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NOTE

New method of isolation of piperine from *Piper retrofractum* Vahl.

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Abstract : Piperine is a major bioactive constituent of the plants of piperaceae family having plethora of therapeutic application. It enhances the bioavailability of many nutraceuticals and drugs. It is generally isolated from *Piper nigrum* (black pepper) with the help of ethanol-KOH, glacial acetic acid-chloroform, dichloromethane-ether etc. The present work describes a new method of extraction of piperine from fruits of *Piper retrofractum* Vahl. by methanol-water in an easy, efficient and cost effective manner.

Keywords : Piperine, bioactive compound, efficient extraction.

Introduction

Piper retrofractum Vahl. (Family : piperaceae) is a glabrous fleshy climber with adhesive roots growing to height of 2 to 4 m. Leaves are papery and short, 6 to 7 cm long and 3 to 8 cm wide. The fruit is united and embedded in the rachis. It is cultivated in various parts of India[1].

The major bioactive constituent of this plant is piperine. Along with this, piperlonguminine, sylvatine, guineensine, filfiline, β-sitosterol, methyl piperate, series of piperine analoges, refractromides[2] and 3-methyl-5-decanoylpyridine[3] are reported. The IUPAC name of piperine is 1-[5-(1,3-benzodioxal-5-yl)]-1-oxo-2,4-pentadienylpiperidine, also called *trans*, *trans*-isomer of 1-piperoyl piperidine. Being an amide piperine is a non-basic alkaloid and is responsible for the pungency of *Piper retrofractum* Vahl. It exhibits anti-inflammatory and anti-arthritic[4], antimutagenic[5], antidiarrhoel[6], antifungal[7] and antioxidant[8] properties. Piperine enhances the bioavailability of a number of therapeutic drugs and many phytochemicals[9]. In human, bioavailability of curcumin is enhanced by 2000% in presence of piperine[10]. Similarly it enhances green tea (*Camellia sinesis*) polyphenol's bioavailability[11]. Piperine's bioavailability enhancing property is due to increase in absorption as a result of its effect on the ultrastructure of intestinal brush border[12].

Piperine is mostly isolated from black pepper (*Piper nigrum*). The common procedure[13] for the isolation of piperine involves its extraction using ethanol (95%) and KOH. However, Epstein *et al.*[14] extracted it without using KOH. Microwave assisted technique (MAT) for isolation of piperine has also been adopted[15] by which 85% pure piperine was obtained. Recently glacial acetic acid has also been used for extraction[16].

Most reported methods are based on several processing steps using many chemicals. As a result, the cost of the piperine becomes high and isolation is difficult. In the present method we have used only methanol for extraction and cold water for precipitation as piperine is insoluble in it. Also the use of basic compounds (like KOH or NaOH) was avoided as piperine may get hydrolysed to piperic acid in presence of these compounds.

Results and discussion

Powdered fruits of *Piper retrofractum* were refluxed in methanol for 1 h. After refluxing and filtering, the extracted solution was concentrated to half of its original volume in order to avoid the co-precipitation of other impurities along with piperine during precipitation with cold water.

Experimental

Fresh fruits of *Piper retrofractum* was purchased from the local market and identified by the Botany Department of our college where a voucher specimen is maintained.

Air dried fruits were ground to a fine powder (100 g), placed in a round bottom flask (1 liter capacity) and 500 ml methanol was added to it. The flask was heated on boiling water bath for 1 h. After cooling, the solution was decanted and filtered. The filtrate was concentrated by a rotary evaporator (Model no. GSI BU 6D) to 250 ml in 10–12 min. The temperature of water bath was maintained between 55–60 °C during concentration.

To the cooled, concentrated extract was added 100 ml ice-cooled distilled water in batches of 10 ml within 2 to 3 min. During addition of water, turbidity started appearing. After keeping overnight, off white precipitate was obtained. The precipitate was separated by decantation, washed twice with ice-cooled water, dried in a vacuum desiccator to furnish 4 g of piperine. After crystallization and recrystallization with absolute alcohol, off white needles (2–2.5 g) of piperine was obtained (m.p. 128–129 °C).

Purified piperine was compared with standard piperine (purchased from Central Drug House Pvt. Ltd., New Delhi) by TLC (silica gel GE 254)) developed with dichloromethane/methanol (9.9 : 0.1) and stained in iodine chamber ($R_f = 0.4$). Identity of the isolated piperine was further confirmed by mix m.p. and Co-TLC.

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