

A rapid method for isolation of piperine from the fruits of *Piper nigrum* Linn.

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Abstract A simple, rapid and efficient method has been developed for the isolation of piperine from the fruits of *Piper nigrum*. The method involves extraction of the fruit powder with glacial acetic acid, from which piperine is partitioned into chloroform and subsequently crystallized. The identity of the compound was confirmed by its melting point, comparison of UV, IR, and mass spectral data with those from a reference standard, and co-chromatography with the reference standard using thin-layer chromatography (TLC). The purity of the compound was ascertained by TLC, by recording UV absorption spectra at the start, middle, and end positions of the spot on the plate, and by differential scanning calorimetry (DSC).

Keywords *Piper nigrum* · Piperine

Introduction

The fruits of *Piper nigrum* (black pepper) have been widely used since time immemorial in household spices and also in various traditional systems of medicine. According to Ayurvedic system of medicine, *P. nigrum* fruits are anthelmintic, antiasthmatic, alterative, and used to treat pain, piles, insomnia, and epilepsy [1]. Studies have revealed anticonvulsant [2] and bioavailability-enhancing properties [3–5] of the drug. The fruits contain 1.0–2.5% volatile oil, 5–9% alkaloids, of which the major ones are piperine, chavicine, piperidine, and piperetine, and a resin

[6]. Most of the pharmacological properties of *P. nigrum* fruits are attributed to a piperidine alkaloid, piperine, which is present in the fruits in amounts of 1.7–7.4% [7]. The structure of piperine is shown in Fig. 1.

Pharmacological and clinical studies have revealed that piperine has CNS depressant, antipyretic, analgesic, anti-inflammatory [8], antioxidant [9], and hepatoprotective [10] activities. Piperine has also been shown to enhance the bioavailability of several drugs, for example sulfadiazine, tetracycline, streptomycin [3], rifampicin, pyrazinamide, isoniazid, ethambutol [4], and phenytoin [5]. Due to its diverse pharmacological properties, piperine is important as a biomarker for standardization of fruit of *P. nigrum* and *Piper longum* and of polyherbal formulations containing these raw materials. The bioavailability-enhancing property of piperine indicates its potential to be used as an adjuvant with therapeutic drugs in chronic ailments, to reduce the effective dose of the drug and, hence, subsequent adverse effects. Inspired by the various pharmacological attributes of piperine, we attempted to develop a rapid and efficient method for isolation of piperine from the fruits of *P. nigrum*.

Most reported methods [11, 12] for isolation of piperine are based on several processing steps, often under difficult operating conditions, which result in a high cost of production.

We report an alternative, rapid, economical, and efficient method for isolation of crude piperine from the fruits of *P. nigrum*.

Results and discussion

A rapid, economical and efficient method has been developed for isolation of piperine from the fruits of *P. nigrum*. Due to the high solubility of piperine in glacial acetic acid,

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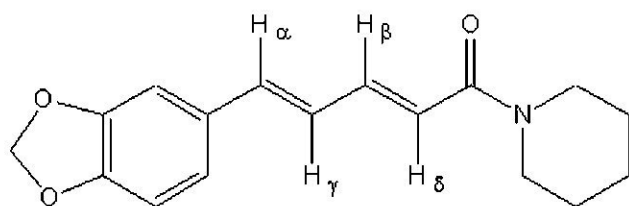


Fig. 1 Chemical structure of piperine (the protons marked α , β , γ , and δ refer to NMR data given in the text)

maceration of the *P. nigrum* fruit powder in glacial acetic acid for a period as short as 5 min was found to be sufficient. Hence the time required for isolation was comparatively short. The yield of crude piperine by the proposed method was found to be about 4.45% w/w as against 2.54% yield by the reported method. The crude piperine content of the fruits of *P. nigrum* is reported to range from 2.8 to 9.0% w/w [7].

The melting point of the isolated compound was found to be 131–132°C which is comparable with the melting point reported for piperine (130°C, Merck Index, 1989). This was further confirmed by determination of the mixed melting point of the isolated compound with standard piperine.

The thin-layer chromatogram of the isolated compound showed a single peak at R_f 0.34 and its UV absorption spectrum exactly overlapped that of the standard.

The UV, IR, and mass spectra of the isolated piperine were superimposable on those of the reference standard.

UV absorption spectra of the isolated piperine, recorded in situ on a thin-layer chromatography (TLC) plate, at the start, middle, and end positions of the spot, were found to overlap exactly, with absorption maxima at 340 nm. DSC analysis of the isolated piperine gave a sharp peak indicating a melting point of 131.01°C, which was comparable with that of the reference standard (130.48°C). The ^1H NMR spectrum of the isolated compound showed characteristic signals (δ , ppm) of piperine at 1.55–1.71 (m, br., 6H, piperidine-H), 3.52 (s, br., 2H, piperidine-H), 3.63 (s, br., 2H, piperidine-H), 5.98 (s, 2H, methylene dioxy-H), 6.44 (d, 1H, α -H), 6.76 (dd, 1H, γ -H), 6.77 (d, 1H, δ -H), 6.89 (dd, 1H, aromatic-H), 6.98 (d, 1H, aromatic-H), 7.40 (ddd, 1H, β -H) (the symbols α , β , γ , δ refer to the protons marked in the piperine structure given in Fig. 1). The above signals are characteristic of piperine and distinguish it from its geometric isomers viz., chavicine, isopiperine, and isochavicine [13]. The ^1H NMR spectrum of the isolated compound did not contain signals characteristic of the geometric isomers of piperine, confirming the purity of the compound [13]. The purity of the isolated crude piperine, as determined by HPTLC, was found to be 90%.

Thus, the reported method was found to give a high yield of piperine of fairly pure quality in a very rapid and economical way. The method can also be used for isolation of piperine from the fruits of *P. longum* and other *Piper* species reported to contain piperine.

Materials and methods

Plant material

Dried fruits of *P. nigrum* were obtained from Trivendrum, Kerala, in the month of March, because black pepper from Kerala is considered superior with respect to its piperine content [7]. They were ground to 40 mesh and stored in airtight container at 15–20°C until further use. The reference standard piperine was purchased from Merck.

Instruments

Melting points were recorded with a Toshniwal (Mumbai, India) melting-point apparatus and with a Perkin–Elmer DSC 7 differential scanning calorimeter. TLC was performed on aluminium plates precoated with silica gel 60 F₂₅₄ (E. Merck, catalogue no. 1.05554.0007) and samples were applied by use of a Camag Linomat V automatic TLC spotter. For recording chromatograms and UV spectra, the TLC plates were scanned on a Camag TLC Scanner 3 using WinCATS software. UV absorption spectra were recorded on a Jasco model 7850 UV–visible spectrophotometer. IR spectra were recorded on a Buck Scientific model 500 IR spectrophotometer. Mass spectra were recorded on Perkin–Elmer LC-MS equipment. ^1H NMR spectra were recorded on a Bruker (Germany) Avance 200 spectrometer, after dissolving the sample in CDCl_3 and adding TMS as internal standard.

Method for isolation of piperine

The fruit powder (25 g) was extracted with glacial acetic acid (6 × 50 ml) by cold maceration for 5 min each time. The extract was filtered and pooled. The pooled acidic extract was diluted with an equal volume of water and extracted with chloroform (3 × 100 ml). The chloroform extracts were pooled and washed thoroughly with 10% sodium bicarbonate solution and then with water. The chloroform extract was then dried over anhydrous sodium sulfate and concentrated to dryness at 60°C on a water bath. The residue was dissolved in minimum quantity of chloroform. Adding diethyl ether to this solution resulted in immediate separation of needle-shaped crystals of crude piperine (yield 1.12 g). The compound was purified by repeated crystallization as described above, to obtain shining yellow needles of piperine with a constant melting point (uncorrected) of 131–132°C (cc. Merck Index, 1989).

Piperine was also isolated from the same sample of *P. nigrum* fruits by the reported method [12].

Confirmation of identity and purity of the isolated compound

The melting point of the compound and its mixed m.p. with reference standard piperine were recorded using DSC (131–132°C). Co-TLC of the compound was performed with reference standard piperine on precoated silica gel plates (E. Merck) using dichloromethane–ethyl acetate (75:10) as the mobile phase.

IR, UV and mass spectra of the isolated compound were recorded and compared with those of the standard piperine. The ¹H NMR spectrum of the compound was also recorded.

The purity of the isolated compound was ascertained by overlaying the UV absorption spectra of the compound recorded in situ on TLC plate at the start, middle, and end positions of the band and by differential scanning calorimetric analysis of the isolated compound. The purity of the isolated piperine was determined quantitatively by high performance thin-layer chromatography (HPTLC).

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