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ORIGINAL ARTICLE

Double bypasses soxhlet apparatus for extraction of piperine from *Piper nigrum*

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Abstract A simple modified soxhlet extractor, double bypasses sidearm soxhlet apparatus (DBSA) was designed and employed for extraction of piperine from *Piper nigrum*. Total extraction time, time taken for a cycle and yield observed in the double bypass sidearm soxhlet apparatus was compared with the soxhlet apparatus. Extraction time, time taken for an extraction cycle and yield of crude piperine obtained in DBSA were 12 ± 1 h, 8 ± 1.00 min, and 3.90 ± 0.10 g whereas the results obtained in the soxhlet method were 22 ± 1 h, 16 ± 1.00 min, and 3.80 ± 0.18 g, respectively. The results obtained in DBSA have demonstrated that this approach is as efficient as the soxhlet apparatus with drastic reduction of extraction time. On the basis of this result, we propose DBSA as the most efficient method and an alternative to the soxhlet extractor.

1. Introduction

Piperine, one of the major constituents of *Piper nigrum* (*P. nigrum*), has received increasing attention in the recent years because of its medicinal properties and as bio-availability enhancer in formulations of several drugs (Reen and Rashmet, 1997). Piperine shows a protective effect against radiation and, therefore, could be administered to cancer patients before radiotherapy (Sharma et al., 2000). Extraction and preparation of crude from plant is the starting point for the isolation and purification of chemical constituents (Romanik et al., 2007). It is an important step in studies involving the discovery of active compounds of plant materials. Ideally, an extraction procedure should be exhaustive with respect to the constituents to be analyzed, rapid, simple and inexpensive (Benthin et al., 1999).

Soxhlet extraction has been the leaching technique, mostly used for long time extraction and has been a standard technique during more than one century and, at present; it is the main reference to which the performance of other leaching methods are compared. Conventional soxhlet apparatus was originally used for the determination of fat content in milk (Soxhlet, 1879). In soxhlet extraction, the sample is repeatedly brought into contact with the fresh portions of the solvent, thereby helping to displace the transfer equilibrium and no

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filtration is required after the leaching step. It is a continuous process and it required minimum amount of solvent, also the basic equipment is inexpensive. The most significant demerits of the soxhlet extractor, as compared to the other conventional techniques for solid sample preparation are, the long time required for extraction which causes solvent loss and is harmful to the environment (Luque de Castro and García-Ayuso, 1998).

Several authors have used the soxhlet apparatus for extraction of pesticides from soil samples and natural products from medicinal plants. Some of the examples for the long extraction process are 16–24, 48–60, 54, and 72 h (Luque-García and Luque de Castro, 2003; Chauhan et al., 2004; Brahmachari et al., 2006; Amzad Hossain et al., 2006; Perwez and Ali, 2009) depending upon the solid matrixes. The merits and demerits of the soxhlet apparatus have been used as starting point for the development of a variety of modifications to improve the extraction efficiencies. Many attempts have been made for the last decades to improve the efficiency of the soxhlet extractor and bring it closer to that of more recent techniques such as microwave-assisted solvent extraction (Mandal et al., 2009) focused microwave-assisted soxhlet extraction (Prados-Rosales et al., 2002) ultrasonic extraction, accelerated solvent extraction (Waksmundzka-Hajnos et al., 2004) high pressure and supercritical fluid extraction solvent extraction (Adil et al., 2008).

Ultrasound-assisted extraction, shaking extraction and stirring extraction methods are in general less effective than the soxhlet (Clarke et al., 1991) as they involve most of the soxhlet's disadvantages, but none of its advantages. Some additional help such as enzymic reaction (Tano-Debrah and Ohta, 1995) solvent mixtures have sometimes been coupled to the shaking or stirring step in order to improve the overall efficiency, it hardly surpasses that of soxhlet extraction (Van-Delft et al., 1994).

The purpose of the present study is to compare soxhlet apparatus extraction with double bypass sidearm soxhlet apparatus extraction with respect to extraction time, extraction cycle, yield of crude piperine and volume of solvent used. Methanol is used as the extracting solvent due to its higher solubilizing capacity. Crude piperine is isolated using column chromatography.

2. Experimental

2.1. Plant sample

Commercial grade sample of *P. nigrum* was purchased from super market, Yerkaud, Salem, India. *P. nigrum* fruit berries were dried and ground to yield powder of 100 mesh size particles and were directly subjected to extraction. Methanol, hexane, petroleum ether, ethyl acetate, diethyl ether and silica gel (60–120 mesh for CC and 200–400 mesh for TLC) were purchased from S.D. Fine Chemicals, Mumbai, India.

2.2. Apparatus

Soxhlet (100 mL capacity) was purchased and modified for our requirement with the help of a glass fabricator. (Soxhlet single bypass: DBSA-double bypasses). Two conventional extraction techniques as given below were used for comparison with DBSA. All extractions were continued until colorless. Percentage extraction yield (w/w) for crude was obtained by using the formula

$$\text{Percentage of crude content} = \frac{\text{Weight of crude obtained}}{\text{Weight of sample taken}} \times 100$$

2.3. Conventional extraction

A weighed sample (40 g) of powdered material was extracted at 70–80 °C for 24 h under reflux with 1000 mL (250 mL × 4) methanol in a round bottomed flask heated in a water bath. After extraction the content was concentrated on a water bath and the yield of crude was calculated.

2.4. Soxhlet and modified soxhlet extractions

Exhaustive extraction with methanol (250 mL) was performed in a soxhlet apparatus, the thimble of which contained a weighed portion of plant material (40 g). Continuous extraction was performed for about 22 h. The obtained extract was concentrated on a water bath and the yield was calculated. A weighed sample (40 g) of the powdered materials in the thimble were introduced in to DBSA which was connected with two distillation flasks through inverted Y shaped joints, as shown in Fig. 3. DBSA extraction Inverted Y-shaped joint was performed with 500 mL of methanol during 12 h.

2.5. Isolation of piperine

The methanol extract on purification over a silica gel (60–120 mesh) column (60 × 3 cm) using hexane/ethyl acetate step gradients (8:2) afforded crude piperine which was crystallized using a mixture of 3:2 acetone/hexane and the yield was calculated.

3. Results and discussion

3.1. Optimization of extraction parameters

In this study, the effects of several influential extraction parameters such as extraction time, volume of solvent and extraction cycle were systematically studied to set up the optimal extraction conditions to obtain the maximum yield of crude. Methanol was used as the extracting solvent owing to its better solubilising capacity for piperine.

3.2. Heat reflux and soxhlet extraction

To compare the extraction efficiencies of various modified soxhlet methods, soxhlet extraction was used as the reference method. In heat reflux and soxhlet procedure, extractions were continued until the solution becomes colorless (up to 24 h) as in the previously reported method (Marion et al., 1966). In heat reflux, material was extracted with 250 mL of the solvent for 6 h and the same was repeated four times. In soxhlet extraction, while heating the flasks, solvent vapors were brought in contact with the extractant freshly every time, and after leaching, it comes back to the distillation flasks. Frequent addition of fresh solvent increases the solubility and leaching. Therefore only one bypass-sidearm exists in

the soxhlet apparatus (Fig. 1) through which, all vapors have to go slowly to the extraction tube and hence, extraction is very slow. About 16 min were required to complete one cycle in soxhlet extraction, hence for an hour, an extractant was washed three times by the hot solvent. But in heat reflux method after 6 h heating followed by filtration, a fresh portion of the solvent was added until colorless. But in soxhlet extraction no filtration was required.

3.3. DBSA extraction

An attempt was made to reduce extraction time and increase the extraction cycle by simple modification in the existing soxhlet apparatus in such a way that it contains a double bypass sidearm (Fig. 2) instead of one bypass sidearm in the soxhlet extractor. For the purpose, firstly, DBSA was connected with two distillation flasks via an inverted Y-shaped joint (Fig. 3). This is called as double bypass soxhlet extractor with inverted Y-shaped joint (DBSAY). The two sides of the inverted Y joint were purposefully designed with equal size. In DBSAY, two distillation flasks were connected at the angle of 60° slope. Hence there was no horizontal link between two flasks. When the flasks were heated, double the volume of vapors directly reached the extraction tube. Subsequently the number of extraction cycles per hour was increased and consequently the extraction time was reduced. Traditionally, extraction of piperine is carried out for extended periods stretching over 16–24 h (Marion et al., 1966). In DBSAY, the extraction was completed in 12 h against 24 h in heat reflux and 22 h in soxhlet extractions. The crude piperine content of the fruits of *P. nigrum* is reported to range from 2.8% to 9.0% w/w (Kanaki et al., 2008; Anonymous, 1998).

3.4. Comparison of DBSA with other conventional extraction techniques

The selection of an extraction method mainly depends on the advantages and disadvantages of the processes, such as extraction yield, complexity, production cost, environmental friendliness and safety. In general, heat reflux extraction is the most frequently used extraction procedure. The drawback of heat reflux extraction is the large amount of solvent, long extraction times and multistep filtrations needed. Considering the excessive solvent consumption and the long extraction period, this extraction method is not favorable for commercial perspectives. The drawbacks of soxhlet extraction are long extraction time and laborious work required. DBSA is a relatively new method, which has received increasing attention as an alternative method. DBSA extraction method is the same as conventional soxhlet but with increased extraction cycles with reduced time. However, compared with the heat reflux and soxhlet extraction methods, DBSA method showed prominent

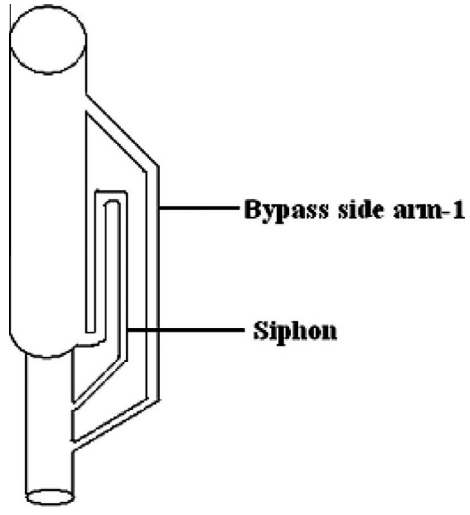

Figure 1 Conventional soxhlet apparatus (single bypass).

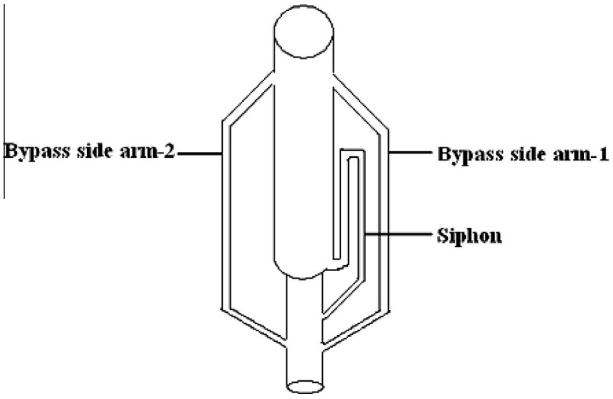

Figure 2 Modified soxhlet apparatus (double bypasses).

Figure 3 Double bypasses sidearm soxhlet extractor connected with two distillation flasks through inverted Y-shaped joint.

Table 1 Extraction time, cycles and yield of crude piperine ($n = 3$, $p < 0.5$).

Method of extraction	Extraction time (h)	Time taken for a cycle (min)	Crude piperine yield (%)
SA[a]	22 ± 1	16 ± 1.00	3.80 ± 0.18
DBSAY[b]	12 ± 1	8 ± 1.00	3.90 ± 0.10

[a] SA: soxhlet apparatus.
[b] DBSA: double bypasses sidearm soxhlet with inverted Y-shaped joint.

advantages with high extraction efficiency, reduced extraction time which can lead to less laborer work. In the current study, DBSA was compared with the other conventional extraction techniques for the extraction of piperine from *P. nigrum*. On extraction time, DBSA was also the fastest method with only 12 h of extraction time and 24, 22 h in heat reflux and soxhlet extractions. An extraction time, cycle and yield of crude piperine were given in Table 1. These features along with an ease of operation and implementation would position DBSA as a simple, fast and economic extraction method with improved efficiency and reduced extraction time suitable for plant crude preparation.

4. Conclusions

Modified soxhlet extraction showed that following results:

(i) DBSA is a rapid extraction method for the extraction of piperine from *P. nigrum* with clear advantages versus heat reflux and conventional soxhlet extraction such as shorter extraction time and lower solvent consumption.
(ii) The recoveries obtained with the described procedure were almost the same as other methods.
(iii) Double bypasses soxhlet apparatus extraction performance was satisfactory for *P. nigrum* extraction hence we propose it is also a suitable and simple method.

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