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Extraction of piperine from *Piper longum* using ultrasound

Sachin S. Rathod, Virendra K. Rathod *

Department of Chemical Engineering, Institute of Chemical Technology, Matunga (E), Mumbai 400019, India

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ABSTRACT

Efforts were made to enhance the yield of piperine using ultrasound assisted extraction (UAE) from fruit of *Piper longum*. The effects of various factors such as solvent, extraction time, solid to solvent ratio, duty cycle, ultrasound frequency, ultrasound power and temperature on the yield of piperine were investigated and optimized. The maximum yield of piperine (5.8 mg/g) from *Piper longum* powder was obtained at optimal UAE conditions such as, ethanol as extracting solvent, extraction time 18 min, solid to solvent ratio 1:10, ultrasound power 125 W, 80% duty cycle, ultrasound frequency 25 kHz and temperature 50 °C. The experimental results revealed the advantage of UAE over traditional method of batch extraction and solvent extraction. The extraction time is reduced from 8 h of batch solvent extraction and 4 h of Soxhlet to 18 min in UAE with enhanced extraction yield of piperine. Extraction yields of piperine obtained from Soxhlet extraction and batch extraction methods were found to be 1.67 mg/g and 0.98 mg/g, respectively, which were much lower than UAE optimized results. Hence, ultrasound assisted extraction of natural phytoconstituents will diminish the problem of lower extractability and higher extraction time over traditional methods.

1. Introduction

Piper longum is a native of North East India and found in tropical areas of India, from central Himalaya's to Khasi, Assam, Mikir hills of West Bengal and the evergreen forests of Western Ghats of Kokan to Travancore (Manoj et al., 2004). Long pepper is a dried fruit of *Piper longum* (Piperaceae) which is slender aromatic climber with perennial woody roots, cultivated for its flavouring properties and unique pharmacological actions. The dried spikes are 1–3 cm in long and 4–6 mm in diameter with greyish to dark in colour. The fruits are pungent and have black pepper flavor resemblance. Traditionally, it is used as a spice in pickles, preserves, soups, meat curry. It is less expensive than the black pepper so, sometimes used as an adulterant in ground black pepper (Govindarajan, 1977). Aqueous extract of root of *Piper longum* is used as food material in western part of India Warrier et al., 1996). On the other hand, it is also used to treat respiratory track diseases like chronic bronchitis, cough, asthma, cold, counter irritant (Warrier et al., 1996), analgesic and also used as antidote to snake bite and scorpion sting (Manoj et al., 2004). The alcoholic extract of the fruits of *Piper longum* and its component piperine showed significant immunomodulatory and antitumor activity (Sunila and Kuttan, 2004). Pipernonaline, a piperidine alkaloid possesses a mosquito larvicidal activity (Sung-Eun, 2000). The maximum piperine content in *Piper longum* is 3–5% (Madhavi et al., 2009). Piperine was the first amide isolated from Piper species which reported CNS depression, anti-inflammatory activity and antipyretic activity (Virinder et al., 1997). In spite of large number of applications, it is not explored by researchers especially to identify efficient extraction process.

The extraction process is a key step in isolation of constituents from spices, so the optimization of the extraction processes for different spices is very important. Batch solvent extraction and Soxhlet extraction processes are traditional extraction methods which are time consuming, labour intensive and require large amounts of solvents. In recent years, novel extraction techniques have been developed to overcome the drawbacks of conventional processes which include supercritical fluid extraction, e.g. Red pepper (Uquiche et al., 2004), microwave assisted extraction, e.g. capsaicinoids from pepper (Gerardo et al., 2006).

Ultrasound assisted extraction (UAE) is one of the novel extraction techniques which has several potential advantages over traditional methods of extraction such as less extraction time, high percentage of extraction yield and easily controllable in terms of process parameter. An enhanced mass transfer across the cell wall due to cavitation and thermal effect, are responsible for the higher extraction yield in UAE. Extraction rate enhancement attributed by ultrasound has been achieved due to propagation of ultrasound

* Corresponding author. Tel.: +91 22 33612020; fax: +91 22 33611020.
E-mail addresses: vk.rathod@ictmumbai.edu.in, virendrakrathod@gmail.com (V.K. Rathod).

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pressure waves resulting in the cavitation. Cavitational bubbles implodes energetically and forms micro-turbulence, perturbation and high velocity inter particle collision which increase the mass transfer further enhancing extraction yield (D'Alessandro et al., 2006; Shirsath et al., 2012; Yang and Zhang, 2008). Ultrasound irradiation also imparts physical and thermal effects which cause liquid circulation, disruption of cell walls, reduction in particle size, and enhanced mass transfer across cell membranes (Yang and Zhang, 2008). Although ultrasound has proved to be an efficient technique for extraction of natural product (Charpe and Rathod, 2012), there is practically no information available on ultrasound assisted extraction of piperine from *Piper longum*.

This work exhibits that UAE aids the efficient extraction of principle pungent component (Piperine) and optimization of different process parameters such as solvent, extraction time, solid to solvent ratio, duty cycle, ultrasound frequency, ultrasound power and temperature with significant reduction in extraction time as compared to traditional extraction processes.

2. Materials and methods

2.1. Materials

Long pepper was purchased from local spice market, Mumbai, India, dried under the sun and grounded to fine powder having average particle size in the range of 0.40–0.55 mm. Moisture content of the raw material determined by using moisture analyser was found to be 5.23%. Standard Piperine was obtained from Total Herb Solutions Pvt. Ltd, Mumbai. Ethanol, Acetone, Hexane were of analytical grade and purchased from S.D. Fine Chemicals, Mumbai. Deionised water (Millipore Milli-Q 50) and acetonitrile (S.D. Fine chemicals, Mumbai) were used as solvent for high pressure liquid chromatography (HPLC), were of analytical grade.

2.2. Apparatus

Ultrasound assisted extraction was carried out in an ultrasound cleaning bath (Model 6.5l200 H, Dakshin, India) of internal dimensions 230 mm × 150 mm × 150 mm and tank capacity 6.5 L approx. having an ultrasonic power of 200 W and frequencies of 25 kHz and 40 kHz. Ultrasonic bath was provided with heater, digital temperature controller/indicator and cooling water recirculation system. Fixed operating frequency can be chosen by the selector switch from the panel as well as Power by varying input AC voltage through auto transformer.

2.3. Ultrasound assisted extraction

The extraction was carried out in a glass vessel with flat bottom having length of 13 cm and internal diameter of 5 cm kept in an ultrasound bath. The distance of the glass vessel from bottom of bath (where transducers are fixed) was kept 2.5 cm and this position was kept constant throughout the experiments. This position and shape of vessel was previously optimized by Kulkarni and Rathod, (2013) for extraction of mangiferin from *mangeferia Indica* by ultrasound. 5 g of *Piper longum* powder was mixed with 50 mL of ethanol (solvent) in a glass vessel and kept in an ultrasound bath. The *Piper longum* powder with solvent was irradiated by ultrasound till 30 min. Samples were withdrawn at 3 min interval in a small aliquot (0.1 mL). Samples were then diluted and analyzed using HPLC. Different process parameters affecting the extraction yield of Piperine have been studied and depicted in Table 1.

Table 1
Process parameters for UAE.

Sr. No	Parameters	Description
1	Solvent	Ethanol, Acetone, Hexane
2	Extraction time (min)	3–30
3	Solid to solvent ratio	1:2.5, 1:05, 1:10, 1:20, 1:30 and 1:40
4	% Duty cycle	20, 40, 60, 80, 100
5	Frequency (kHz)	25 and 40
6	Ultrasonic power (W)	100, 125, 150, 175 and 200
7	Temperature (°C)	30, 40, 50, 60, and 70

2.4. Soxhlet extraction

Extraction was carried out using a conventional Soxhlet apparatus. It consists of distillation flask placed in oil bath, thimble holder and condenser. 5 g of *Piper longum* powder was packed in a cellulose filter pouch, placed in thimble holder which is filled with the condensed fresh solvent from the distillation flask. The extraction of Piperine took place in thimble chamber. When liquid reaches the overflow level, liquid moves through siphon and unloads it back into the distillation flask, carrying extracted solutes into the bulk liquid. In a solvent flask, solute was separated from the solvent by distillation. Solute was left in the flask and fresh solvent passed back into the solid bed material. The operation was repeated until maximum extraction was achieved. Approximately 150 mL of solvent was used in a typical run. The temperature of oil bath was maintained at 80 °C and the samples were collected after 2 h of intervals. The collected samples were further analyzed for piperine content using HPLC.

2.5. Batch solvent extraction

In order to study the batch solvent extraction, 5 g of *Piper longum* powder and 50 mL of ethanol was taken in a simple glass reactor of 100 mL capacity. This mixture was stirred for 8 h at 40 °C and 600 rpm and very small amount of samples were collected after 1 h of interval to analyze the piperine content.

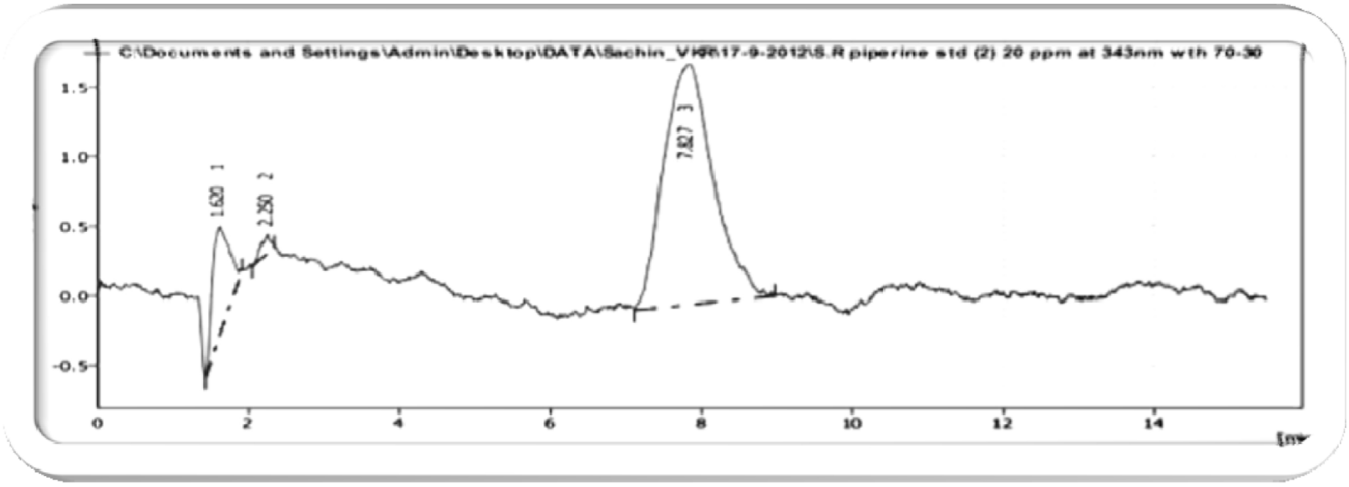
2.6. Analytical method

The Piperine was analyzed using High Performance Liquid Chromatography. The HPLC instrument was of KNAUER make, Spectra system SCM 1000, gradient HPLC pump Spectra system K 501 and Spectra system K 2501 as UV detector. The column used was C 18 (reverse phase) vertex column of 250 × 4 mm cross section packed with Eurosphere 100-5C 18. The wavelength was set at 343 nm, mobile phase used was 90:10 (ACN: H_2O), the total injection volume for analysis was 20 μL and the flow rate was 1.5 mL/min. Fig. 1(a) and (b) shows the chromatogram of standard piperine and ethanolic extract of *Piper longum*, respectively, with piperine retention time of 7.2 min.

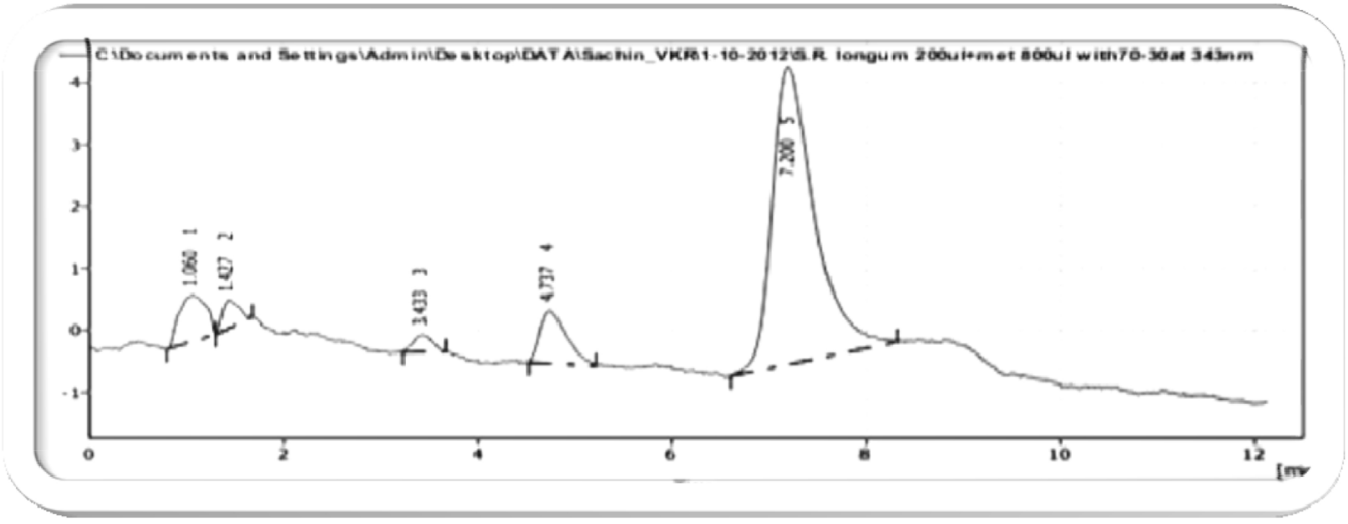
3. Result and discussion

3.1. Effect of different solvents

The selection of the most suitable solvent for extracting the compounds of interest from the sample is a crucial step for any extraction method especially for UAE. Extraction of alkaloids was generally performed by using alcoholic solvents. Thus, three different solvents (ethanol, acetone, and hexane) were screened for the extraction of piperine from *Piper longum* by batch extraction and UAE. The process parameters for UAE were kept constant for all solvents, which were as follows 5 g dried sample, temperature 40 °C, solid to solvent ratio 1:10, ultrasound power 175 W, extraction time 20 min. Similarly, the process parameters for batch



(a) HPLC analysis of standard piperine



(b) HPLC Analysis of ethanolic extract of *Piper longum*

Fig. 1. (a and b) HPLC analysis of standard piperine and ethanolic extract of *Piper longum*.

extraction were 5 g dried sample, temperature 40 °C, solid to solvent ratio 1:10, extraction time 8 h. Results obtained for both the extraction are reported in Fig. 2. It shows that the maximum yield of piperine (4.53 mg/g in UAE and 1.01 mg/g in batch extraction) was obtained with acetone followed by ethanol (4.32 mg/g in UAE and 0.98 mg/g in batch extraction) and hexane (4.08 mg/g in UAE and 0.84 mg/g in batch extraction). In the case of batch extraction, extraction yield is affected by the polarity and viscosity of the solvent. Table 2 depicts the various properties of selected solvents. Although the polarity indices for the ethanol and acetone are nearly same, the lower viscosity of acetone is responsible for higher diffusion and thus higher yield using acetone. On the other hand, the yields obtained for UAE are higher for all the solvent as

Table 2
Physical properties of solvents.

Solvent	Surface tension (mN/m)	Polarity index	Vapour pressure (kPa)	Viscosity (cP)
Ethanol	23.27	5.2	5.2	1.2
Acetone	23.7	5.1	24	0.32
Hexane	18.4	0.001	17.3	0.33

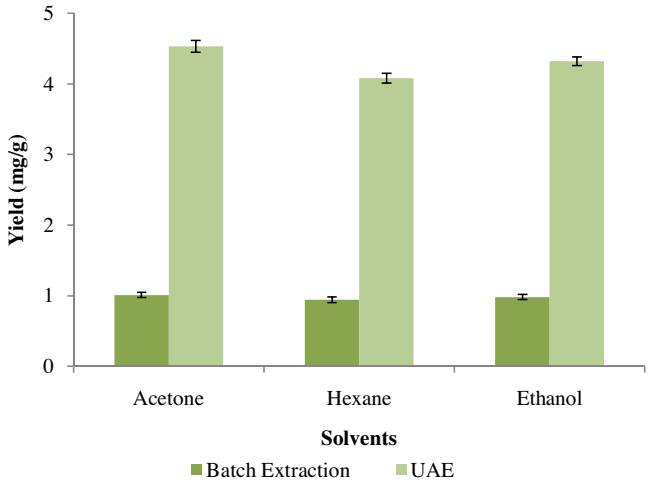


Fig. 2. Effect of different solvent on extraction yield of Piperine from *Piper longum* (For batch extraction-solid/solvent ratio of 1:10, temperature of 40 °C, time 8 h, agitation 600 rpm, For UAE-solid/solvent ratio of 1:10, temperature of 40 °C, ultrasonic power of 175 W and frequency of 25 kHz, time 20 min).

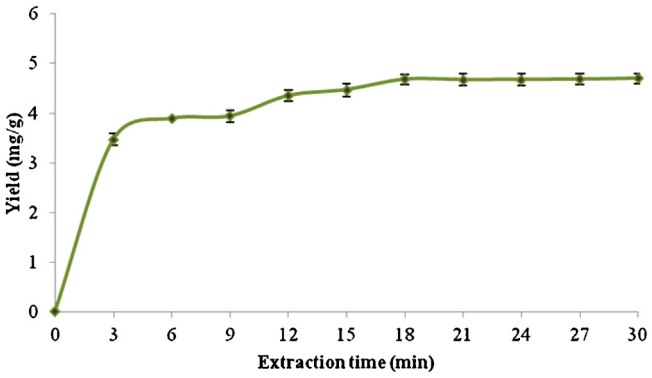


Fig. 3. Effect of time on extraction yield of Piperine from *Piper longum* using UAE (ethanol as solvent, solid/solvent ratio of 1:10, extracting time of 30 min, temperature of 40 °C, ultrasonic power of 175 W, frequency of 25 kHz).

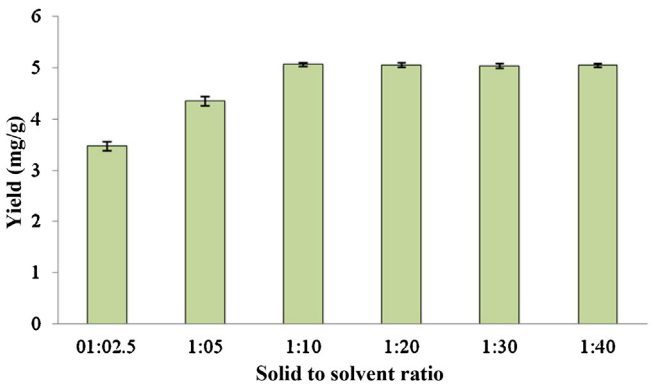


Fig. 4. Effect of solid to solvent ratio on extraction yield of piperine from *Piper longum* (ethanol as solvent, extracting time of 18 min, temperature of 40 °C, ultrasonic power of 175 W, frequency of 25 kHz).

compared to batch process. However, both the techniques show similar extraction trend for solvents. In presence of Ultrasound, the variation in the yield of piperine is also attributed to their different solubility and physico-chemical properties, i.e. surface tension, viscosity and vapour pressure. Generally, vapour pressure of the solvents plays an important role in generating cavitation and it is found that the lower vapour pressure solvent generates few bubbles but they implode with higher force. Thus, the solvent having lower vapour pressure helps in extracting more solute. Further in lower viscosity solvent the cavitation occurs easily as well as diffusion of solvent takes place faster. Table 2 clearly shows that hexane has higher vapour pressure and low polarity index which resulted in lower extraction yield. Although, the polarity index of ethanol and acetone is similar, the acetone has lower viscosity as compared to ethanol and thus gives higher extraction yield. Since, the difference in the yield obtained with acetone and ethanol is not very significant and considering the acetone handling problem and cost, ethanol was selected as solvent. Jadhav et al. (2009) have performed ultrasound assisted extraction of vanillin from vanilla pods by using ethanol, acetone, chloroform, methanol, water as extracting solvent and obtained higher vanillin yield (140 mg/L) in ethanol.

3.2. Effect of time

Time is a very important parameter for better process development and to minimize energy and cost of process. The ultrasound assisted extraction of piperine from *Piper longum* was studied with respect to time using ethanol as a solvent. The other process parameter were kept constant which were as follows, solid to solvent ratio 1:10, temperature 40 °C and ultrasound power of 175 W. Fig. 3 shows the amount of piperine extracted per gram of *Piper longum* powder with time. It has been observed that the yield of piperine extraction increases logarithmically till 18 min thereafter gradually increases for extended time. The initial sharp increase in extraction yield was due to higher concentration gradient of piperine between extracting solute and solvent. Further increase in extraction time reduces the concentration gradient which is responsible for decrease in mass transfer and after that there was extraction yields remain the same. Hence, 18 min was chosen as optimum time for extraction of piperine.

3.3. Effect of solid to solvent ratio

Solid to solvent ratio is varied from 1:2.5 to 1:40 and results are shown in Fig. 4. It was observed that the amount of extracted piperine per gram of *Piper longum* powder increased with decrease in solid to solvent ratio till 1:10. An increase in extraction yield by changing solid to solvent ratio from 1:2.5 to 1:10 was due to the fact that the amount of solvent increases with decrease in ratio which increases the concentration gradient and further helps to enhance the mass transfer and extraction yield. As solute concentration was increased, the solvent slowly becomes saturated and there is no increase in yield after a specific ratio (1:10). From Fig. 4, it is clearly found that extraction of piperine is higher at 1:10 as compared to other solid to solvent ratios (1:2.5–1:5 and 1:20–1:40) used for extraction. Thus, to avoid the wastage of solvent and bulky handling of the process, the solid to solvent ratio of 1:10 was taken as optimized ratio for the further extraction study.

3.4. Effect of duty cycle

Since continuous exposure of material with ultrasound may degrade the material, it is always recommended to use the ultrasound for optimum time and not in continuous mode. Duty cycle of ultrasonic irradiation was varied by changing the ON-OFF time. Other operational parameters were kept at constant value such as ultrasound power (175 W), time (18 min), temperature (40 °C) and solid to solvent ratio (1:10), at 25 kHz frequency. Fig. 5 shows that there was increase in extraction yield with an increase in the duty cycle until the equilibrium was achieved at duty cycle of 80% (48 s on 12 s off). At 100% duty cycle, no further enhancement in the extraction yield of piperine was observed. Hence, 80% duty cycle was found to be optimum for higher extraction yield. Dey et al. (2013) has also reported the similar results for the extraction of β-carotene from *Spirulina platensis* and reported that maximum extraction yield can be obtained by proper manipulation of duty cycle in UAE.

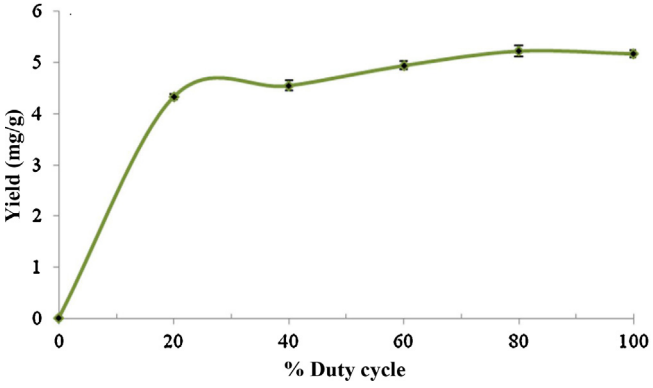


Fig. 5. Effect of duty cycle on extraction yield of piperine from *Piper longum* (ethanol as solvent, extracting time of 18 min, solid/solvent ratio of 1:10, temperature of 40 °C, ultrasonic power of 175 W, frequency of 25 kHz).

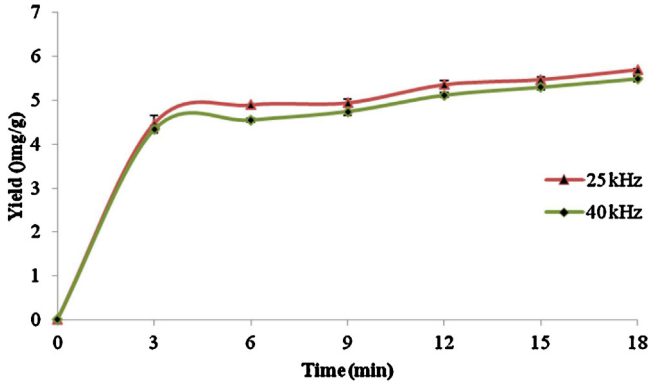


Fig. 6. Effect of ultrasound frequency on extraction yield of piperine from *Piper longum* (ethanol as solvent, extracting time of 18 min, solid/solvent ratio of 1:10, temperature of 40 °C, ultrasonic power of 175 W).

3.5. Effect of ultrasound frequency and ultrasound power

The effect of two different frequencies, i.e. 25 kHz and 40 kHz on amount of piperine extracted per gm of *Piper longum* powder was studied at ultrasound power of 175 W and result are reported in Fig. 6. Calorimetric studies were also carried out and the dissipated power was calculated from the rise in temperature of the solvent after particular time at both the frequencies for similar input power. Based on the calorimetric study, it is observed that values of power dissipation at 25 and 40 kHz were 30 W and 49 W, respectively. Although the power dissipation at both the frequencies was different, a marginal difference in the extraction yield of piperine was observed at 40 kHz (4.95 mg/g) and 25 kHz (5.17 mg/g). Similar results were obtained when extraction of Glycyrrhizic acid from licorice was performed at two different frequencies (Charpe and Rathod, 2012).

Further, experiments were also performed to identify the effect of input power at 25 kHz keeping all the other parameters constant and results are depicted in Fig. 7. It shows that the extraction yield increases from 5.17 to 5.5 mg/g with an increase in the ultrasound power till 125 W. Highest extraction yield was obtained at 125 W with an energy dissipation of 18 W as determined by calorimetric study. As the ultrasound power increases, large amplitude of waves travels through the liquid medium and the bubbles collapse more violently (Dey and Rathod, 2013). However, further increase in ultrasound powers, i.e. 150 W and 200 W does not show indicative difference in the extraction yield which have energy dissipation of 24 W and 56 W, respectively. Hence, considering the electrical energy consumption 125 W was considered as an optimized ultrasound power for the further experimentation.

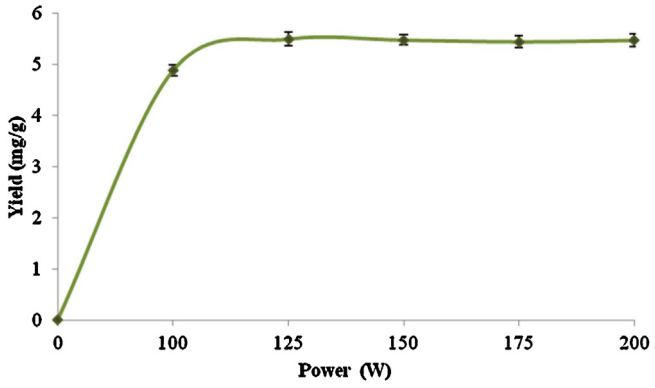


Fig. 7. Effect of ultrasonic power on extraction yield of piperine from *Piper longum* (ethanol as solvent, extracting time of 18 min, solid/solvent ratio of 1:10, temperature of 40 °C, frequency of 25 kHz).

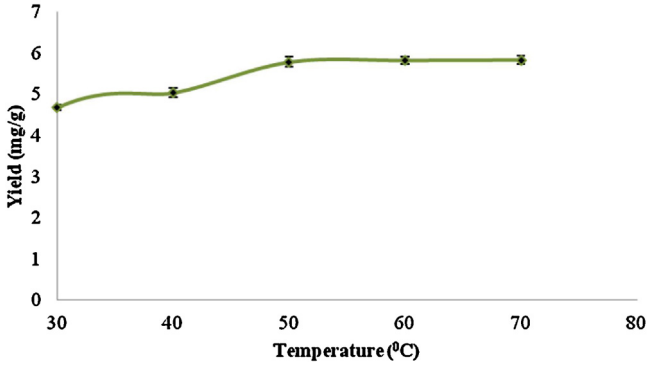


Fig. 8. Effect of temperature on extraction yield of piperine from *Piper longum* (ethanol as solvent, extracting time of 18 min, solid/solvent ratio of 1:10, frequency of 25 kHz, ultrasonic power of 125 W).

3.6. Effect of temperature

The effect of temperature on extraction was investigated, since it affects the solubility, mass transfer rate of target compounds in solvent and also cavitation phenomenon. In this study, five different temperatures (30, 40, 50, 60 and 70 °C) with ethanol as a solvent were selected to study the influence of temperature on the extraction of piperine from *Piper longum*. Other conditions were 5 g dried sample, solid to solvent ratio: 1:10, ultrasound power: 125 W and frequency 25 kHz. Both cavitation and thermal effect showed predominant effect on extraction yield. Cavitation causes implosion of cavitation bubbles which is responsible for higher turbulence while thermal effect is responsible for higher solubility of solute as well as reduction in viscosity of solvent for increased mass transfer across the cell membrane (Romdhane et al., 1995; Raso et al., 1999; Entezari and Kruus, 1996).

At a temperature of 50 °C cavitation and thermal effects were equally dominant hence, the highest piperine yield of 5.8 mg/g was obtained at this temperature. However, further increase in extraction temperature from 60 °C to 70 °C does not show significant increase in the extraction yield (Fig. 8). This is due to the fact that cavitation effect reduces as the temperature increases (Yu-Chio Yang et al., 2013) and thermal effect alone is not capable of contributing largely to the extraction yield. Finally, 50 °C was taken as optimized extraction temperature. Sun et al. (2011) has extracted isoflavones by UAE and obtained similar trend of extraction.

3.7. Comparison of ultrasound assisted extraction with batch and Soxhlet extraction

In order to compare the ultrasound assisted extraction with conventional extraction process, the extraction was performed using batch extraction and Soxhlet extraction process. The results obtained for batch extraction process are reported in Fig. 9 and compared with Fig. 3. This shows that the time required for ultrasound extraction is only 18 min while that of batch extraction is 8 h. In Soxhlet extraction, the time required for maximum extraction was 4 h. Similarly, the maximum yield obtained using UAE was compared with batch extraction and Soxhlet extraction and reported in Fig. 10. UAE process gives 5.8 mg/g of extraction yield which is significantly higher than a batch (0.98 mg/g) and Soxhlet extraction (1.67 mg/g). The higher extraction yield by ultrasound is due to cavitation effect. When ultrasound is passed through the liquid, cavities or microbubbles are formed. The temperature and pressure is very high inside the bubble. When bubbles are collapsed the

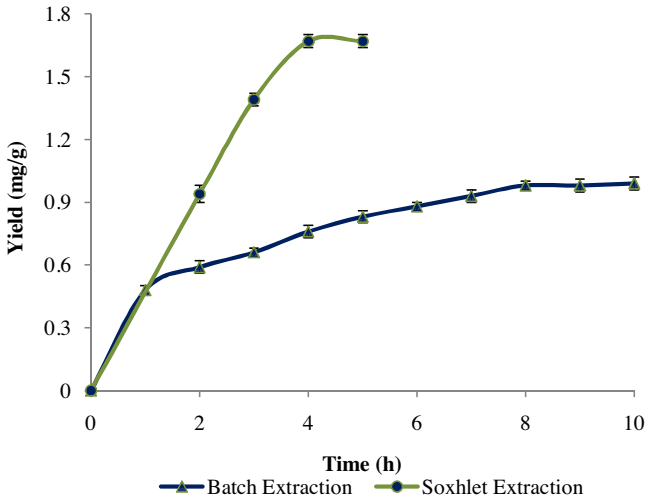


Fig. 9. Extraction yield of Piperine from *Piper longum* with respect to time using traditional methods.

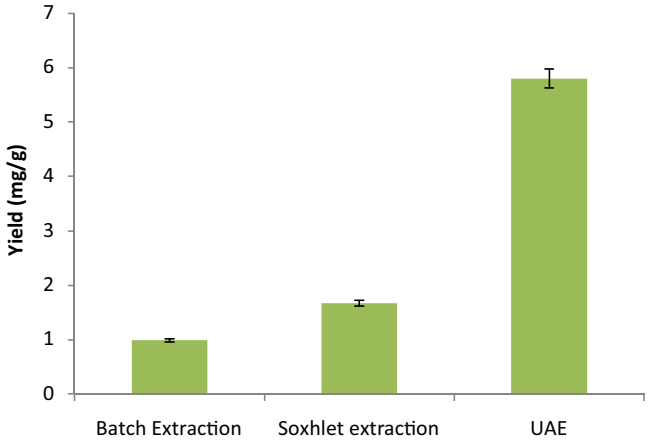


Fig. 10. Comparison of extraction methods with respect to yield of piperine.

violent shock wave and high speed jet are generated which enhances the penetration of the solvent into the cell wall and releases the intracellular compounds into the medium by disrupting the cell wall (Rhianna and Larysa, 2013). It is observed that there is a huge destructive morphological change in UAE extracted sample as compared to traditional methods and results are following the same trend observed by Tao et al. (2012). In UAE, both (cavitation and thermal) effects contribute to the extraction process dominantly unlike traditional methods where as only thermal effect is crucial in conventional extraction process which is not sufficient to increase the extraction yield of piperine, alone. Hence ultrasound assisted extraction has significantly increased the extraction yield of piperine with reduced time of operation.

4. Conclusion

In the present study, we have successfully intensified extraction process of piperine from *Piper longum* by ultrasound assisted extraction. The process parameters which affect UAE yield like extracting solvent, time, solid to solvent ratio, ultrasound power, duty cycle, ultrasound frequency and temperature have been thoroughly investigated. It is observed that ultrasound assisted extraction needs less extraction time with higher yield of piperine. As it can be seen temperature increment up to 50 °C showed exponential increase in extraction yield. Further increment in temperature could not result in increased extraction yield due to reduction of cavitation phenomenon. Under optimum conditions the highest extraction yield of piperine obtained was 5.8 mg/g in 18 min only. The result of present study indicates that the UAE significantly decreases extraction time and increases the extraction yield of **piperine from *Piper longum*** as compared to the traditional extraction methods and proved as an attractive alternative technique for extraction of natural phytoconstituents.

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