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Ultrasound assisted extraction of polyphenols from *Polyalthia longifolia* leaves

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This study was designed to improve phenolic yield using ultrasound assisted extraction from leaves of *Polyalthia longifolia* used for medicinal applications. The extraction of polyphenols was performed using probe type and bath type ultrasound sources to obtain maximum efficiency by optimizing the condition of extraction process like, type of solvent, powdered leaf to solvent ratio, extraction time etc. The results have showed better yield for an extraction time of 60 min using ultrasound bath with 50% methanol solvent and 1:25 powdered leaf to solvent ratio. Ultrasound assisted extraction yielded total polyphenols of 2756.86 (probe type) and 2954.36 (bath type) mg GAE/100 g of dry leaves whereas, in conventional method it was 2021.31 mg GAE/100 g of dry leaves. Also, the DPPH, FRAP and ABTS radical scavenging activity was measured to be 92.63, 86.99 and 92.18% (probe type) and 95.76, 88.98 and 95.76% (bath type) from the optimized samples. Total flavonoid content obtained was 260.52 (bath type); and 189.66 (probe type) mg/100 g of dry leaves. Therefore, it could be concluded that ultrasound assisted extraction process using methanol solvent showed highest polyphenolic yield and maximum antioxidant capacity when compared to conventional extraction process.

Keywords: *Polyalthia longifolia* leaves, ultrasound probe, ultrasound bath, extraction, polyphenols, flavonoids.

Introduction

Polyhydroxyphenols, commonly known as polyphenols are the naturally occurring compounds but these are also available in the market in the form of synthetic as well as semi-synthetic organic chemicals. The polyphenols are classified based on the number of carbon atoms and benzene rings which accommodate the peculiar physico-chemical and biological properties of specific members of the class. Examples include Gallic acid, Quercetin, Rutin, Catechin, Hesperetin, etc. Polyphenols can be categorized into four groups depending on the number of phenolic rings available namely Flavonoids, Stilbenes, Lignans and Phenolic acids. In general, the outer layers of plants like bark, stem, leaves, etc. contain a higher percentage of phenolic compounds compared to inners parts like fruits, roots, etc.

Flavonoids are a significant category of natural products having a polyphenolic structure, intensively found in fruits, vegetables, and certain beverages. Flavonoids have a huge effect on human health and are absolutely necessary components in a variety of nutraceutical, pharmaceutical, therapeutic and restorative applications. This is the result of their anti-inflammatory, anti-mutagenic, anti-carcinogenic and antioxidative properties combined with their ability to adjust key cell compound capacities[1]. Flavonoids are utilized by vegetables for their development and safeguard against plaques. They shield plants from different biotic and abiotic stresses and act as excellent UV filters[2].

Polyalthia longifolia is a plant that has many medicinal properties which is much safer and proved to be the best medicine in the cure of many ailments. Moreover, the abundant availability of this tree makes it very special in the field of medicine. It belongs to the family Annonaceae which is an evergreen Ashoka tree found densely in central and eastern Himalayas, Kerala and the Western Ghats of Maharashtra. Ashokarishta is an ayurvedic preparation from *Polyalthia longifolia* to treat different ailments in ladies. It helps in reducing excessive bleeding, leucorrhea, and headache. Different polyphenols that have anti-oxidant properties available in this leaves are Rutin, Quercetin, Gallic acid, Catechin, etc. Out of all those available, gallic acid is one of the major

compounds which possesses antimutagenic and antigenotoxic properties[3].

There are numerous methods available for identification, extraction, and isolation of polyphenolic compounds which have been developed in the last few decades. The most widely used method is solvent extraction which is easy and cheap to use but they are highly time taking as well as solvents used are harmful to human health. The other method used these days is microwave assisted extraction which is comparatively efficient but the major disadvantage of this method is that it uses high pressure and high temperature which poses safety risks also at high-temperature materials may degrade. To overcome the disadvantages of the above-mentioned methods ultrasound assisted extraction could be a better alternate.

High quantities of bioactive compounds like polyphenols, flavonoids, lipids, etc. can be extracted easily within a shorter extraction time using ultrasound assisted extraction (UAE). Ultrasound assisted extraction is a suitable strategy for quick extraction of various compounds from food and biological materials by its extreme conditions developed at the time of bubble collapse such as high temperature and pressure conditions, micro-jets and micro-streaming[4]. Considering the above beneficial properties of ultrasound, this study is mainly focussed on exploring the extraction of polyphenols from *Polyalthia longifolia* using ultrasound for the first time to obtain better yield, and were compared with conventional and Soxhlet extraction method.

Experimental methodology

Pretreatment of Polyalthia longifolia leaves:

Polyalthia longifolia leaves were collected from National Institute of Technology, Tiruchirappalli campus. The leaves were washed with tap water followed by double distilled water. Then, they were dried in a hot air oven at 60°C for 4 days followed by grinding into fine powder using a mixer-grinder. The obtained dried leaves powder was then stored in an air-tight containers for further study.

Extraction of polyphenols using different methods

Conventional method:

5 g of dried leaf powder was taken in a beaker containing 100 mL of solvent. The mixture was stirred continuously for 4 days, and the sample was collected, centrifuged and filtered for analysis.

Ultrasound assisted extraction method:

Fine powder of the leaves of different weights was taken in a beaker and 100 mL of solvent was added. The entire mixture was sonicated with using either an ultrasound bath (ELMA 4910DH, Transsonic digitals) or horn (Vibra Cell-VCX 500, Sonics and material). Samples were collected at an interval of 15 min for polyphenols concentration measurement.

Soxhlet extraction method:

6 g of dried leaf powder was kept into a tea bag and used for extraction using methanol as solvent. Extraction was performed for 24 h, and the supernatant liquid was taken for analysis.

Determination of total polyphenolic and flavonoid content:

Folin-Ciocalteau method[5] was used to calculate total polyphenol content using Gallic Acid as standard. Aluminium chloride colorimetric method[6] was used to determine total Flavonoid content by using Quercetin as standard. UV/Vis spectrophotometer (Shimadzu-2600) was used to measure absorbance 760 nm for polyphenol and 442 nm for flavonoid.

Determination of DPPH radical scavenging activity:

This was performed using 0.5 mL of 0.3 mM DPPH methanol solution in which 0.1 mL of leaf extracts were mixed. The mixture was kept in dark at 37°C for 30 min. The absorbance was measured spectrophotometrically at 517 nm.

Determination of ferric reducing anti-oxidant power (FRAP) assay:

The FRAP reagent was prepared using acetate buffer (3.6 pH) with 10 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ) solution added in 40 mM HCl and 20 mM ferric chloride solution in proportion of 10:1:1 (v/v) respectively. The extracted sample of 0.1 mL was added to 3 mL the FRAP reagent and mixed well for absorbance measurement at 593 nm using spectrophotometer.

Determination of ABTS radical scavenging capacity:

The ABTS assay study was carried out using 7 mM ABTS and 2.4 mM ammonium persulfate solution by mixing them in equal quantities and allowing them to react for 12 h at 30°C in the dark. To this 1 mL of the extracted sample was

added and allowed to react, and its absorbance was measured at 734 nm using spectrophotometer.

Results and discussion

Effect of material to solvent ratio using ultrasound sources:

In the case of an ultrasound bath, as shown in Fig. 1, for 1:10 ratio the polyphenolic yield was 998.5 mg GAE/100 g of dry leaves and increased to 2238.25 mg GAE/100 g of dry leaves for 1:25 ratio and decreased to 1798.53 mg GAE/100 g of dry leaves for 1:30 ratio. With ultrasound probe also, the maximum polyphenolic yield of 2689.56 mg GAE/100 g of dry leaves with 1:25 ratio. Based on the mass transfer principles occurred in ultrasound assisted extraction, the driving force for mass transfer was considered to be the concentration gradient between the solid and solvent. The results revealed that the optimal ratio leading to the strongest driving force was with 1:25 ratio for phenolic extraction in *Polyalthia longifolia* leaves.

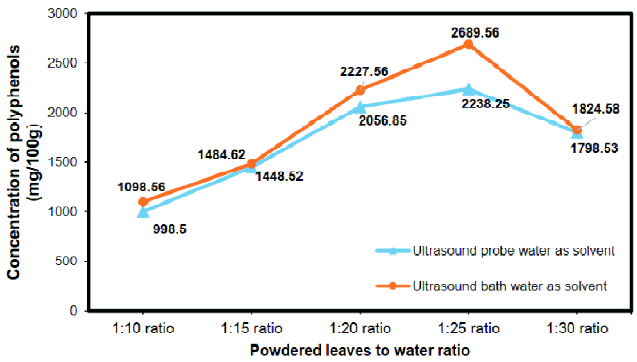

Fig. 1. Effect of material to solvent ratio on polyphenols using ultrasound sources (Time: 60 min.; Solvent: Water).

Effects of extraction time using ultrasound sources:

The polyphenol yield results using different ultrasound sources at varying extraction time were shown in Fig. 2. With ultrasonic bath operated at 235 W and 40 kHz using double distilled water as the solvent, there was a continuous increase in the polyphenolic yield of 1098.56 mg GAE/100 g of dry leaves upto 60 min it decreased to 988.91 mg GAE/100 g of dry leaves at 75 min. The reason for the decrease in yield beyond 60 min might be due to prolonged extraction time leading to lowered permeability of solvent into the cell walls because of higher leaf material. Moreover, prolonged extraction time may increase the chances of decomposition of phenolics and potentially increase the loss of solvent by vaporization.

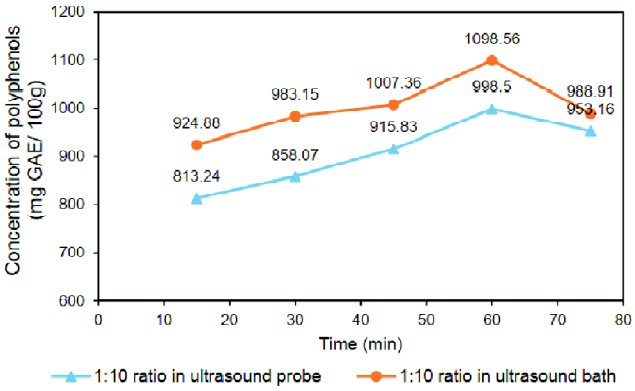

Fig. 2. Effect of extraction time on polyphenols using ultrasound sources (1:10 ratio; Solvent: Water).

Similar trend was observed for ultrasound probe operated at 100 W and 20 kHz. At 15 min the yield of polyphenols was 813.24 mg GAE/100 g of dry leaf which increased to a maximum of 998.5 mg GAE/100 g of dry leaves at 60 min and decreased to 953.16 mg GAE/100 g of dry leaves at 75 min. This shows that maximum yield was obtained at 60 min of extraction time using ultrasound sources with a better yield achieved with ultrasound bath. The reason of higher yield with ultrasound bath could be explained that it leads to higher area of exposure to ultrasound wave with lower ultrasound power reaching the extraction solution whereas in the case of ultrasound probe, the effective area of ultrasound wave was confined near to the probe with higher ultrasound power induced directly into the solution which might lead to the degradation of the produced polyphenol.

Extraction of polyphenols using conventional method:

In conventional method, the yield obtained for 60 min was 1127.869 mg GAE/100 g of dry leaves and later on yield increased to a maximum of 2062.29 mg GAE/100 g of dry leaves for an extraction time of 4 days as shown in Fig. 3. The total polyphenolic content thus obtained from ultrasound sources were compared with conventional method and were shown in Fig. 4.

Polyphenols extraction using inorganic solvents:

The polyphenols extracted using ethanol as solvent with ultrasound sources were shown in Fig. 5. Using ultrasound bath, there was a gradual increase in yield from 968.87 to

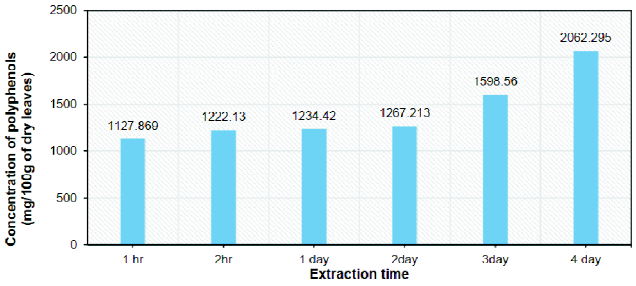

Fig. 3. Polyphenolic yield at different extraction time using conventional method (1:25 ratio; Solvent: Water).

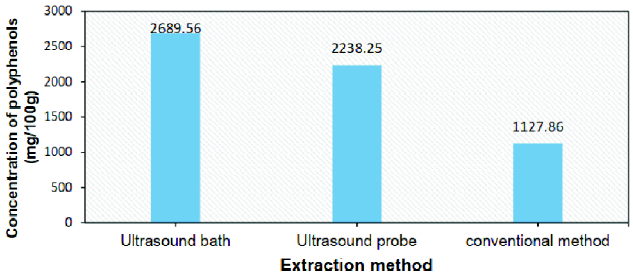

Fig. 4. Comparison of polyphenolic yield between different extraction methods (Time: 60 min; 1:25 ratio; Solvent: water).

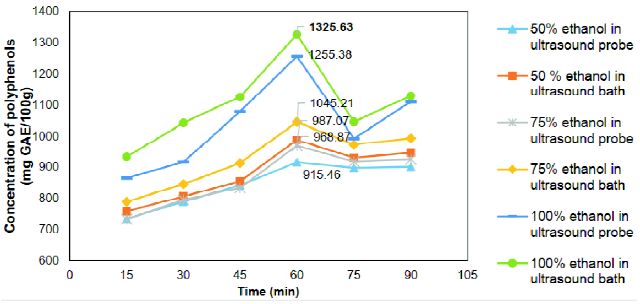

Fig. 5. Polyphenolic yield using ethanol as solvent (1:25 ratio).

1325.63 mg GAE/100 g of powdered leaves when the concentration was varied from 50% to 100%. Similarly, in the case of ultrasound probe, maximum of 1255.38 mg GAE/100 g of powdered leaves was obtained for 100% ethanol.

When methanol solvent was used drastic increase in the polyphenolic yield was obtained using ultrasound sources irrespective of their type. The results are shown in Fig. 6. In both the ultrasound sources, at 50% methanol maximum yield of 2945.36.21 (bath) and 2756.86 (probe) mg GAE/100 g of powdered was obtained.

While in conventional method (from Fig. 7), it was 2021.31

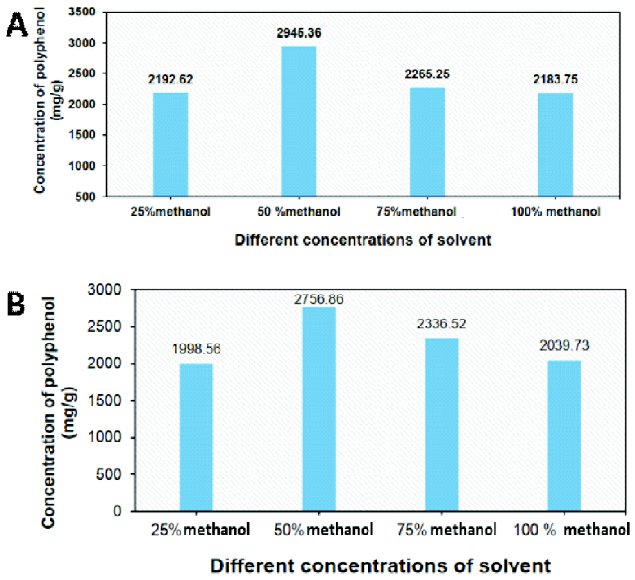

Fig. 6. Extraction of polyphenols using methanol solvent using ultrasound sources: (A) Ultrasound bath, (B) Ultrasound probe (Time: 60 min; 1:25 ratio).

and 2853.29 mg GAE/100 g of powdered leaves for Soxhlet extraction. Hence, from the above studies it was understood that phenolic content of methanol extract was higher when compared to yields with water and ethanol extract. Methanol solvent had exhibited closest polarity to the phenols in *Polyalthia longifolia*. Overall, it could be concluded that ultrasound-assisted extraction method was better than conventional and Soxhlet method.

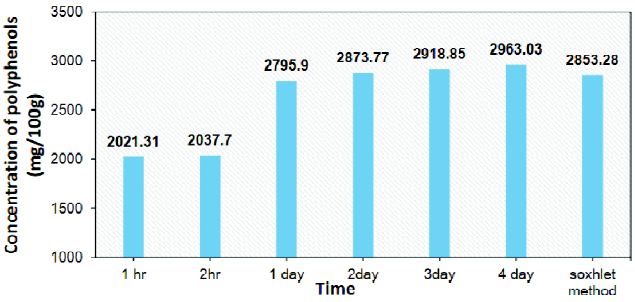

Fig. 7. Extraction of polyphenols conventional and Soxhlet method (Solvent: 50% methanol; 1:25 ratio).

Total flavonoid content:

The results obtained from the extracts conducted in ultrasound sources using water solvent were shown in Fig. 8. The results indicates that the flavonoid content yielded a

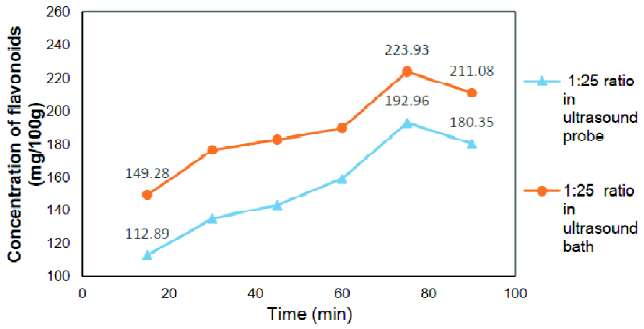

Fig. 8. Total flavonoid concentration for different time intervals using ultrasound sources (Solvent: water; 1:25 ratio).

maximum of 192.96 (probe) and 223.93 (bath) mg/100 g of dry leaves at 75 min.

With variation in the material to solvent ratio from Fig. 9, maximum flavonoid content of 223.93 (bath) and 192.96 (probe) mg/100 g of dry leaves was obtained for 1:25 ratio.

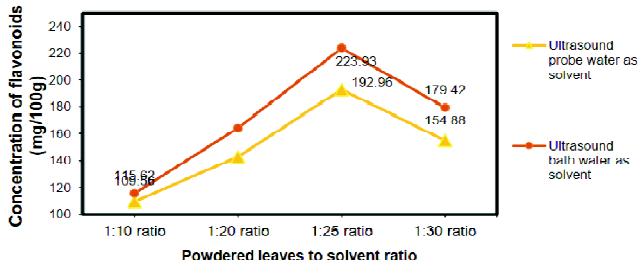

Fig. 9. Total flavonoid concentration for different solvent to material ratio using ultrasound sources (Solvent: water; Time: 75 min).

Using the same methodology, the extraction of flavonoid was performed using methanol and ethanol as solvent, and the results were compared with water solvent using ultrasound sources and conventional method under the condition of 1:25 ratio and 75 min time. Using ethanol solvent, the flavonoid yield obtained were 105.36 (probe) and 120.35 (bath) mg/100 g of dry leaves for 100% ethanol. When methanol solvent was used, 189.66 (probe) and 260.52 mg/100 g of powdered leaves was obtained with 50% solvent. Whereas, it was very less with conventional (26.39) method under similar extraction condition.

DPPH free radical scavenging activity and ferric reducing antioxidant activity:

From Fig. 10, the maximum percentage free radical scavenging obtained was 95.76% for ultrasound bath. From Fig. 11, the maximum percentage FRAP assay obtained was 88.98% using ultrasound bath.

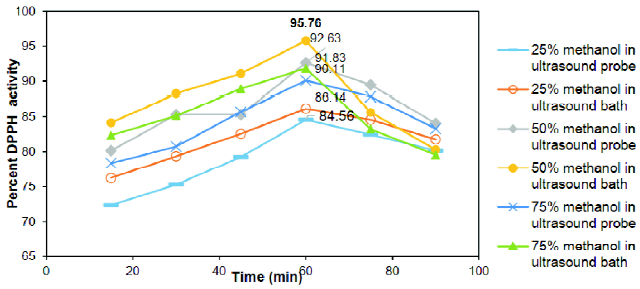

Fig. 10. Percentage DPPH radical scavenging activity (1:25 ratio).

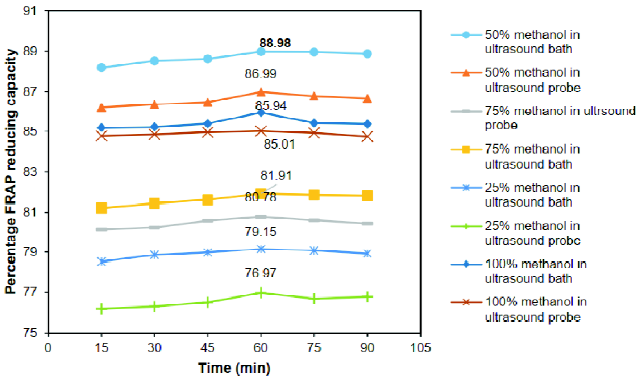

Fig. 11. Percentage antioxidant activity of FRAP reagent (1:25 ratio).

The maximum ABTS radical scavenging activity obtained was 97.31% using ultrasound bath (when compared to other methods) with 50% methanol solvent, 60 min time and 1:25 ratio.

Conclusions

Maximum polyphenolic yield of 2945.36 mg GAE/100 g of dry leaves was obtained for an extraction time of 60 min and powdered leaves to solvent (methanol) ratio of 1:25 using ultrasound bath. Maximum flavonoid content of 260.52 mg/100 g of dry leaves was obtained for an extraction time of 75 min and powdered leaves to solvent (methanol) ratio 1:25. Methanol proved to be the best solvent compared to other solvents used in the study. Both ultrasound sources provided better results but bath type showed higher due to higher area of exposure to ultrasound waves when compared to probe type. Also, sonication had proven to be the better

method compared to conventional as well as Soxhlet method for extracting polyphenols effectively providing higher yield.

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