



A STUDY ON THE EXTRACTION, ISOLATION AND CHARACTERIZATION OF PIPERINE FROM *PIPER NIGRUM*

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ABSTRACT

The prolific chemical constituent of Black Pepper, Piperine, is known for its ample biological activities. In the present study an attempt has been made to isolate Piperine using different solvents like Methanol, Chloroform and Isopropyl alcohol in order to prepare its semi-synthetic derivatives bearing 1, 3, 4-oxadiazole scaffolds. Methanolic extract had resulted Piperine equivalent to 7% yield. The percent yield of Piperine from isopropyl alcohol and chloroform extracts were found to be 5% and 3% respectively. 10% w/v potassium hydroxide was used to co-precipitate the resins and acids which are likely to be present along with Piperine. The methanolic extract was subjected to

purification and isolation of Piperine by column chromatography using toluene:ethylacetate (8:2) as mobile phase. The isolated Piperine was characterized by melting point, TLC, IR, HPTLC and ¹H NMR.

KEYWORDS: Piperine, Methanol, column chromatography, yield, HPTLC.

INTRODUCTION

Piper nigrum (Black pepper) is one of the most abundantly grown spices, used for dietary, medicinal, preservative and perfumery purposes. Black pepper is also known as “king of spices” hails from south India *i.e* from Western Ghats of Kerala. As of now Black pepper is grown in many countries, and few of such countries includes Vietnam (produces 34% of total production of black pepper in the world) and Indonesia (19%), India (11%), Brazil (9%) and China (6.5%)^[1] The ethnobotanical survey reveals the fact that it is known with many names in different languages. Sans. – Maricha; Bengali- Golmirch, Kalimirch; Gujarat-Kalamari, kalimirch; Hindi-Golmirch, Kalimirch; Kannada-karemenasu, Malayalam-Kurumulaku;

Marathi-Kalimirch; Tamil-Milagu; Telugu-Miriyalu, Maichanu *etc.*, Kingdom: Plantae; Class: Equisetopsida; Subclass: Magnoliidae; Super order: Magnolianae; Order: Piperales; Family: Piperaceae; Genus: Piper; Species: Nigrum^[1]

Piper nigrum is a thick glabrous climber, whose fruit is 6 mm in diameter. It is externally blackish brown, with raised reticulated wrinkles. The seeds are white and hollow. Black pepper has got an aromatic odour and the taste is aromatic and strongly pungent. However, the fruit is initially green and turns black upon drying.^[2]

Piper nigrum's different parts and the secondary metabolites are used as drug, preservative, insecticidal and larvicidal control agents.^[3] They are also used as antiapoptotic, antibacterial, anticolon toxin, antidepressant, antifungal, antidiarrhoeal, anti-inflammatory, antimutagenic, antimetastatic, antioxidative, antipyretic, antispasmodic and Bioenhancers *etc.*,^[4,5,6]

In the present study an effort has been made to isolate and compare the yields of a most therapeutically useful alkaloid *i.e* Piperine, extracted using Methanol, Chloroform and Isopropyl alcohol from Black pepper dry seeds.

MATERIALS AND METHODS

Commercially available fruits of *Piper nigrum* were procured from Amruth Kesari, Bengaluru and authenticated by Regional Ayurveda Research Institute for Metabolic Disorders, Bengaluru and the same were used to extract Piperine using different solvents such as Methanol, Chloroform and Isopropyl alcohol. The voucher specimen is kept in our laboratory for future reference.

Extraction and Isolation of Piperine

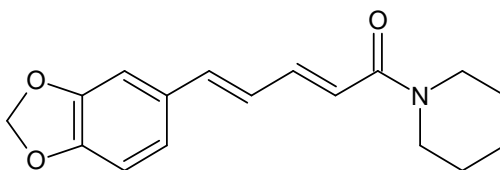
The Soxhlet apparatus was set by preparing a bed of cotton. 10 g of Black pepper was grounded to coarse powder and added into the Soxhlet apparatus and this was covered with another bed of cotton. This apparatus was fitted to a 500ml RBF which contained 250ml of Methanol. Above the Soxhlet apparatus a condenser was fitted. And this was refluxed to 40°C for about 4 to 5 hours. After the extraction, the solvent was filtered and was concentrated under vacuum to half the quantity. To this 10% w/v of potassium hydroxide solution was added with stirring. Later distilled water was added till the precipitation of Piperine lasted. Refrigerated the mixture for 2 days and filtered, recrystallized from Methanol. The above procedure was further followed using Chloroform and Isopropyl alcohol to extract Piperine.^[2]

Isolation of Piperine using column chromatography

A glass column (15X1cm) was taken and washed with toluene, plugged with cotton at the end. Silica gel of mesh size 60 to 120 was mixed with toluene, poured into column with a light tapping on the column so that all the silica particles are uniformly settled. About 2/4th of the column was packed with silica gel. The sample was dissolved in Methanol, introduced into the column. About 10ml of the mobile phase toluene:ethylacetate (7:3) was poured into the column, ran till the 5 fractions were collected. All the fractions were subjected to TLC analysis using Piperine standard (gift sample from Natural remedies) and the R_f value was compared. The 2nd and 3rd fractions collected were matched with standard R_f value of Piperine.

PIPERINE

Structure



IUPAC name: 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine.

Molecular formula: C₁₇H₁₉NO₃

Density: 1.193g/cm³[1]

The isolated Piperine was characterized for its physical and chemical characteristics by:

1). **Solubility:** Soluble in Ethanol, Methanol, and Chloroform, Isopropyl alcohol.

2). **Melting point:** 129⁰C (128-129⁰C)[1]

3). **Chemical test:**

Wagner's test: The isolated sample was treated with Wagner reagent in an alkaline medium.

A reddish brown precipitate obtained indicated the presence of Alkaloid-maybe Piperine.

4) **TLC**

Stationary phase: precoated silica gel

Visualizing chamber/reagent: UV chamber (256nm) / saturated Iodine chamber.

R_f (standard) = 0.25^[1]; R_f (isolated piperine) = 0.25

5). **IR spectra:** It is recorded using Shimadzu IR Affinity-1S spectrophotometer.

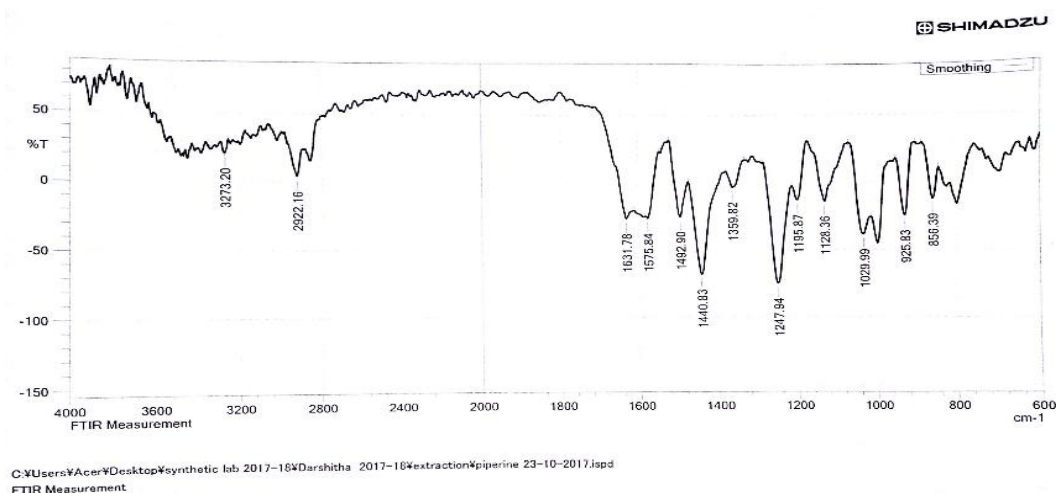


Fig. 1: IR spectroscopy of Piperine.

Table 1: IR spectral values of isolated Piperine in cm^{-1} .

Groups	Standard values (cm^{-1})	Observed values (cm^{-1})
Alkane C-H stretching	3000-2850	2922
Aromatic C-H (stretch)	3150-3050	3273
Aromatic C-H (bend)	900-690	925
C-O stretching	1300-1000	1128
Asymmetric stretching =C-O-C	1250, 1190	1247, 1195
Symmetrical stretching =C-O-C	1030	1029
Aromatic stretching of C=C (benzene ring)	1600 and 1475	1492
Symmetric and Asymmetric stretching of C=C (diene)	1680-1600	1575
Stretching of -CO-N-	1680-1630	1631
C-N (Amine)	1350-1000	1359

5). HPTLC: High performance Thin Layer Chromatography.

Extracts used : Methanol, Isopropyl alcohol, Chloroform extracts.

Application mode : CAMAG Linomet V.

Development mode : CAMAG Twin Trough chamber.

Software used : Win CATS 4 software

Sample application

The samples were dissolved in respective solvents used for extraction [Piperine (25mg/ml in Methanol, 3mg/ml in Isopropyl alcohol and 1mg/ml in Chloroform)] separately. Filtered through a 0.45 micron syringe filter and 2 μ l quantity of sample was applied on the HPTLC silica gel 60F 254 graded plate with size (10cm \times 10cm) with 0.25mm layer thickness; Merck using CAMAG Linomet 5 injector.

Chromatogram Development

CAMAG Twin Trough chambers were used to develop the plates. After elution, plates were taken out of the chamber and air dried.

Scanning

Plates were scanned under UV at 254nm and 364nm. The data's obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each extract and chromatograms were recorded fig.2, 3, 4, 5 and R_f values were recorded.

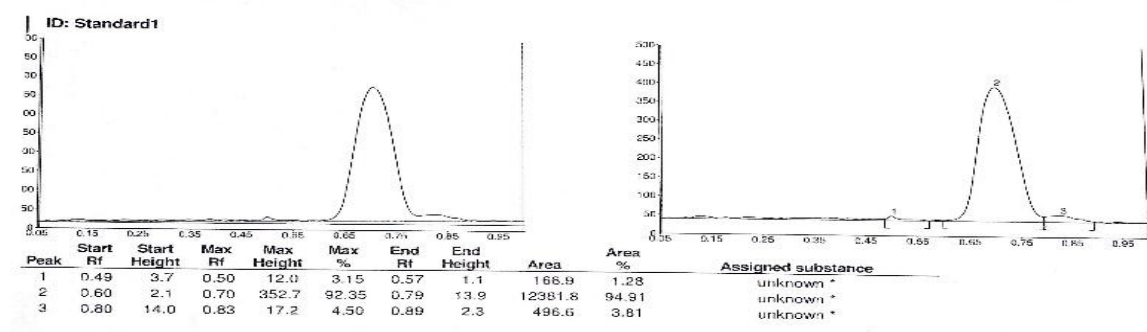


Fig.2: Chromatogram of standard Piperine.

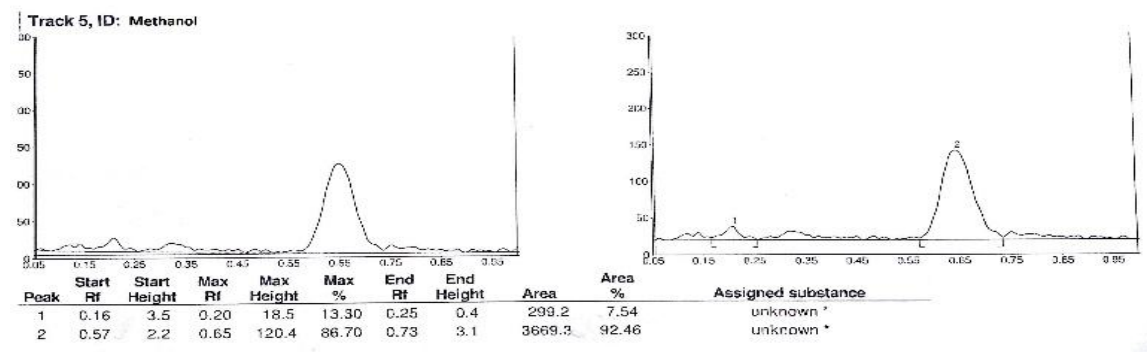


Fig.3: Chromatogram of Methanolic extract of Piperine.

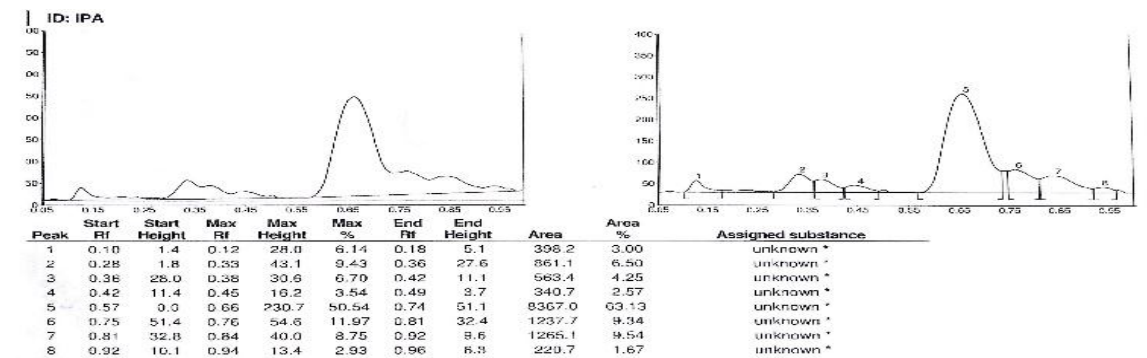


Fig.4: Chromatogram of Isopropyl alcohol extract of Piperine.

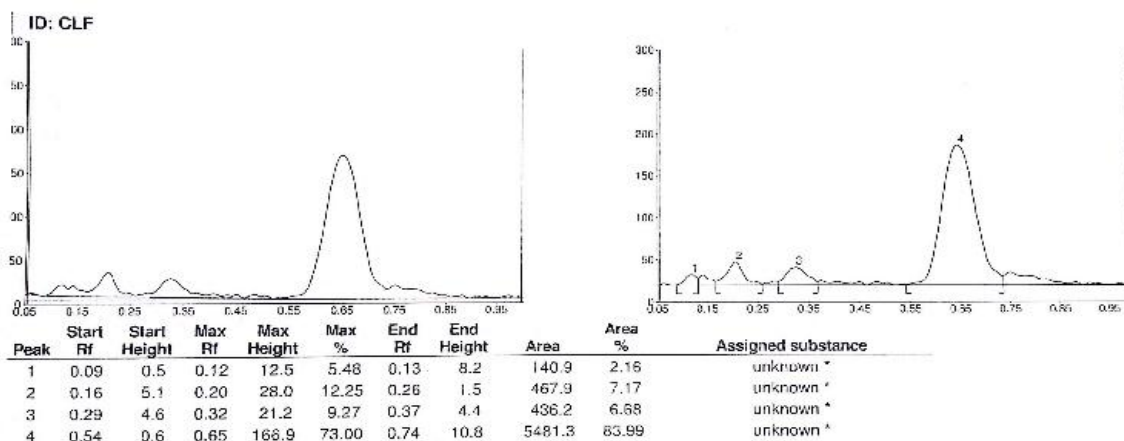


Fig.5: Chromatogram of Chloroform extract of Piperine.

6). ^1H NMR

The proton NMR (fig. 6) of isolated Piperine was recorded using spect NMR instrument at 400MHz in deuterated Methanol in presence of TMS as internal standard. The chemical shift δ values are recorded in ppm (Table 2).

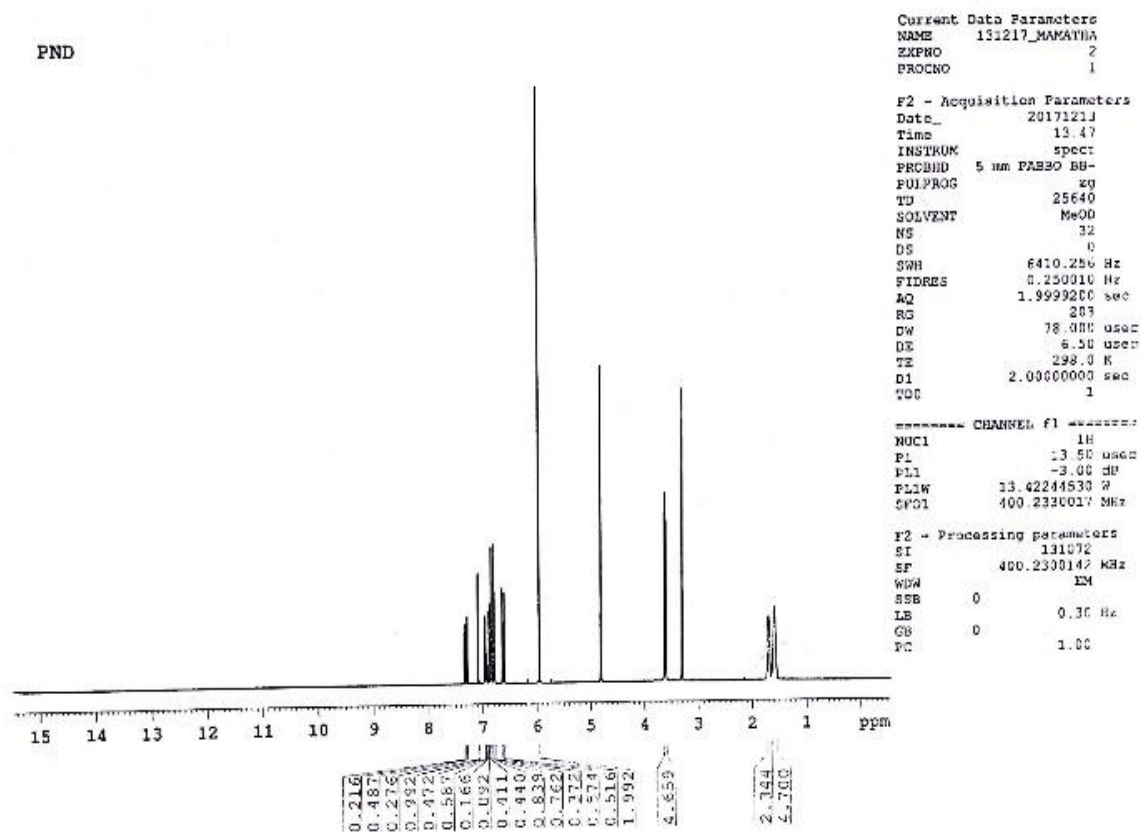


Fig.6: ^1H NMR of isolated Piperine.

Table 2: δ values of observed protons in ppm.

Type of proton	Observed chemical shift δ in ppm	Reference chemical shift in ppm
Cycloaliphatic, 10H	1.68 ~ 1.70	1.2 ~ 1.4
Alkenic, 4H	4.8 ~ 5.9	4.6 ~ 5.9
Aromatic, 3H	6.77 ~ 7.34	6.8 ~ 7.3
Methylene attached to oxygen atoms, 2H	3.60 ~ 3.63	3.2 ~ 3.8

RESULTS AND DISCUSSION

Piperine was isolated from *Piper nigrum* using different solvents such as Methanol, Chloroform and Isopropyl alcohol. 10g of *Piper nigrum* (Black pepper) seeds were used in common for extraction. The yield of Piperine was found to be highest in Methanol (7%) compared to Isopropyl alcohol (4%) and Chloroform (3%) extracts. A pure Piperine was isolated using column chromatographic procedures from all the extracts separately, where 2nd and 3rd fractions were found to contain Piperine. Then it was characterized by solubility, melting point, chemical test, TLC, IR, HPTLC and ¹H NMR studies. The Wagner's test carried out on extracts of *Piper nigrum* gave positive results indicating the presence of an Alkaloid. Melting point of the extracted Alkaloid was determined using open capillary tube and is uncorrected. It was found to be 129^oC equivalent to the melting point of standard Piperine 128-129^oC. The R_f value of isolated Piperine was found to be 0.25 which is matching with 0.25 R_f value of standard Piperine. In IR spectra (fig 1) the peak observed at 1631 cm⁻¹ indicates the presence of amide carbonyl group of Piperine (Table 1). In ¹H NMR (Table 2) the multiplet δ 6.8 ~ 7.3 ppm indicates 3 aromatic protons of Piperine, 2 protons of methylene attached to oxygen are indicated by a singlet at δ 3.60 ~ 3.63 ppm. A triplet at δ 1.68 ~ 1.70 ppm represents the presence of 10 protons of Piperidine nucleus of Piperine. Doublet at δ 4.8 ~ 5.9 ppm indicates the presence of alkenic 4 protons (fig.6). The results of these characterization parameters were in good agreement with the Piperine literature.^[7] HPTLC studies on all the extracts was carried out to quantify the Piperine (fig.2,3,4,5) in comparison to standard Piperine. The results of these studies confirmed the presence of highest Piperine content in Methanol extract 92.43% (the peak area) compared to Isopropyl alcohol extract 60.13% and Chloroform extract 83.99% This isolated and characterized Piperine will be used in our further studies as starting material to synthesize 1, 3, 4-oxadiazole derivative of Piperine for Antidepressant activity.

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