Journal of Essential Oil Bearing Plants

ISSN: 0972-060X (Print) 0976-5026 (Online) Journal homepage: http://www.tandfonline.com/loi/teop20

Chemical Composition of the Essential Oils from Stem, Root, Fruit and Leaf of Piper longum Linn

Titto Varughese, Prakash Kumar Unnikrishnan, M. Deepak, Indira Balachandran & A.B. Rema Shree

To cite this article: Titto Varughese, Prakash Kumar Unnikrishnan, M. Deepak, Indira Balachandran & A.B. Rema Shree (2016) Chemical Composition of the Essential Oils from Stem, Root, Fruit and Leaf of Piper longum Linn, Journal of Essential Oil Bearing Plants, 19:1, 52-58, DOI: 10.1080/0972060X.2015.1119065

To link to this article: http://dx.doi.org/10.1080/0972060X.2015.1119065

Published online: 07 Mar 2016.

Taylor & Francis
Taylor & Francis Group

ISSN Print: 0972-060X
ISSN Online: 0976-5026

Chemical Composition of the Essential Oils from Stem, Root, Fruit and Leaf of *Piper longum* Linn.

Titto Varughese [1], Prakash Kumar Unnikrishnan [2], M. Deepak [2], Indira Balachandran [2], A.B. Rema Shree [2]*,

[1] Department of Chemistry, Christ College, Irinjalakuda, Kerala
[2] Centre for medicinal Plants Research, Arya Vaidya Sala Kottakkal, Malappuram, Kerala--676503, India

Received 19 March 2014; accepted in revised form 24 November 2014

Abstract: The essential oil obtained from the fresh parts of root, stem, fruit, and leaf of *Piper longum* growing wild in Western Ghats, Kerala was analyzed by GC-MS. The study led to the identification and quantification of 38 (root), 36 (stem), 29 (fruit) and 37 (leaf) chemical constituents belonging to different classes of compounds accounting for 96.9 %, 97.2 %, 97.1 % and 76.7 % of the total oil composition respectively. The essential oil production, yield of total volatile oil content in fruit and leaf was higher than that of root and stem. The principal components in root, stem, and fruit were comparable. The oil composition of root (62.0 %), fruit (71.5 %), and stem (69.2 %) was dominated by the presence of monoterpene hydrocarbons while the leaf had sesquiterpene hydrocarbons (68.7 %) as the major components. The high amount of monoterpene, especially pinene and camphene in the root, stem and fruit due to the freshness of the sample.

Key words: *Piper longum* Linn, Piperaceae, Pippali, GC-MS, monoterpene hydrocarbons, nerolidol, caryophyllene.

Introduction

Piper longum Linn. (Sanskrit name pippali) a slender, aromatic, creeping perennial climber belonging to the family Piperaceae is native to the North-Eastern and Southern parts of India. It is a common Indian dietary spice and a traditional medicine. In Ayurvedic system of medicine, *P. longum* is used as a carminative, a therapeutic agent for the treatment of respiratory tract diseases, inflammation [1], and as medhyarasayana for improving intellect and memory power [2]. A large number of compounds such as lignans, steroids, alkaloids, and oil have been isolated and identified from the *P. longum* using various extraction techniques [3].

Majority of the phytochemical and ethnopharmacological analysis of *P. longum* was carried out on the fruit, and to a lesser extent on other parts of the plant [4]. The characteristic aroma and flavor of *P. longum* is mainly due to the chemical composition of its essential oil components. Literature survey showed contradicting reports on the composition of fruit volatile oil and no significant study was available for the volatile components on other parts of this plant [4, 5, 6, 7]. All of these studies have been carried out after completely drying the fruit which could affect the actual essential oil composition. Moreover, it is difficult to distinguish the fruits of *P. longum* and *P. retrofractum* Vahl. (syn. *P. chaba* L., *P. officinarum* C. DC.) in dried form [8], whereas in fresh form they can be differentiated with their colour. During

*Corresponding authors (A.B. Rema Shree)
E-mail: < remashree@gmail.com >

the course of our ongoing project on medicinal plants, we have carried out an extensive phytochemical and pharmacognostic analysis on *P. chaba* L. [9] and *P. longum* L.[10]. It is easy to distinguish the fresh mature fruits of both these plants as *P. chaba* are reddish yellow and *P. longum* is black in colour. Due to the difficulty in distinguishing the dried fruits of these two, *P. longum* purchased from the markets often littered with *P. chaba* [11]. Above mentioned issues along with the differences in geographic locations of the collected samples could be the reason for the conflicting literature reports on the essential oil constituents available so far.

Though literature reports are available on isolation and bioactivity studies of essential oil of other pats of the plant, there is complete lack of studies on the composition of volatile oils of stem, root and leaf of this plant. This lack of systematic research on the essential oil composition of various parts of this economically important plant led us to carry out an analysis on the distribution pattern of its essential oil throughout the plant. Hence, the objective of the present work is to carryout a comparative study on the distribution pattern of essential oil constituents of stem, root leaf and fruit of *Piper longum* Linn.

Experimental

Plant material and extraction procedure

The leaves (350 g), roots (160 g), fruit (120 g), and stem (350 g) of *Piper longum* was collected on 2nd August 2012 from Western Ghats region of Kerala, India. The botanical identifications were carried out at the Herbarium of Centre for Medicinal Plants Research and the voucher specimens (No.4177/12) were deposited in the herbarium of the institute. 100 g each of fresh plant materials were used for the isolation of essential oils. Prior to the isolation, samples were cut into small pieces to ensure maximum yield. To obtain essential oil of the greenish fruits of *P. longum* were subjected to hydrodistillation for 6 h using a Clevenger-type apparatus on the next day of collection. The root, stem and leaf essential oils were isolated using the same method on subsequent days in that order. The essential oils then separated from water, diluted to 1 mg/mL using hexane and dried over anhydrous sodium sulfate. The dried volatile oils transferred to 10 mL standard flasks, sealed and stored in refrigerator at 4°C until tested and analyzed. The yields (w/w) were calculated according to the weight of fresh plant material.

Gas chromatography-Mass spectrometry

GC-MS analysis was performed on an Agilent Gas chromatograph series 6850 System fitted with a HP-5 MS fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 μm) coupled with an Agilent 5975C VL-MSD with Triple-Axis detector under the following conditions: 1 μL of diluted samples (1 mg/ml in Hexanes, w/v) were injected automatically and in a split mode (1:25); Helium as carrier gas at 1 mL/min constant flow mode, injector temperature 230°C, oven temperature 60°C to 180°C at 2.5°C /min, hold at 180°C for 5 min followed by 180°C to 250°C at 10°C / min . Mass spectra: electron impact (EI+) mode, 70 eV and ion source temperature 230°C. Mass spectra were recorded over 50-500 amu range; electronmultiplier 1460 eV; scan rate, 2.96 scan/ s. The retention indices were used for identifying the chemical constituents based on homologous series C_8-C_{20} n-alkanes. Further identification of the components was made by the comparison of mass spectra with the literature [12] or by the comparison of their mass spectra with those stored in NIST-8 library [13]. Component relative percentages were calculated based on GC-MS peak areas.

Chemical analysis

Quantification of caryophyllene and nerolidol was performed on Agilent 6890 gas chromatograph with flame ionization detector (FID), equipped with a HP-5 fused silica capillary column, (30 m x 0.25 mm, 0.25 μm film thicknesses) using multiple point external standard method. The temperature programme is as follows: 60-180°C at 2.5°C/min, hold at 180°C for 5 min followed by 180-250°C at 20°C/min. N_2 carrier gas is at a flow rate of 1.0 mL/min; injector port and detector temperature were 230°C and 250°C, respectively. Samples were injected by splitting and the split ratio is 1:25. The diluted standard solutions (nerolidol purity ≥ 97.0 %, β-caryophyllene ≥ 80.0 % from Sigma Aldrich) were prepared in the range

of 10 µg/ml to 250 µg/ml. 10 µl of the standard solutions were injected, from the respective peak areas a calibration curve is prepared by plotting peak area *vs* concentration of standards applied. The sample solutions were prepared in the range of 1 mg/ml and 10 µL of neat samples were injected to determine the area of the peak corresponding to the standards and the amount of nerolidol and caryophyllene present in the samples were calculated from calibration curve of standards applied.

Qualitative analysis

The identification of the constituents was assigned on the basis of a comparison of their retention indices, based on a series of n-alkanes (C_8–C_{20}), their mass spectra found in the literature and supplemented by the NIST-08 Mass Spectral Library Upgrade (provided by Agilent with the GC-MS control and data processing software).

Results and discussion

The chemical composition of the essential oil from the stem, root, fruit, and leaves of *P. longum* L. is shown in Table I according to the order of their retention time from HP-5 column. The essential oil production, yield of total volatile oil content in fruit and leaf was higher than that of root and stem. The oil yield from stem (0.026 %), root (0.054 %), fruit (0.1 %) and leaf (0.077 %) was calculated on fresh weight basis (w/w). GC-MS analysis of stem, root, fruit and leaves of *P. longum* led to the identification of 36, 38, 29 and 37 chemical constituents accounting for 97.2 %, 96.9 %, 97.1 % and 76.7 % of the total oil composition respectively.

The gas chromatograms of the essential oils are shown in Figure 1. The root oil was dominated by the presence of monoterpene hydrocarbons accounting for 62.0 % while the sesquiterpene hydrocarbons constitute 27.5 % of the total oil composition. The oil composition of stem was comparable to that of root with the mono- and sesquiterpene which were 69.2 % and 25.6 % respectively. 71.5 % of the oil from fruit contained monoterpene hydrocarbons in which β-pinene was the single largest component. However the leaf essential oil was dominated by sesquiterpene (68.7 %) and the monoterpenoids were present only in small quantities (2.6 %). The principal components of fruit, root and stem were β-pinene, camphene and α-pinene of the total volatile constituents.

Comparison of fresh fruit volatile oil constituents with that of dried fruit oil data available in literature reveals significant differences in the monoterpene constituents [4, 5, 6, 7]. The pinene and camphene were present in high amount and eugenol or isoeugenol were absent in our data. It is reasonable to assume that the fresh fruit oil composed of pinene and camphene and has been lost completely or partially during the process of drying. If the amount β-pinene, camphene and α-pinene are deducted, then percentage of monoterpene will be 9.9 (root), 13.8 (stem) and 12.4 (fruit). Since the isolation of fruit carried out

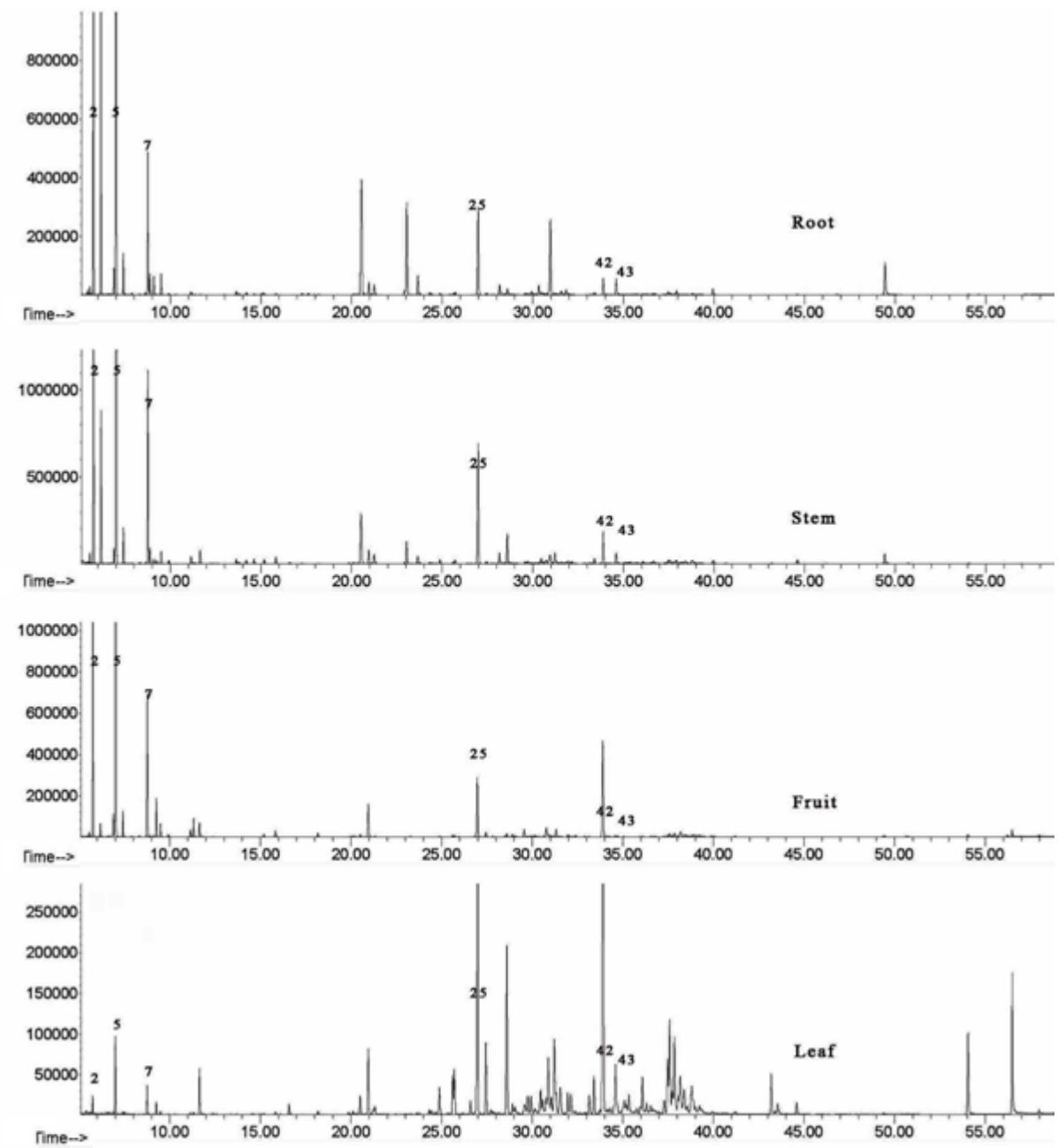
Table I. Chemical composition of root, stem, fruit and leaf oils of *P. longum*

No.	Compound	R_t	RI	Root %	Stem %	Fruit %	Leaf %
1	α-Pinene	5.5	941	11.8±0.07	14.0±0.10	15.3±0.20	0.3±0.07
2	Camphene	5.7	953	13.9±0.05	6.6±0.06	0.7±0.03	-
3	β-Pinene	6.1	978	26.4±0.10	34.8±0.09	43.1±0.80	1.6±0.03
4	β-Myrcene	6.9	993	1.5±0.08	1.6±0.01	1.4±0.01	tr
5	α-Phellandrene	7.0	1005	0.2±0.03	0.4±0.03	tr	-
6	β-Phellandrene	7.4	1029	1.0±0.03	0.7±0.04	1.4±0.01	-
7	Limonene	8.7	1030	6.3±0.02	10.3±0.04	9.6±0.02	0.7±0.01
8	Eucalyptol	8.8	1034	0.9±0.06	0.8±0.01	tr	-
9	*trans*-Ocimene	9.1	1038	0.7±0.06	0.6±0.01	tr	-
10	*cis*-Ocimene	9.2	1049	0.9±0.01	tr	0.8±0.01	tr
11	Terpinolene	9.5	1086	tr	0.4±0.01	0.5±0.01	tr
12	Linalool	11.1	1097	-	0.8±0.01	1.1±0.01	1.2±0.02

table 1. (continued).

No.	Compound	R_t	RI	Root %	Stem %	Fruit %	Leaf %
13	1-Methylhexyl acetate	11.3	1128	tr	tr	2.5±0.02	0.3±0.01
14	2-Nonanone	11.6	1176	-	-	1.3±0.04	-
15	Terpineol	15.8	1189	tr	0.4±0.01	0.5±0.01	tr
16	Decanal	16.5	1207	-	-	-	0.3±0.01
17	Bornyl acetate	20.5	1285	10.0±0.04	5.0±0.04	-	0.6±0.03
18	2-Undecanone	20.9	1295	0.8±0.01	1.0±0.01	2.9±0.1	2.0±0.01
19	Tridecane	21.2	1300	0.6±0.01	0.7±0.01	-	tr
20	δ- Elemene	23.0	1338	5.8±0.10	1.6±0.03	-	-
21	γ-Elemene	23.6	1338	-	-	-	1.4±0.03
22	Terpinyl acetate	24.9	1354	1.1±0.01	0.5±0.07	-	-
23	Copaene	25.7	1377	tr	tr	-	0.9±0.01
24	β-Cubebene	26.6	1382	tr	tr	tr	2.3±0.07
25	β-Elemene	27.0	1387	0.1±0.01	tr	tr	1.4±0.09
26	Dodecanal	27.4	1411	-	-	-	0.4±0.08
27	Caryophyllene	28.1	1415	5.6±0.03	9.3±0.04	5.7±0.2	16.8±0.10
28	Guaiene	28.6	1425	tr	tr	-	0.6±0.01
29	Gurjunene	28.9	1432	tr	0.8±0.01	-	2.6±0.02
30	Humulene	29.7	1455	0.4±0.01	2.3±0.02	tr	5.8±0.04
31	(Z)-â-Farnesene	30.3	1467	-	-	tr	0.3±0.01
32	γ-Muurolene	30.7	1478	tr	-	tr	0.6±0.05
33	1-Pentadecene	30.8	1492	0.6±0.03	-	-	-
34	2-Tridecanone	30.9	1497	tr	-	0.9±0.1	0.4±0.01
35	Pentadecane	31.0	1500	5.0±0.04	0.7±0.01	-	-
36	α-Muurolene	31.2	1503	tr	tr	tr	1.8±0.06
37	β-Patchoulene	31.3	1503	0.7±0.01	0.8±0.01	-	tr
38	Bisabolene	31.9	1510	-	-	0.6±0.01	-
39	cadinene	32.1	1532	tr	tr	tr	0.8±0.01
40	Nerolidol	33.1	1540	1.0±0.07	2.2±0.05	8.8±0.3	22.5±0.30
41	Elemol	33.4	1551	tr	tr	-	0.6±0.01
42	8-Isopropenyl-1,5-dimethylcyclodeca-1,5-diene	33.9	1570	-	tr	-	0.5±0.01
43	Caryophyllene oxide	34.6	1579	1.1±0.03	0.9±0.01	-	2.1±0.03
44	δ-Cadinol	37.3	1640	0.1±0.07	tr	tr	1.9±0.03
45	α-Cadinol	37.4	1648	-	-	-	0.5±0.01
46	β-Eudesmol	37.6	1659	tr	tr	tr	3.3±0.05
47	α- Eudesmol	37.7	1662	-	-	-	0.7±0.01
48	Heptadecane	38.1	1700	0.4±0.05	-	-	-
49	9-Eicosyne	39.9	2000	-	-	-	1.5±0.07

Note- Components are listed in order of their elution from HP-5 column; RI: retention indices were based on homologous series C_8-C_{20} n-alkanes;
Values represent the average of three measurements
tr: traces;
Rt: Retention time



2: α-Pinene; **5**: β-Pinene; **7**: Limonene; **25**: Caryophyllene; **42**: Nerolidol; **43**: Caryophyllene oxide

Figure I. GC-MS Chromatogram of Root, Stem, Fruit and Leaf oils of *P. longum*

immediately after collection, the percentage of caryophyllene oxide, often produced by the oxidation of caryophyllene was very low compared to literature. The absence of pinene in earlier reports is little intriguing because the aroma model of *piper* species consists of pinene [14]. The presence of eugenol or isoeugenol in fruit oil was noted only by Liu *et al.*, and has been questioned by other researchers on the basis of adulteration [11].

The major active constituents of *P. longum* were nerolidol and caryophyllene. They have extensive applications in the pharmaceutical industry. The composition percentage of nerolidol varied from 1.0 to 22.5, whereas that of caryophyllene from 5.6 to 16.8 in various parts. Caryophyllene is found to be distributed almost uniformly through-

Table 2. GC-FID based Quantitative Analysis

Parts	Nerolidol	Caryophyllene
Root	1.1±0.1	5.9±0.1
Stem	2.2±0.2	9.1±0.2
Fruit	9.3±0.4	5.8±0.0
Leaf	23.4±0.7	17.7±0.6

out the plant. This is in agreement with the Hosana *et al*. observation that caryophyllene was the most general representative of many Piper species [15].

Caryophyllene is known to have local anesthetic activity and an anti-inflammatory sesquiterpene [16]. Nerolidol is used as a flavouring agent in perfumes and a natural pesticide against mites. It is also currently under testing as a skin-penetration enhancer for the trans-dermal delivery of therapeutic drugs activity [17].

In conclusion, this report compares the essential oil composition of fresh parts of *Piper longum* Linn. plant. Though a quantitative difference between volatile oils from various parts of the plant was apparent, the root, stem, and fruit oils showed more similarity in their chemical composition. By comparing the fresh parts of the plant especially fruits, we came to the conclusion that the monoterpene like α- and β- pinene, and camphene are invariably present in the fruit and probably released out through drying. This is also the first report on the complete analysis of the plant essential oil composition. The appreciable amount of α- and β-pinene in stem, fruit and root essential oil of *Piper longum* is economically important as they have great demand in perfumery industry.

Acknowledgements

The authors are thankful to the Department of AYUSH, ICMR Govt. of India, New Delhi for providing fund and The Arya Vaidya Sala – Dorabji Tata Trust for the facilities.

References

1. **Warrier, P.K., Wambia, V.P.K., Ramankutty, C., Nair, R.V. (1993).** Indian Medicinal Plant- A Compendium of 500 spices. Orient Longman Pvt Ltd, Chennai., Vol-III, 444.
2. **Sivarajan, V.V. and Balachandran, I. (1994).** Ayurvedic Drugs and Their Plant Sources. Oxford and IBH Publishing Co Pvt Ltd, New Delhi., 374-376.
3. **Parmar, V.S., Jain, S.C., Bisht, K.S., Jain. R., Taneja, P., Jha, A., Tyagi, O.D., Prasad, A.K., Wengel, J., Olsen, C.E., Boll, P.M. (1998).** Phytochemistry of the genus Piper. Phytochemistry, 46: 597-673.
4. **Zaveri, M., Khandhar, A., Patel, S., Patel, V. (2011).** Chemistry and Pharmacology of *Piper longum* L. International Journal of Pharmaceutical Sciences Review and Research, 5: 67-76.
5. **Handa, K.L., Nigam, N.C., Sharma, M.L. (1963).** The essential oil of *Piper longum*, properties of the components and isolation of two monocyclic sesquiterpenes, Perfume Kosmetik, 44: 233-235.
6. **Tewtrakul, K.S., Has, S.K., Namba, K.T., Komatsu, K., Tanaka, K. (2000).** Fruit Oil Composition of *Piper chaba* Hunt., *P. longum* L. and *P. nigrum* L. Journal of Essential Oil Research, 12: 603.
7. **Liu, L., Song, G., Hu, Y. (2007).** GC-MS Analysis of the Essential Oils of *Piper nigrum* L. and *Piper longum* L. Chromatographia, 66: 785-790.
8. **Manoj, P., Sonia, E.V., Banerjee, N.S., Ravichandran, P. (2004).** Recent studies on well known spice, *Piper longum* Linn. Natural product radiance, 3: 222-227.
9. **Tandon, N. and Sharma, P. (2013).** Quality Standards of Indian Medcinal Plants, Indian Council of Medical Research, New Delhi., Vol-XI, 305-315.

10. **Gupta, A.K. (2003).** Quality Standards of Indian Medicinal Plants. Indian Council of Medical Research, New Delhi., Vol. I: 168-173.
11. **Mammen, D. and Daniel, M. (2014).** A critical review on the pharmacognosy and chemistry of the fruiting spikes of *Piper longum* L. Indian journal of applied research, 3: 429-432.
12. **Adams, R.P. (2007).** Identification of Essential Oil Components by Gas chromatography/ Mass Spectrometry. 4th Edition. Allured Publishing Corporation, Carol Stream, Il 60188-2787, USA.
13. **National Institute of Standards and Technology Library (2008).** PC version of the NIST/EPA/ NIH Mass Spectral Database. U.S. Department of Commerce, Gaithersburg, MD.
14. **Jagella, T. and Grosch, W. (1999).** Flavour and off-flavour compounds of black and white pepper (*Piper nigrum* L.). European Food Research and Technology, 209: 16-21.
15. **Hosana, M.D.N., Andreia de, A.M., Alberto, C.A., Luis, O.R., Debora, C.B.B., Marcelo, T., Alberto, J.C., Marcia, N.L., Vanderlan da, S.B., Maysa, F., Marcia, O.M.M., Maria, C.M.Y., Massuo, J.K. (2006).** Composition and antifungal activity of essential oils from *Piper aduncum*, *Piper arboreum* and *Piper tuberculatum*. Química Nova, 29: 467-470.
16. **Santra, M., Santra, D.K., Rao, V.S., Taware, S.P. and Tamhankar, S.A. (2005).** Inheritance of β-carotene concentration in durum wheat (*Triticum turgidum* L. ssp. *durum*). Euphytica, 144: 215-221.
17. **Parthasarathy, U., Ashish, G.R., Zachariah, T.J., Saji, K.V., George, J.K., Jayarajan, K., Mathew, P.A., Parthasarathy, V.V. (2008).** Spatial influence on the important volatile oils of *Piper nigrum* leaves. Current Science, 94: 1632-1635.