ORIGINAL ARTICLE

Antioxidant Activity and Total Phenolics Content of Brazilian Spinach (*Alternanthera sissoo*) and Spinach Cultivar in Malaysia

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ABSTRACT

Introduction: Brazilian spinach and Malaysian spinach are from the Amaranthaceae family but under different genera. Thus, there may be differences in terms of chemical and nutrition composition in vegetables as they are influenced by geographical distribution, regional differences, and meteorological aspects. Thus, the objective of this study is to quantify the antioxidant activity of Brazilian spinach and Malaysian spinach and investigating the effects of cooking on their antioxidant activity. **Methods:** Raw, boiled, and stir-fried samples of Brazilian spinach and Malaysian spinach were extracted by water and 80% methanol solvents. Then, the antioxidant activity and total phenolic content of extracts from Brazilian spinach and Malaysian spinach were evaluated by measuring their scavenging abilities to 2,2-diphenyl-1-1-picrylhydrazyl (DPPH) and the Folin-Ciocalteu method. **Results:** The results demonstrated that boiling yielded the highest scavenging activity followed by stir-frying, whereas boiling and stir-frying significantly decreased the total phenolics content of *A. sissoo* and *A. sp.* in selected extraction solvents. **Conclusion:** Present findings would serve as a database providing information for the impact of various cooking treatments on the antioxidant potential of Brazilian spinach and Malaysian spinach. Consequently, this may encourage the food industry and households in choosing the most appropriated cooking treatment in dietary applications to help preserve the antioxidant activity of the vegetables.
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Keywords: Antioxidant activity, Total phenolics content, Brazilian spinach, *Alternanthera sissoo*, *Amaranthus sp*.

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INTRODUCTION

Vegetables notably represent one of the most important parts of the staple diet as they are recognized as having significant health-promoting activities related to the functional properties of their nutrients and non-essential chemical compounds, namely phytochemicals (1, 2). In a study of food analysis, Roberts and Moreau (2) stated that there is overwhelming evidence that indicates diets rich in vegetables have protective properties against chronic diseases such as cancer, obesity, and cardiovascular disease. Other studies have also discovered the exact evidence that vegetables exhibit a vast range of bioactivities that include anti-diabetic, anti-tumour, anti-inflammatory, antihypertensive, and hypoglycaemic properties (3 – 6). Therefore, being consciously informed of their nutritional characteristics may improve health status of a population. Even though consumption of vegetables among the population in Malaysia has increased over the years, a recent survey by National Health and Morbidity Survey (7) discovered that 94.9% of adults do not consume adequate fruits and or vegetables of 5 servings daily as recommended.

Brazilian spinach (*Alternanthera sissoo*) and Malaysian spinach cultivars (*Amaranthus gangeticus, Amaranthus tricolor,* and *Amaranthus sp.*) are the common tropical perennial plants currently used as the sources for leafy vegetables that can be prepared either by stir-fry, soup, or stew with minimal heat cooking. Brazilian spinach goes by the name sissoo spinach, poor man's spinach, or samba lettuce while Malaysian spinach cultivars that can be divided into two distinct spinach variants, namely green spinach and red spinach or Chinese spinach (8, 9). Malaysian spinach cultivars are typically consumed during the main part of the meal rather than as a snack or dessert, while Brazilian spinach, which has recently gained traction and acceptance being an affordable, healthy snack among the locals. Furthermore, both crops are grown on a large scale commercially, especially Malaysian spinach cultivars followed by Brazilian spinach that is currently in constant demand within a rural locality (10). Despite both vegetables having spinach in their respective names, these two are distant relatives of the actual spinach (*Spinacia oleracea*) albeit from the same Amaranthaceae family, well-known in media and cultural references. As many do not aware of this, several websites have been promoting the health benefits of the former by citing information regarded for the latter which is misleading as they may have a difference in nutritional and pharmaceutical values.

Brazilian spinach is recently famed as healthy snacking food in Malaysia. Spinach (*Amaranthus sp.*) is naturally an excellent source of vitamin K, magnesium, calcium, and phosphorus (11, 12). Brazilian spinach and spinach cultivars in Malaysia are from the Amaranthaceae family, but different genera i.e., the genus of spinach is Amaranthus, while the genus of Brazilian spinach is Alternanthera. Hence, the assumption is the former may have a significant difference in nutritional values as the latter. Vegetables are mostly cooked before consumption, which is known to induce significant changes to its chemical composition that can affect the concentrations of nutrients and retention of antioxidant activity (13). Many foods composition databases have simply quantified vegetables in the raw state without considering any effects of cooking practices such as boiling, steaming, or frying. This holds great importance for the field of nutrition and public health as most vegetables consumed are usually treated first whilst only a small amount is ever eaten raw.

The extent of difference between the nutritional values of Brazilian spinach and spinach cultivars in Southeast Asian countries especially in Malaysia has not yet been established. There may be differences in terms of nutritional composition, bioactive components, and antioxidant activity in vegetables as they could be affected by geographical distribution and regional differences especially from the use of local varieties, different quality of soil, or meteorological aspects (14, 15). In addition, cooking practices or heat treatments of vegetables may also induce significant changes to their chemical composition. Consequently, Amin et al. (16) reported that many food composition databases had not taken into consideration that cooking practices may change the concentrations of nutrients and their antioxidant activity. By quantifying and evaluating the potential antioxidant activity of Brazilian spinach and spinach cultivar in Malaysia under controlled environment testing, the approximate numbers of its nutritive compounds and phytochemicals obtained will serve as dietary reference intakes in the field of nutrition and public health and lay the foundations for further study to investigate its beneficial effects towards population health.

MATERIALS AND METHODS

Sample

The sampling that was used in this study is non-probabilistic sampling method, namely convenience sampling. It is the most common method of collecting samples in exploratory research such as food analysis or lab work and is performed by taking samples that are conveniently located around a designated location (17). In addition, since there is no definitive pattern in acquiring the samples, this type of sampling provides the most flexibility in the quantitative analysis (17, 18). Thus, the acquisition of selected vegetables will be from the local supermarkets located close to the laboratory.

Preparation of Plant Samples

The selected commercially available Brazilian spinach (*A. sissoo*) (Fig. 1) and Malaysian spinach (*Amaranthus sp.*) (Fig. 2) were purchased from local supermarkets in Selangor, Malaysia. They were randomly selected from the market shelves, purchased in batches of approximately 500 g, and immediately transported to the laboratory. The samples were washed clean with tap water in order to remove any dirt and foreign materials. Next, the inedible parts of the samples were removed before they are divided into two portions (250 g for each application); raw and cooking treatments.

Figure 1: Brazilian Spinach (*Alternanthera sissoo*)

Figure 2: Malaysian Spinach (*Amaranthus sp.*)

Cooking Treatments

The cooking treatments that were used in this study are based on the two most common cooking methods for Brazilian spinach and Malaysian spinach, namely boiling, and stir-frying. The cooking time was set at 5 minutes for boiling and 2 minutes for stir-frying. For boiling, 250 g of homogenous vegetable samples were immersed in 1000 ml of boiling water at 100 °C in a covered stainless-steel pot. The ratio of vegetable samples to water is 1:4. The samples were drained off after 5 minutes of being boiled. For stir-frying, 250 g of vegetable samples were added to 15 ml of palm oil preheated to 130 °C in a wok. The samples were stir-fried for 2 minutes before they were drained off and dabbed with blotting paper to remove access oil. After each cooking treatment, the samples were immediately soaked with cold water with ice for approximately 1 minute to avoid further heating.

Preparation of Extracts

Preparation for sample extraction that will be used in the present study are outlined according to Al-Sayyed et al. (19) with slight modifications. Raw and treated samples of *Alternanthera sissoo* and *Amaranthus sp.* were rinsed with deionized water and dried using towel paper. Next, the samples were finely chopped and weighed to get a predetermined weight of 750 mg. The weighed samples were then placed into test tubes and added with 15 ml of two extraction solvents which include aqueous methanol (methanol: water, 80:20 v/v) and water respectively. The mixtures were heated in water bath at 50 °C and 90 °C respectively for two hours while agitated intermittently for 30 seconds every 30 minutes by using vortex mixer. The extracts were centrifuged at 3000 rpm for 15 minutes and filtered through a filter paper (Wattman filter paper No.4) in order to remove any precipitation and fine solid particles. Next, the extracts were added into Eppendorf tube and stored at -80 °C in laboratory freezer until analysed. Deionized water was used for the preparation of all extraction solutions and to complete the reactions. In addition, preparation of sample extracts was conducted under a minimal light setting as antioxidant activity of the sample may be affected by the light intensity.

Antioxidant Activity by DPPH Radical Scavenging Assay

The antioxidant activity of plant extracts from Brazilian spinach and Malaysian spinach cultivars was observed and evaluated by measuring their scavenging abilities to 2, 2-diphenyl-1-1-picrylhydrazyl (DPPH) stable radical as outlined by Amin et al. (16). The established DPPH method started with the preparation of positive and negative controls. Four milliliters of ascorbic acid solution (0.05 mg/ml) were added into 1 mL DPPH solution (0.4 mg/mL) as preparation of positive control while negative control is prepared by mixing 4 ml of distilled water with 1 ml DPPH (0.4 mg/ml) solution for water extraction and 4 ml of 80% methanol with 1 ml of DPPH (0.4 mg/ml) for methanol extraction. Next, 4 ml of the sample extraction (50 mg/ml) was added with 1 ml of DPPH (0.4 mg/ml). The mixture was homogenized gently and incubated for 30 minutes in the dark at room temperature. The absorbance of the sample was measured at 520 nm by using a spectrophotometer. The scavenging activity was calculated based on the percentage of scavenged DPPH.

Determination of Total Phenolic Content by Folin-Ciocalteu

The total phenolic content of selected vegetable samples will be determined spectrophotometrically by using the Folin-Ciocalteu method by Amin et al. (16) with slight modification. 0.75 mL of 10-fold diluted Folin–Ciocalteu reagent will be mixed with 100 µL of sample extracts (50 mg/ml) into a test tube. The mixture will be mixed and allowed to stand at room temperature for about 5 minutes only. Next, 0.75 mL of 20% sodium carbonate solution will be pipetted into the mixture. The mixture was adjusted to 10 ml with distilled water before it is homogenized and kept in the dark for 90 minutes at room temperature. Total absorbance of the samples was measured by using a spectrophotometer at 725 nm. Gallic acid was used as a reference for constructing a standard curve. The total phenolic content was expressed as mg of Gallic Acid Equivalents (GAE) / g of vegetable samples.

Data Analysis

Data was analysed statistically by using Microsoft Excel and Statistical Package for Social Sciences (SPSS) software version 20.0. The analysis was repeated for three times of each experiment, and the reading was collected for the data analysis. Two-way analysis of variance (ANOVA) was used to compare mean differences between cooking treatments that have been split on two independent variables (plant samples; *A. sissoo* and *Amaranthus sp.*). The analysed data was expressed as mean and standard deviation (SD) and significance of differences were determined at $p < 0.05$.

RESULT

DPPH Free Radical Scavenging Assay

The antioxidant activity of *Alternanthera sissoo* and *Amaranthus sp.* leaves were determined by using the DPPH free radical scavenging assay. In this process, polyphenols can quench DPPH radicals and convert them to a colourless substance by offering hydrogen atoms or electron donation (20). Thus, the greater percentage of inhibition of free radical activity, the more potent the antioxidant capacity of the extract in terms of hydrogen atom-donating capacity. Accordingly, both water and methanol extracts of the vegetable samples were evaluated to determine their total antioxidant capacity. Theoretically, the absorbance of samples yielded from spectrophotometer should reflect the antioxidant capacity of *Amaranthus sp.* and *Alternanthera sissoo*. The data were expressed as the percentage of scavenging activity wherein the higher the percentage of DPPH free radical scavenging activity, the higher the antioxidant content.

Table I shows the results of DPPH free radical scavenging activity for water and methanol extraction for control and treatment groups. Raw Brazilian spinach leaves (69%) exhibit lower percentage of scavenging activity compared to the raw Malaysian spinach leaves (97%). The difference in between these samples is 28%. Hence, there is a significant difference between raw Brazilian spinach and Malaysian spinach leaves samples (p=0.011) for water extraction. Boiled Brazilian spinach leaves (90%) exhibit slightly higher percentage of scavenging activity than boiled spinach leaves (94%). The difference in between these two samples is about 4%. Hence, there is no significant difference between boiled Brazilian spinach and spinach leaves (p=0.011) for water extraction. Furthermore, stir-fried Brazilian spinach leaves (35%) exhibit comparably lower percentage of scavenging activity than stir-fried spinach leaves (37%). The difference in between these samples is 2%. Hence, there is no significant difference between stir-fried Brazilian spinach and spinach leaves (p=0.011) for water extraction.

Table I : DPPH Free radical scavenging activity for water and methanol extraction

Extraction	Plant sample	DPPH Free Radical Scavenging Activity (%)	*p*-value**
Water	Raw Brazilian spinach	69.07^{c}	0.011
	Raw Malaysian spinach	96.90^{a}	
	Boiled Brazilian spinach	90.03^{b}	0.408
	Boiled Malaysian spinach	93.81^{ab}	
	Stir-fried Brazilian spinach	34.71^{d}	0.107
	Stir-fried Malaysian spinach	37.46^{d}	
Methanol	Raw Brazilian spinach	27.84^{c}	0.006
	Raw Malaysian spinach	20.66^{c}	
	Boiled Brazilian spinach	92.81^{a}	0.903
	Boiled Malaysian spinach	93.11^{a}	
	Stir-fried Brazilian spinach	76.95^{b}	0.005
	Stir-fried Malaysian spinach	89.22^{a}	

*Values represent mean ± standard deviation (SD) from triplicate readings.
**Significant difference is at *p*-value < 0.05.
Superscript letters within the same row indicate significant differences of means within the extracting solvent where *p*-value < 0.05.

For methanol extractions, raw Brazilian spinach leaves (28%) exhibit slightly lower percentage of scavenging activity compared to the raw Malaysian spinach leaves (21%). The difference in between these samples is 7%. Hence, there is a significant difference between raw Brazilian spinach and spinach leaves samples (p=0.006) for methanol extraction. Boiled Brazilian spinach leaves (93%) exhibit similar percentage of scavenging activity boiled spinach leaves (93%). There is no significant difference between boiled Brazilian spinach and spinach leaves (p=0.903) for methanol extraction. Furthermore, stir-fried Brazilian spinach leaves (77%) exhibit comparably lower percentage of scavenging activity than stir-fried spinach leaves (89%). The difference in between these samples is 12%. Hence, there is no significant difference between stir-fried Brazilian spinach and spinach leaves (p=0.005) for methanol extraction.

Fig. 3 illustrates the scavenging activity of *Alternanthera sissoo* and *Amaranthus sp.* by using two types of extraction that are water and aqueous methanol in control and two cooking treatments groups which are boiling and stir-frying. Based on observation, water is evidently showing better extraction capacity compared to aqueous methanol. The overall pattern for vegetable samples in both solvents is provisionally different. Raw *Amaranthus sp.* in water extraction exhibited the significantly higher (p<0.05) scavenging activity compared to the ones that are boiled and stir-fried while raw *Alternanthera sissoo* in water extraction is significantly lower than one that was boiled but higher than one that was stir-fried. On the contrary, raw *Amaranthus sp.* and *Alternanthera sissoo* in methanol extraction exhibited the lowest scavenging activity compared to both samples that were boiled and stir-fried as these cooking methods may have liberated much more antioxidant compounds from insoluble parts of the plants. Results from ANOVA of two factors with replication analysis have indicated that there is a significant difference (p=0.000) of scavenging activity between vegetables in water extraction but there is no significant difference (p=0.216) of scavenging activity between vegetables in methanol extraction. Furthermore, there is also a significant difference (p=0.000) of scavenging activity between samples in control and cooking treatments in both water extraction and 80% methanol extraction.

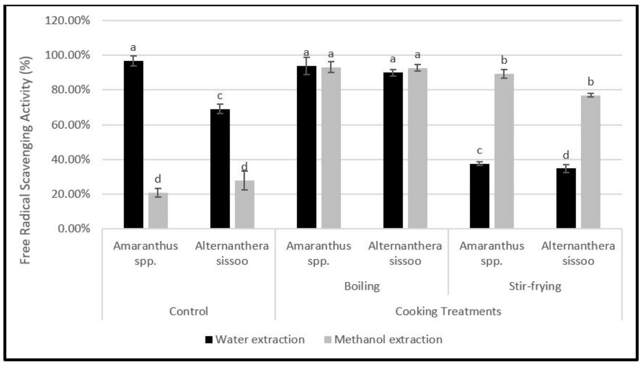

Figure 3: Scavenging activity of Amaranthus sp. and Alternanthera sissoo by using two types of extractions in control and two cooking treatments. Data obtained are the means of triplicate readings. Different letters indicate significant difference for scavenging activity within each type of plant samples where p-value < 0.05.

Total Phenolic Assay

The determination of total phenolic content of *Amaranthus sp.* and *Alternanthera sissoo* were determined spectrophotometrically by using Folin-Ciocalteu method. Accordingly, this assay was performed for both water and methanol extraction of samples. Theoretically, the total phenolic compounds present in the samples are reflected by intensity of the blue colour solution. The absorbance of the vegetable samples was expressed as mg Gallic Acid Equivalents (GAE) per g of sample after being calculated from an established standard curve.

Table II displays the results of total phenolic contents of water and methanol extraction samples for both control and treatment groups. It is shown that raw Brazilian spinach leaves exhibit the lowest total phenolic content with 3.450 mg GAE/g of the sample followed by raw Malaysian spinach leaves with 3.496 mg GAE/g of the sample for control group. Consequently, there is a significant difference between raw Brazilian spinach leaves and raw Malaysian spinach leaves samples (p=0.003). Next, boiled Brazilian spinach showed higher total phenolic content with 3.386 mg GAE/g compared with boiled Malaysian spinach with 3.312 mg GAE/g of the sample. Consequently, there is no significant difference between boiled Brazilian spinach and boiled Malaysian spinach (p=0.051). Moreover, it is also found that stir-fried Brazilian spinach with 3.394 mg GAE/g has comparably similar amount of total phenolic content compared with stir-fried spinach with 3.404 mg GAE/g. Thus, there is no significant difference between stir-fried Brazilian spinach and stir-fried spinach (p=0.811). Meanwhile, results for total phenolic content of samples for methanol extraction shows that raw Brazilian spinach exhibit the highest total phenolic content with 3.680 mg GAE/g of the sample followed by raw spinach with 3.358 mg GAE/g sample for control group, respectively. Consequently, there is a significant difference between raw Brazilian spinach and raw Malaysian spinach samples (p=0.035). Next, boiled Brazilian spinach with 3.297 mg GAE/g is slightly lower compared to boiled Malaysian spinach with 3.312 mg GAE/g. Thus, there is no significant difference between boiled Brazilian spinach and boiled Malaysian spinach (p=0.306). Next, it is shown that stir-fried Brazilian spinach with 3.419 mg GAE/g is higher than stir-fried Malaysian spinach with 3.281 mg GAE/g. Subsequently, there is a significant difference found between stir-fried Brazilian spinach and Malaysian stir-fried spinach (p=0.004).

Table II: Total phenolic content for water and methanol extraction.

Extraction	Plant sample	Total Phenolic Content (mg GAE/g sample)	*p*-value**
Water	Raw Brazilian spinach	3.450^{a}	0.035
	Raw Malaysian spinach	3.496^{a}	
	Boiled Brazilian spinach	3.389^{b}	0.051
	Boiled Malaysian spinach	3.312^{c}	
	Stir-fried Brazilian spinach	3.394^{b}	0.811
	Stir-fried Malaysian spinach	3.404^{b}	
Methanol	Raw Brazilian spinach	3.680^{a}	0.003
	Raw Malaysian spinach	3.358^{c}	
	Boiled Brazilian spinach	3.297^{d}	0.306
	Boiled Malaysian spinach	3.312^{d}	
	Stir-fried Brazilian spinach	3.419^{b}	0.004
	Stir-fried Malaysian spinach	3.281^{d}	

*Values represent mean ± standard deviation (SD) from triplicate readings.

**Significant difference is at *p*-value < 0.05.

Superscript letters within the same row indicate significant differences of means within the extracting solvent where *p*-value < 0.05.

Fig. 4 illustrates the total phenolic content of *Amaranthus sp.* and *Alternanthera sissoo* by using two types of extraction that are water and aqueous methanol in control and two cooking treatments groups which are boiling and stir-frying. Based on observation, both water and aqueous methanol demonstrated relatively similar extraction capacity. Overall, raw vegetable samples in both extraction solvents showed the highest total phenolic content than samples that underwent cooking treatments. Results from ANOVA of two factors with replication analysis have indicated that there is no significant difference (p=0.562) of total phenolic content between vegetables in water extraction but there is a significant difference (p=0.000) of total phenolic content between vegetables in methanol extraction. Furthermore, there is also a significant difference (p=0.000) of total phenolic content between samples in control and cooking treatments.

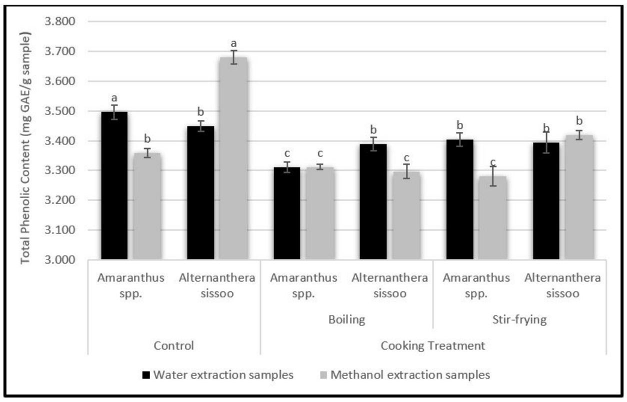

Figure 4: Total phenolic content of Amaranthus sp. and Alternanthera sissoo by using two types of extraction in control and two cooking treatments. Data obtained are the means of triplicate readings. Different letters indicate significant difference for total phenolic content within each type of plant samples where p-value < 0.05.

DISCUSSION

Antioxidant Content of *Alternanthera sissoo* and *Amaranthus sp.*

Oxidative stress in human body is capable of causing a variety of health problems such as aging, cardiovascular disease, diabetes, and cancer (21). Many epidemiological studies done in the past discovered that the consumption of vegetables rich of antioxidants is provisionally linked with several health benefits as it hinders many degenerative processes to a certain extent and can significantly reduce the incidence of cancer and cardiovascular diseases (22, 23). Supplementation of plants containing beneficial phytochemicals acting as natural antioxidant that scavenge and neutralize free radicals from body cells and tissues will prevent and reduce the damage caused by oxidation. For this reason, the study and use of natural antioxidants from plants, fruits and vegetables has garnered considerable attraction in recent years. Natural antioxidant compounds such as phenolics, carotenoids, anthocyanins and tocopherols are universally known to be found in fruits and vegetables. In particular, several kinds of spinach were evidently reported to have a high concentration of antioxidant compounds (16). Spinach is one of the most common leafy vegetables commercially available in Malaysia and consumed by majority of the population. There have been many studies conducted in the past to determine the antioxidant activity of spinach particularly *Amaranthus sp.,* yet not much information is available in the literature relating to antioxidant of Brazilian spinach. The present study was conducted to analyse the antioxidant activity of Brazilian spinach and commercially available spinach in Malaysia. The most apparent findings revealed from the analysis of the present study is that both *Amaranthus sp.* and *Alternanthera sissoo* have verified great free radical scavenging activity.

Influence of Cooking Treatment on Antioxidant Activity

Many studies have reported that cooking treatment affects the antioxidant activity of plant samples differently. These effects may depend upon the morphological and chemical characteristics of the plants (24, 25). Furthermore, Hwang et al. (26) suggested that cooking parameters such as technique, temperature, cooking duration and portion size may greatly alter the antioxidant activity of the samples. In the present study, the cooking treatment that yielded the highest scavenging activity is boiling followed by stir-frying. Even though *Amaranthus sp.* exhibited a slight decline of scavenging activity in boiling compared to control which is in agreement with previous study by Amin et al. (16), *Alternanthera sissoo* contrastingly had incremental changes. Additionally, results from ANOVA analysis verified that there is a significant difference ($p=0.000$) of scavenging activity between raw and treated samples. Other studies have also reported similar findings where antioxidant capacities on tomatoes, broccoli, spinach, cruciferous vegetables and other selected vegetables were significantly elevated after cooking treatments (27 – 31). One of the possible elucidations for this finding is that antioxidant compounds were liberated from insoluble parts of the plants like cell walls and sub-cellular compartments (27). Additionally, it is also suggested total antioxidant activity comes from the natural synergistic combination of phytochemicals (28). Moreover, it is also possible that the enhancement in antioxidant activity of *Alternanthera sissoo* after boiling process was due to thermal chemical reaction, suppression of the oxidation capacity of antioxidants by thermal inactivation of oxidative enzymes or formation of novel compounds such as Maillard reaction to antioxidant activity (26, 31). However, it is not certain to what extent that each suggested possibility factor has contributed to the incremental change in scavenging activity of *Alternanthera sissoo*.

The total phenolic assay indicated stir-frying yielded the highest number of total phenolic content compared to boiling for both plant materials as opposed to scavenging activity trend discussed earlier. This result is in parallel with a study by Hwang et al. (26), that showed stir-frying did not significantly affect the total phenolics level in plant materials whereas boiling significantly decreased the level. Additionally, the authors also affirmed that similar trend was observed for scavenging activity for their extracts as opposed to analysis of the present study. In other words, boiling can cause a significant reduction in antioxidant activity related to the loss of water-soluble polyphenols and vitamins. Moreover, this may also be due to degradation of certain types of phenolic compounds (16). For this reason, stir-frying preserved antioxidant compounds relatively better than boiling. Interestingly, *Alternanthera sissoo* showed a great scavenging activity in boiling than in raw sample extracts yet the total phenolic content proved quite the contrary which evidently suggest that other potential antioxidant compounds apart from polyphenols such as carotenoids and vitamins were mostly retained. This affirmed that a complex system of synergistical effects and distinctive symbiosis of dietary phytochemicals such as polyphenols are essential for antioxidant activity (32).

Influences of Extraction Solvents on Antioxidant Activity

Recovery of antioxidant compounds from plants, fruits or vegetables is normally performed through different method or techniques depending on their chemistry composition and distribution in the plant matrix. For instance, outer parts of fruits and grains namely epidermal and sub-epidermal layers are known to have presence of higher soluble phenolics than in inner parts such as mesocarp and pulp (33, 34). Solvent extraction is the most widely used technique for recovering plant antioxidant compounds. In the present study, water and aqueous methanol are the extraction solvents used for the investigation of antioxidant capacity. Water is often traditionally used to extract compounds while aqueous methanol was known to be effective in bioactive compound extraction for phenolics compounds (35). As antioxidant compound contained in plant material are distinctly varied in chemical characteristics and polarities, the selected extraction solvents for the present study would give better range of the extract yields and the influence of extraction solvents on antioxidant activity the plant materials.

In the present study, it is shown that water extracts had demonstrated slightly higher readings for scavenging activity and total phenolics content than methanol extracts. These results indicate water extraction samples to have excellent antioxidant or antiradical activity on DPPH free radicals which stems from the greater amount of antioxidant compounds present in them that could act rapidly with DPPH free radicals and diminish greater DPPH radical molecules. However, the findings of the present study are not consistent in notion with most other studies that reported polar solvent such as methanol or ethyl extract of plants was found to have better extraction compared to aqueous solvent. In fact, they are frequently used for the extraction of secondary metabolites of plants (polyphenols) from the innermost plant matrices. A study by Do et al. (36) stated that highest total antioxidant activity and phenolic content was obtained absolute ethanol extract while another study by Monteiro et al. (37) mentioned that methanol extracts were the most effective in scavenging DPPH. In addition, a study by Butsat and Siriamornpun (38) determined that extracting phenolic compounds from Amomum Chinese C. leaf by aqueous methanol provided a greater amount of phenolics and antioxidant activity compared to other solvent types. The findings of the present study and previous studies reflect on the variety and distribution of antioxidant compounds in with hydrophilic and lipophilic properties in which might influence the scavenging activity and total phenolics content in different types of solvent extraction. From the present data, this suggest that *Alternanthera sissoo* and *Amaranthus sp.* may have high hydrophilic antioxidant compounds such as glutathione, ascorbic acid, phenolics acid and flavanols.

CONCLUSION

The present study indicates that the extracts obtained from leafy parts of Brazilian spinach (*Alternanthera sissoo*) and Malaysian spinach cultivar (*Amaranthus sp.*) have significant free radical scavenging activity on stable DPPH and high reactive hydroxyl radical, the extent of which depends on the cooking treatments and extraction solvents. The two different cooking treatments (boiling and stir-frying) changed the antioxidant activity and the total phenolics content of both vegetables. Cooking treatment that yielded the highest scavenging activity is boiling followed by stir-frying, whereas boiling and stir-frying significantly decreased the total phenolics content of *A. sissoo* and *A. sp.* in selected available extraction solvents. In conclusion, the study's findings would serve as a database providing information for the impact of various cooking treatments on the antioxidant potential of Brazilian spinach and Malaysian spinach. In this sense, this may encourage the food industry and households to select the most appropriated cooking treatment in dietary applications to help preserve the antioxidant activity of the vegetables.

For further improvement of knowledge gap in this research, it is recommended to analyse the macronutrient and micronutrient content of the sample to provide profound insight and better view for exploitation and utilization of Brazilian spinach as alternative valuable sources of dietary fibre, functional foods, and nutraceuticals. It is desirable to employ more research which include a phytochemical screening to further explore potential antioxidant compounds in *Alternanthera sissoo* while also assigning certain antioxidant effects to specific compounds recovered from the resulting extracts. Implementation of more experimental conditions that can specifically reflect the impact of various cooking treatments on antioxidant activity *in vivo* will also be beneficial to the application of nutrients and plant secondary metabolite in the human biological system.

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