at 344 nm wavelengths and compared with authentic standard piperine sample for confirmation [15]. The reproducibility of quantitative analysis was verified by carrying out ten replicate injections of standard and three replicate injections of each extract.

#### 2.2.5. Validation of HPLC Analysis

The HPLC system was validated for accurate result profile. The precision, accuracy, LOD, LOQ and correlation coefficient etc were calculated to know the proficiency of the HPLC system and given in (Table 1).

**Table 1: Shows the Statistical Data for Validation of HPLC**

Parameters	Values
Absorption maxima	344 nm
Correlation coefficient (r <sup>2</sup> )	0.997
Regression equation (Y=bx+c)	Y=34346x+0
Intercept (c)	0
Slope (b)	34346
LOD mg/ml	0.534
LOQ mg/ml	1.62
Retention Time	1.4-1.5
Precision (% RSD)	0.01
Accuracy (%)	98.64%



**Figure 4: Standard Curve of Piperine through HPLC Analysis**

### 3. RESULTS

#### 3.1. Piperine Content as estimated by spectrophotometer

Methanol extracted *P. longum* plants showed piperine content within a range of 0.0176-1.424% dry wt. in root samples.

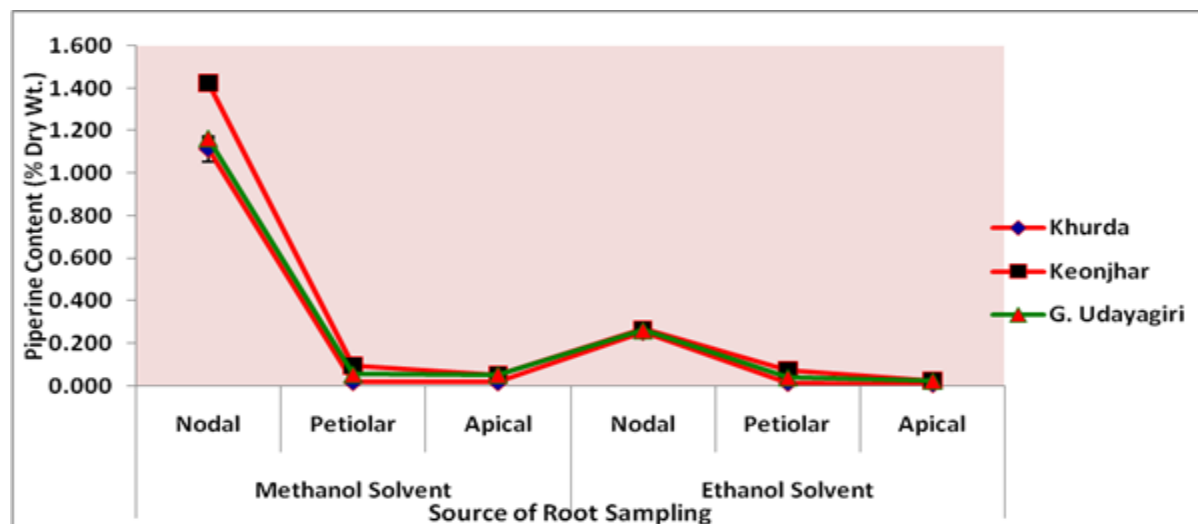
Highest amount of piperine content was found in Nodal root samples collected from Keonjhar (1.424% dry wt.) whereas Apical root sample from Khurda gave the minimum amount of piperine content (0.0176% dry wt.). However Ethanol extracted root samples showed piperine content in a range of 0.0094-0.267% dry wt. Highest amount of piperine was found in Nodal root samples collected from Keonjhar (0.267% dry wt.) whereas Apical root sample from Khurda yielded the minimum amount of piperine content (0.0094% dry wt.). In both the cases the highest amount of

piperine content was found in Keonjhar region followed by G. Udayagiri and then in Khurda. In all the three Agro-Climatic zones the percentage of piperine content was found to follow a peculiar pattern of Nodal > Petiolar > Apical (**Table 2, Fig. 5**). All the results are expressed as Mean  $\pm$  SD. The results were analyzed statistically through Two-way RM ANOVA followed by Sidak's multiple comparisons test using GRAPH PAD PRISM 6.0 and variations in Spectrophotometric results were observed at 95% significant level.

**Table 2: Piperine content in various roots of *Piper longum* estimated through Spectrophotometer**

Solvent Used	Root Source	Piperine Content (% dry wt.)		
		Khurda	Keonjhar	G. Udayagiri
Methanol	Nodal	1.113 $\pm$ 0.061 ****	1.424 $\pm$ 0.011 ****	1.165 $\pm$ 0.001 ****
	Petiolar	0.0191 $\pm$ 0.0018	0.095 $\pm$ 0.003 *	0.0521 $\pm$ 0.0056 *
	Apical	0.0176 $\pm$ 0.00017 *	0.0523 $\pm$ 0.0025 ****	0.051 $\pm$ 0.0021 ****
Ethanol	Nodal	0.254 $\pm$ 0.003 ****	0.267 $\pm$ 0.006 ****	0.26 $\pm$ 0.013 ****
	Petiolar	0.015 $\pm$ 0.002	0.074 $\pm$ 0.003 *	0.037 $\pm$ 0.0061 *
	Apical	0.0094 $\pm$ 0.0002 *	0.023 $\pm$ 0.0006 ****	0.022 $\pm$ 0.009 ****

Results expressed as Mean  $\pm$  SD. The statistical differences were tested by Two-way RM ANOVA followed by Sidak's multiple comparisons test, where \*\*\*\*P < 0.0001, \* P=0.0327



**Figure 5: Piperine content in various roots of *Piper longum* estimated through Spectrophotometer**

### 3.2. Piperine Content as estimated by HPLC

During the course of isolation of pure piperine for HPLC, the R<sub>f</sub> values of the standard and the extracted samples were found to be 0.25 through TLC (**Fig. 7**) [16]. Methanol extracted *P. longum* plants

showed piperine content within a range of 0.104-0.74% dry wt. in root samples. Highest amount of piperine content was found in Nodal root samples collected from Keonjhar (0.74% dry wt.) whereas Apical root sample from Khurda gave the minimum amount of piperine content

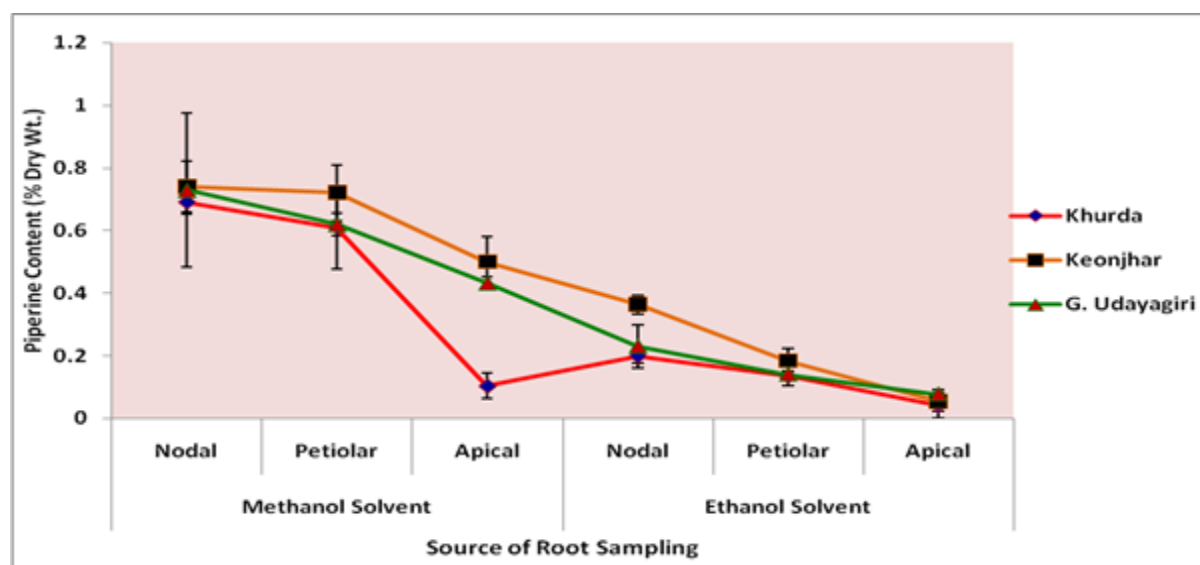
(0.104% dry wt.). However Ethanol extracted root samples showed piperine content in a range of 0.042-0.363% dry wt. Highest amount of piperine was found in Nodal root samples collected from Keonjhar (0.363% dry wt.) whereas Apical root sample from Khurda yielded the minimum amount of piperine content (0.042% dry wt.). In both the cases the highest amount of piperine content was found in Keonjhar region followed by G. Udayagiri and then in Khurda. In all the three Agro-Climatic zones

the percentage of piperine content was found to follow a peculiar pattern of Nodal vine roots then Petiolar leaf roots and finally Apical leaf roots (**Table 3, Fig. 6**). All the results are expressed as Mean  $\pm$  SD. The results were analyzed statistically through Two-way RM ANOVA followed by Sidak's multiple comparisons test using GRAPH PAD PRISM 6.0 and variations in HPLC results were observed at 95% significant level.

**Table 3: Piperine content in various roots of *Piper longum* estimated through HPLC**

Solvent Used	Root source	Piperine Content (% dry wt.)		
		Khurda	Keonjhar	G. Udayagiri
Methanol	Nodal	0.69 $\pm$ 0.036 *	0.74 $\pm$ 0.081	0.73 $\pm$ 0.246 *
	Petiolar	0.61 $\pm$ 0.133 **	0.72 $\pm$ 0.09 **	0.62 $\pm$ 0.035 **
	Apical	0.104 $\pm$ 0.04	0.5 $\pm$ 0.08	0.432 $\pm$ 0.019
Ethanol	Nodal	0.2 $\pm$ 0.0231 *	0.363 $\pm$ 0.029	0.23 $\pm$ 0.07 *
	Petiolar	0.135 $\pm$ 0.031 **	0.183 $\pm$ 0.041 **	0.141 $\pm$ 0.010 **
	Apical	0.042 $\pm$ 0.04	0.053 $\pm$ 0.03	0.077 $\pm$ 0.015

Results expressed as Mean  $\pm$  SD. The statistical differences were tested by Two-way RM ANOVA followed by Sidak's multiple comparisons test, where \*\*P=0.0010, \*P=0.0169



**Figure 6: Piperine content in various roots of *Piper longum* estimated through HPLC**

**Table 4: Two-way RM ANOVA Showing Piperine content in various roots of *Piper longum* estimated through Spectrophotometric and HPLC Method**

ANOVA table	Methods used	SS	MS	DF	P value
Source of Roots Collected from various geographic locations Piperine Content (% dry wt.) in various solvent system	Spectrophotometric Method	157.9	19.74	8	P < 0.0001
		23.72	23.72	1	P < 0.0001
Source of Roots Collected from various geographic locations Piperine Content (% dry wt.) in various solvent system	HPLC Method	28.98	3.623	8	P = 0.0010
		51.40	51.40	1	P = 0.0169

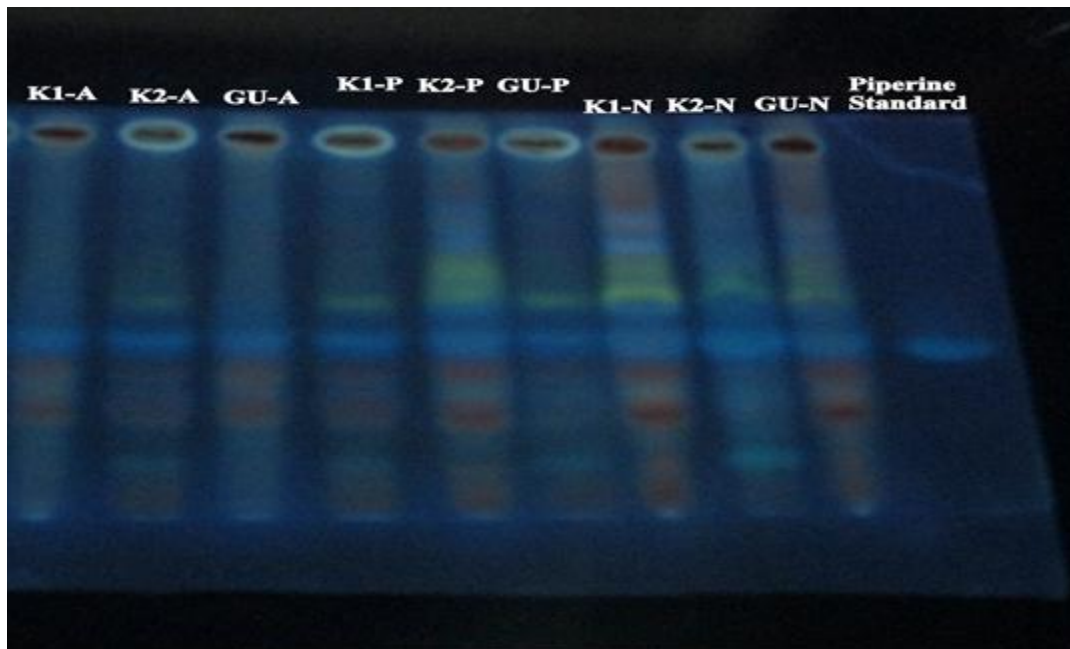


Figure 7: TLC Sheet Results of piperine Standard and Samples

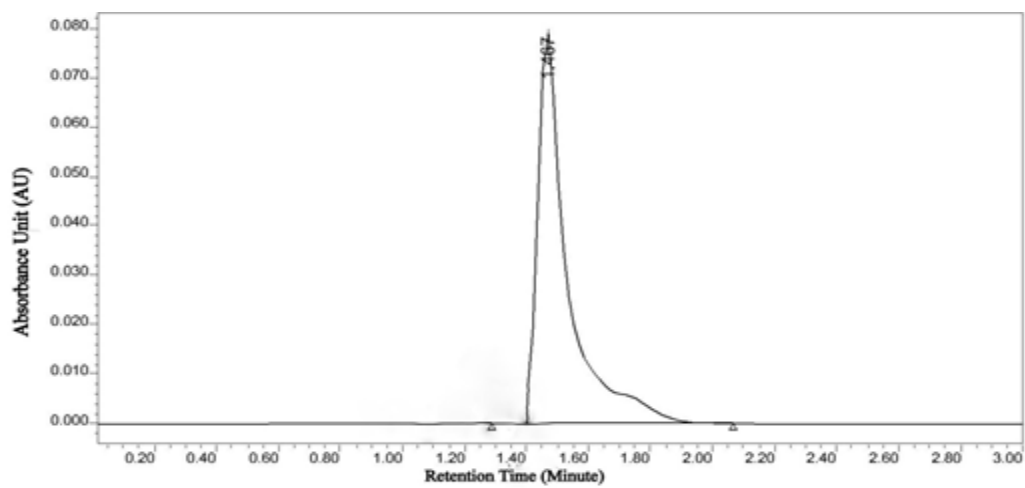


Figure 8: HPLC Chromatograms of Piperine Standard

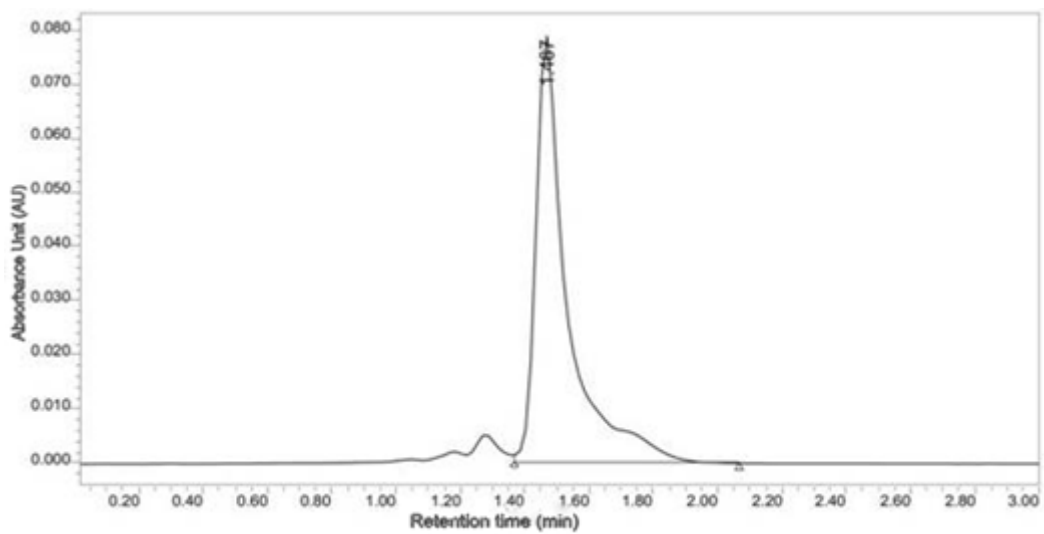
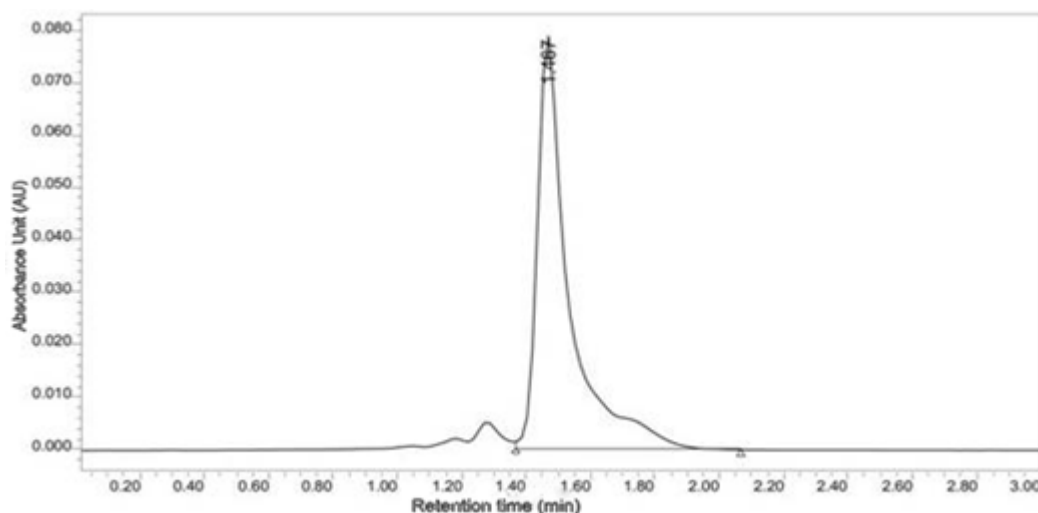


Figure 9: HPLC Chromatograms of Methanol Extracted Piperine Sample



**Figure 10: HPLC Chromatograms of Ethanol Extracted Piperine Sample**

#### 4. DISCUSSION

This present investigation is an attempt to isolate piperine from the roots of *P. longum*, developed from various root origin sources (viz. nodal stem cutting, Apical and Petiolar leaf cutting) and its quantification along with the comparative assessment of zonal differentiation through HPLC analysis, to evaluate the percentage of piperine for herbal drug formulations. Mainly three geographic locations were selected for this process viz. Keonjhar, Khurda and Phulbani (G. Udayagiri) and two different solvent types for extraction procedure.

Through Spectrophotometric method, Methanol extracted *P. longum* root samples showed piperine content within a range of 0.0176-1.424% dry wt. and Ethanol extracted samples showed piperine content in a range of 0.0094-0.267% dry wt. Evaluation of piperine content through Spectrophotometric analysis was validated by other researchers [17, 18, 19]. According to their study, Piperine content in the roots of *P. longum*, through Spectrophotometric analysis was found to be 1.63% w/w, which supports our research work. Similarly in case of HPLC analysis, Methanol extracted *P. longum* root samples showed piperine content within a range of 0.104-0.74% dry wt. and Ethanol extracted samples showed piperine content in a range of 0.042-0.363% dry wt. Similar type of results were observed by many others [10, 11, 12, 20, 21]. According to their studies, piperine content in the roots of *P. longum*, through

HPLC method was found to be in a range of 0.31-1.02% w/w that validate our work.

Comparative account on piperine content from the root samples of *P. longum* collected from various agro-climatic locations was not reported so far with special reference to Odisha, India. In our experiment, the Keonjhar region showed higher yield of piperine in the root samples followed by G. Udayagiri and finally by Khurda. When the piperine content in the roots of *P. longum* were compared in case of methanol extracted samples through Spectrophotometric analysis, the Keonjhar region showed approximately 1.2 fold more piperine content than in comparison to G. Udayagiri and Khurda samples. In case of HPLC analysis, the piperine content in the roots of *P. longum*, methanol extracted samples through Spectrophotometric analysis was found to be approximately one fold more in Keonjhar region than in comparison to G. Udayagiri and Khurda samples. In general the roots originated from nodal vine cuttings showed approximately two fold higher piperine content in comparison to the roots originated from Petiolar leaf cuttings and apical leaf cutting process. Moreover among the selected solvent systems, methanol showed promising result in extraction process of piperine than the ethanol.

#### 5. CONCLUSION

Piperine content was assessed from the various types of roots of *P. longum*, collected from different agro-climatic zones of Odisha, through Spectrophotometric and

HPLC methods. From this experiment comparative analysis of two types of extraction methods was done.

From the experiment Piperine content was appraised to be the highest in the roots of plants grown through nodal vine cuttings. However the plants grown through Petiolar and apical cuttings also showed presence of Piperine content in a visible quantity that can be utilised by pharmacists for drug formulation. Last but not least HPLC system is found to be the precise and accurate method for quantitative estimation of piperine as compared to crude Spectrophotometric method.

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