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Impact of drying and extraction method on antioxidant capacity, phenolic compounds and betalain pigment of *Alternanthera sessilis* red

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This study attempts to determine the effect of drying and extraction methods on the antioxidant capacity, phenolic compounds, and betalain pigment of *Alternanthera sessilis* red. Evaluation of extraction yield showed that superheated steam drying exhibited a higher yield than freeze drying. A combination of superheated steam drying and ultrasonic-assisted extraction resulted in the highest 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity (39.48%). Ferric reducing antioxidant power analysis revealed that ultrasonic-assisted extraction enhanced the antioxidant capacity (878.86 mg Trolox equivalents/100g dried sample) of freeze-dried *Alternanthera sessilis* red on par with superheated steam dried sample. The increase in the concentration of major phenolic compounds was also reported in all superheated steam drying samples (gallic acid: 1556.98 -1715.75 mg/100 g dried sample; vanillic acid: 217.21 - 230.41 mg/100 g dried sample; *p*-coumaric acid: 1422.10 – 1559.96 mg/100 g dried sample). As for betalain pigment, superheated steam drying caused its degradation but the impact was reduced after combination with ultrasonic-assisted extraction (betanin : 9.77 mg/100 g dried sample; betaxanthin: 9.07 mg/100 g dried sample). The present result showed that superheated steam drying enhanced the extraction yield, antioxidant capacity and concentration of phenolic compounds of *Alternanthera sessilis* red, regardless of the extraction method.

Keywords: *Alternanthera sessilis* red, Betalain, Freeze drying, Superheated steam drying, Ultrasonic-assisted extraction

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Introduction

Nowadays, there is wide commercialisation of plants traditionally used to prevent or treat illnesses. These medicinal plants are transformed into herbal drinks, supplement pills, and beauty products, as well as incorporated into premix drinks like coffee. Plants that are commonly used in South East Asia include 'daunrerama' (*Christia vespertilionis)*, 'misai kucing' (*Orthosiphon aristatus*), 'daun kelor' (*Moringa oleifera*), 'daun hempedu bumi' (*Andrographis paniculata*) and 'kerema kmerah' (*Alternanthera sessilis* red). Among these plants, *Alternanthera sessilis* red (ASR) is the least commonly explored species. ASR, also known as red sessile joyweed, is considered peculiar to Malaysians, except to older generations of Chinese. Studies revealed that ASR has excellent antioxidant properties that are contributed by phenolic compounds in it[1-3]. Moreover, the antioxidant properties of ASR could also be due to its betalain pigment. Betalain is a secondary metabolite derived from the amino acid L-tyrosine via the formation of L-dihydroxyphenylalanine (L-DOPA). It can be categorised into betacyanins and betaxanthins. Betacyanins include the reddish to violet betalain pigments, while betaxanthins are those betalain pigments that appear yellow to orange. In a study, betalain extract of *Talinum triangulare* had successfully inhibited DPPH radical[4]. However, the relationship between betalain content in ASR and its antioxidant capacity is yet to be known.

On an industrial scale, converting raw materials, especially medicinal plants, into commercial products such as herbal drinks and supplements involves physical processes like drying and extraction. Drying can prolong the shelf life of plant materials and reduce the bulk weight, leading to lower logistic costs[5]. Natural drying, whether sun or shade drying, is not recommended as it is influenced by weather and can lead to loss of the product ingredients[6]. Drying at high temperatures could maintain the yield of phenolic compounds along with the antioxidant capacity[7]. Nevertheless, hot air drying consumes energy and causes loss of nutrients, giving a product

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of low quality. Superheated steam (SHS) drying is an innovative method that has begun to be explored due to its positive effect. It is considered superior to hot air drying as it is conducted in the absence of air[8], consumes less energy and emits less heat and odour to the environment[9]. Although SHS drying involves heat application, the oxygen-free environment of the system can prevent the degradation of bioactive compounds that are susceptible to oxidation. Studies even implied that the heat caused the breakage of the cell wall, allowing better extraction of the antioxidant compounds[10]. Even though there is substantial research on the effect of SHS drying on various types of products[8], there still needs to be more data on its effect on individual phenolic compounds and betalain pigment.

Ethanol is one of the solvents widely used to extract bioactive compounds from plants due to its high purity, low price and completely biodegradable[11]. However, the use of ethanol in halal product production raises debate[12]. Although it is possible to remove the ethanol through evaporation, the percentage of ethanol residue in the extract will remain unknown. The method of detection of ethanol in the sample also still has low reliability and precision apart from requiring a unique set of laboratory instruments and material[13].

Even though alcohol such as ethanol and methanol are stated to give a good yield, a similar result can also be achieved in water extraction. The TPC of water extracts of petiole and leaves from water hyacinth were reported higher than its ethanolic extracts[14]. The antioxidant capacity of water extracts (leaf and flower of water hyacinth) was also the highest. However, some studies reported that water extraction resulted in lower phenolic and antioxidant capacity[1,2]. On top of that, the lack of data on individual phenolics of water extract had resulted in its scarcity of use in research. To further enhance the capability of water extraction, a more advanced extraction method could be applied. Ultrasonic-assisted extraction (UAE) is one of the advanced extraction methods that can give efficient extraction. For this study, an ultrasonic probe was used mainly as the ultrasound was directly transferred in the extraction medium via the tip of the probe rather than indirect sonication by the ultrasonic bath. To date, the capability of water as an extraction solvent for phenolic compounds and betalain pigment extraction, aided by a more effective extraction method like UAE, still needs to be explored.

This study aims to assess the effect of the combination of drying and extraction methods on the quality of ASR extract in terms of antioxidant capacity, the concentration of phenolic compounds, and total betalain content. UAE, with a combination of innovative drying methods, SHS drying is expected to enhance the antioxidant capacity, the concentration of phenolic compounds, the betalain pigment, and the colour of ASR.

Materials and Methods

Collection and identification of sample

ASR was obtained from the wet market in Kepong Baru, Selangor, Malaysia, from June to October 2020. The plant was identified by Dr Khairil Mahmud, Coordinator of the Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia (voucher no.: KM 0043/22). All parts of ASR (leaves, stems, and flowers) were used except the roots. The samples were washed and chopped into smaller parts before drying.

Drying method

The chopped ASR was divided into two portions, each subjected to freeze and SHS drying. In freeze drying, the samples were kept at −20ºC before lyophilisation at −60°C in a freeze dryer (Alpha 1-4 LD plus, Martin Christ, Germany) for three days. Meanwhile, SHS drying was conducted in an SHS oven (DC Quto QF-5200C, Naomoto, Japan) at 170°C for 1 h. Dried ASR from both methods was ground using a laboratory blender, sieved, and stored in an airtight opaque plastic jar.

Extraction

Dried ASR from each drying method was then subjected to two different extraction methods: Conventional and ultrasonic-assisted extraction (UAE). The sample was extracted with distilled water at a ratio of 1:15 in both methods. In conventional extraction, the mixture was extracted for 30 min at 40°C in an incubator shaker (Innova 40, Eppendorf, Germany) at 200 rpm. Meanwhile, in UAE, the mixture was ultrasonicated for 30 min using a 20 kHz ultrasonic probe (CP 505, Cole-Parmer, USA). Sonication occurred at a temperature of 40°C. The temperature and extraction period for both methods were kept similar to ensure a comparable result. Once the extraction process ended, the mixture from each extraction method was individually filtered through vacuum filtration. The liquid extract was collected,

transferred into a glass jar, and kept at -20ºC before lyophilisation.

Total Phenolic Content (TPC)

TPC of ASR extracts was evaluated through Folin-Ciocalteu assay[1]. As stated in the method, 200 µL of aqueous extract was transferred into an amber test tube and 1.5 mL Folin-Ciocalteu reagent, priorly diluted to 10-fold with distilled water. The test tube was then subjected to vortex (REAX Control, Heidolph, Germany) for 10 s. The mixture was incubated for 5 min at room temperature. Then, 1.5 mL of 0.56 M sodium carbonate (Na_2CO_3) solution was added into the test tube and incubated for 90 min at room temperature. The absorbance value of the mixture was measured at 725 nm with a UV-Visible Thermo Spectrophotometer (Thermo Scientific, Thermo Fisher Scientific, USA). Gallic acid was used as standard, and the results were expressed as mg of gallic acid equivalents (GAE)/100 g dried sample.

DPPH Radical Scavenging activity

DPPH Radical Scavenging activity was measured by adding 1 mL of aqueous extract to 2 mL of 0.15 mM of DPPH, which was then mixed thoroughly using a vortex mixer (REAX Control, Heidolph, Germany)[15]. After that, the mixture was allowed to stand for 30 min in the dark at room temperature. The absorbance of the mixture was measured after 30 min at 517 nm using a UV-Vis Thermo Spectrophotometer (Thermo Scientific, Thermo Fisher Scientific, USA) with ascorbic acid as a comparative standard. The mixture of distilled water and DPPH solution served as a control. The result was expressed as a percentage of scavenging activity, calculated using the formula:

$$\text{Percentage of scavenging activity [\%]} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was performed according to the procedure of Benzie and Strain,[16]. In brief, the FRAP reagent was prepared from 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM hydrochloric acid, and 20 mM iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh daily and incubated at 37°C for 10 min in a water bath before usage. 100 µL aqueous extract was added to 8.7 mL of the FRAP reagent. The mixture was then placed in a dark condition at 50°C for 1 h. The absorbance of the reaction mixture was then recorded at 593 nm after a 1 h incubation period. The standard curve was plotted using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and the result was expressed as mg Trolox equivalent (TE)/100 g dried sample.

Identification and quantification of individual phenolic compound

The identification and quantification of individual phenolic compounds in ASR extracts were conducted according to a method by Othman *et al.*[1]. Before analysis, the extract was hydrolysed with 1.2 M hydrochloric acid in 70% ethanol at 90°C for 2 h. The hydrolysed sample was then filtered through 0.2 µm nylon filters. The phenolic compounds were analysed by an HPLC system composed of a liquid chromatographic system. A reverse phase with a ZORBAX Eclipse XDB-C18 column (4.6 x 150 mm, i.d. and particle size 5 µm; Agilent, USA) was used for separation. The mobile phases used were 0.5% acetic acid (v/v) (A) and methanol (B). The applied gradient elution profile was: 0–2 min, 18% B; 2–5 min, 40% B; 5–6 min, 60% B, 6–15 min, 70% B and finally, washing and reconditioning of the column. The sample was injected at an injection volume of 6 µL into the HPLC system with flow rate and column temperature set at 0.5 mL/min and 30°C, respectively. The detection of phenolic compounds was performed at a wavelength of 280 nm. The major phenolic compounds in ASR were identified by comparing their retention times with the reference standards, namely gallic acid, vanillic acid, and *p*-coumaric acid.

Total betalain content (TBC)

The total betalain content in the extract was determined using the spectrophotometric method[17] with slight modification. The extract was redissolved and diluted with McIlvaine buffer (pH 6.0, citrate-phosphate) to obtain absorption values of $0.9 \leq A \leq 1.0$ at their respective absorption maxima. The betalain content was calculated using the equation:

$$\text{Betalain content (betanin/betaxanthin) [mg/g]} = \frac{(A \times MW \times DF)}{(\mathcal{E} \times 1)}$$

where A = Absorbance at 536 or 480 nm; MW = molecular weight (betanin=550 g/mol; betaxanthin= 308 g/mol); DF = dilution factor; ε = extinction coefficient (betanin: 60000 M^{-1} cm^{-1}; betaxanthins (vulgaxanthin I) : 48000 M^{-1} cm^{-1}).

Colour

The colour of the extract was measured with a chromameter[18] (CR-400, Konica Minolta Sensing Inc., Japan). At the end of the measurements, results were recorded in accordance with the CIELAB system. The parameters determined were L* (L*=0 (black) and L*=100 (white)), a* (negative values correspond to greenness and positive values to redness), and b* (negative values correspond to blueness and positive values to yellowness).

Statistical analysis

Extractions were conducted in duplicates, and each experiment was conducted in triplicates. The results were expressed as the mean±standard deviation. The statistical differences between different groups (FC, FU, SSC, SSU) were calculated by one-way analysis of variance (ANOVA) using SPSS software, version 26 (SPSS Inc., Chicago, Illinois, USA) with the level of significance at P <0.05. The homogeneity of variance test was used to determine the suitable post-hoc multiple comparisons. A post-hoc Tukey test was employed when equal variances were assumed, while the post-hoc Games-Howell was applied when equal variances were not assumed. Pearson correlation was used to assess the relationships between TPC and extraction yield, antioxidant capacity (DPPH and FRAP), and betanin and betaxanthin relation with antioxidant capacity. The significance level was set at P <0.01.

Results and Discussions

Extraction yield

Based on the study conducted, the extraction yield of ASR extracts, which are FC, FU, SSC and SSU, was 22.18^{b} ± 2.12, $27.65^{a,b}$± 1.27, 31.09^{a} ± 2.88 and 32.19^{a} ± 2.18 respectively. The result shows that the drying method significantly affected the extraction yield, with SHS drying being superior to freeze drying. In another study, SHS treatment on perilla seeds had increased the oil yield by 2.5 times from untreated seeds[19]. SHS treatment had ruptured the structure of the seed, crushing the cell membrane and outer seed coat, exposing the seed's inner portion and easing the oil transfer from vacuoles to the outer surface[19]. A similar explanation applies to the finding in this study, where SHS drying might have caused the breaking of the cell wall of ASR, resulting in more compounds released in the extraction medium, giving a higher extraction yield. Apart from that, higher extraction yield in the SHS drying sample could also be due to a greater rehydration ratio as the effect of the drying process. It had been seen that thermal drying had led to greater rehydration capacity than vacuum freeze drying[20]. Rehydration capacity represents the degree of damage, either cellular or structural, to food due to drying[8]. With greater rehydration capacity, more water was able to be absorbed into the sample during the extraction process and later carried more analytes with it. It was found that samples subjected to heat treatment had a small amount of intercellular air in the structure[20]. Hence, when water was added, the plant structure expanded, and the volume of the sample pumped the water into the cell, increasing its absorption. In contrast, the network structure of the freeze-dried sample was filled with air; hence, water potential was not enough to remove all air inside it, preventing water from being absorbed in the tissue[20].

Despite this, no significant difference was seen between the extraction yield of the FU extract and both of the SHS drying extracts. This could be due to the extraction method used, which was UAE. It produced a higher yield in a shorter time than conventional techniques (Soxhlet extraction and batch extraction) due to cavitation, mechanical, and thermal effects. Production of bubbles, generation of shockwaves, the greater contact surface between water and sample, and an increase in temperature and pressure due to compression of bubbles, had improved the extractability of compounds.

Total Phenolic Content (TPC)

Fig. 1 shows a significant difference between ASR extracts undergoing different drying methods. It can be seen that the TPC of both SHS drying extracts were significantly higher than freeze drying extracts. Similarly, the TPC of lightly milled rice increased when subjected to SHS treatment at 120°C at 2.5 min compared to the untreated sample[21]. The authors implied that the increase of the TPC of lightly milled rice exposed to SHS processing was parallel to the increase of its soluble phenolic compounds. The increment of soluble phenolics could result from releasing insoluble phenolic compounds.

Nonetheless, the insoluble phenolic compounds did not decrease[21], leading the authors to conclude that SHS might have improved the extractability of insoluble phenolic compounds from the food matrix, leading to the increase in TPC. Besides that, the increment of phenolics by SHS processing was also linked to shorter processing time and oxygen

availability. A shorter drying time of ASR in SHS drying, which was 1 h might have prevented the loss of phenolic compounds in the extract compared to freeze drying, which was conducted for three days. In addition, the oxygen-free environment of SHS drying contributed to higher TPC in SHS drying extracts by preventing the degradation of phenolics.

As for the extraction method, there was a significant difference between FC and FU extracts. The TPC of the FU extract was 28.3% higher than that of the FC extract, which could be contributed by higher extraction yield. Higher extraction yield means more phenolic compounds being extracted. The present result shows a positive correlation (R^2 = 0.808) between extraction yield and TPC. This positive correlation made it not remarkable when there was no significant difference in the TPC of SSC and SSU extracts. No significant difference is attributed to the drying method applied. SHS drying might have caused the cell structure of ASR to rupture to the maximum. As a result, when UAE was applied in combination with SHS drying, the extraction yield and TPC only increased slightly without reporting a significant change.

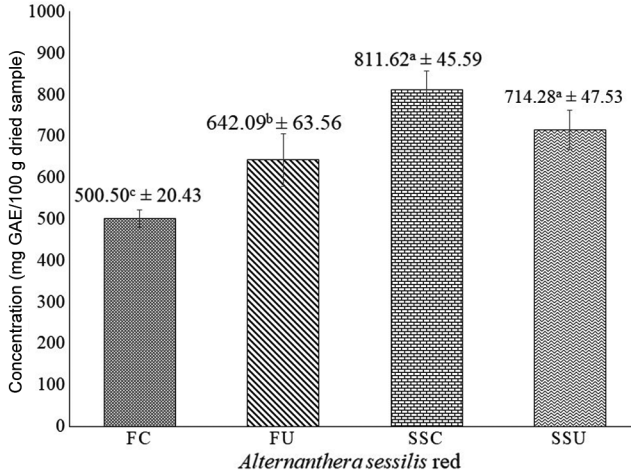

Fig. 1 — Total phenolic content (TPC) of ASR extracts. Values were expressed as the mean ± standard deviation (n = 6). Mean with different letters ($^{a, b, c, d}$) were significantly different at the level of P <0.05. FC: ASR subjected to freeze drying and conventional extraction method, FU: ASR subjected to freeze drying and ultrasonic-assisted extraction, SSC: ASR subjected to superheated steam drying and conventional extraction method, SSU: ASR subjected to superheated steam drying and ultrasonic-assisted extraction.

DPPH Radical Scavenging Activity

Drying of ASR in SHS produced two times higher percentage of DPPH radical scavenging activity than freeze drying extracts (Table 1). A similar positive effect of SHS on DPPH scavenging activity has also been reported in other studies[10,19]. It was proposed that higher antioxidant capacity could have been due to increased dry matter with increasing temperature and time of SHS treatment[19]. A strong positive correlation between TPC and DPPH (R^2 = 0.884) in this study also showed greater antioxidant capacity in plants with higher TPC. On the other hand, compounds like melanoidin from the Maillard reaction induced by heat processing[22] might have contributed to the antioxidant capacity. Melanoidin contributes to antioxidant capacity by 'its ability to trap positively charged electrophilic species, scavenge oxygen radicals, or carry out metal chelation to form inactive complexes[22]. In contrast, drying in a hot air oven at 60ºC for 16 h resulted in a lower DPPH scavenging effect of hawthorn than its freeze-dried sample[23]. These contradictory findings might be due to the type, parameters and application stage of heat processing, as well as the nature of the sample itself, as both positive and negative effects of the drying method were reported in various types of research.

A comparison between conventional extraction and UAE on the DPPH scavenging effect of ASR extracts was also found to be significant. UAE increased the

Table 1 — Antioxidant capacity and total betalain content of ASR extract

Sample	Antioxidant capacity		Total betalain content	
	DPPH (%)	FRAP (mg TE/100 g dried sample)	Betanin content (mg/100 g dried sample)	Betaxanthin content (mg/100 g dried sample)
FC	16.26^{d}±2.27	441.82^{b}±112.71	27.73^{a}±2.44	21.88^{a}±0.27
FU	19.19^{c}±1.60	878.86^{a}±133.23	30.99^{a}±4.60	25.02^{a}±5.39
SSC	35.79^{b}±1.10	1068.22^{a}±151.52	7.18^{c}±0.02	6.15^{c}±0.02
SSU	39.48^{a}±1.19	1015.02^{a}±166.01	9.77^{b}±0.02	9.07^{b}±0.39

Values were expressed as the mean±standard deviation (n = 6). Mean with different letters ($^{a, b, c, d}$) within the same column were significantly different at the level of P <0.05.

FC: ASR subjected to freeze drying and conventional extraction method, FU: ASR subjected to freeze drying and ultrasonic-assisted extraction, SSC: ASR subjected to superheated steam drying and conventional extraction method, SSU: ASR subjected to superheated steam drying and ultrasonic-assisted extraction.

percentage of DPPH scavenging activity, regardless of the drying method employed. The increase was attributed to the mechanism of UAE[24]. The high intensity and frequency ultrasound waves in the UAE damage the plant cell walls, enabling better extraction of phenolic compounds. This was further supported by the strong positive correlation between TPC and DPPH scavenging activity in this study, as mentioned above. Replacing magnetic stirring with ultrasound treatment in three-phase partitioning of green chiretta also increased the antioxidant capacity[24].

Ferric Reducing Antioxidant Power (FRAP)

Variation in the antioxidant assay can lead to different results as the effect of different reaction kinetic[25]. This explained why the resulting trend for FRAP was slightly different than the DPPH assay for this study. For the FRAP assay, there was no significant difference between the SHS drying extracts and FU extract (Table 1). The possible reason behind such indifference is the low drying temperature of freeze drying. Due to the slow freezing at -20ºC in freeze drying, the cell structures of the sample might have been damaged, which in turn released more extractable phenolic compounds[25]. The authors added that the low drying temperature of freeze-drying reduced the degradation of thermal-sensitive phenolic compounds. However, the FRAP of FC extract significantly differed from FU, even though it has undergone a similar freeze-drying method. The only difference between FC and FU extracts was the extraction technique employed. The butterfly pea flower extracted through UAE also showed higher FRAP than conventional extraction, although similar parameters (150 min, 50ºC) were employed[26]. The authors asserted that high shear forces and other physical forces generated by acoustic cavitation enhance mass transfer, leading to better extractions. Like DPPH radical scavenging activity, FRAP recorded a positive correlation ($R^2 = 0.663$) with TPC. A higher amount of phenolic compounds in the extract will give higher FRAP because phenolics can donate electrons to Fe^{3+}-TPTZ complex that is initially colourless, causing it to reduce to blue coloured complex, Fe^{2+}-tripyridyltriazine. A positive correlation between TPC and antioxidant capacity (DPPH and FRAP) showed that phenolic compounds are the main contributor to the antioxidant capacity of ASR extracts. The following section has further investigated and discussed individual phenolic compounds that contribute to the antioxidant capacity.

Identification and quantification of individual phenolic compounds

As explained in the methods section, all extracts were subjected to acid hydrolysis for identifying and quantifying individual phenolic compounds. Acid hydrolysis was suggested prior to HPLC identification of phenolic compounds to minimise problems in detection. For instance, in this study, the non-hydrolysed sample showed only one unknown peak in the chromatogram. Meanwhile, all peaks shown from non-hydrolysed samples cannot be identified in different study[2]. Acid hydrolysis allows easier identification of aglycone or free polyphenols as it cleaves the sugar bound to polyphenols[2].

Fig. 2 shows the HPLC–DAD profiles of major phenolic compounds in ASR extracts. These major

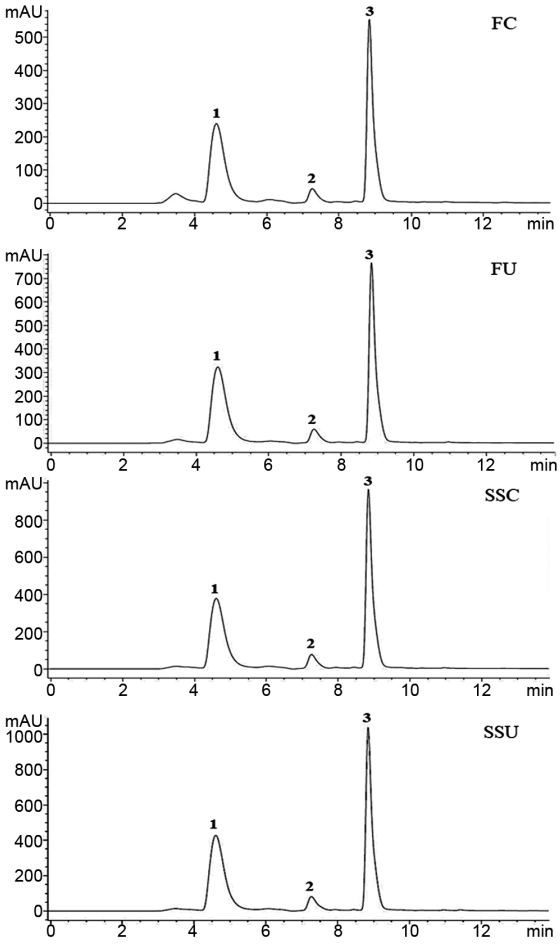

Fig. 2 — Chromatograms of major phenolic compounds in ASR extracts. Individual phenolic compound was numbered accordingly; **(1)** gallic acid; **(2)** vanillic acid; and **(3)** *p*-coumaric acid. FC: ASR subjected to freeze drying and conventional extraction method, FU: ASR subjected to freeze drying and ultrasonic-assisted extraction, SSC: ASR subjected to superheated steam drying and conventional extraction method, SSU: ASR subjected to superheated steam drying and ultrasonic-assisted extraction.

Table 2 — HPLC profile showing phenolic composition (mg/100 g dried sample) in ASR extracts

Peak no.	Retention time (min)	Compound	Concentration of compound in sample (mg/100 g dried sample)			
			FC	FU	SSC	SSU
1	4.6	Gallic acid	773.45^{b}±135.11	835.43^{b}±29.15	1556.98^{a}±367.86	1715.75^{a}±378.99
2	7.3	Vanillic acid	114.87^{b}±8.04	133.30^{b}±15.90	217.21^{a}±32.93	230.41^{a}±29.49
3	8.8	*p*-coumaric acid	596.88^{b}±99.02	729.87^{b}±42.54	1422.10^{a}±284.12	1559.96^{a}±370.65

Values were expressed as the mean±standard deviation (n = 6). Mean with different letters ($^{a, b}$) within the same row were significantly different at the level of P <0.05.

FC: ASR subjected to freeze drying and conventional extraction method, FU: ASR subjected to freeze drying and ultrasonic-assisted extraction, SSC: ASR subjected to superheated steam drying and conventional extraction method, SSU: ASR subjected to superheated steam drying and ultrasonic-assisted extraction.

compounds were based on compounds reporting peak areas higher than 590 mAU*s. Based on Fig. 2, major phenolic compounds in ASR extracts were identified as gallic acid, vanillic acid and *p*-coumaric acid (Table 2), which were all categorised as phenolic acids. Gallic acid was the dominant phenolic compound in ASR for this study. The result obtained from this study differs from other studies[1-3], even though it uses a similar plant species. Two phenolics from Othman *et al.*[1], were identified in this study, but none of the phenolics reported in other research was seen. Moreover, *p*-coumaric acid, a compound not detected in previous research, was detected in ASR extracts in this study. The availability of compounds detected could be influenced by the location of sample collection, agronomic conditions and variation in extraction parameters[2]. For instance, chlorogenic acid was detected[27] in *Gynura bicolour* DC, while it was not detected in a study by different researchers[28], although the same plant species were used. The samples were harvested from different locations and extracted under different parameters in both studies. *p*-coumaric acid was previously reported in *Alternanthera sessilis*[29], yet no specification on whether ASR or green was used.

Since extracts underwent conventional extraction and UAE showed similar major phenolic compounds, it can be said that UAE neither caused the formation nor degradation of phenolic compounds. It also did not show a significant increment in the concentration of all phenolic compounds compared to conventional extraction. This means that a combination of drying, whether SHS drying or freeze drying with UAE, did not produce a synergistic result in enhancing the phenolic compound concentration. As displayed in Table 2, UAE did improve the concentration of phenolics in the sample, but it was not high enough to report a significant difference.

As for the drying method, SHS drying did not cause the degradation of phenolic compounds. Currently, there is no study reported on the effect of SHS drying on individual phenolic compounds. Hence, the result was compared to the effect of the thermal drying process on phenolics. Oven drying at 40°C had been seen to increase the caffeic acid in banana flour when compared to freeze-dried sample[30]. This is because phenolic acids are commonly bound to the cellulose and pectin inside the cell wall. Thus, when thermal processing is applied, the cell wall breaks, causing more phenolics to be extracted.

On the other hand, SHS drying had caused evolution of vapour inside the foodstuff which causes cells to expand[8]. These structural changes facilitated the extraction of bioactive compounds from sample matrices into an extraction medium. However, drying of the apple peel by-products at a temperature higher than 110ºC resulted in the degradation of phenolic compounds[31]. The authors relate the degradation of phenolic compounds to the oxidation reactions or the cleavage of covalent bonds. Phenolic compounds can undergo oxidation with molecular oxygen, yielding degradation products[32], lowering the concentration of phenolic compounds detected. However, SHS drying was conducted without air or in an oxygen-free environment[8]. Elimination of oxygen in SHS drying may prevent oxidation, hence enhancing the concentration of phenolic compounds detected. Moreover, the temperature of 170°C in SHS drying was only meant for the steam temperature, while the oven temperature was still set at 100ºC. This might explain why this research did not observe the reduction of phenolic compounds.

Total Betalain Content

Based on Table 1, it can be seen that the drying method had a negative impact on the betalain pigment of

ASR. The sample dried under SHS drying reported lower betanin and betaxanthin content by three times than those dried in the freeze dryer. Heat treatment was able to degrade betanin and isobetanin to a different structure through 1) decarboxylation, causing the change in maximum wavelength absorption, giving the orange-red appearance; 2) dehydrogenation, forming neobetanin that explained the yellow hue; and 3) cleavage, leading to the development of the bright yellow betalamic acid and the colourless cyclo-Dopa 5-*O*-β-glucoside[33]. These might explain the lower amount of betanin compound in SSC and SSU extracts. As for betaxanthin, its stability is not well-specified[34]. Still, its degradation upon thermal processes could be related to hydrolysis and isomerisation process[35]. It was reported that the retention of betaxanthin in cactus decreased as storage temperature increased up to 80ºC in a 4 h stability study[34]. Thus, betaxanthin degradation in SHS drying extracts might have occurred because the drying was conducted at 100ºC oven temperature. Although heat was known to be causing betalain degradation, drying in SHS drying was predicted to prevent the degradation as it was conducted in an oxygen-free environment without light. However, findings from this study proved that the exclusion of light and oxygen in heat processing was not able to prevent the degradation of betalain. Adding food additives like antioxidants (ascorbic acid) and chelating agents (citric acid) along with excluding oxygen in heat processing[36] may give a better result.

Nevertheless, the application of UAE slightly reduced the deleterious effect of SHS drying on betanin and betaxanthin content. The betanin and betaxanthin content of SSU extract was higher by 36 and 47%, respectively, than SSC extract. This finding is tallied with another study where the betanin and betaxanthin content of *Bougainvillea glabra* bracts extracted through UAE was significantly higher than those extracted in conventional extraction[37]. The *B. glabra* bracts had been dried in a dryer with forced air circulation for 8 h at 30ºC. An increase in the yield of betalain pigment through UAE was linked to its cavitation effect. The cavitation effect is where small bubbles are formed, grow, and collapse in the medium during extraction. As the bubbles collapse, disruption of the plant matrix occurs, accelerating the diffusion of extraction solvent into it. Furthermore, sonication also generates strong shock waves, causing the swelling and enlargement of pores of the plant materials, which improves the penetration of solvent into the plant tissue.

Betanin, which made up betalain pigment, was reported to own antioxidant capacity due to its ability to scavenge free radicals[38]. However, the present result reported a strong negative correlation between DPPH radical scavenging activity with betanin (R^2= -0.928) and betaxanthin (R^2= -0.888). A strong negative correlation was also obtained between FRAP with betanin (R^2= -0.588) and betaxanthin (R^2= -0.533). Still, to confirm this negative correlation, further isolation and purification of betalain pigment using instruments like preparative HPLC need to be done. Identifying individual betalain presented in ASR might help explain such a contrary finding from other studies and the mechanisms behind it.

Colour

Data from Table 3 showed that the L* value of ASR extracts increased with the application of SHS drying. The higher L* value of dried and powdered samples is relatable to the thermal decomposition of the pigments and the interaction of the pigments with other compounds[39]. The authors also reported a higher L* value in sweet red pepper dried through hot air drying, infrared and intermittent microwave drying. Since ASR was chosen for its red-purple colour due to the presence of betalain pigment, evaluation of its a* and b* values after drying and extraction is crucial. It was found that SHS drying resulted in greater redness (a*) and yellowness (b*) of ASR extract compared to

Table 3 — CIE L* a* b* colour parameters of ASR extract

Sample	L*	a*	b*
FC	$42.13^{a,b}$±1.24	5.05^{d}±0.89	7.25^{b}±1.44
FU	38.92^{b}±5.65	7.01^{c}±1.03	9.49^{b}±1.66
SSC	49.81^{a}±6.05	19.25^{a}±0.88	15.62^{a}±0.55
SSU	48.15^{a}±0.60	16.36^{b}±0.28	16.84^{a}±0.89

Values were expressed as the mean±standard deviation (n = 6). Mean with different letters ($^{a, b, c, d}$) within the same column were significantly different at the level of P <0.05.

FC: ASR subjected to freeze drying and conventional extraction method, FU: ASR subjected to freeze drying and ultrasonic-assisted extraction, SSC: ASR subjected to superheated steam drying and conventional extraction method, SSU: ASR subjected to superheated steam drying and ultrasonic-assisted extraction.

L*: lightness, a*: red or green coordinate, b*: yellow or blue coordinate.

freeze drying. The higher value of redness in samples that underwent heat treatment at 140-150°C was due to a non-enzymatic browning reaction that speeds up with temperature[8].

There is no particular trend on whether the application of UAE enhanced or reduced the lightness, redness and yellowness of ASR extract. UAE did cause lower lightness in freeze-drying extract, but not for SHS drying extracts. It also reduced the redness of ASR undergoing SHS drying while increasing the redness when ASR is freeze-dried. It also did not cause a significant increase in yellowness for both SHS drying and freeze-drying samples. The colour changes in samples subjected to UAE could be due to cavitation that permitted various physical, chemical or biological reactions that include speeding up chemical reactions, enhancing diffusion rates, disseminating aggregates or breakdown of particles like enzymes and microorganisms[40].

Initially, it was predicted that ASR extract that reported higher betanin and betaxanthin content would have higher a* and b* values as the values represented redness and yellowness, respectively. However, a contrasting finding was observed in this study. A strong negative correlation was found between betanin and a* value (R^2= -0.949), and a moderate negative correlation was seen between betaxanthin and b* value (R^2= -0.797). Based on direct observations on the dried ASR, obvious colour differences between samples dried through SHS and freeze drying can be seen. The SHS drying sample appeared more reddish than the freeze-drying sample, which looked more purplish. This might have explained the higher a* and b* values of the SHS drying sample. Still, the sample did not show a higher betalain concentration. Therefore, it can be said that colour analysis could not be used as a direct estimation of betalain pigment concentration when various processes are involved in the sample. To measure the effectiveness of pigment addition in food, the purpose will determine the appropriate analysis to be performed. If the product is focused more on having an appealing red colour, determination using a chromameter can be conducted. In contrast, if the product needs to have a high concentration of betalain pigment, total betalain content analysis needs to be performed.

Conclusion

In conclusion, the combination of SHS drying and UAE enhanced the extraction yield, phenolic compounds concentration and antioxidant capacity of ASR extracts while maintaining the visual acceptability of the extract. However, a combination of SHS drying and UAE still decreased the total betalain content. Hence, this innovative method should be investigated further to ensure its maximum reliability in producing natural colourants. This study also showed that extraction of phenolic compounds can be accomplished through water extraction, with one condition: acid hydrolysis with ethanol addition must be performed before identification through HPLC analysis.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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