

Chapter 15

Extraction of Phenolic Compounds from the Waste of *Borassus flabellifer*: A Step Toward Waste Valorization



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Introduction

Consumption of fruits and vegetables by humans has been observed from ancient times. Scientific studies have shown the positive effect of increased consumption of such fruits and vegetables toward preventing disease-causing conditions because of the presence of phytochemicals [1–3]. Polyphenols, vitamins, minerals, proteins, flavonoids and phenolic acids are the examples of phytochemicals that provide numerous immunity benefits due to antioxidant, antifungal, antibacterial activities and so on. Chemicals like polyphenols act as antioxidant and help in scavenging active oxygen species [4]. Reactive oxygen species (ROS) and various oxygen-centered free radicals keep on developing in human body which resulted in the death of cell and damage to the tissue. Cancer, cardiovascular problems and so on are implicated due to such oxygen species and radicals [5]. Oxidation is a process which is naturally occurring in the human body; hence, there is a need to balance the functioning of body by introducing antioxidants to maintain good health. Butylated hydroxyl anisole (BHA) and butylated hydroxy toluene (BHT) are the majorly used synthetic antioxidants which are effective but with some side effects [6]. Hence, there is a growing interest in searching antioxidants from natural resources. Further, promotion of the national health care programs by Government of India encourages the use of traditional herbal medicine since such drugs are easily available, show less or no side effects and have earned trust [7]. It has, therefore, become important to classify and estimate the required components in fruits and vegetables so as to obtain the benefits for health. One such natural source of phenolic contents is *Borassus flabellifer* Linn, commonly known as palmyra palm or doub palm. It is cultivated around the tropical

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regions of India. Some countries like Thailand, Malaysia, Indonesia, Myanmar and Sri Lanka also cultivate palmyra palm on a large scale due to favorable climatic conditions [8].

B. flabellifer is known to be used for multiple purposes, viz., anti-laprotic, diuretic, analgesic, antipyretic, antiarthritic antiphlogistic and anti-inflammatory actions. The different parts of the tree have been used in curing various diseases, like fruit is useful in curing hyperdipsia, dyspepsia, skin diseases and fever [9–13]. The extract of the roots of this tree contained various phenolic compounds, that is, tannins, alkaloids, glycosides, terpenoids, flavonoids and saponins [14]. The phytochemical analysis of fruit of *B. flabellifer* has shown the presence of carbohydrates, amino acids, saponins, tannins, flavonoids and phenolic compounds in the extract [15]. All the parts of the tree like roots, leaves, fruits and seeds possess antioxidant activity due to the presence of phenolic compounds [14–18]. Various articles have reported on the useful activities and phytochemical analysis of various parts of *B. flabellifer* [10–18]. The fruit contains majorly two parts: a hard cover known as shell and gelatinous eatable endosperm, often called a tender fruit. However, there is no work reported on the shell of the fruit and it is the major waste generated after obtaining the fruits.

The objective of the present work was to extract the phenolic compounds from the shell of the fruits of *B. flabellifer*. The effect of various parameters like type of solvent (methanol, ethanol, acetone and water), solid loading (5–20 g), volume of solvent (50–200 mL), concentration of solvent (0–100%), temperature (25–65 °C) and extraction time (2–10 h) on extracting the phenolic compounds has been studied using solvent extraction technique. Based on parametric study, a combination of optimum conditions has been obtained. The process was then further improved by incorporating sonication in the system since ultrasound-assisted extraction has the ability to achieve a faster rate of extraction with comparable yield.

Experiments

Materials

The shells of the fruit of *B. flabellifer* were obtained from the local cultivator in Surat, India. The shells were dried under sunlight for about 4–5 days and then chopped into small pieces. Those pieces were ground in the laboratory grinder and screened to have size of the powdered material around 500 μm . The size reduction would help to improve the mass transfer rate during extraction. Methanol (Rankem Laboratory), ethanol (Advent Chembio Pvt. Ltd.), acetone (Loba Chemie Laboratory Reagents), sodium carbonate (Finar Chem), Folin-Ciocalteu phenol reagent (Sisco Research Laboratories), gallic acid (Finar Chem), tannic acid (S. D. Fine Chem Ltd.) were used for extraction as well as for analysis purposes along with distilled water.

Method

Extraction of Phenolic Compounds. The powdered material having an average size of 500 μm was subjected to extraction using conventional extraction technique. In this process, a fixed amount of powdered material was subjected to extraction along with the solvent. The mixture was kept in a round-bottom flask and a condenser was connected to it to prevent the loss of volatile solvents. The phenomenon of transfer of solute from solid material to solvent is termed as leaching. The leaching operation focuses on extracting valuable compounds, known as solute, from the solid feed using a suitable solvent. In case of natural product extraction, the solvent from the bulk volumes moves toward liquid film surrounding the solid material after which it diffuses through the film toward the solid surface. Once the solvent reaches the surface, it permeates through the cell wall toward the inner part of the cell, wherein the solute resides. The solvent upon reaching the cell dissolves the solute and when it becomes saturated moves outward through cell wall and then back diffusion. Finally, it reaches back to the bulk solution. The phenomenon continues till the whole solution becomes saturated and the driving force becomes zero.

Based on the solubility of the phenolic compounds in the solvent and its saturation, temperature, and extraction time, recovery of phenolic compounds changes. Therefore, the effect of various parameters, like type of solvent, solid loading, volume of solvent, concentration of solvent, temperature and extraction time on extracting the phenolic compounds has been studied while power was kept constant. Agitation was kept in the range of 350–400 rpm to ensure uniformity in temperature and mass transfer. The procedure along with the processing variables is presented in Fig. 15.1. Upon completion of the extraction, the solution was filtered to remove solid material and the filtrate was then stored for determining the total phenolic compounds (TPC). In the present work, TPC was measured in terms of gallic acid and tannic acid. Each experiment was conducted twice to ensure repeatability.

Ultrasound-Assisted Extraction. The sonicator bath from Aqua Scientific Instruments (Surat, India) was used for performing extraction in the presence of sonication. Since sonication has the ability to improve the process performance primarily by reducing the extraction time, the aim of this study was to estimate the time required for extracting the phenolic compounds using sonication process. All the parameters except time were kept constant and the extraction was carried out for time period in the range of 2–10 min.

Determination of Total Phenolic Compounds. The filtered extract was analyzed for TPC in terms of gallic acid (as mg of gallic acid equivalent weight (mg GAE)/g of feed material). The sample after dilution if required was assessed using UV-visible spectrophotometer (DR600, HACH). The method for determining TPC using UV-visible spectrophotometer was developed based on Folin-Ciocalteu method [19]. The standard solution of gallic acid was used to prepare the calibration curve (Fig. 15.2).

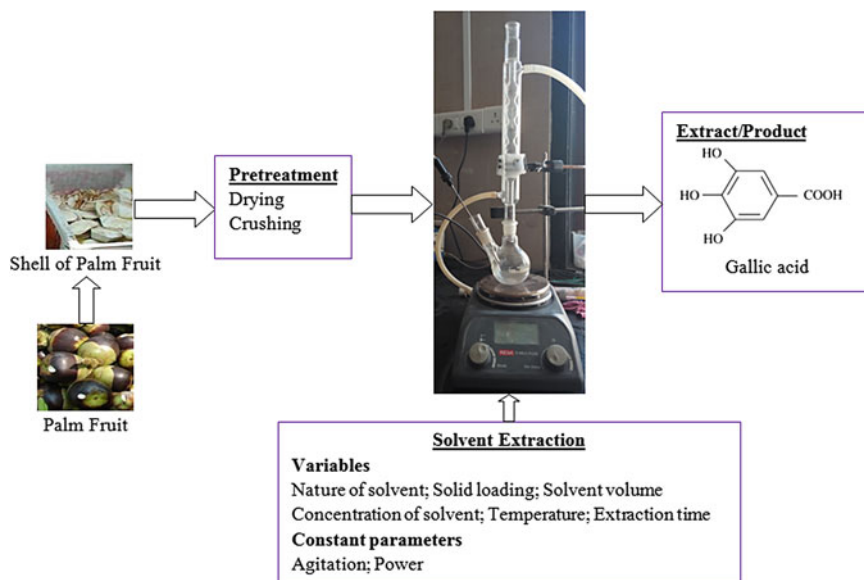


Fig. 15.1 Extraction of phenolic compounds using solvent extraction

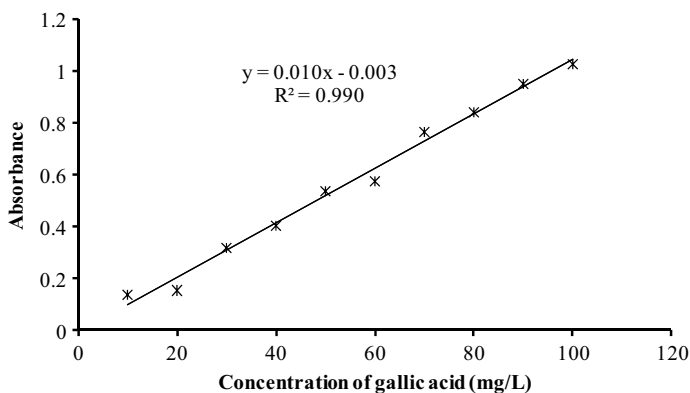


Fig. 15.2 Calibration curve for gallic acid using methanol as a solvent

In addition, TPC was determined in terms of tannic acid (mg of tannic acid equivalent weight (TAE)/g of feed material). The method used UV-visible spectrophotometer (DR600, HACH) for determining the concentration of TAE [20]. Using standard solution of tannic acid, the calibration curve was prepared having $R^2 = 0.992$.

Results and Discussion

Parametric Study

Effect of Nature of Solvent. It is always preferable to select a solvent which has a higher affinity toward the targeted compound, hence different solvents have been used to extract phenolic compounds from the shell and other parameters were kept constant at 5 g of solid loading, 50 mL pure solvent, 25 °C temperature and 6 h of extraction time (Fig. 15.3). As seen from the figure, methanol has provided the highest recovery of phenolic compounds (29.46 mg GAE/g) compared to other solvents while acetone has exhibited the lowest recovery. The solubility of phenolic compounds was more in methanol due to its polarity, and also the methanol was able to penetrate through the solid material easily compared to other solvents. Hence, a higher extraction of phenolic compounds was observed in case of methanol and was used for further studies. Similar results were obtained while extracting rosmarinic acid using methanol [21, 22].

Effect of Solid Loading. Solid loading assists in determining the capacity of the process and it is always desirable to operate the process at its maximum capacity. In order to observe the effect of solid loading, it was varied from 5 to 20 g while volume of the solvent, solvent concentration, temperature and extraction time were maintained at 100 mL, 100% methanol, 25 °C and 6 h, respectively. The influence was observed by determining the phenolic contents in terms of gallic acid and tannic acid (Fig. 15.4). A recovery of phenolic compounds has experienced a decreasing trend with a higher solid loading due to decrease in solvent to solid loading ratio. A higher amount of solid material in a fixed volume of solvent increases the solution viscosity which may impart resistance in mass transfer leading to reduced recovery of the solute [23]. TPC in terms of gallic acid equivalent was higher compared to TPC in terms of tannic acid equivalent. However, both have shown similar behavior upon

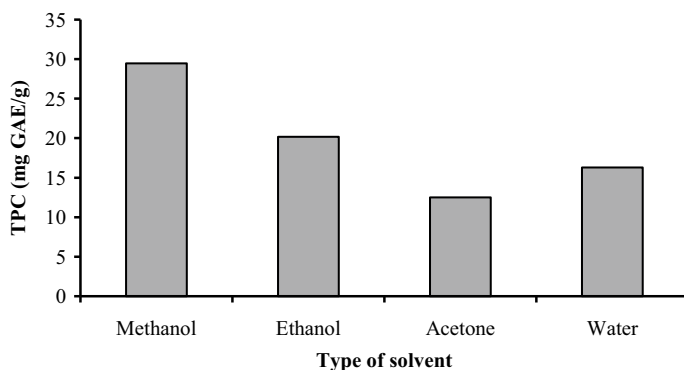


Fig. 15.3 Effect of nature of solvent on phenolic compounds

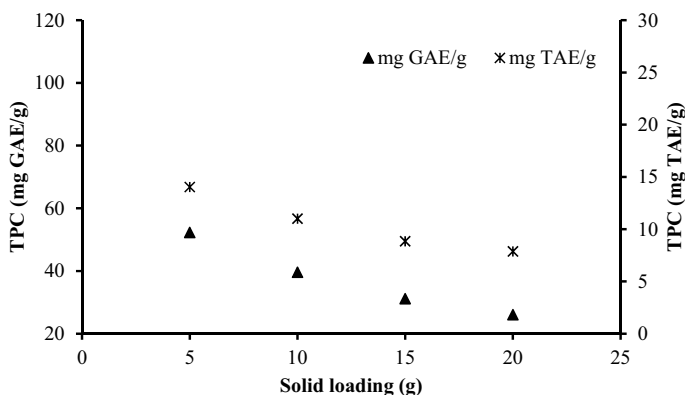


Fig. 15.4 Effect of solid loading on phenolic compounds

increasing the solid amount. The highest recovery was observed at 5 g (52.33 mg GAE/g and 14.04 mg TAE/g), and therefore, it was used for rest of the experiments. Similar observations have been made while isolating geraniol from the leaves of palmarosa [24].

Effect of Solvent Volume. In order to observe the influence of solvent volume on extraction of phenolic compounds, it was varied in the range of 50–200 mL while maintaining other parameters constant, that is, solid loading: 5 g, solvent concentration: 100% methanol, temperature: 25 °C and extraction time: 6 h. A higher solvent volume provides a higher solubilization capacity as well as improved driving force for mass transfer. It is based on the principle of mass transfer between solid and liquid medium, which provides increased concentration gradient [25]. As seen from Fig. 15.5, the highest extraction (67.29 mg GAE/g and 17.3 mg TAE/g) was observed for 200 mL.

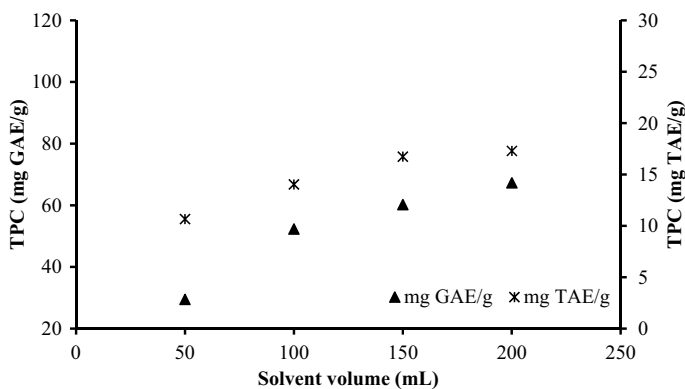


Fig. 15.5 Effect of solvent volume on phenolic compounds

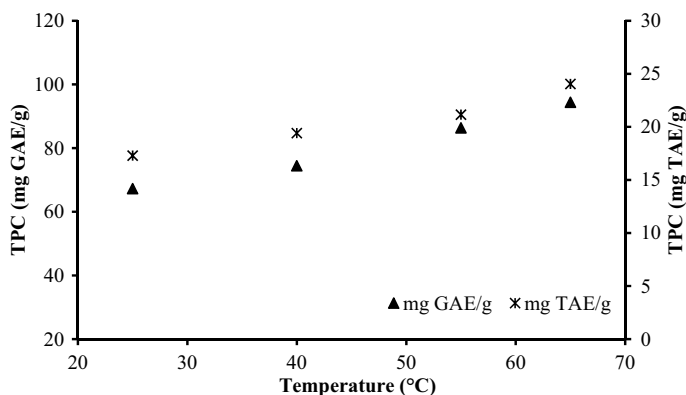


Fig. 15.6 Effect of temperature on phenolic compounds

Effect of Temperature. Diffusivity of solute toward solvent from solid and solubility of solute in solvent increase when temperature is increased [25, 26]. Hence, leaching is always preferred at an elevated temperature. Also, a higher temperature reduces the viscosity and surface tension of solvent which further enhances the rate of extraction [27]. However, one needs to ensure that there is no degradation of the solute in case of heat-sensitive compounds [25, 28]. In the present study, the temperature was varied from 25 to 65 °C and the other parameters were kept constant at 5 g of solid loading, 200 mL pure methanol and 6 h of extraction time (Fig. 15.6). At 65 °C, boiling of the solvent was observed; hence, the highest recovery might be expected. The phenolic compounds have exhibited a higher extraction upon increasing the temperature and the highest extraction was observed at 65 °C (94.42 mg GAE/g and 24.06 mg TAE/g).

Effect of Solvent Concentration. A change in the concentration of the solvent may lead to a change in the recovery of phenolic compounds due to the presence of two solvents (methanol and water) at the same time. The concentration of methanol was varied from 0 to 100% (v/v) while keeping other parameters constant, viz., solid loading: 5 g, solvent volume: 200 mL, temperature: 65 °C and extraction time: 6 h (Fig. 15.7). There was an increase in the TPC as the purity of solvent was changed from 0 (pure water) to 100 (pure methanol) % (v/v) which might be due to the higher affinity of the phenolic compounds toward methanol. However, at 75% (v/v) concentration the extraction efficiency was similar to that with the pure methanol, that is, 93.53 mg GAE/g and 23.67 mg TAE/g against 94.42 mg GAE/g and 24.06 mg TAE/g, respectively. Since the solubility of phenolic compounds was improved because of methanol, desorption of solute has become easier due to the presence of water [29], and such extraction efficiency could be expected. Hence, 75% (v/v) methanol was used for next experiments.

Effect of Extraction Time. A process should be provided with sufficient time so that the completion of the extraction can be achieved. A lesser time may lead to incomplete extraction, while a higher time may result in degradation of the solute

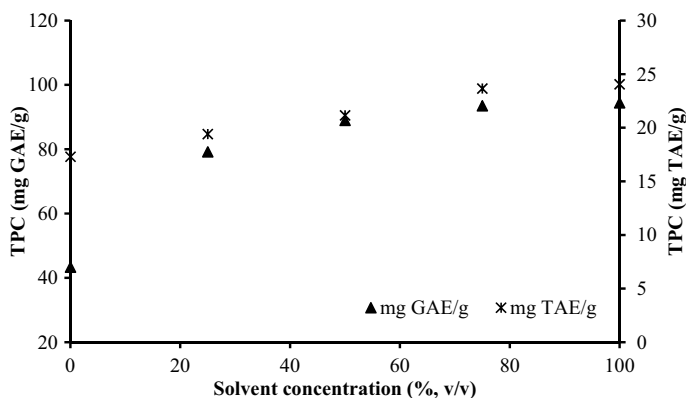


Fig. 15.7 Effect of solvent concentration on phenolic compounds

due to its exposure to the elevated temperature for a longer period. In the present work, the extraction time was varied in the range of 2–10 h, while keeping other parameters constant (5 g of solid loading, 200 mL methanol with 75% (v/v) concentration and 65 °C temperature). The highest recovery of phenolic compounds was achieved at 8 h that is, 110.3 mg GAE/g and 25.6 mg TAE/g, as shown in Fig. 15.8. As the time of extraction was further increased, a decrease in the extraction efficiency was observed. Since the compounds were exposed to elevated temperature for a longer time as well as they were prone to oxidation, a degradation of the compounds has been observed [30].

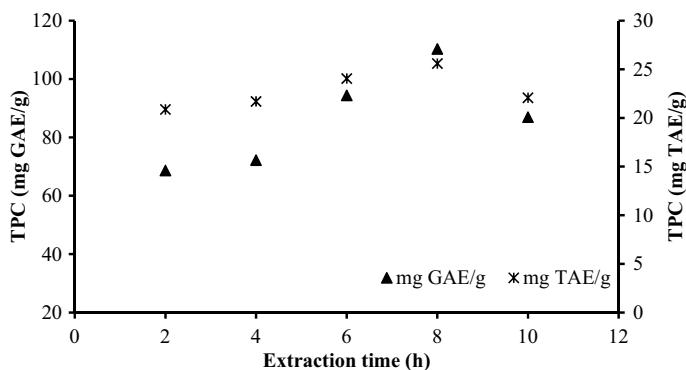


Fig. 15.8 Effect of extraction time on phenolic compounds

Ultrasound-Assisted Extraction

In conventional extraction (CE), solvent has to penetrate through the cell wall to solubilize the solute and then transfer back to the bulk solution. This step is regarded as the slowest step in the extraction process and leads to extended time of extraction [23, 24, 27]. Ultrasound-assisted extraction (UAE) has the ability improve the mass transfer by damaging the cells. In sonication, the propagation of sonic waves leads to the formation of bubbles, which in turn gets ruptured after attaining the maximum size. This rupture results in release of tremendous amount of energy at a molecular level. The energy has the sufficient intensity to damage the cell wall either by creating cracks, pores, or by breaking the cell walls. Also, a micro-jet phenomenon may assist in erosion of the cell wall or detexturation. These phenomena allow easy access of solute, better interaction between solute and solvent, and finally accelerate the dissolution of the solutes which are either residing in the cell or able to come out from the cell [27, 31]. Thus, the rate-limiting step observed in the convention extraction can be eliminated using sonication and the extraction time can be reduced which may further help in improving the production capacity [31].

In the present study, extraction of phenolic compounds was carried out in sonication bath at different time intervals (Fig. 15.9) with the combination of parameters which have provided a better extraction in conventional method except temperature, that is, solid loading: 5 g; solvent volume: 200 mL, concentration of solvent: 75% (v/v) methanol and temperature: 30 °C. As seen from the figure, the extraction time was greatly reduced from 8 h to 8 min when sonication was employed in the process and TPC was obtained as 92.08 mg GAE/g and 25.35 mg TAE/g.

Apart from the benefit of reduction in extraction time, other advantages are also obtained upon employing UAE like reduced power consumption, shrunken carbon footprint and almost negligible utility requirement in terms of cooling water. The comparison of both the techniques is shown in Fig. 15.10, where conventional method

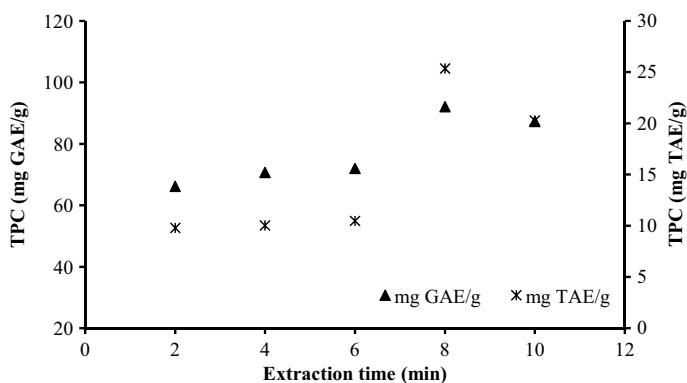


Fig. 15.9 Effect of extraction time on phenolic compounds in ultrasound-assisted extraction

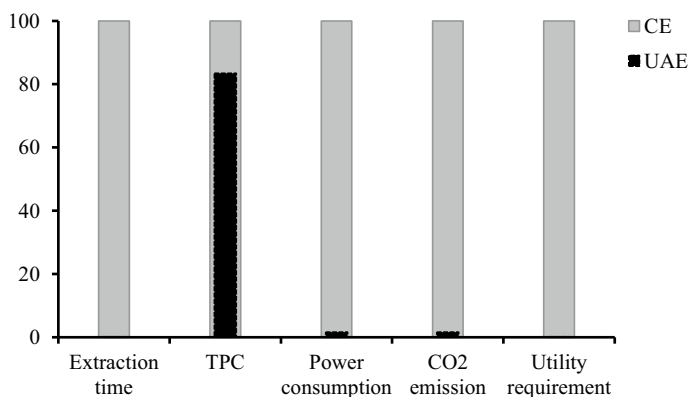


Fig. 15.10 Comparison of ultrasound-assisted extraction (UAE) with conventional extraction (CE)

has been considered as a reference (100%). Further improvement in the sonication process can be achieved by varying the sonication parameters, like frequency, amplitude and pulse ratio.

Conclusion

Borassus flabellifer is the natural source of phenolic compounds that can be used as an antioxidant for different medicinal purposes. The waste material, that is, shell left after using the fruit of this plant was evaluated for its possible utilization as the source of phenolic compounds. Among various solvents like methanol, ethanol, acetone and water, extraction of phenolic compounds was efficiently carried out using methanol. Various parameters like solid loading, volume of solvent, temperature, concentration of solvent and extraction time have been assessed to understand their impact on the recovery of phenolic compounds. The phenolic contents have been estimated in terms of gallic acid equivalent and tannic acid equivalent. From the one factor at a time study, a combination of optimum parameters was obtained as solid loading: 5 g, solvent volume: 200 mL, temperature: 65 °C, concentration of solvent: 75.5 (v/v) methanol and extraction time: 8 h. Under the set of optimized conditions, TPC was obtained as 110.3 mg GAE/g and 25.6 mg TAE/g. In order to overcome the limitation of the conventional technique, ultrasound-assisted extraction was employed which has reduced the extraction time from 8 h to 8 min, thereby providing sustainable alternative to the conventional technique. A detailed study on the process parameters of ultrasound-assisted extraction will further add value to the process.

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