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NEW METHOD DEVELOPMENT FOR EXTRACTION AND ISOLATION OF PIPERINE FROM BLACK PEPPER

P.N. Shingate, P.P. Dongre and D.M. Kannur*

Indira College of Pharmacy, New Pune- Mumbai Highway, Tathwade, Pune-411033, Maharashtra, India

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Correspondence to Author:

D. M. Kannur

Associate Professor & Head, Department of Pharmacognosy, SCES'S, Indira College of Pharmacy, Tathwade, Pune, Maharashtra, India

E-mail: dmkannur@rediffmail.com

ABSTRACT: Piperine, a major alkaloid in black pepper is one of the most promising bioenhancers till date. Other methods used for its extraction suffer drawbacks such as poor extraction efficiency, tedious and expensive isolation methodology, piperine photodegradation, *etc.* Hence a simple, rapid and efficient method has been developed for the isolation of piperine from the fruits of *Piper nigrum*. The methods under study involve extraction of piperine with various solvents such as ethanol, glacial acetic 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 glacial acetic acid proved to be effective in isolating piperine with higher yield and in higher purity. Hence extract derived using glacial acetic acid was further subjected to column chromatography. Finally raisins removal and crystal formation were facilitated by recrystallization with solvent ether after alkali wash. 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%) [1, 2, 5].

Piperine is major alkaloid [5] of black pepper belonging to family Piperaceae which has anti-inflammatory [3, 4], analgesic [4], antiarthritic [3], CNS depressant [6] ,anticonvulsant [6] etc.

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Literature review also revealed that piperine is one of the bioenhancer which can be isolated from black pepper [8].

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 [9]. Piperine increases bioavailability of drugs like Barbiturates, Coenzyme Q10 (CoQ10), Curcumin (extract from turmeric), Dapsone, Ethambutol, Atenolol, Phenytoin, Propranolol, Pyrazinamide, Rifampicin, Ampicilin, etc. [7, 10, 11, 22]. Bioavailability of Rifampicin increases about 60% due to Piperine *i.e* dose reduces 450mg to 200mg of Rifampicin. 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 [11].

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 [12]. 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 [13].

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 [14, 15].

Extraction by using various solvents is another method in which piperine is extracted by using solvents like ethanol [16], dichloromethane [17] and glacial acetic acid [18]. 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) [19] as a solvent can be used.

MATERIALS AND METHODS:

Materials: Black pepper was procured from local market while authentic piperine procured from Yucca Enterprises, Mumbai-400 037. All chemicals obtained from the college laboratory.

Methods:

Methods of extraction of piperine:

1. **Extraction with ethanol:** 10 gm of black pepper powder extracted with 150ml 95% ethanol in Soxhlet extractor for 2 hours. The solution was filtered and concentrated on the water bath at 60^{o}C. 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:** 10g of ground pepper powder was refluxed with 20ml of dichloromethane for 20 min 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 Glacial Acetic Acid:** Cold maceration of 25gm black pepper powder was done by using 300ml glacial acetic 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 toluene: 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 Glacial acetic acid extract (GE) for the column chromatography was done by adsorption of GE on activated Silica Gel (60-120) (105^{o}C, 30 minutes) with ratio 1:10 respectively. It was kept for drying in an oven 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, 8 drops/minute) were collected, and the solvent was removed to reduce volume of fraction by evaporation in vacuum at 35^{o}C. Fractions were monitored by TLC method with same solvent system and concentrated H_2SO_4 was used as spraying reagent as shown in **Fig. 1**.

FIG. 1: COLUMN CHROMATOGRAPHY OF GLACIAL ACETIC ACID EXTRACT

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) [19]. 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 (**Fig. 2A**), Fraction no. 4 (**Fig. 2B**) and Authentic piperine (**Fig. 2C**) were showed almost same R_f value.

TABLE 1: ANALYSIS OF FRACTIONS BY TLC

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 spots	Blood-red	0.57, 0.42
3	Single Spot	Blood-red	0.51
4	Single Spot	Blood-red	0.53
5	Single spots	-	0.44
6-10	-	-	-

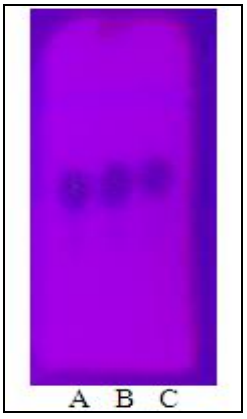

FIG. 2: ANALYSIS OF FRACTIONS ON PRECOATED TLC PLATE UNDER UV. (A) FRACTION NO. 3, (B) FRACTION NO. 4, (C) AUTHENTIC PIPERINE

RESULT AND DISCUSSION: Piperine was isolated from black pepper by using three different solvents ethanol, dichloromethane, glacial acetic 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 glacial acetic acid produced clear needle shaped piperine crystals (**Fig. 3**). Hence, the method of extraction with glacial acetic acid and recrystallization with solvent ether after alkali wash proved to be effective in isolating piperine with higher purity.

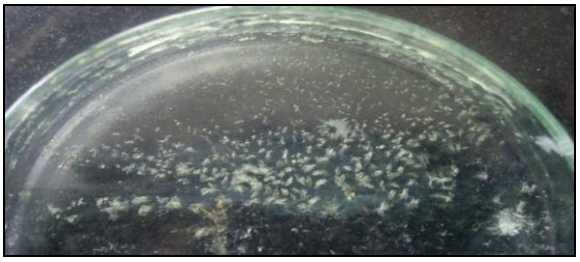

FIG. 3: NEEDLE SHAPED CRYSTALS OF PIPERINE

After isolation identification of the product was done by TLC, melting point, IR, HPLC and UV-visible spectrophotometer and compared it with standard 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 [23].

2. **Melting point** – 131°-132°C.

3. **UV analysis of Piperine** [21]**:** 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.

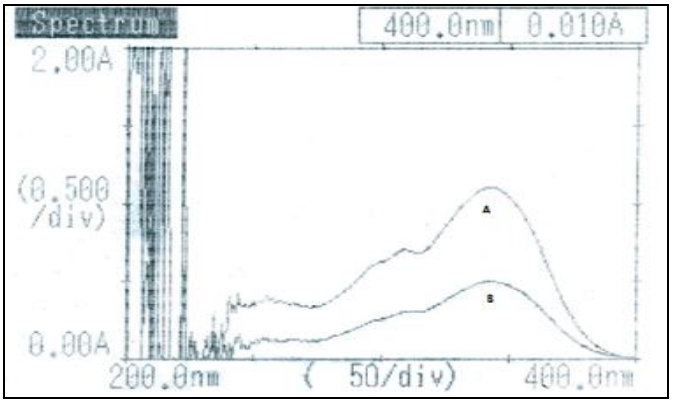

FIG. 4: λ_{max} OF AUTHENTIC PIPERINE (A) AND ISOLATED PIPERINE (B) IN METHANOL

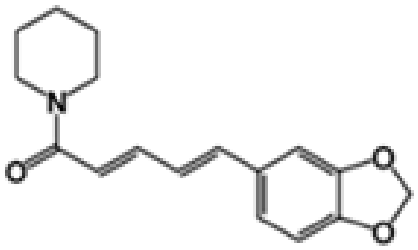

FIG. 5: PIPERINE STRUCTURE

IR Spectra: Figure 6

4. Confirmation by IR:

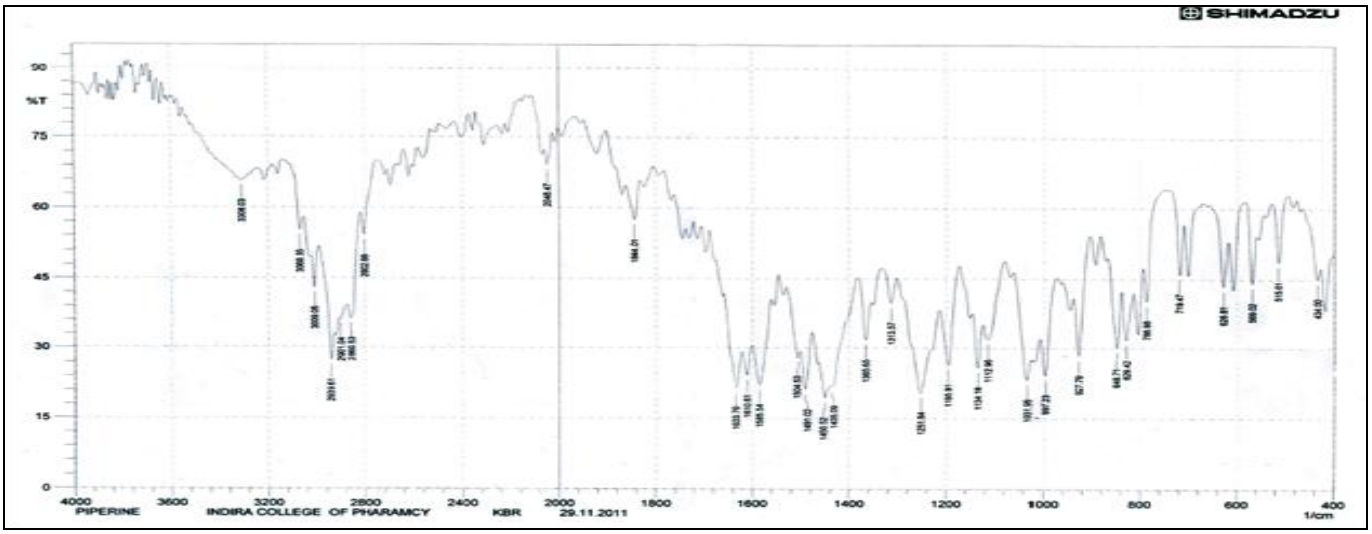

FIG. 6: IR OF PIPERINE CRYSTALS

TABLE 2: COMPARATIVE STUDY OF ABSORBANCE BANDS OF PIPERINE [6, 20]

Type of phenomenon	Standard IR values	Isolated piperine IR values
Aromatic C-H stretching	3000	3009
Symmetric and asymmetric stretching of C=C (diene)	1635 1608	1633 1610
Aromatic stretching of C=C (benzene ring)	1608 1580 1495	1610 1585 1491
Stretching of –CO-N	1635	1633
Methylenedioxy group :-		
Asymmetric and symmetric CH_2 stretching, aliphatic C-H stretching	2925; 2840	2939 2860
CH_2 bending	1450	
Asymmetrical stretching =C-O-C	1250 1190	1251 1195
symmetrical 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; 830; 805	848; 829; 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 HiQ Sil C_{18}W and mobile phase methanol: water (70: 30) with 1ml/min flow rate. Retention time for authentic piperine was found to be 6.246 min while retention time of isolated piperine was 6.232min. Hence from these graphs, we confirm that needle shaped crystals was of piperine (**figure 7A & B**).

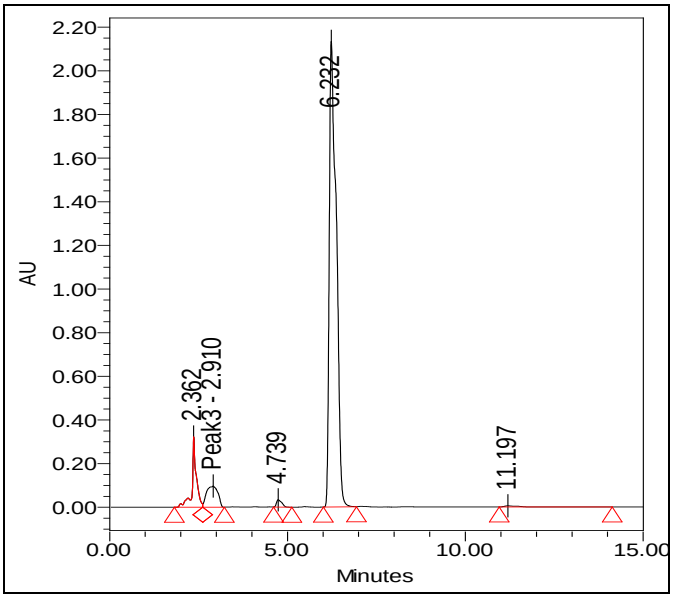

FIG. 7A: AUTHENTIC PIPERIN

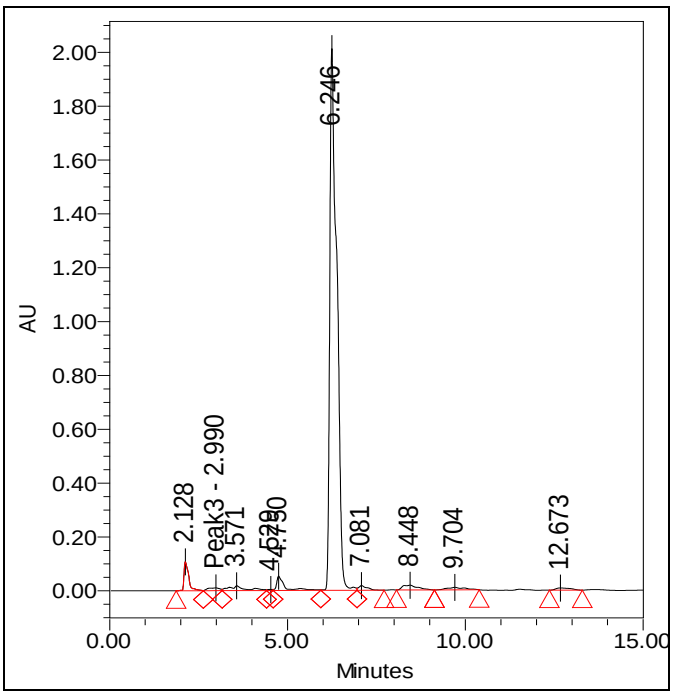

FIG. 7B: ISOLATED PIPERINE

CONCLUSION: As discussed above, the novel method for isolation of piperine was found to be extraction with glacial acetic 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.

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