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Research paper

Effect of Post-Harvest Preservation and Extraction Methods on Antioxidant Properties of Alternanthera Sessilis Red

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Abstract

Herbs with antioxidant properties are usually preserved and extracted before being converted into commercial products. The main focus of this study was to determine the effect of preservation of *A. sessilis* red as well as extraction method on its antioxidant properties. *A. sessilis* red was preserved using two different methods; freeze drying and superheated steam drying, followed by extraction with 70% ethanol using conventional extraction and ultrasonic-assisted extraction. Drying in the superheated steam oven displayed shorter drying period of 1 hour compared to freeze drying (several days). Combination of superheated steam drying and ultrasonic-assisted extraction showed the highest extraction yields (12.99%). Results showed that superheated steam drying and ultrasonic-assisted extraction displayed an increase in the total phenolic content. In terms of antioxidant capacity, *A. sessilis* extracts obtained from superheated steam drying has higher radical scavenging activity (72.39% - 76.70%) than those freeze-dried (60.68% - 65.33%). Meanwhile, ultrasonic-assisted extraction had negatively impacted the radical scavenging activity of the extracts due to the formation of free radicals that are related to acoustic cavitation. As for ferric reducing antioxidant power, both superheated steam drying and ultrasonic assisted extraction yielded extracts with greater capacity. Present result shows that the combination of superheated steam drying and ultrasonic-assisted extraction enhanced total phenolic content by 60% and improved antioxidant activity based on ferric reducing antioxidant power assay

Keywords: *ASR – Alternanthera sessilis red; CE – Conventional extraction; Freeze drying; Superheated steam drying; UAE – Ultrasonic-assisted extraction*

1. Introduction

Alternanthera sessilis red (ASR) or keremak merah is a weed that possesses vast medicinal properties, yet under exploited (Shreshtha *et al.*, 2017). The weed was reported in many studies to provide a wide range of benefits, from treating illness and diseases, healing wounds (Singh *et al.*, 2009) to neutralizing venoms (Nadkarani, 2000). In Sri Lanka, Mukunuwenna (*Alternanthera sessilis*) is very popular, widely produced and consumed by the natives (Kananke *et al.*, 2016). The author added that, since it is cheap and convenient as well as contain nutrients like fibre, vitamins, minerals and antioxidants, its consumption is increasing. On the contrary, keremak merah is not widely known and consumed in Malaysia, except to several Chinese communities; the old generation. These locals consume ASR or Hung Teen Wu to reduce cholesterol as well as detoxification and improving blood circulation. The Chinese will boil keremak merah with the addition of honey, let it simmered for 2 hours, and the water from it will be consumed (Cheah, 2010).

In the varieties of food preservation, drying is one of commonly used technique. Drying reduces deterioration in food which is caused by water content, by removing it thus improving the food quality (Karam *et al.*, 2016). Dewanto *et al.* (2002) added that higher biological activities were seen in thermally processed foods, especially fruits and vegetables, as the heat being applied resulted in various chemical changes in the food. However, conventional hot air drying caused high energy-consumption, non-uniform drying, unacceptable product quality with degradation of nutrient (Sehrawat *et al.*, 2016). Although freeze drying is more common in preserving product with high volatile nutrients, setting up the equipment will incur high initial investment cost and the process also involves high energy consumption. Sehrawat *et al.* (2016) stated that superheated steam drying (SSD) is an innovative drying technology, which eliminates excess water from the sample by utilization of heated steam (the drying medium) beyond its boiling point. Employing SSD to dehydrate food, results in shorter drying period (Jangam, 2011) as well as reduced cost (Moreira, 2001). The heat also may affect the cell structure of food, cause it to damage, leading to an increased antioxidant component.

The suitable extraction method is crucial in order to obtain a higher extraction yield, thus higher content of desirable phytochemicals in the extract (Chekroun *et al.*, 2015). González-Centeno *et al.* (2015) suggested that conventional extraction (CE) is an effective extraction technique, but it is considered not economical because of high solvent and energy consumption as well as long extraction time. The emergence of new extraction methods that are environmental-friendly made it possible to overcome the downside of CE by reducing extraction time and amount of solvent, giving a better extract. Ultrasonic-assisted extraction (UAE) is one of the green extraction methods that can give efficient extraction (Mane *et al.*, 2015; Yu *et al.*, 2017) mainly due to the transmission of sound waves which breakdown the cell, encourag-

ing mass transfer. UAE had been applied in the extraction of various bioactive compounds and many reported a positive result.
This study centred on the effect of two different drying methods; freeze drying (FD) and superheated steam drying (SSD) on antioxidant properties of ASR. The SSD was seen to be able to increase phenolic compounds and antioxidant activities of the plant (Husen *et al*., 2014; Rumruaytum *et al*., 2014) even it involves the application of heat. Once the ASR had been dried, extraction follows, using two different techniques; conventional extraction (CE) or ultrasonic-assisted extraction (UAE). UAE is said to result in higher extraction yield of better quality than CE. The effect of drying as well as extraction methods on ASR was investigated by evaluation on total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity based on radical scavenging activity (DPPH) and ferric reducing antioxidant power (FRAP).

2. Materials and methods

2.1. Samples

Fresh *Alternanthera sessilis* red (ASR) was purchased from the wet market in Kepong. Samples were washed and the roots were discarded, leaving the leaves, stems and flowers intact. The remaining part was then chopped into smaller portions, prior to the drying process.

2.2. Post-harvest preservation

Samples were subjected to two different drying methods; freeze-drying (FD) and superheated steam drying (SSD). For SSD, samples were spread thinly on aluminium rectangle trays and put into a superheated steam oven (DC Quto QF-5200C, Naomoto, Japan), exposed to superheated steam at a temperature of 170°C for one hour. Meanwhile, for FD, the samples were kept at −30°C before lyophilisation at −60°C in a freeze dryer (Alpha 1-4 LD plus, Martin Christ, Germany). These dryings were done according to method conducted by Husen *et al*. (2014). Then, the dried ASR were powdered using a grinder, sieved and stored in an air-tight container.

2.3. Extraction of phenolic compounds

Phenolic compounds from both SSD and FD extracts were extracted with 70% ethanol (v/v) at a ratio of 1:15. In the conventional extraction (CE), the samples were subjected to a direct solvent extraction of 70% ethanol (v/v). The mixture was prepared in a conical flask (wrapped with an aluminium foil) and shaken for 30 mins at 200 rpm at a temperature of 40°C (Innova 40, Eppendorf, Germany). In ultrasonic-assisted extraction (UAE), the samples were mixed with 70% ethanol in a beaker (wrapped with an aluminium foil). Then, the ultrasonic probe (CP 505, Cole-Parmer, USA) was inserted into the beaker, without touching the bottom surface. Sonication occurred at 20 kHz at 40°C for 30 mins. After 30 minutes, the mixture from each extraction method was individually filtered through vacuum filtration. After that, the filtrate was evaporated under vacuum with a water bath temperature of 40°C using a Rotavapor (RE21, Büchi, Switzerland). Sample from each extraction was collected and placed in wrapped glass jars and stored at -30°C. The liquid extracts were lyophilised at -60°C in a freeze dryer (Alpha 1-4 LD plus, Martin Christ, Germany). The dried extracts were kept at -20°C until further analyses. The sample that had undergone FD, extracted in CE was labelled as FDC, where the one extracted by UAE labelled as FDU. On the other hand, a sample that had been dried through SSD and extracted by CE and UAE were labelled as SSDC and SSDU respectively.

2.4. Total phenolic content (TPC)

TPC was determined using the method by Othman *et al.* (2016). According to the method, 200 µL of each ethanolic extract was pipetted into an amber test tube. Then, 1.5 mL Folin–Ciocalteu reagent, which had been diluted by 10-fold dilution with distilled water, was added into each test tube and subjected to vortex (REAX Control, Heidolph, Germany) for 10 s. The mixture was allowed to stand at room temperature for 5 mins. After that, 1.5 mL of 0.56 M sodium carbonate (Na_2CO_3) solution was added to the mixture and left to stand for 90 mins at room temperature. After 90 mins, the absorbance of the mixture was read using a UV-Vis Thermo Spectrophotometer (Thermo Scientific, Thermo Fisher Scientific, USA) at 725 nm. Ferulic acid had been used as a standard for TPC determination. The results were expressed as mg ferulic acid equivalents (FAE) per 100 g dried sample.

2.5. Total flavonoid content (TFC)

TFC was determined using a method by Djeridane *et al*. (2006), based on the formation of flavonoid-aluminium. One millilitre of sample extract was mixed with 1 mL of 2% aluminium chloride-6-hydrate ($AlCl_3.6H_2O$) solution. The mixture was incubated at room temperature for 15 mins. After the incubation period, the absorbance of the mixture was measured using UV- Vis Thermo Spectrophotometer (Thermo Scientific, Thermo Fisher Scientific, USA) at 430 nm. Rutin was used as a standard to plot the calibration curve. TFC of the sample was expressed as milligrams (mg) of rutin equivalents (RE) per 100 g dried sample.

2.6. Antioxidant capacity

2.6.1. DPPH radical scavenging activity

Free radical scavenging activity against 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical was measured using the method described by Oboh (2005). In the method, 1 mL of ethanolic extract was added to 2 mL of 0.15 mM of DPPH and mixed thoroughly with a vortex mixer (REAX Control, Heidolph, Germany). The mixture was left in a dark place for 30 mins. After 30 mins incubation time, the absorbance of the mixture was measured at 517 nm using UV-Vis Thermo Spectrophotometer (Thermo Scientific, Thermo Fisher Scientific, USA) against 70% ethanol as blank. The mixture of 70% ethanol and DPPH solution acted as a control, where ascorbic acid was used as a comparative standard. Antioxidant activity was expressed as the percentage of scavenging activity. The percentage of scavenging activity was calculated as in (1).

$$\% \text{ of scavenging activity} = \frac{(A \text{ control} - A \text{ sample})}{A \text{ control}} \times 100 \quad (1)$$

2.6.2. Ferric reducing antioxidant power (FRAP)

The determination of FRAP of the sample was based on the method by Benzie & Strain (1996). FRAP is based on the reduction of Fe^{3+}-TPTZ (2,4,6-tripyridyl-s-triazine) complex into a ferrous compound that is blue coloured at low pH. In the method, FRAP reagent was freshly prepared by mixing 300 mM of acetate buffer (pH 3.6) with 10 mM TPTZ solution and 20 mM of ferric chloride hexahydrate ($FeCl_3.6H_2O$) solution in the ratio of 10:1:1. The reagent was then incubated at 37°C for 10 mins. After that, 100 µL ethanolic extract was mixed with 8.7 mL of the freshly prepared FRAP reagent and incubated in a dark place at 50°C for an hour. The absorbance of the mixture was read after an hour at 593 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) had been used as standards for preparing a

calibration curve. The result was expressed as mg Trolox equivalent (TE) per 100 g dried sample.

2.7. Statistical analysis

All data were expressed as a mean ± standard deviation and were done in triplicates. A one-way analysis of variance (ANOVA) with significant differences between means defined as $p < 0.05$ and measured with post-hoc multiple comparisons and Duncan test were performed with SPSS version 23 (SPSS Inc., Chicago, Illinois, USA). Pearson correlation was used to assess the relationships between TPC and TFC and antioxidant capacity (DPPH and FRAP). The significance level was set at $p < 0.05$.

3. Results and Discussions

3.1. Extraction yield

In this study, fresh ASR had been dried using two methods which are freeze drying (FD) and superheated steam drying (SSD) to a moisture content of 4.51% and 4.90% respectively. Each dried sample then had undergone two different extraction processes; conventional extraction (CE) and ultrasonic-assisted extraction (UAE). The drying of ASR through SSD took one hour, where FD took a longer time in drying ASR to desirable moisture content.
The extraction yield of ASR obtained for FDC, FDU, SSDC and SSDU is 6.86%, 9.62%, 10.76% and 12.99% respectively. Based on the extraction yield, it can be seen that SSD resulted in higher yield compared to FD. A study by Stamatopoulos *et al.* (2012) showed that steam processing increased the extractability of oleuropein in olive leaves by application of steam blanching for 10 minutes. Structural changes in plant tissue because of steam or thermal treatment might contribute to the increase in the extraction yield. In terms of extraction methods, UAE provides a higher extraction yield compared to CE. This data coincides the purpose of employing UAE as suggested by several researchers, where UAE was able to enhance the efficiency of extraction (Da Porto *et al.*, 2013; Deng *et al.*, 2017; Ma *et al.*, 2009). The increase in the extraction yield could be due to mechanical vibration of the ultrasound probe, leading to better contact between solid and liquid phase, hence improving solvent penetration into the sample (Pan *et al.*, 2012; Samaram *et al.*, 2015). Chekroun *et al.* (2015) stated that higher extraction yield would give the higher content of desirable phytochemicals, such as total flavonoid and polyphenols in the extract.

3.2. Total phenolic content (TPC) and total flavonoid content (TFC)

Fig. 1 depicts TPC in ASR subjected to different drying and extraction methods. SSDU reported the highest TPC (723.87 mg FAE/100 g dried sample) above all. The result indicates that SSD resulted in an increase in the TPC of ASR within same extraction method. Similar findings had been seen in Husen *et al.* (2014), where SSD had been seen to increase the TPC of avocado pulp by 0.4 to 0.8 folds, with 170°C SSD resulted in the highest TPC. Huang *et al.* (2006) and Husen *et al.* (2014) suggested that SSD may damage the cell structures of fruits, releasing more antioxidant components from the pulp. Other than that, inactivation of oxidative and/or hydrolytic enzymes due to steaming could also lead to higher extraction of phenolic compounds (Dadan *et al.*, 2018; Husen *et al.*, 2014)

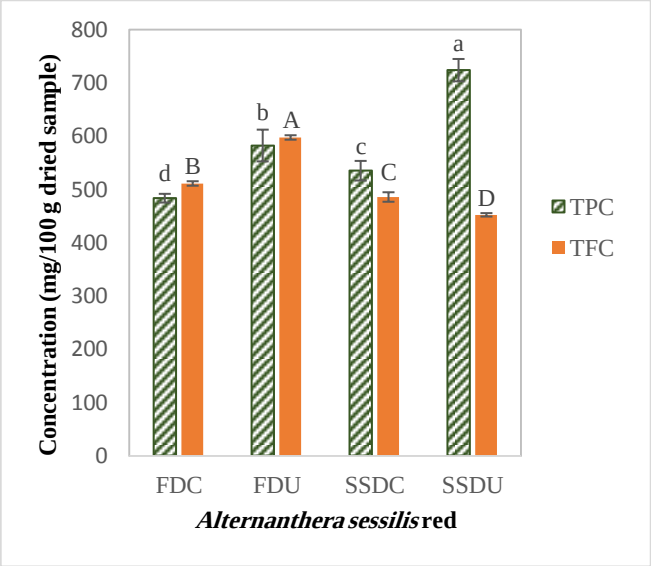

Fig. 1: Total phenolic content (TPC) and total flavonoid content (TFC) of *Alternanthera sessilis* red. Results were expressed as ferulic acid equivalents (FAE) for TPC and rutin equivalents (RE) for TFC. Values were expressed as the mean ± standard deviation (n = 6). Means with different letters were significantly different at the level of $p < 0.05$.
FDC: *ASR* subjected to freeze drying and conventional extraction method, FDU: *ASR* subjected to freeze drying and ultrasonic-assisted extraction, SSDC*: ASR* subjected to superheated-steam drying and conventional extraction method, SSDU: *ASR* subjected to superheated-steam drying and ultrasonic-assisted extraction.
a,b,c,d showed significant difference between extracts in terms of TPC ($p<0.05$).
A,B,C,D showed significant difference between extracts in terms of TFC ($p<0.05$).

However, FDU appeared to have higher TPC compared to SSDC. This condition could be influenced by the extraction method, since both ASR extracted by UAE showed higher TPC, even though undergone different drying technique. A study by Deng *et al.* (2017) displayed alike result where UAE of fresh olive at optimum condition of 47˚C for 30 mins gives a higher yield of TPC compared to those subjected to maceration extraction. The yields of phenolic compounds in citrus peel extract also found to be significantly higher, after UAE at 15˚C for one hour than those subjected to maceration extraction at 40˚C for 8 hours (Ma *et al.*, 2009). Higher content of TPC in the extracts from UAE could be due to acoustic cavitation that leads to the destruction of cell walls, releasing more bioactive compounds into the extraction medium.
In terms of total flavonoid content (TFC), the TFC of extracts being previously freeze-dried, for both extractions are higher compared to those dried in superheated steam oven. The decrease in the TFC of superheated steam-dried ASR could be due to the leaching of flavonoid in the extracts, into the steam or residual water which were discarded at the end of the process (Harris *et al.*, 2015; Lombard *et al.*, 2005). Meanwhile, Prommuak *et al.* (2008) relate the decrease in flavonoid to the high temperature condition. There are several studies reported the similar result as this study. Roy *et al.* (2009) reported that steam processing of broccoli for 5 and 10 minutes had decreased total flavonoid content in its lipophilic extracts. The result also similar to findings by Harris *et al.* (2015) where steam processing of onion for 5, 10 and 15 minutes had decreased the amount of quercetin, which is also a flavonoid. Other than that, steaming cauliflower lowers kaempferol content, although quercetin increases (dos Reis *et al.*, 2015; Mazzeo *et al.*, 2011).
For comparison between extraction techniques, it can be seen that UAE resulted in the highest TFC on the freeze-dried sample, but reported the lowest TFC on superheated steam-dried sample. Possible clarification for the different effect of UAE on TFC of dried extract could be the involvement of heat during the drying process. In SSD, ASR was exposed to heat, where no heat was introduced

to sample in FD. The drying methods may not yet cause degradation of some compounds that contribute to TFC. However, as the dried ASR underwent extraction with UAE, it was once again exposed to heat, produced from the mechanisms of UAE. UAE or sonication involves a process called acoustic cavitation. Acoustic cavitation refers to the formation, growth and collapse of the microbubbles within the ultrasonic field. Application of high-intensity ultrasound to liquid medium causes the formation of bubbles or clouds which then develops to a critical size and violently collapsed, producing a condition of extreme temperature and pressure (Ashokkumar *et al.*, 2008; Leighton, 1994). Further exposure to heat in UAE might have destroyed certain bioactive compounds in superheated steam-dried ASR.

3.3. Antioxidant capacity

Table 1 shows that SSD gives a positive effect to the antioxidant capacity of ASR in terms of free radical scavenging activity and ferric reducing power. The percentage of DPPH scavenging activity of ASR dried using superheated steam oven increased by more than 10% than the one dried in a freeze dryer. Increase in the antioxidant activity of superheated steam-dried ASR with respect to the increased percentage of DPPH scavenging activity could be due to increasing phenolic compounds (Dewanto *et al.*, 2002; Ju *et al.*, 2010). Rice-Evans and Miller (1996) and Zhang *et al.* (2014) supported that fruits having higher TPC usually showed stronger antioxidant capacity. Yet, the correlation between TPC and DPPH radical scavenging activity was found to be a weak positive correlation (R^2=0.185), whereas the correlation between TFC and DPPH scavenging activity displays moderate negative correlation (R^2=-0.793). The increase in the antioxidant activity could also be due to the increase of solubilized flavonoids from the cell structures of the fruit as an effect of thermal processing (Roy *et al.*, 2009). Other than that, the same authors added that increased total antioxidant activity could also possibly due to the liberation of non-phenolic substances during thermal processing. Degraded polysaccharides and pyrolysis products such as furfural and 5-HMF may contribute to antioxidant activity along with the phenolic compounds (Noda *et al.*, 2013).

Table 1: The antioxidant capacity of *Alternanthera sessilis* red.

Sample	Method	
	DPPH (%)	**FRAP (mg TE/100 g dried sample)**
FDC	65.33 ± 1.49^{c}	217.30 ± 3.59^{d}
FDU	60.68 ± 2.70^{d}	229.83 ± 9.84^{c}
SSDC	76.70 ± 1.28^{a}	288.92 ± 12.43^{b}
SSDU	72.39 ± 1.33^{b}	331.90 ± 10.73^{a}

Values were expressed as the mean ± standard deviation (n = 6). Means with different letters were significantly different at the level of $p < 0.05$.
FDC: ASR subjected to freeze drying and conventional extraction method, FDU: ASR subjected to freeze drying and ultrasonic-assisted extraction, SSDC: *ASR* subjected to superheated steam drying and conventional extraction method, SSDU: ASR subjected to superheated steam drying and ultrasonic-assisted extraction.
a,b,c,d showed significant difference between extracts within same column ($p<0.05$).

In terms of extraction methods, ASR being extracted using CE shows greater free radical scavenging activity than the other. UAE resulted in a 4.3% difference in DPPH free radical scavenging activity between superheated steam-dried ASR, while 4.7% between freeze-dried ASR. The lower scavenging activity presented by extracts from UAE could be due to its mechanism as mentioned earlier. Acoustic cavitation caused the formation of microbubbles that grow and collapse as it reached a critical size. According to Ashokkumar *et al.* (2008), this condition also leads to decomposition of solvent and solute molecules in the bubbles, releasing highly reactive radicals, such as H• and •OH for medium containing water. The author revealed that the formation of •OH radicals was found to be minimal at 20kHz, but increases as the frequency increases from 20 kHz to 358 kHz. However, in this study, the formation of •OH radicals at ultrasonication frequency of 20 kHz has already begun to adversely impact the DPPH free radical scavenging activity of both ASR extracted through UAE. As a result, some antioxidant compounds in ASR may have reacted with free radicals produced, resulting in fewer compounds available to react with free radicals of DPPH. A study by Ashokkumar *et al.* (2008) unveiled the reduction of antioxidant capacity (based on radical-scavenging activity) of the sample being treated with sonication by one-fifth from the original value, after 4 hours of sonication at 358 kHz.

Meanwhile, for ferric reducing antioxidant power (FRAP), dehydration by superheated steam on ASR showed a higher antioxidant activity than those dried in a freeze dryer. The antioxidant capacity based on FRAP assay of lightly milled rice treated in superheated steam at 120˚C was also higher compared to milled rice exposed to other treatments (Wu *et al.*, 2016). Besides that, there are also several types of research that showed a similar result, where common steaming procedures had been seen to enhance FRAP of samples. As studied by Mazzeo *et al.* (2011), steaming treatments in the oven at 100˚C for 20 minutes on spinach and carrot reported an increase in the FRAP compared to untreated and boiled samples. Steaming also affects the FRAP of *Brassica* vegetables, where 5 minutes of steaming reported an increasing trend in all samples than uncooked vegetables (Wachtel-Galor *et al.*, 2008). All the researches relate the increase in FRAP of the steam-treated sample to its phenolic compounds. Superheated steam was proposed to positively affect the extractability of insoluble phenolics from the food matrix explaining the increase in the amount detected (Wu *et al.*, 2016). A positive correlation was observed in this study, between TPC and FRAP (R^2=0.765), conforming to the statement of other researches that phenolic compounds do contribute to FRAP of extracts.

For the effect of extraction methods on the reducing power of ASR, UAE gives yield with greater ferric reducing antioxidant power than the one extracted using CE. A research by Zekovi´c *et al.* (2017) reflects the benefit of UAE compared to microwave-assisted extraction, where FRAP of sage extracts obtained through UAE is approximately doubled than extracts of the later. Huang *et al.* (2009) imply an increase in the flavonoid concentration to be the contributor to an increased antioxidant activity. A study by Zekovi´c *et al.* (2017) also displayed the good correlation between total phenolic and total flavonoid content with FRAP of sage herbal dust extracts. However, in this study, a negative correlation had been seen between TFC and FRAP (R^2=-0.740). Other compounds such as ascorbic acid (Min *et al.*, 2007) and reducing sugars are also reducing agent that might have reduced the iron (III) to iron (II), leading to an increased in antioxidant capacity.

4. Conclusion

This study showed that the preservation of ASR through superheated steam-drying resulted in shorter drying time compared to the commonly used method; FD. It is also found out that SSD was able to not just retain, but enhanced the phenolic compounds and antioxidant activity of ASR. On the other hand, ultrasonic-assisted extraction was seen to be an effective extraction method since it produced higher extraction yield compared to conventional method. This alternative extraction method had also increased the ferric reducing antioxidant power of the extracts possibly due to the increase in the total phenolic content. The combinations of superheated steam drying and ultrasonic-assisted extraction resulted in the highest extractability of phenolic compounds as well as remarkable ferric reducing antioxidant power. Superheated steam drying as well as ultrasonic-assisted extraction should be studied more in depth as it may lead to other significant findings.

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