

## ANTIOXIDANT POTENTIAL OF *Fragrea acuminatissima* Merr. BY DIFFERENT EXTRACTION TECHNIQUES

Nurul Amira Buslima

Department of Agro Based Industry, Faculty of Agro Based Industry  
Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia  
Email: amira.b@umk.edu.my

Mohammad Afiq Faiz Shaharudin

Department of Agro Based Industry, Faculty of Agro Based Industry  
Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia  
Email: afiqfaiz@yahoo.com

Tang Bee Eik

Department of Agro Based Industry, Faculty of Agro Based Industry  
Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia  
Email: tangbeeik@yahoo.com

Shamsul Mohamad

Department of Agro Based Industry, Faculty of Agro Based Industry  
Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia  
Email: shamsul.m@umk.edu.my

### ABSTRACT

Developing countries are still counting on herbal medicine which aims to promote primary health care with better cultural, human acceptability and fewer side effects. *Fragrea acuminatissima* is one of underutilized herb consumed by Temiar tribe in Malaysia which able to give the similar benefits. The most important attribute in developing herbal based product is the extraction condition and selection of the technique used will potentially affect the expression of antioxidant compound in herbal plant. Technical problem arising from the extraction phase also urge the needs to elucidate the best techniques for respective herbal product. Therefore, this study was aimed to investigate the best extraction techniques (maceration, orbital shaker assisted, ultrasonic assisted and microwave assisted) on Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and antioxidant activity of *F. acuminatissima* stem and root parts by using methanol solvent. Folin-Ciocalteu method was used for determination of TPC, aluminium chloride colorimetric method for TFC, while antioxidant activity was determined by DPPH radical scavenging assay. Overall, the best extraction method of *F. acuminatissima* was ultrasonic assisted extraction which exhibited the highest percentage yield, TPC and TFC values for both plant parts. Based on the DPPH assay, *F. acuminatissima* have procured the excellent IC<sub>50</sub> of 29.954 µg/ml by ultrasonic assisted extraction for stem while maceration techniques showed the best IC<sub>50</sub> for root at 76.996 µg/ml. Positive moderate and strong correlation between TPC, TFC and DPPH was detected which support the result obtained. In brief, ultrasonic assisted technique was suggested to be used in future research of this plant for phytochemical profiling, bioactivity assays and herbal product development.

Keywords: *Fragrea acuminatissima*, extraction techniques, Total Phenolic Content (TPC); Total Flavonoid Content (TFC), DPPH radical scavenging effect.

## INTRODUCTION

Plant extracts such as of fruits, herbs, vegetables and cereals which had been scientifically proven to contain abundant of secondary metabolites are currently become a major interest in the food industry because they able to impede the oxidative degradation of macro nutrients and thus improve the quality and nutritional value of food (Žugić *et al.*, 2014). Herbs and spices have been used not only as food preservatives and flavouring, but also as traditional medicines for thousands of years (Yi & Wetzstein, 2011). These herbal medicine are being used extensively because they are considered safe since they are from natural resources (Aziz *et al.*, 2014). The usage of herbs as antioxidant in processed food is becoming very popular in food industry as an alternative to synthetic antioxidants as they are cheaper, easily consume and locally available in raw or involve only conventional medicinal preparations (Nićiforović *et al.*, 2010).

The plant species *Fragrea acuminatissima* (Loganiaceae) or locally known as Tengkok Biawak grows wild in Peninsula region of Malaysia. Their best habitats are at inland forest and lowland area. This plant is an epileptic climber and falls under the shrub tree category. *Fragrea acuminatissima* are widely been used by Temiar tribe, one of the local indigenous population in Kelantan for treatment of fever and body ache.

Currently, there are no other studies had been done on this plant especially on the antioxidant and phytochemical aspect. However, under the same family tree of Loganiaceae, there were some studies that had been conducted such as in *Anthocleista nobilis* which was claimed to be able to cure fever, diarrhea and stomach ache in West Africa (Ngwoke *et al.*, 2015). According to the researchers, antioxidant activity of stem bark showed good antioxidant activity extracted by acetone and methanol solvents as well as by using maceration technique. This finding give the vision that *F. acuminatissima* also able to exhibit the same potential as it falls under the same family of Loganiaceae.

The extraction yield of active secondary metabolites from plant materials is influenced mainly by the process of extraction with aqueous and/or organic solvent (Cacace & Mazza, 2003). Effective separation of antioxidants with high extraction yield and concentration of bioactive compound from a complex plant matrix is a difficult procedure due to co-extraction of other compound, which are undesirable in antioxidant extract (Bimakr *et al.*, 2010).

Maceration technique had been conventionally used to extract the bioactive compounds from plant material. This technique need no complicated instrumentation and involving a few simple procedures. However, the main drawback of this technique is it need longer time to complete the extraction (Azmir *et al.*, 2013). In order to increase time efficiency and extraction yield, shaking technique had been incorporated in the conventional maceration method and some studies showed better results (Amorim-Carrilho *et al.*, 2014). Other than that, ultrasound has been recognized for potential industry application in the phyto-pharmaceutical extraction for a wide range of herbal extract as it creates forces that destruct cell walls mechanically and enhance material transfer (Truta *et al.*, 2010). Simple, inexpensive and efficient ultrasonic assisted extraction have major benefits over conventional techniques as it has faster kinetic and able to increase the extraction yield (Wang & Weller, 2006). Microwave assisted extraction has been also considered as a potential alternative to solid-liquid extraction of the metabolites from plants. It has been used to extract nutraceutical for various reason such as, reduce solvent usage, improve extraction yield and reduce extraction time (Wang & Weller, 2006). Heating effect which occurs due to the dipole rotation of the solvent in microwave field are generally attributed of enhancement of product recovery where as the solvent temperature rise, the solubility of the compound of interest will increase (Zaheer, 2011).

Various extraction techniques had been innovated mainly to increase the efficiency and ease the extract production. However, different extraction techniques might exhibit different number of potential antioxidant compound in respective plant. Since there are scarcity of information regarding the antioxidant potential of this underutilized plant, this preliminary study was conducted over different extraction techniques on root and stem of *F. acuminatissima* by using total antioxidant content and activity assays.

## MATERIALS AND METHODS

### Materials

Chemical used in this study were Folin-ciocalteu reagent, NaH<sub>2</sub>PO<sub>4</sub>, Na<sub>2</sub>HPO<sub>4</sub>, sodium carbonate, aluminium trichloride, gallic acid, trichloroacetic acid, potassium persulfate ascorbic acid and methanol as supplied by Merck,

Germany. Whereas quercetin, gallic acid and 2,2-diphenyl-picrylhydrazyl (DPPH) were obtained from Sigma Aldrich, USA. Plant samples were collected at Kuala Betis, Gua Musang in Kelantan, Malaysia.

## Methods

### Sample preparation

The samples were separated into stem and root parts, thoroughly washed and blot dried. Then, samples were course ground into flakes and dried in the electric oven (Lab Companion, Korea) for 3 days at 50 °C. The dried samples were further ground to fine powder using electrical grinder (Panasonic, Japan) and kept in 4 °C chiller prior extraction.

### Sample extraction

Sample extraction was done by using simple maceration technique, and assisted with orbital shaker (Lab Companion, Korea), ultrasonic bath (Lab Companion, Korea) and microwave oven (Sharp, Japan) according to the methods described by Savita & Prakashchandra (2011) with a slight modification. Overall, 50 g of powdered samples were mixed with 250 ml of 95 % methanol. Then, mixtures were proceeded for incubation by using incubation protocols stated in Table 1. After the incubation period, the mixtures were filtered by Whatman no. 4 filter paper and samples were extracted again using the same protocols. Then, the solutions obtained were pooled and evaporated to obtain the semi solid crude sample, respectively.

Table 1: Incubation protocols of different extraction techniques

Techniques	Temperature	Period	Condition
Maceration	Room temperature	24 hours	-
Orbital Shaker Assisted (OAE)	Room temperature	3 hours	Shaking frequency at 150 rpm
Ultrasonic Assisted (UAE)	Room temperature	1 hour	Medium sonication (17 kHz sound wave)
Microwave Assisted (MAE)	-	15 minutes	Power of 90 W

### Total Phenolic Content

Total phenolic content was determined by using Folin-Ciocalteu assay based on method described by Singleton & Rossi (1965) with slight modification by Ghazi *et al.* (2012). Folin-Ciocalteu reagent was prepared by diluting the stock reagent in distilled water with ratio of 1:10. Then, 50 µl of sample extract at concentration of 1 mg/ml were mixed with 250 µl of 10 % Folin-Ciocalteu reagent in the test tubes. The mixtures were incubated for 5 minutes at room temperature and 750 µl sodium carbonate were added into the test tube. The mixtures were shaken and incubated in the dark for 2 hours at room temperature. Then, absorbance values were recorded at 765 nm using UV-Vis spectrophotometer (Thermoscientific, USA). Gallic acid was used as a standard following the similar procedures and graph was constructed in range of 6.25 to 400 µg/ml. Linear equation obtained was used to calculate the total phenolic content and values were expressed as µg gallic acid equivalent (GAE)/mg extract.

### Total Flavonoid Content

Total flavonoid content was evaluated by using aluminium chloride colorimetric method described by Christ & Muller (1960) with slight modification by Maisarah *et al.* (2013). Briefly, 0.3 ml of sample extracts at concentration of 1 mg/ml were mixed with 3.4 ml of 30 % aqueous methanol, 150 µl sodium nitrite solution (0.5 M) and 150 µl aluminium chloride solution (0.3 M). After 3 minutes, 1 ml of NaOH solution (1 M) was added. Then, absorbance values were measured at 506 nm against blank. Standard graph of quercetin was constructed in range of 50 to 1600 µg/ml following the similar procedures and linear equation was obtained. Total flavonoid content of plant extracts were calculated and expressed as µg quercetin equivalent (QE)/mg extract.

### Free Radical Scavenging activity

Free radical scavenging activity was done by using 2,2-diphenyl-picrylhydrazyl (DPPH) reagent following the method described by Brand-Williams *et al.* (1995) with slight modification by Gan *et al.* (2013). DPPH solution was prepared

by diluting in 95 % methanol at concentration of 0.1 mM. Sample extracts were prepared in 95 % methanol at different concentrations ranged from 6.25 µl/ml to 400 µl/ml. Amount of 2 ml of diluted extracts were added with 2 ml DPPH solution and incubated in the dark for 30 minutes. Then, absorbance values were measured at 517 nm using UV-Vis spectrophotometer against blank. Control containing 2 ml of 95 % methanol and 2 ml DPPH was prepared following the similar procedures. Scavenging activity was calculated according to following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left[ \frac{(\text{Ac}-\text{As})}{\text{Ac}} \right] \times 100,$$

where Ac is the absorbance of the control and As is the absorbance of the sample extracts. Graph of scavenging activity was plotted and inhibition concentration at 50 % (IC<sub>50</sub>) was estimated. Ascorbic acid was used as standard comparison.

### Statistical Analysis

Data were reported as mean ± standard deviation using triplicate measurement values. Statistical analysis was done using Statistical Packages for Social Science (SPSS) version 20.0 (IBM Corp., USA). One-way analysis of variance (ANOVA) and Duncan's test were performed to compare the mean between samples. Pearson correlation coefficient was done to correlate between antioxidant activity and content. Data were considered significant differences at  $p \leq 0.05$ .

## RESULT AND DISCUSSION

### Sample Extraction

The ability of different extraction techniques in exhibiting the antioxidant potential were investigated. In brief, the time of extraction was ranged from 15 minutes to 24 hours, where MAE had the lowest extraction time while maceration technique had the highest extraction time. MAE, UAE and OAE are the instrumental techniques while maceration extraction is only using gravitational force. After solvent removal, the colour produced by all crude extracts was dark brown in colour and the extracted condition were in highly viscous or semi solid state.

Table 2 shows the extraction yield of *F. acuminatissima* stem and root by using different extraction techniques. From the result, the UAE for both stem and root of *F. acuminatissima* showed the highest yield, meanwhile the lowest yield for stem extract was obtained through maceration and MAE technique for the root extract.

Table 2: Extraction yield of stem and root of *F. acuminatissima*

Extraction Techniques	Stem			Root		
	Raw (g)	Yield (g)	Yield (%)	Raw (g)	Yield (g)	Yield (%)
Maceration	50	2.26	4.53	50	3.81	7.62
Orbital Shaker Assisted (OAE)	50	3.20	6.40	50	3.26	6.52
Ultrasonic Assisted (UAE)	50	3.93	7.87	50	4.82	9.65
Microwave Assisted (MAE)	50	2.93	5.86	50	3.13	6.27

Ultrasonic assisted extraction decreases the inner and external mass transfer limitation, thus gaining the yield percentage (Gimbun *et al.*, 2014). A comparison study on different extraction techniques in yellow tea had suggested that ultrasound assisted extraction could successfully have been used for extraction of polyphenol and methylxanthines thus prove that this technique provide more energy efficient (Horžić *et al.*, 2012). Although microwave assisted extraction have shown higher percentage of recovery effect of anthraquinonines extraction but after 60 minutes of extraction time, the yield become plateau and constant (Hemwimon *et al.*, 2007).

### Total Phenolic Content (TPC)

TPC of stem and root of *F. acuminatissima* at different extraction techniques were estimated by Folin-Ciocalteu's reagent and using gallic acid as standard. The graph of gallic acid concentration ranged from 6.25 µg/ml to 400 µg/ml at 750 nm was plotted with a regression co-efficient ( $R^2$ ) = 0.9967 and linear equation of  $y = 0.008x + 0.078$ . The absorbance was steadily increased with the incremental of concentration.



Table 3 shows TPC content of plant parts at different extraction techniques. The highest TPC for the both stem and root parts were obtained from UAE and the lowest TPC for both stem and root parts were acquired from MAE. Statistical analysis showed that there were significant differences between the TPC value of entire sample ( $p < 0.05$ ).

Table 3: Total Phenolic Content of stem and root of *F. acuminatissima* at different extraction techniques

Extraction Techniques	Total Phenolic Content ( $\mu\text{g GA /mg extract}$ )	
	Stem	Root
Maceration	205.33 $\pm$ 0.78 <sup>g</sup>	137.99 $\pm$ 0.33 <sup>b</sup>
Orbital Shaker Assisted (OAE)	188.63 $\pm$ 0.13 <sup>f</sup>	156.13 $\pm$ 0.63 <sup>c</sup>
Ultrasonic Assisted (UAE)	206.88 $\pm$ 0.13 <sup>h</sup>	184.33 $\pm$ 0.20 <sup>e</sup>
Microwave Assisted (MAE)	171.00 $\pm$ 0.13 <sup>d</sup>	83.46 $\pm$ 0.20 <sup>a</sup>

\* The values are means  $\pm$  standard deviation ( $n = 3$ ). Values with different superscripts were significantly different by Duncan test at level of  $p < 0.05$ .

The findings were supported by other study where ultrasonic assisted extraction technique also showed higher yield of phenolic compounds in *Nigella sativa* crude extract (Gimbun *et al.*, 2014). Cavitation phenomena in ultrasonic was possibly occurred by propagation of ultrasound pressure waves through the solvent and the plant materials (Shirsath *et al.*, 2012). Phenolic component reacts differently depending on the extraction condition and composition (Ksouri *et al.*, 2008). Therefore, antioxidant capabilities are influenced by several factors that cannot be evaluated by single condition where other criterias such as solvent extracting power, duration of the extraction and their interaction are also important (Falleh, 2012). Many researchers also noted that TPC assay are very not specific to phenols and also sometimes oxidized another component (Escarpa & Gonzalez, 2001).

#### Total Flavonoid Content (TFC)

TFC for stem and root of *F. acuminatissima* at different extraction techniques were measured with the aluminium chloride colorimetric assay using quercetin as standard. The graph of quercetin concentration ranged from 50  $\mu\text{g/ml}$  to 1600  $\mu\text{g/ml}$  at 510 nm was plotted with a regression co-efficient ( $R^2$ ) = 0.9802 and linear equation of  $y = 0.0003x + 0.0379$ .

Table 4 showed TFC of stem and root of *F. acuminatissima* at different extraction techniques. The highest TFC for the extraction of *F. acuminatissima* stem was obtained by using UAE technique and the lowest TFC was gained through extraction using maceration technique. Meanwhile, for the root part, the highest TFC was obtained from ultrasonic UAE technique and OAE technique showed the lowest TFC value. Overall, there were significant differences between the TFC value of entire sample ( $p < 0.05$ ). However, further analysis using Duncan test showed no significant different between TFC in UAE and MAE of root part.

Table 4: Total Flavonoid Content of stem and root of *F. acuminatissima* at different extraction techniques

Extraction Techniques	Total Flavonoid Content ( $\mu\text{g QE/mg extract}$ )	
	Stem	Root
Maceration	479.67 $\pm$ 3.33 <sup>c</sup>	449.67 $\pm$ 3.3 <sup>b</sup>
Orbital Shaker Assisted (OAE)	581.89 $\pm$ 5.09 <sup>e</sup>	443.00 $\pm$ 3.33 <sup>a</sup>
Ultrasonic Assisted (UAE)	873.00 $\pm$ 3.33 <sup>g</sup>	566.33 $\pm$ 1.92 <sup>d</sup>
Microwave Assisted (MAE)	609.81 $\pm$ 3.33 <sup>f</sup>	563.00 $\pm$ 3.33 <sup>d</sup>

\* The values are means  $\pm$  standard deviation ( $n = 3$ ). Values with different superscripts were significantly different by Duncan test at level of  $p < 0.05$ .

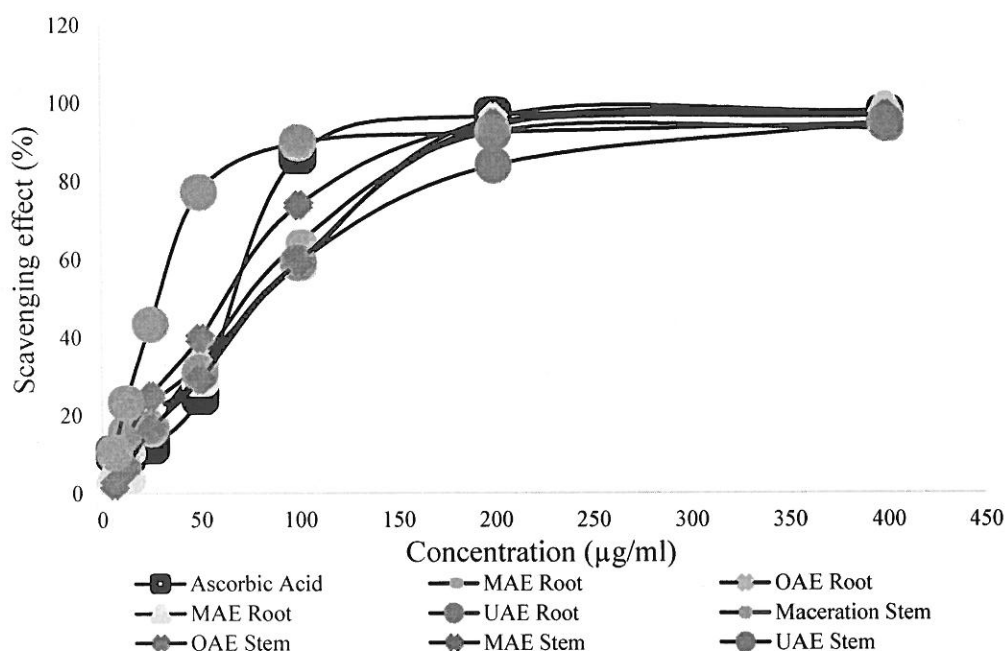
This higher efficiency of UAE in exhibiting the flavonoid compounds could be attributed to acting sonic vibration, which produce particle size reduction and cell disruption. Moreover, the ultrasonic vibration towards solid's surface lead to greater contact area between solid and liquid phase (Chen *et al.*, 2007). Ultrasonic assisted extraction gives the highest extraction yield of some flavonoid components such as- tectoridin, iristectorin A, iristectorin B, tectorigenin, iris-tectorigenin A and total isoflavones in shorter time next to soxhlet extraction and maceration extraction (Sun *et al.*, 2011). However, study done by Gimbun *et al.* (2014), showed otherwise where, maceration techniques revealed higher

yield of TFC when compared to UAE. This results support the previous statement by Falleh (2012) which stated that there are other extraction conditions that need to be considered in order to exhibit the antioxidant capabilities.

### DPPH Radical Scavenging Assay

Figure 1 shows the graph of DPPH scavenging effect of ascorbic acid and different parts of *F. acuminatissima* at different extraction techniques. Generally, standard and samples showed a plateau pattern starting from concentration of 200 µg/ml except for ultrasonic assisted root which only reached the plateau stage at 400 µg/ml.

Figure 1: DPPH scavenging activity of ascorbic acid and samples at different plant parts and extraction techniques



This study revealed that all samples had exhibited  $IC_{50}$  value of DPPH radical scavenging effect as showed in Table 5. The value of  $IC_{50}$  was ranging from 29.33 µg/ml to 82.00 µg/ml for stem extracts where, UAE was found to be the best and OAE was the weakest extract to scavenged 50 % of DPPH free radicals. Other than that,  $IC_{50}$  of root extract ranged from 74.17 µg/ml to 82.51 µg/ml where, extract from maceration technique need lowest amount of concentration to obtain 50 % scavenging effect and MAE need much higher concentration. Meanwhile, the standard ascorbic acid showed the  $IC_{50}$  value of 70.483 µg/ml. Generally, all the samples were statistically different at  $p < 0.05$ . Despite of that, further Duncan analysis showed there were no significant differences between OAE and UAE of root extracts as well as between OAE of stem extract and MAE of root extract.

Table 5:  $IC_{50}$  of *F. acuminatissima* stem and root

Extraction Techniques	$IC_{50}$ (µg/ml)	
	Stem	Root
Maceration	62.83 ± 0.577 <sup>c</sup>	74.17 ± 0.577 <sup>d</sup>
Orbital Shaker Assisted (OAE)	82.00 ± 0.500 <sup>f</sup>	81.00 ± 0.500 <sup>e</sup>
Ultrasonic Assisted (UAE)	29.33 ± 0.289 <sup>a</sup>	80.17 ± 0.289 <sup>e</sup>
Microwave Assisted (MAE)	50.67 ± 0.577 <sup>b</sup>	82.51 ± 0.500 <sup>f</sup>

\* The values are means ± standard deviation (n = 3). Values with different superscripts were significantly different by Duncan test at level of  $p < 0.05$ .

DPPH assay is widely being used in the determination of free radical scavenging activity of natural antioxidants, mainly due its conventional reproducibility and high sensitivity (Wijekoon *et al.*, 2011). Ultrasonic assisted extraction does not have long extraction time compared to other parameters, as ultra-sonication could induce free radical's formation within the liquid medium thus causing degradation and oxidation of the sample (Hemwimon *et al.*, 2007).

The lower the value of  $IC_{50}$  indicated more strength of the antioxidant potential. According to the current and previous findings, the antioxidant activity of the extracts also varies from one to another (Alothman *et al.*, 2009). There are two factors that affect the result collected by the antioxidant assays such, some phytochemicals available in the extracts may contain molecular weight antioxidants or antioxidants bound to complex molecules and some phenolic compound might not possess the antioxidant strength (Gursoy *et al.*, 2009).

### Correlation between TPC, TFC and DPPH radical scavenging assay

Table 6 shows the correlation of TPC, TFC and DPPH radical scavenging activity assay analysed by using Pearson's correlation coefficient test. Statistically, the correlations between all antioxidant content and activity were significantly different. From the table, TPC r-value incated a positive and moderate correlation with DPPH, meanwhile, TFC showed a positive and strongly correlated with DPPH activity.

Table 6: r-value of Pearson Correlation Test

	DPPH Radical Scavenging Activity
Total Phenolic Content	0.478*
Total Flavonoid Content	0.841**

\* Correlation is significant at the level  $p < 0.05$ .

\*\* Correlation is significant at  $p < 0.01$ .

The medium correlation between TPC and DPPH assay was possibly caused by the present of non-phenolic compound, where Folin-Ciocalteu reagent also react with simple phenol which is not compelling antioxidant. Moreover, distinctive phenolic component may also show different antioxidant activity depending on their structure, with the present of other compound that included in the crude extract (Nićiforović *et al.*, 2010).

Moderate and slightly strong correlation coefficient between total phenolic and flavonoid content with DPPH assay respectively might be due to the instability of DPPH free radical. Previous research by Molyneux (2004) described that the stability of DPPH solution can last for 3 days. Nevertheless, this hypothesis was considered inaccurate due to the inconsistency of the results. Therefore, preparation of fresh DPPH-methanol stock solution is recommended to be prepared before an experiment is conducted.

In brief, the ultrasonic assisted extraction process proved to produce higher amount of TPC and TFC for both stem and root part of *F. acuminatissima*. This is probably because during sonication, production of sound waves caused the generation of bubbles (pit), close to the tissue of the plant cell, cleaved and disrupt the cell walls, thus caused the solvent to penetrate and thereby releasing the contents of cells. Besides, sonication also accelerates the movement of the molecules, thus bringing together the molecules of solvent with those of the sample (Roidaki *et al.*, 2015).

Microwave assisted extraction method is not a good option for extracting antioxidant compounds in *F. acuminatissima*, where high temperature may have caused enzymatic oxidation and led to the denaturation, or destruction of plant secondary metabolites (Khoddami *et al.*, 2013). Besides, other operational conditions (e.g., high extraction pressure) of microwave assisted extraction may modify the chemical structures of the target compounds (Zhang *et al.*, 2011). Acceleration of chemical reactions or changes of some target metabolites caused by microwave irradiation (Ghani *et al.*, 2008).

### CONCLUSION

This study demonstrated that *Fragrea acuminatissima* able to reveal significant amount of antioxidant compounds and activity by using different extraction techniques. Suprisingly, all findings on TPC and TFC showed that UAE had the highest antioxidant content. Similar result was also found in antioxidant activity of stem sample. Hence, it can be concluded that UAE is a highly efficient technique not only good in extract yield but also excellent in revealing the antioxidant capacities. In addition, recent findings also support the principles of this technique which able to minimize

the extraction time and solvent usage as compared to the conventional techniques. In contrast, despite of shorter extraction time and solvent usage, MAE was proved to be a poor method for crude extraction of *F. acuminatissima* stem and root, with respect to extraction efficiency and antioxidant capacity.

Since there are a lot of other factors that may influence the potential of antioxidant, further research on UAE extract should be investigated on the other extraction condition such as solvent extracting power, duration of the extraction and compounds interaction. Moreover, water based extract had been increasing in interest of natural product developers respect to the safety reason to commercialize the plant. Not to mention, further research is needed to delve into the major or individual polyphenolic group and other bioactive compound of *Fragrea acuminatissima* stem and root.

## ACKNOWLEDGEMENT

This research was financially funded by Research Aculturism Grant Scheme (RAGS) from Ministry of Higher Education, Malaysia.

## REFERENCES

- Alothman, M., Bhat, R., & Karim, (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry*, 115(3), 785–788.
- Amorim-Carrilho, K. T., Cepeda, A., Fente, C., & Regal, P. (2014). Review of methods for analysis of carotenoids. *TrAC Trends in Analytical Chemistry*, 56, 49-73.
- Aziz, M. M., Raza, M. A., Saleem, H., Wajid, M., Bashir, K., & Ikram, M. (2014). Medicinal values of Herbs and Plants. Importance of Phytochemical evaluation and Ethnopharmacological Screening : An Illustrated review essay, *Journal of Pharmaceutical and Cosmetic Sciences* 2(1), 6–10.
- Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., ... & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 117(4), 426-436.
- Bimkr, M., Abdul, R., Saleena, F., Ganjloo, A., Salleh, L., Selamat, J. Zaidul, I. S. M. (2010). Food and Bioproducts Processing Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint leaves (*L. Mentha spicata*). *Food and bioproducts processing*, 89(1), 67–72.
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science Science and Technology*, 28(1), 25-30.
- Cacace, J. E., & Mazza, G. (2003). Mass transfer process during extraction of phenolic compounds from milled berries. *Journal of Food Engineering*, 59(4), 379–389.
- Chen, F., Sun, Y., Zhao, G., Liao, X., Hu, X., Wu, J., & Wang, Z. (2007). Optimization of ultrasound-assisted extraction of anthocyanins in red raspberries and identification of anthocyanins in extract using high-performance liquid chromatography-mass spectrometry. *Ultrasonics Sonochemistry*, 14(6), 767–778.
- Christ, B., & Muller, K. H. (1960). Determination of the amount of flavonol derivatives in drugs. *Archives of Pharmacol Research*, 293, 1033-1042.
- Escarpa, A. & Gonzalez, M. C. (2001). Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic and spectrophotometric methods, 427, 119–127.
- Falleh, H., Ksouri, R., Lucchessi, M. E., Abdelly, C., & Magné, C. (2012). Ultrasound-assisted extraction: Effect of extraction time and solvent power on the levels of polyphenols and antioxidant activity of *Mesembryanthemum edule* L. Aizoaceae shoots. *Tropical Journal of Pharmaceutical Research*, 11(2), 243–249.
- Gan, C. H., Nurul Amira, B., & Asmah, R. (2013). Antioxidant analysis of different types of edible mushrooms (*Agaricus bisporous* and *Agaricus brasiliensis*). *International Food Research Journal*, 20(3), 1095-1102.
- Ghani, S. B. A., Weaver, L., Zidan, Z. H., Ali, H. M., Keevil, C. W., & Brown, R. C. D. (2008). Microwave-assisted synthesis and antimicrobial activities of flavonoid derivatives. *Bioorganic & Medicinal Chemistry Letters*, 18, 518-522.
- Ghazi, F., Rahmat, A., Yassin, Z., Ramli, N. S., & Buslima, N. A. (2012). Determination of total polyphenols and nutritional composition of two different types of *Ficus carica* leaves cultivated in Saudi Arabia. *Pakistan Journal of Nutrition*, 11(11), 1061.
- Gimbun, J., Ishak, N. F., Muhammad, N. I. S., Pang, S. F., Kadir, M. A. A., Ramli, H., ... & Khadisah, Z. (2014). Ultrasonic assisted extraction polyphenols and antioxidant from *N. Sativa* Seed, *Journal of Engineering and Technology*. 5(2), 17–26.



- Gursoy, N., Sarikurkcu, C., Cengiz, M., & Solak, M. H. (2009). Antioxidant activities, metal contents, total phenolics and flavonoids of seven *Morchella* species. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, 47(9), 2381–8.
- Hemwimon, S., Pavasant, P., & Shotipruk, A. (2007). Microwave-assisted extraction of antioxidative anthraquinones from roots of *Morinda citrifolia*, 54, 44–50.
- Horžić, D., Jambrak, A. R., Belščak-Cvitanović, A., Komes, D., & Lelas, V. (2012). Comparison of Conventional and Ultrasound Assisted Extraction Techniques of Yellow Tea and Bioactive Composition of Obtained Extracts. *Food and Bioprocess Technology*, 5(7), 2858–2870.
- Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, 18(2), 2328–2375.
- Ksouri R, Megdiche W, Falleh H, Trabelsi N, Boulaaba M, Smaoui A, Abdelly C. (2008). Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of *Tunisian halophytes*. *Comptus Rendus Biologie*, 331, 865–873.
- Maisarah, A. M., Amira, B. N., Asmah, R., & Fauziah, O. (2013). Antioxidant analysis of different parts of *Carica papaya*. *International Food Research Journal*, 20(3), 1043–1048.
- Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, 26(2), 211–219.
- Ngwoke, K., Anusi, I., Eze, P., Okezie, U., Abba, C., & Abonyi, D. (2015). Phytochemical and Antioxidant Properties of Stem Bark Extracts of *Anthocleista nobilis*. *European Journal of Medicinal Plants*, 8(2), 107–111.
- Nićiforović, N., Mihailović, V., Mašković, P., Solujić, S., Stojković, a., & Muratspahić, D. P. (2010). Antioxidant activity of selected plant species; potential new sources of natural antioxidants. *Food and Chemical Toxicology*, 48(11), 3125–3130.
- Roidaki, A., Zoumpoulakis, P. G., & Proestos, C. (2015). Comparison of Extraction Methods for the Determination of Antioxidant Activity in Extracts of *Hippophae Rhamnoides* L. and *Lippia Citriodora*. *The Effect of Seasonal Collection*. *Austin Journal of Nutrition and Food Science*, 3(1), 1057.
- Savita K & Prakashchandra K. (2011). Optimization of extraction conditions and development of a sensitive HPTLC method for estimation of wedelolactone in different extracts of *Eclipta alba*. *International Journal of Pharmaceutical Science and Drug Research*, 3(1), 56–61.
- Shirsath, S. R., Sonawane, S. H., & Gogate, P. R. (2012). Chemical Engineering and Processing: Process Intensification Intensification of extraction of natural products using ultrasonic irradiations — A review of current status. *Chemical Engineering & Processing: Process Intensification*, 53, 10–23.
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144–153.
- Sun, Y., Liu, Z., & Wang, J. (2011). Ultrasound-assisted extraction of five isoflavones from *Iris tectorum* Maxim. *Separation and Purification Technology*, 78(1), 49–54.
- Truta, D. M., Tofana, M., & Socaci, S. A. (2010). The influence of two extraction methods of basil volatile compounds on the aroma profile of apple vinegar. *Journal of Agroalimentary Processes and Technologies*, 16(2), 159–162.
- Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science and Technology*, 17(6), 300–312.
- Wijekoon, M. M. J. O., Bhat, R., & Karim, A. a. (2011). Effect of extraction solvents on the phenolic compounds and antioxidant activities of bunga kantan (*Etilingera elatior* Jack.) inflorescence. *Journal of Food Composition and Analysis*, 24(4-5), 615–619.
- Yi, W., & Wetzstein, H. Y. (2011). Effects of drying and extraction conditions on the biochemical activity of selected herbs. *Horticulture Science*, 46(1), 70–73.
- Zaheer Z. (2011). Optimization of extraction process and phytochemical investigations of *Spathodea campanulata* flowers. *African Journal of Pharmacy and Pharmacology*, 5(20), 2226–2231.
- Zhang, H. F., Yang, X. H., & Wang, Y. (2011). Microwave assisted extraction of secondary metabolites from plants: current status and future directions. *Trends in Food Science & Technology*, 22(12), 672–688.
- Žugić, A., Đorđević, S., Arsić, I., Marković, G., Živković, J., Jovanović, S., & Tadić, V. (2014). Antioxidant activity and phenolic compounds in 10 selected herbs from Vrujci Spa, Serbia. *Industrial Crops and Products*, 52, 519–527.