Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 1, 2013

Research Article

CHARACTERIZATION AND HPLC QUANTIFICATION OF PIPERINE ISOLATED FROM *PIPER GUINEENSE* (Fam. PIPERACEAE)

REIMMEL KWAME ADOSRAKU[1*], JAMES OPPONG KYEKYEKU[1], ISAAC YAW ATTAH[1]

Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana[1]. *Email: etorwu@yahoo.com

Received: 30 Oct 2012, Revised and Accepted: 28 Nov 2012

ABSTRACT

Piperine (1 - piperoyl piperidine) is the major alkaloid responsible for the pungent smell of the West African pepper, *Piper guineense,* a plant whose parts are well known for their traditional indications for various ailments. The amount of piperine in the various parts of *Piper guineense* are however unknown. This project, seeks to develop a RP-HPLC method to standardize the various parts of *Piper guineense* using isolated piperine as a biomarker and a secondary reference. All samples of *Piper guineense* parts were obtained from the Physic garden of KNUST and authenticated by Pharmacognosy Department, KNUST, Kumasi. Piperine was isolated from the dried fruits of *Piper guineense* with ethanolic KOH and recrystallized from acetone: hexane (3:2). The melting point was determined to be 128ºC - 130ºC and the crude yield was 2.07%w/w. Thin Layer Chromatography gave R_f values of 0.285 ± 0.013 for hexane: ethylactate: glacial acetic acid (3: 1: 0.3) and 0.70 ± 0.010 for chloroform: ethylacetate (1: 1). The isolated piperine was characterized by carrying out NMR, UV and mass spectroscopy analyses. A validated reverse phase HPLC with methanol: water (80:20) at a flow rate of 1.40ml/min on a Phenomenex Kromosil 5 C_8 (250mm x 4.6mm, 5 micron i.d.) column with detection at 343nm gave a retention time of 3.78 ± 0.06 min. Piperine was found to be 0.0054 ± 0.00009%w/w in the dried leaves, 0.0437 ± 0.000816%w/w for fresh leaves, 0.115 ± 0.00228%w/w for dried stem and 3.345 ± 0.0339%w/w for dried fruits.

Keywords: *Piper guineense*, Piperine, RP-HPLC, Validation, Quantification

INTRODUCTION

Medicinal plants have been used since medieval times for treating ailments and spices are no of exception. The extended use and demand for spices have led to high market value and adulteration of such spices and their herbal products.

Piper guineense, (fam. Piperaceae) a spice commonly known as West African Black Pepper, is a climbing plant climbing up to 12m high by its adventitious rootlets. Various parts of the plant have been indicated for the treatment of ailments such as boils, bronchitis, catarrh, chest pains, coughs, dyspepsia, impotence, insect repellant, lumbago and rheumatism. It is also used for treating uterine fibroids and wounds [1].

Piperine, the major alkaloid in piper species, has been shown to have antimycobacterial activity [2] and several pharmacological activities such as antihyperlipidemic [3], antiandrogenic [4], immunoregulatory [5], antidepressant [6]. Piperine has also been shown to have certain serious toxicities such as antifertility [7], respiratory paralysis, hemorrhagic necrosis and edema in gastrointestinal tract, urinary bladder and adrenal glands [8] and immunotoxicological effects [9].

Piperine content is 3-9% and 3-5% (on dry weight basis) in the fruits of *Piper nigrum* and *Piper longum* of commence respectively [10]. The amount of piperine in *Piper guineense* is however not known. Chromatographic fingerprinting could sometimes be used to standardize herbal preparations. The developed fingerprint pattern of components can be used to determine the presence of marker compounds [11]. Chromatographic fingerprinting may however be insufficient to quantitatively analyze herbal preparations. The lack of knowledge of the amount of piperine has led to a reduced market premium in international commerce of the plant parts of *Piper guineense*. In African traditional medicine, herbal preparations such as those containing extracts of *Piper guineense* are dispensed usually without any fair estimation of the amount of active constituents being consumed. Over-dosage and under-dosage may result in various adverse effects. This work therefore, seeks to develop a RP-HPLC method that could readily quantify the amount of piperine in the fruits, leaves and stem of *Piper guineense*. This method can then be used to standardize herbal preparations containing *Piper guineense* and the plant parts for international trade.

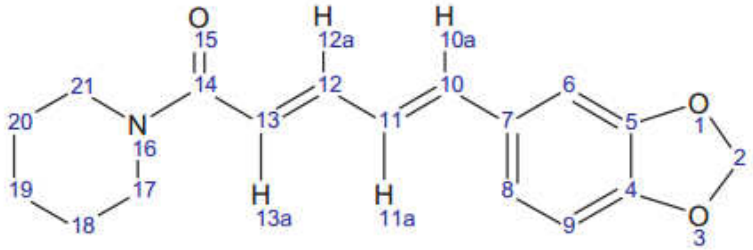

Fig. 1: Labelled structure of piperine [(E,E)1- [5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine]

MATERIALS AND METHODS

Extraction and Purification of Piperine

All samples of *Piper guineense* parts were obtained from the Physic garden of KNUST and authenticated by Pharmacognosy Department, KNUST, Kumasi and were phytochemically screened. 120g of the finely ground dried fruits of *Piper guineense* was refluxed with about 350ml of ethanol for 3hrs. The mixture was then allowed to cool and the ethanol filtered off. For exhaustive extraction, the marc was refluxed again with another 350ml of ethanol for 3hrs. The combined ethanolic extract (about 700ml) was then concentrated under vacuum in a rotary evaporator at 70ºC to about 25ml of concentrate which was transferred into a beaker. 50ml of 10% ethanolic KOH was then added stirred thoroughly and the mixture allowed standing for about 2hr. The mixture was then decanted leaving the insoluble residue in the beaker. The supernatant solution

was then allowed to stand undisturbed for 48hrs for fine yellowish crystals of piperine to precipitate out. The crystals were recrystallized in acetone: hexane 3:2.

Characterization of isolated Piperine

The melting point of piperine was determined using the Stuart melting point apparatus.NMR spectra of the isolated piperine were obtained on Mercury-300BB Varian Spectrometer with sample dissolved in deuterated chloroform ($CDCl_3$). Mass spectra data were obtained on LCQ Advantage MAX mass spectrometer (Thermo Electric Inc) with ACPI probe. UV spectra data was obtained on T90+ UV/VIS Spectrometer (PG instruments Ltd.) with UVWin 5.2.0. software. TLC of isolated piperine was run alongside the extract samples using chloroform: ethylacetate 1:1 and Hexane: ethylacetate: glacial acetic acid 3: 1: 0.3. The detection was under both UV light using Dragendorff reagent as an indicator.

Sample preparation for HPLC

The powdered dried fruits (**DF**) of *Piper guineense,* 0.3000g, was weighed into a glass mortar and triturated with a few milliliters of methanol into a smooth paste, 20ml of methanol was added, mixed and allowed to stand for 5 minutes. It was then filtered into a 100ml volumetric flask using a Whatman No. 1 filter paper. The residue was washed thoroughly with methanol and the filtrate made up to volume. The same procedure was used for 3.0g of the leaves (fresh and dried) and stem extracts of *Piper guineense* prior to HPLC analysis. The samples were coded as **DL** (dried leaves), **FL** (fresh leaves), **DS** (dried stem) and **DF** (dried fruits)

HPLC analysis of Piperine

The samples prepared above were analysed by a chromatograph consisting of Kontron instruments HPLC pump 422 with a programmable Perkin Elmer UV/Visible detector and a Power Chrom integrator. The column used was a Phenomenex Kromosil 5 C_8 250mm x 4.6mm 5 micron i.d. The mobile phase consisted of methanol: water (80:20) pumped at a flow rate of 1.40ml/min. 100µl of the samples were injected unto the column at ambient temperature. A maximum run time of 6.5min was allowed during analysis and the eluent was monitored at 343nm. An appropriate calibration curve was plotted from which the content of piperine in the samples was determined.

The HPLC method was validated according to the ICH guidelines [11] and the results were statistically analyzed.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening revealed the presence of alkaloids in the various parts of *Piper guineense* investigated. This indicates piperine, which is an alkaloid, may be present.

Characterization of isolated Piperine

The melting point range of 128ºC - 130 ºC determined was consistent with stated values in literature [12]**.** The percentage yield of 2.07%w/w of isolated piperine from *Piper guineense* compares favorably with the 2.50%w/w isolated from *Piper longum* [13] considering the different methods of extraction. TLC analysis confirmed the presence of piperine in all the sample extracts except in **DL.** This could be as a result of the concentration of piperine in **DL** being below the TLC detection limit. The Rf values determined for the two solvent systems could be used for qualitative analysis of piperine. The UV spectrum of piperine was consistent with literature [14], with a maximum wavelength of absorption at 343nm. The C-13 chemical shifts of NMR spectra obtained were consistent with the position of carbons in piperine. Mass Spectroscopy gave an m/z value of 286.03 to be the molecular peak ion of piperine. The reverse phase HPLC method developed for analysis of piperine gave a retention time of 3.78 ± 0.05656 min.

RP-HPLC method validation

The calibration curve was found to be linear over the concentration range **0.0002%w/v – 0.004%w/v**. The Coefficient of Correlation, R^2, was **0.998** for the equation of the line **y = 1535x – 0.034**. The LOD and LOQ were determined to be **1.872 x 10^{-4}%w/v** and **5.672 x 10^{-4}%w/v** respectively. The method was determined to be precise with RSD (%) of **1.217% (n=6)** and **1.704% (n=18**) for intraday and interday precisions respectively. The percentage recoveries ranged from 94.61% to 102.21% with RSD ≤2% hence implying a high method accuracy. Using One - way ANOVA to statistically compare results from moderate modifications of the method, the HPLC method was found to be robust for the wavelength range of 343 ± 3nm, methanol: water composition from 78: 22 to 82: 18 and flow rates of 1.30 – 1.60ml/min.

Piperine content

Piperine was found to be **0.0054 ± 0.00009%w/w** in the dried leaves (DL), **0.0437 ± 0.000816%w/w** in the fresh leaves (FL), **0.115 ± 0.00228%w/w** in the dried stem (DS) and **3.345 ± 0.0339%w/w** in the dried fruits (DF). The value of **3.345 ± 0.0339%w/w** of piperine determined for dried fruits (**DF**) of *Piper guineense* is comparable to the 3- 9%w/w and 3-5%w/w (on dry weight basis) reported respectively for *Piper nigrum* and *Piper longum* [10] sold on the international market.

Table 1: Phytochemical tests on *Piper guineense*

Phytochemical Test	Seed	Leaves	Stem
Alkaloid	Positive	Positive	Positive
Glycosides	Positive	Positive	Negative
Tannins	Positive	Positive	Positive
Saponins	Positive	Positive	Positive

Table 2: R_f values of piperine in different mobile phases

Mobile phase	Average R_f value (n = 4)
Hexane: ethylacetate: glacial acetic acid 3: 1: 0.3	0.285 ± 0.01291
Chloroform: ethylacetate 1: 1	0.70 ± 0.009574

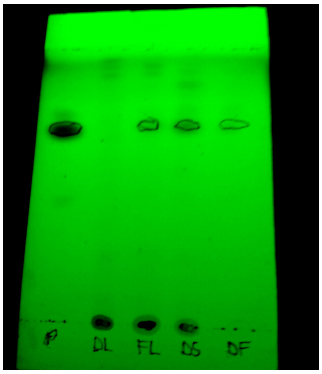

Fig. 2: TLC chromatogram of isolated piperine (P), dried leaves extract (DL), fresh leaves extract (FL), dried stem extract (DS) and dried fruits extract (DF)

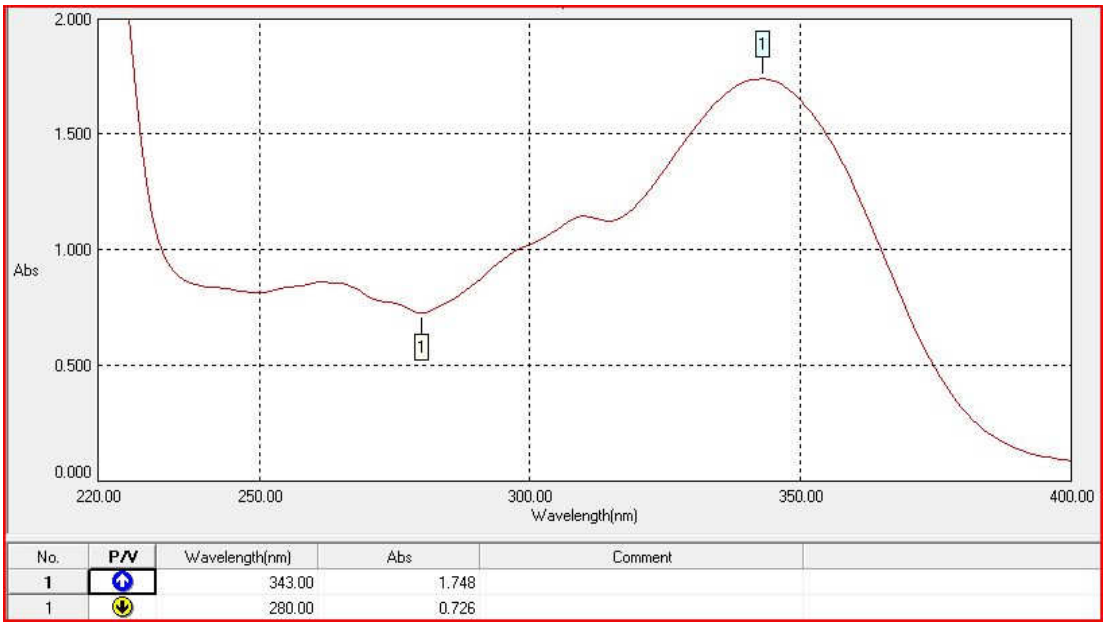

Fig. 3: UV/V is spectrum of piperine

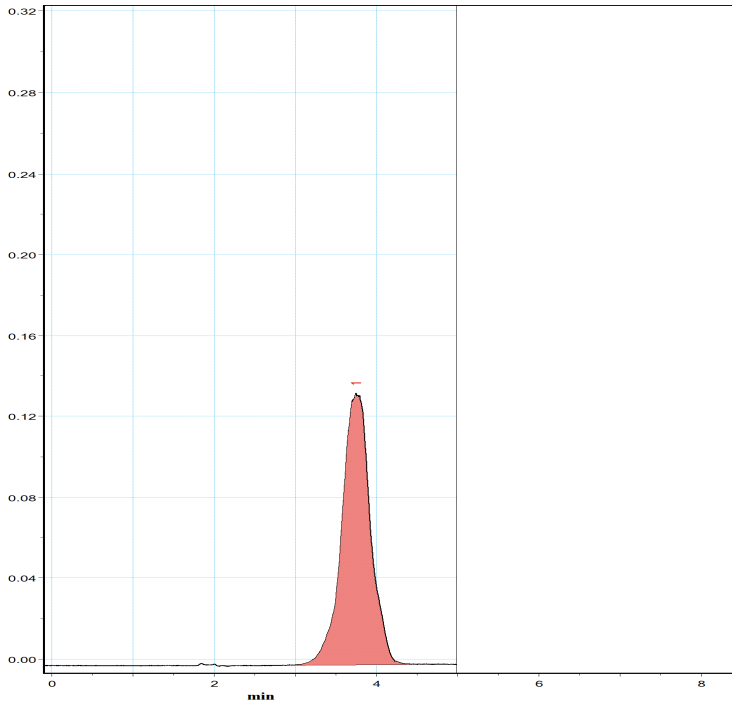

Fig. 4: Chromatogram of isolated piperine

Table 3: Percentage content of piperine in various parts of *Piper guineense*.

Type of extract	Mean % content (n= 6)	Standard Deviation	% RSD
DL (3g/100ml)	5.44×10^{-3}	9.24×10^{-5}	1.697
FL (3g/100ml)	0.0437	0.000816	1.87
DS (3g/100ml)	0.115	0.00228	1.98
DF(0.3g/100ml)	3.345	0.0339	1.0138

Table 4: Percentage recovery of spiked extracts of *Piper guineense*

Type of Extract	Spiked piperine Conc. (%w/v)	Mean % recovery (n=3)	Standard deviation	% RSD
DL	0.001	98.84	2.0724	2.097
FL	0.001	96.14	1.527	1.589
DS	0.001	97.27	1.861	1.912
DF	0.002	100.36	1.853	1.847

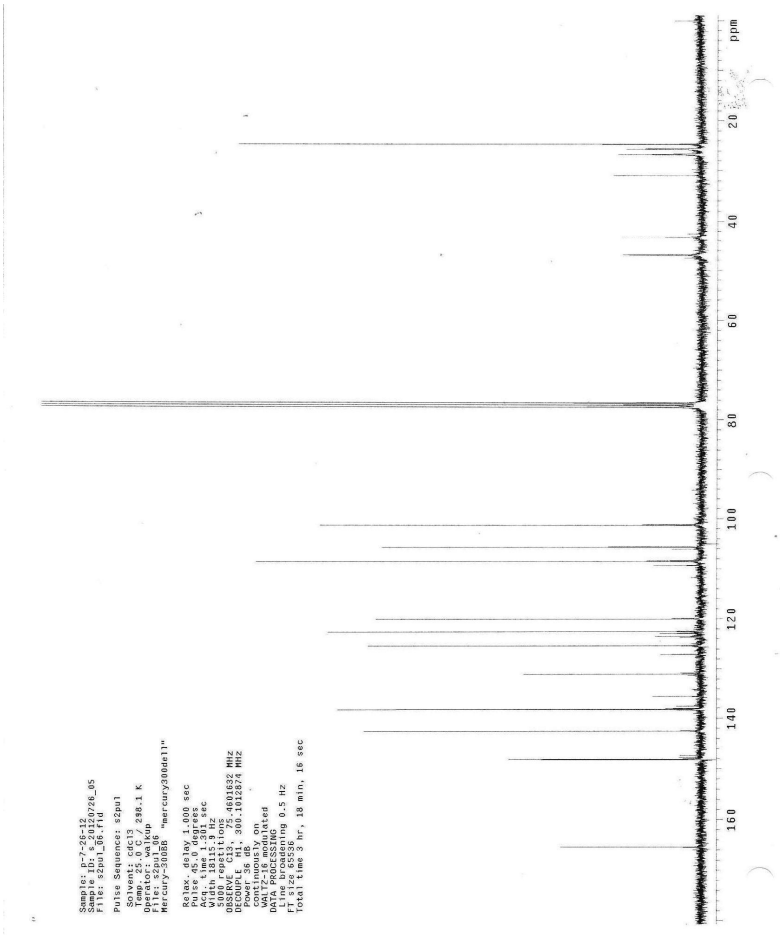

Fig. 5: C-13 NMR of Isolated Piperine

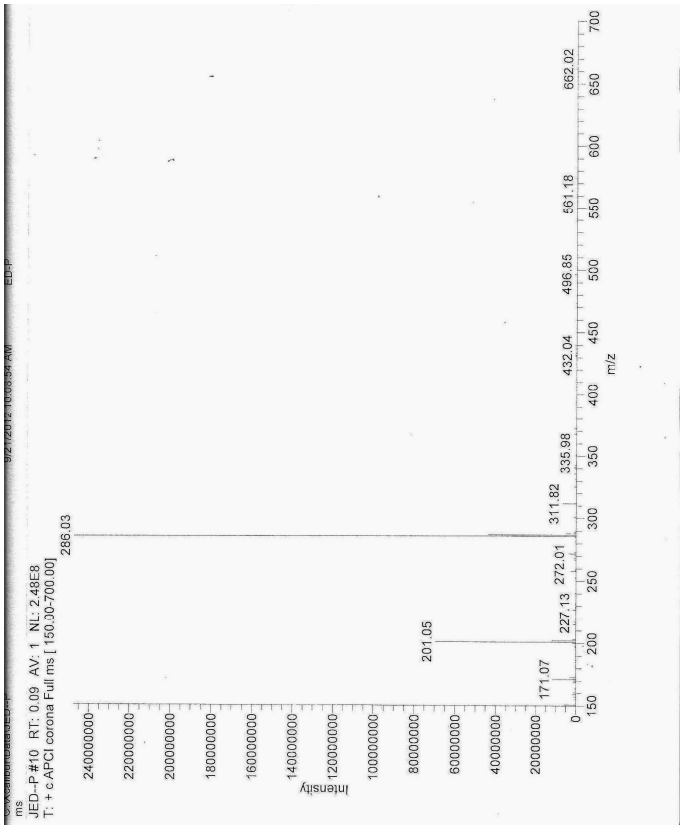

Fig. 6: Mass Spectrum of isolated Piperine

CONCLUSION

Piperine was isolated, purified and characterized for use as a reference. The developed RP-HPLC method was validated according to the ICH guidelines [15]. The validated RP-HPLC with methanol: water (80:20) at a flow rate of 1.40ml/min on a Phenomenex Kromosil 5 C_8 (250mm x 4.6mm, 5 micron i.d.) column with detection at 343nm gave a retention time of **3.78 ± 0.06 min.** The percentage content of Piperine was estimated to be **0.0054 ± 0.00009%w/w** in the dried leaves, **0.0437 ± 0.000816%w/w** for fresh leaves, **0.115 ± 0.00228%w/w** for dried stem and **3.345 ± 0.0339%w/w** for dried fruits. The proposed method could therefore be used to standardize *Piper guineense* for export in order to increase its market premium and in quality control analysis for the estimation of piperine content of herbal preparations containing *Piper guineense* extracts.

ACKNOWLEDGEMENTS

The Authors are grateful to the College of Pharmacy and Pharmaceutical Sciences of Florida A&M University for running the NMR and mass spectroscopy analysis of the isolated piperine.

REFERENCES

1. Busia K. *Ghana Herbal Pharmacopoeia*. Science and Technology Policy Research Institute (STEPRI), Printed by QualiType Limited, Accra- North, Ghana; 2007; 36-40
2. Jin J., Zhang J., Guo N., Feng H., Li L., Liang J., Sun K., Wu X., Wang X., Liu M., Deng X. and Yu L. The plant alkaloid piperine as a potential inhibitor of ethidium bromide efflux in Mycobacterium smegmatis. *J Med Microbiol* 2011; 60, 223-229.
3. Jin Z., Borjihan G., Zhao R., Sun Z., Hammond G.B. and Uryu T. Antihyperlipidemic compounds from the fruit of Piper longum L. *Phytother Res* 2009; 23, 1194-1196.
4. Hirata N., Tokunaga M., Naruto S., Iinuma M. and Matsuda H. Testosterone 5alpha-reductase inhibitory active constituents of Piper nigrum leaf. *Biol Pharm Bull* 2007; 30, 2402-2405.
5. Pradeep C.R. and Kuttan G. Piperine is a potent inhibitor of nuclear factor-kappaB (NF-kappaB), c-Fos, CREB, ATF-2 and proinflammatory cytokine gene expression in B16F-10 melanoma cells. *Int Immunopharmacol* 2004; 4, 1795-1803.
6. Lee S.A., Hwang J.S., Han X.H., Lee C., Lee M.H., Choe S.G., Hong S.S., Lee D., Lee M.K. and Hwang B.Y. Methylpiperate derivatives from Piper longum and their inhibition of monoamine oxidase. *Arch Pharm Res* 2008;31, 679-683.
7. Daware M.B., Mujumdar A.M. and Ghaskadbi S. Reproductive toxicity of piperine in Swiss albino mice. *Planta Med* 2000; 66, 231-236.
8. Piyachaturawat P., Glinsukon T. and Toskulkao C. Acute and subacute toxicity of piperine in mice, rats and hamsters. *Toxicology Letters* 1983; 16, 351-359.
9. Dogra R.K.S., Khanna S. and Shanker R. Immunotoxicological effects of piperine in mice. *Toxicology* 2004;196, 229-236.
10. Madhavi B.B., Nath A.R., Banji D., Madhu M.N., Ramalingam R. and Swetha D. Extraction, identification, formulation and evaluation of piperine in alginate beads. *International Journal of Pharmacy and Pharmaceutical Sciences* 2009; 1, 156-161.
11. Venkatachalapathi S. and Subban R. Isolation and quantification of lupeol in *Strobilanthes ciliatus* Nees by HPTLC method. International Journal of Pharmacy and Pharmaceutical Sciences 2012; (4) 4: 405-408.
12. Henry T.A. *THE PLANT ALKALOIDS (4th Edition)*. J. & A. Churchill Ltd.104 Gloucester Place, W.I., London; 1949; 1,2.
13. Deepthi S.P.R., Junise V., Shibin P., Senthila S. and Rajesh R.S. Isolation, identification and antimycobacterial evaluation of piperine from Piper longum. *Der Pharmacia Lettre* 2012; 4, 863-868.
14. Wood A.B., Barrow M.L. and James D.J. Piperine determination in pepper (Piper nigrum L.) and its oleoresins—a reversed-phase high-performance liquid chromatographic method. *Flavour and Fragrance Journal* 1988; 3, 55-64.
15. ICH validation of analytical procedures: text and methodology. in *topic q2(r1)*. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf; 2005.