Research Article

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Novel Methodology for the Isolation of Pure Piperine from Plant Source through Sonication

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Abstract

Piperine was discovered in 1819 by Hans Christian, who isolated it from the fruits of Piper nigrum, the source plant of both the black and white pepper grains [1]. Fluckiger and Hanbury found piperine in Piper longum and Piper officinarum ***Piperine****, along with its isomer chavicine, is the alkaloid[1b] responsible for the pungency of black pepper and long pepper [2]. Piperine is extracted from black pepper using dichloromethane [3]. Aqueous hydrotropes can be used in the extraction to result in high yield and selectivity [4]. The amount of piperine varies from 1-2% in long pepper, to 5-10% in commercial white and black peppers [5]. Further, it may be prepared by treating the solvent-free residue from an alcoholic extract of black pepper, with a solution of potassium hydroxide to remove resin (said to contain chavicine, an isomer of piperine) and solution of the washed, insoluble residue in warm alcohol, from which the alkaloid crystallises on cooling [6]. It has been used in some forms of traditional medicine. Piperine, a major alkaloid in black pepper is one of the most gifted bioenhancers till date.*

Additional methods used for its isolation suffer disadvantages such as poor extraction efficiency, tedious and pricey isolation methodology, piperine photode gradation, etc. Hence a simple, rapid and well-organized method has been developed for the extraction of piperine from the fruits of Piper nigrum. The methods under study involve extraction of piperine with various solvents such as ethanol, propionic acid and dichloromethane. Then isolation and purification were followed by separate classical methods for respective extracts. Compared to other two methods, the novel method using propionic acid proved to be valuable in isolating piperine with higher yield and in higher purity. Hence extract derived using propionic acid was further subjected to alkali wash and passing out through small silicagel bed. Then identification of the compound was confirmed by various analytical methods TLC, melting point, UV-visible spectrophotometer, FT-IR, HPLC and compared it with authentic piperine which resulted into better pure piperine crystals as that of authentic piperine.

Introduction

The fruits of Piper nigrum (black pepper) have been widely used in household spices and also in various traditional systems of medicine. Pepper consist of piperine alkaloid (3-9%), pungent resin (6.0%), volatile oil (1-2.5%), piperidine and starch (about 30%) [7,8]. Piperine is major alkaloid of black pepper belonging to family Piperaceae which has anti-inflammatory, analgesic, antiarthritic, CNS depressant, anticonvulsant etc [9-11].

Literature review also revealed that piperine is one of the bioenhancer which can be isolated from black pepper [12]. A bioenhancer is an agent which increases bioavailability and bioefficacy of a particular drug with which it is combined; it does not have any typical pharmacological activity of its own at the dose used [13]. Piperine increases bioavailability of drugs like Barbiturates, Coenzyme Q10 (CoQ10), Curcumin (extract from turmeric), Dapsone, Ethambutol, Atenolol, Phenytoin, Propranolol, Pyrazinamide, Rifampicin, Ampicilin, etc. [14-17]. Bioavailability of Rifampicin increases about 60% due to Piperine i.e dose reduces 450mg to 200mg of Rifampicin. By the joint endeavor of our Institute (IIIM) and Cadila Pharma already launched Anti-TB formulation named Risorine containing 200 mg of Rifampicin, 300 mg of Isoniazid (INH) and 10mg of Piperine, in November 2009 [16].

Extraction of piperine using hydrotropes is good alternative for solvent extraction method. Hydrotrope penetrates into cell and increases permeability of cell membrane. This allows easy transport of piperine outside cell facilitating increase extraction rate of piperine [18]. Thus, hydrotropes like Sodium alkyl benzene sulfonates, Sodium butyl monoglycol sulfate show selective and rapid extraction of Piperine from black pepper. The recovered Piperine can be 90% pure [19]. One of the popular methods used for essential oil extraction in Piper nigrum is supercritical fluid extraction. In this method carbon dioxide is used as a solvent at critical temperature

and pressure. But this technique is relatively very expensive due to requirement of high-pressure equipment. Though high-pressure steam treatment enhances extraction rate it is relatively slow and consumes a large amount of steam [20,21]. Extraction by using various solvents is another method in which piperine is extracted by using solvents like ethanol, dichloromethane and glacial acetic acid [22-24]. In this method extraction of the fruit powder with glacial acetic acid is done. The piperine extract obtained is further partitioned into chloroform. Purification of piperine is done by column chromatography along with toluene and ethyl acetate (7:3) as a solvent can be used.

Materials and Methods

Materials: Black pepper was procured from local market. All chemicals obtained from our departmental chemical store in IIIM Jammu. Solvents and other chemicals of reagent grade were used without further purification. Laboratory grade solvents were purified and dried by reported methods. All melting points were determined by capillary method on a Buchi technical apparatus (BUCHI-510) and are uncorrected. Bandelin Sonorex RK 510 H used for sonication. NMR spectra (^{1}H NMR and ^{13}C NMR) were obtained on Bruker Supercon 500 MHz instruments and are expressed in δ values down field from tetramethylsilane (TMS) as the internal standard. Mass spectra were recorded with a JEOL MS-D 300 mass spectrometer, IR (KBr pellet or neat sample) were recorded on Perkin Elmer-377 and Shimadzu IR-435 spectrophotometers. Column chromatography was performed on silica gel (100-200 mesh) and TLC was performed on Silica gel 60 F_{254} (Merck) plates. For the visualization of spots either UV or iodine vapor, or Ceric ammonium sulfate spraying reagent (10% aqueous sulfuric acid containing 2% Ceric ammonium sulfate).

Methods

Methods of extraction of piperine

1. **Extraction with ethanol:** 20 gm of black pepper powder extracted with 180ml 95% ethanol in Soxhlet extractor for 2 hours. The solution was filtered and concentrated on the water bath at 50oC. 10 ml 10% of alcoholic potassium hydroxide was added to the filtrate with continuous stirring. The insoluble residue was filtered and alcoholic solution was left overnight and filtered through a membrane filter.
2. **Extraction with dichloromethane:** 20g of ground pepper powder was refluxed with 40ml of dichloromethane for 25min in a round bottom flask. Condenser was attached and water was allowed to run through to condense dichloromethane vapors. Later on the flask was cooled and filtered through Buchner funnel. The extract was treated with acetone and hexane.
3. **Extraction with Propionic Acid:** Cold maceration of 20gm black pepper powder was done by using 250ml propionic acid. The extract was diluted with equal volume of water and partitioned with chloroform in separating funnel. Chloroform extract was washed with 10% sodium bicarbonate and then with water. The extract was concentrated in Rota evaporator and then dried on anhydrous sodium sulphate. Purification of extract was done by column chromatography by using Pet. ether: ethyl acetate (7:3) as a solvent. Resinous impurity was washed with Sodium hydroxide solution and then with water to remove excess of Sodium hydroxide. The extract was recrystallized by using diethyl ether.

Isolation and purification by Column Chromatography

1. Preparation of sample: Preparation of sample of Propionic acid extract (GE) for the column chromatography was done by adsorption of GE on Silica Gel (60-120) with ratio 1:10 respectively. It was run for drying on rotator evaporator till free flowing material was formed.
2. Column specification and Solvent system: The dried prepared sample was subjected to column chromatography (CC), using a 38 X 4.5 cm glass column filled with silica gel 60 (mesh size: 60-120#) in toluene: ethyl acetate (7:3). Prepared sample of GE extract was added to the free volume at the head of the column. After settling down of the material, Fractions (20ml, 6 drops/minute) were collected, and the solvent was removed to reduce volume of fraction by evaporation in vacuum at 35°C. Fractions were monitored by TLC method with same solvent system and concentrated H_2SO_4 was used as spraying reagent as shown in (Figure 1).

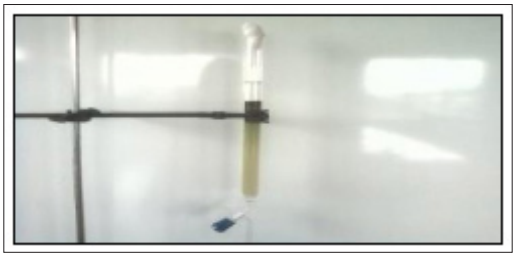


Figure 1: Column Chromatography

3. Analysis of fractions by Thin Layer Chromatography (TLC): Study of isolated fraction of GE was performed by precoated TLC plate (Silicagel GF254 plates 20cmX10cm, Merck) with solvent system toluene: ethyl acetate (7:3) [25]. Separation pattern of GE on TLC was observed by putting TLC plate in iodine chamber and confirmed with concentrated H_2SO_4 solution. Rf values were calculated for the each spot on TLC plate. The authentic sample was also used for its comparison of Rf- value with isolated piperine Fraction no. 3 (Figure 2A), Fraction no. 4 (Figure 2B) and Authentic piperine (Figure 2C) were showed almost same Rf value.

After isolation identification of the product was done by TLC, melting point, IR, HPLC and UV-visible spectrophotometer and compared it with standard piperine.

Table 1: Analysis of Fractions by Thin Layer Chromatography

Fraction no.	No. of Spots	Chemical test with Conc. H_2SO_4	R_f Value
Authentic	Single Spot	Blood-red	0.55
1	Trail	-	0.62
2	Two spot	Blood-red	0.57, 0.42
3	Single Spot	Blood-red	0.51
4	Single Spot	Blood-red	0.53
5	Single Spot	-	0.44
6-10	-	-	-

Result and Discussion

Piperine was isolated from black pepper by using three different solvents ethanol, dichloromethane, propionic acid which gave piperine yield 3.2%, 5% and 4.6% respectively. The yield of piperine

was found out to be more in dichloromethane extract but the final product obtained was not crystalline in nature. The product obtained from the other method where piperine was extracted with propionic acid produced clear needle shaped piperine crystals (Figure 3). Hence, the method of extraction with propionic acid and recrystallization with solvent ether after alkali wash proved to be effective in isolating piperine with higher purity.

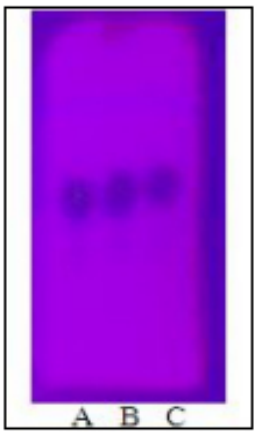


Figure 2: Analysis of fractions on precoated tlc plate under uv. (a) Fraction no. 3, (b) Fraction no. 4, (c) Authentic piperine

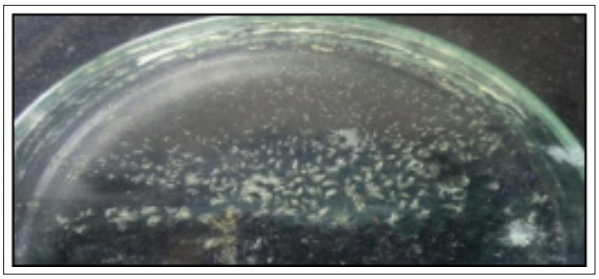


Figure 3: Needle Shaped Crystals of Piperine

Analysis of isolated Piperine Crystals

1. Chemical tests: All Chemical Tests for alkaloids were positive and especially with concentrated H_2SO_4 blood red color was obtained. Reddish brown precipitate was obtained with Dragendroff's Reagent [26].
2. Melting point - 131°-132°C.
3. UV analysis of Piperine [27]: The lambda max of authentic piperine (Fig. 4A) in methanol was 343.5nm also showed peak of 310nm. λ_{max} of isolated piperine (Fig. 4B) was found to be 344nm and this isolated piperine also showed peak of 310nm which is similar to the authentic piperine.

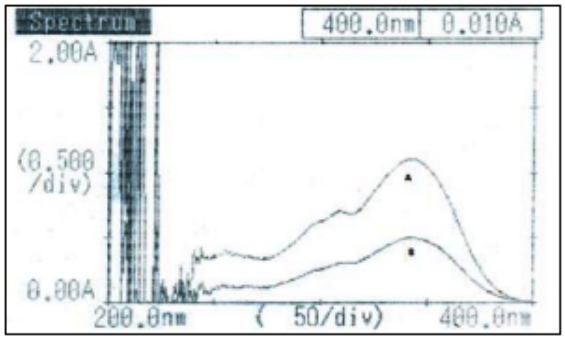


Figure 4: λ_{max} of Authentic Piperine (a) and Isolated Piperine (b) in Methanol

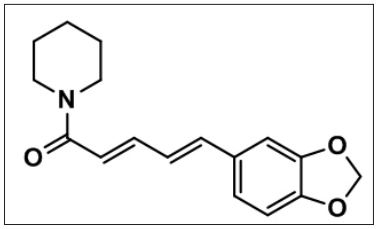


Figure 5: piperine structure

4. Confirmation by IR:

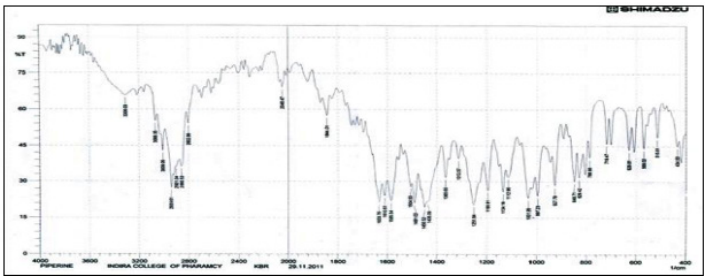


Figure 6: IR of Piperine Crystals

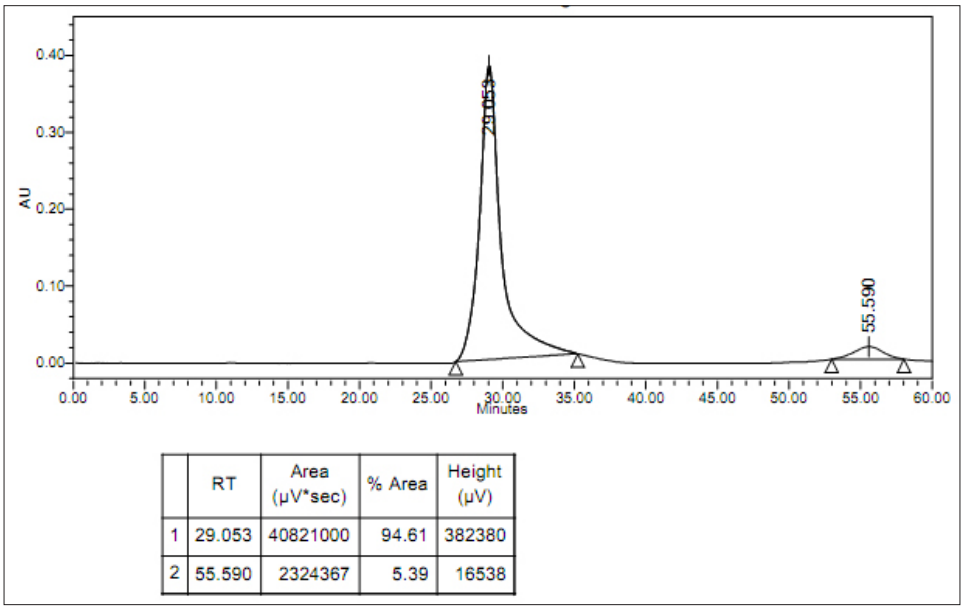
Table 2: Comparative Study of Absorbance Bands of Piperine [11,28].

Type of phenomenon	standard IR values	isolate piperine IR values
Aromatic C-H stretching	3000	3009
Symmetric and asymmetric stretching of C=C (diene)	1635	1633
	1608	1610
Aromatic stretching of C.0 (benzene ring)	1608	1610
	1580	1585
	1495	1491
	1635	1633
Methylenedioxy group:-		
Asymmetric and Symmetric CH_2 stretching, aliphatic C-H stretching	2925;	2939
	2840	2860
CH_2 bending	1450	
Asymmetrical stretching =C-O-C	1250	1251
	1190	1195
Symmetric stretching =C-O-C	1030	1031
C-O stretching (most characteristic)	930	927
in-plane bending of phenyl C-H	1132	1134
C-H bending of trans -CH=CH-	995	997
Out-of plane C-H bending 1,2,4-trisubstituted phenyl (two adjacent hydrogen atoms)	850;	848;
	830;	829;
	805;	786;

As shown in table 2; peak of all bonds were present in isolated piperine crystals which is nearby to Standard IR values of each functional group.

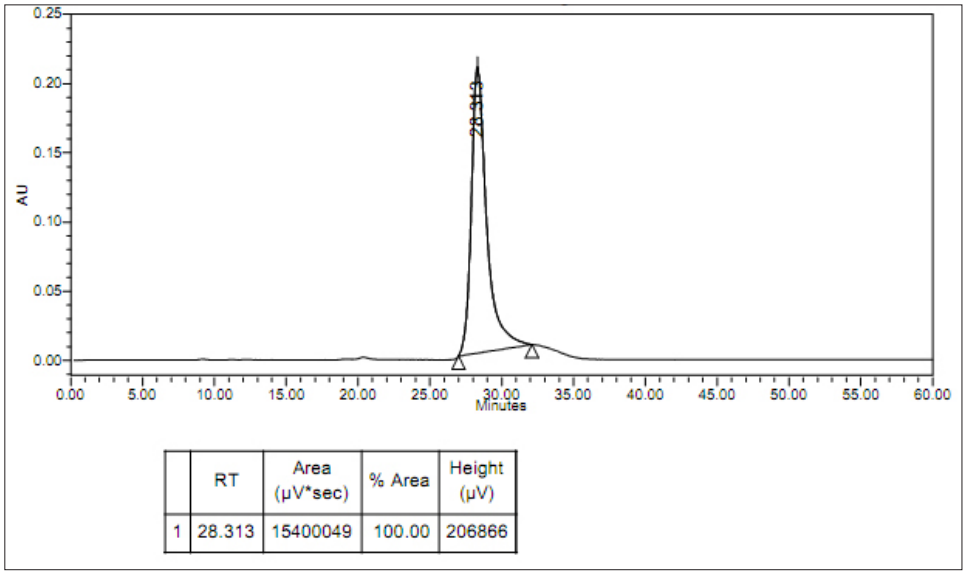
HPLC analysis: HPLC analysis was done by using column RP-18,5um and mobile phase ACN: MeOH:H2O(43:5:52) (43: 5:52) with 0.5ml/min flow rate. Retention time for authentic piperine was

found to be 29.053 min while retention time of isolated piperine was 28.313min. Hence from these graphs, we confirm that needle shaped crystals was of piperine (figure 7A & B).



	RT	Area (µV*sec)	% Area	Height (µV)
1	29.053	40821000	94.61	382380
2	55.590	2324367	5.39	16538

Figure 7A: Authentic Piperin



	RT	Area (µV*sec)	% Area	Height (µV)
1	28.313	15400049	100.00	206866

Figure 7b: Isolated Piperine

Conclusion

As discussed above, the novel method for isolation of piperine was found to be extraction with propionic acid and recrystallization with solvent ether after alkali wash proved to be effective in isolating piperine with higher yield and purity. Isolated piperine was identified by chemical test, M.P, TLC, UV and IR and compared with authentic piperine which resulted into better pure piperine crystals as that of authentic piperine.

References

1. Oersted, "Über das Piperin, ein neues Pflanzenalkaloid" [On piperine, a new plant alkaloid], (Schweigger's) Journal für Chemie und Physik, vol. 29: 80-82.
2. Merck Index, 11th Edition, 7442.
3. Epstein WW, Netz DF, Seidel JL (1993) "Isolation of piperine from black pepper". J. Chem. Ed 70: 598.
4. Gaikar (2002) Process for extraction of piperine from piper species. US 6365601.
5. Ikan R (1991) Natural Products: A Laboratory Guide 2nd Ed. San Diego: Academic Press, Inc. 223-224.
6. 1f. Gorgani Leila, Mohammadi Maedeh, Najaf Pour Ghasem D, Nikzad Maryam (2017) Comprehensive Reviews Volume 16: 124-140.
7. Kokate CK, Purohit AP (2010) Gokhale SB: Pharmacognosy. Nirali prakashan, Edition 45, 2: 1.56-1.58.
8. Evan WC (1997) Trease and Evans' Pharmacognosy. W.B.

Saunders London, Edition 14: 363-364.
9. Bang JS1, Oh DH, Choi HM, Sur BJ, Lim SJ, et al. (2009) Anti-inflammatory and antiarthritic effects of piperine in human interleukin 1β-stimulated fibroblast like synoviocytes and in rat arthritis models. Arthritis Research &Therapy 11: R49.
10. Sudjarwo SA (2005) The potency of piperine as anti-inflammatory and analgesic in rats and mice. Folia Medica Indonesiana 41: 190-194.
11. Deepthi SP, Junis V, Shibin P, Senthiil S, Rajesh RS (2012) Isolation, identification and antimycobacterial evaluation of piperine from Piper longum. Der Pharmacia Letter 863-868.
12. Acharya SG, Momin AH, Gajjar AV (2012) Review of piperine as bioenhancer. American Journal of Pharmtech Research 2: 2249-3387.
13. Randhawa GK, Kullar JS, Rajkumar (2011) Bioenhancers from Mother Nature and their applicability in modern medicine. International journal of applied and basic medical research 1: 5-10.
14. Zutshi RK, Singh R, Zutshi U, Johri RK, Atal CK (1985) Influence of piperine on rifampicin blood levels in patientsof pulmonary tuberculosis. J Assoc Physicians India 33: 223-224.
15. Janakiraman K, Manavalan R (2011) Compatibility and stability studies of Ampicillin trihydrate and piperine mixture. International Journal of Pharma Sciences and Research 2: 1176-1181.
16. Atal N, Bedi KL (2010) Bioenhancers: Revolutionary concept to market. Journal of Ayurveda and integrative Medicine 1: 96-99.
17. Lee EB, Shin KH, Woo WS (1984) Pharmacological study on piperine. Archives of Pharmacal Research 7: 127-132.
18. Dongre PP, Kannur DM, Kosambiya V, Desai M (2011) Significant role of Hydrotropes in extraction of phytoconstituents- A review. International Journal Pharma Sciences and Research 2: 730-734.
19. Raman G, Gaikar VG (2002) Extraction of Piperine from Pipernigrum (Black Pepper) by Hydrotropic Solubilization. Ind. Eng. Chem. Res 41: 2966-2976.
20. Ferreira SR, Nikolov ZL, Doraiswamy LK, M.Angela A.Meirelesd, Ademir J.Petenatee et al. (1991) Supercritical fluid extraction of black pepper (Piper nigrun L.) essential oil. Journal of Supercritical Fluids 14: 235-245.
21. Hamrapurkar PD, Jadhav K, Zine S (2011) Quantitative estimation of piperine in Piper nigrum and Piper longumusing High performance thin layer chromatography. Journal of Applied Pharmaceutical Science 2: 117-120.
22. Kolhe SR, Borole P,Patel U (2011) Extraction and evaluation of piperine from Piper nigrum Linn. International Journal of Applied Biology and Pharmaceutical Technology 2:144-149.
23. Madhavi BB, Nath RA, Banji D, Madhu MN, Ramalingam R, et al. (2009) Extraction, identification, formulation and evaluation of piperine in alginate beads. International Journal of Pharmacy and Pharmaceutical Sciences 1: 156-161.
24. Kanaki N, Dave M, Padh H, Rajani M A (2008) Rapid method for isolation of piperine from the fruits of Piper nigrum Linn. Journal of Natural Medicines 62: 281-283.
25. Gupta V, Jain UK (2011) Status of piperine content in Ayurvedic formulation: Method standardization by HPTLC. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2: 524-532.
26. Sreevidya N, Mehrotra S (2003) Spectrophotometric method for estimation of Alkaloids precipitable with Dragendorff's reagent in plant materials. Journal of AOAC International 86: 1124-1127.
27. Gupta V, Jain UK (2011) Quantitative analysis of piperine in ayurvedic formulation by UV spectrophotometry. International Journal Pharma Sciences and Research 2: 58-61.
28. Silverstein RM, Webster FX, Kiemle DJ (2005) Spectrometric identification of organic compounds, John Wiley & Sons, Edition 7: 80-108.