



Comparison of Microwave and Ultrasonic Assisted Extraction of Kaempferol from Cassia Alata

Yi Ling Yeong^{1,2}, Sook Fun Pang³, Sin Yee Chong², Jolius Gimbut^{1,2*}

¹Centre of Excellence for Advanced Research in Fluid Flow, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia.

²Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia

³Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia.

*Corresponding author E-mail: jolius@ump.edu.my

Abstract

The effect of auxiliary energy to the kaempferol extraction yield from *Cassia alata* is presented in this paper. The effect of auxiliary energy ranging from 0.045 to 20 W/ml was studied by performing extraction using ultrasonic assisted extraction (UAE), maceration (ME) and microwave assisted extraction (MAE). A ultra-performance liquid chromatography coupled photodiode array (UPLC-PDA) was used for kaempferol identification and quantification. Matching of residence time and UV spectrum between the authentic standard and plant extract was observed to confirm the presence of kaempferol. It was found that MAE method has the highest extraction yield of kaempferol at 21.55 mg/g DW followed by UAE (18.60 mg/g DW) and ME (12.01 mg/g DW). Besides that, the extraction time of MAE was the shortest which is only 4 minutes. The optimum conditions to obtain a high kaempferol yield was achieved using 100% ethanol to extract *C. alata* powdered leaves with PSD $\leq 125\mu\text{m}$ and plant solid to solvent ratio of 1:20. The findings in this work may serve as a useful guide to obtain highest extraction yield of kaempferol from *C. alata*.

Keywords: Auxiliary energy; *Cassia alata*; gelenggang; kaempferol; microwave assisted extraction.

1. Introduction

Cassia alata (ver. name: candlestick cassia) is a shrub with flower resembling a yellow candlestick that often grows in the tropics such as Malaysia, India and Indonesia. The plant was used for generation in many countries such as Malaysia, India and Indonesia for skin problems therapy, including eczema, ringworm and itching [1-4]. Juice from *C. alata* leaves is used for skin treatments in Nigeria, whereas in South America the plant is used to treat fever, asthma, skin infections and stomach problems. A decoction from the plant leaves is used to cure high blood pressure in Africa, and traditionally used to hasten childbirth in Thailand. *C. alata* also used for treatment of poisonous bites and venereal diseases in India [4]. The leaf extract has many benefits such as antifungal, anti-inflammatory, antibacterial and antioxidant functions. Owing to its medicinal properties, it has been used in the formulation of skin care products [3]. The aforementioned medical effect is due to the existence of bioactive components in the leaf of *C. alata*. The main bioactive components of *C. alata* are anthraquinones such as chrysophanol, rhein, physcion, aloe-emodin, emodin and polyphenols such as kaempferol. These anthraquinones act as an antifungal, antioxidant, antimicrobial, antitumor and cytotoxic. Apart from that, various studies show kaempferol has anticancer properties [2]. *C. alata* is accepted for primary health care programmes by the World Health Organization and also included in the Thai National List of Essential Drugs as herbal medicines according to Drug Act B.E. 2510 [5] owing to its phytomedicine effect. The bioactive component from *C. alata* can be extracted before it can be routinely used for phytomedicinal purpose. The

extraction method affects the quality, recovery and yield of the bioactive components in the extract [4].

In the past, researchers employed maceration and soxhlet extraction to extract bioactive components from *C. alata* [6-8]. Both the maceration and soxhlet extraction are a conventional and traditional method which requires longer extraction time. Besides that, thermal degradation of bioactive components may occur due to the inherently long exposure to higher temperature process in the conventional extraction method. Ultrasonic Assisted Extraction (UAE) is newer method that enhanced extraction by reducing the both internal and external mass transfer limitation of bioactive components from the plant matrix and in the solute. The sonication breaks the cell membrane of the plant and hence facilitates release of bioactive components to the bulk liquid [9]. In the Microwave Assisted Extraction (MAE), interaction between the microwave and the polar molecules in the extraction media cause a rapid increase in temperature and pressure causing the plant cell to burst and enhance the release of the bioactive component from the plant cells [10]. MAE is a more efficient extraction method for bioactive components compared to maceration, soxhlet and UAE because it has shorter duration [4]. Yet, there is no previous study on the MAE application to *C. alata*, and therefore this is one of the objectives of this work. The main difference between the conventional method, i.e. ME with the newer extraction method e.g. MAE and UAE are the auxiliary energy used. Hence, the effect of auxiliary energy used on the yield of kaempferol extraction is elucidated in this work.

2. Material and Methods

2.1. Chemicals and Plant Materials

The standard reference for kaempferol was obtained from Sigma Alrich (St. Louis, MO), 0.1% formic acid was obtained from Merck (Darmstadt, Germany), whereas HPLC grade acetonitrile and ethanol were obtained from Fisher Scientific (Pittsburgh, PA). *C. alata* plant used in this experiment was collected from Paloh Hinai, Sg. Pahang. The freshly collected *C. alata* leaves are washed and dried in an oven at 40 °C for 4 days before ground into powder with size ranging from about 100 µm to 2 mm. The ground plant is sieved into several size classes i.e. ≤ 125 µm, 125 µm to ≤ 500 µm, 500 µm to ≤ 800 µm and ≥ 800 µm. The moisture content of the plant powder was determined using a moisture analyzer (AND MS-70, Japan) and then kept in an air tight plastic bag at room temperature to prevent moisture absorption before further use.

2.2. Ultrasonic Assisted Extraction (UAE)

The extracts were prepared by using ultrasonic processor Qsonica Sonicators Q700 (Newtown, USA) equipped with a standard probe. *C. alata* dried powder with particle size ranging from 125 to 500 was weighed accurately. 50 mL of solvent (50% methanol or 50% ethanol) was added into weighted powder (dry weight) at the solid to solvent ratio of 1:10 as reported in [11]. The mixture was sonicated at the amplitude of 90% for the extraction time of 10 min. Once the suitable solvent type is determined, it will be employed in the study of particle size (less than 125 µm, 125-500 µm, 500-800 µm and more than 800 µm), solvent ratio (0%, 20%, 50%, 70% and 100%), solid to solvent ratio (1:5, 1:10, 1:15, 1:20 and 1:25), extraction time (5min, 10 min, 15min, 20min and 25min) and amplitude (10%, 30%, 50%, 70% and 90%). These parameters are determined based on the literature concerning on UAE in extracting polyphenols of *C. alata* and limitation of the equipment. After extraction, the supernatant was separated using a refrigerated (4 °C) centrifuge (Eppendorf 5810 R, Hamburg, Germany) at 10000 rpm for 15 min. The samples were filtered using 0.22 µm nylon syringe filter and stored in a -80 °C freezer to prevent the degradation of bioactive components prior to UPLC analysis.

2.3. Microwave Assisted Extraction (MAE)

The extracts were prepared by using SP-Microwave (CEM) which is controlled by Synergy™ software. The best solvent, optimum particle size, optimum solid-to-solvent ratio and optimum solvent ratio were determined then employed in the study of MAE. The extraction time (1min, 2min, 3min, 4min and 5 min) and microwave irradiation power (10W/ml, 20W/ml, 30W/ml, 40W/ml and 50W/ml) were studied. These parameters are determined based on literature for MAE of polyphenols from leafy plant and the limitation of the equipment. After extraction, the supernatant was separated using a refrigerated (4 °C) centrifuge (Eppendorf 5810 R, Hamburg, Germany) at 10000 rpm for 15 min. Subsequently, the samples were filtered using 0.22 µm nylon syringe filter and stored in a -80 °C freezer to prevent the degradation of bioactive components prior to UPLC analysis.

2.4. Maceration (ME)

The best solvent, optimum particle size, optimum solid-to-solvent ratio and optimum solvent ratio were determined then employed in the study of ME. Maceration was performed by continuous constant stirring with solvent heated to 70 °C for 2 hours in Water Bath Memmert. After extraction, the supernatant was separated using a refrigerated (4 °C) centrifuge (Eppendorf 5810 R, Hamburg, Germany) at 10000 rpm for 15 min. Then, The samples were filtered using 0.22 µm nylon syringe filter and stored in a -80 °C

freezer to prevent the degradation of bioactive components prior to UPLC analysis.

2.5. Identification and Quantification of Kaempferol Using UPLC-PDA

A UPLC-PDA analysis was performed using a Water Acquity UPLC™ H-class fitted with photodiode array detector. A Luna Omega C18 Column 100A (100 x 2.1mm, 1.6µm inner diameters) fitted with a column guard (SecurityGuard Ultra Cartridge) were used. The mobile phase consists of solvent A (0.1 % formic acid in water) and solvent B (0.1 % formic acid in acetonitrile). The performed elution scheme were: 0-4min, 80-62% A; 4-7min, 62-49% A; 7-13.5 min, 49-15% A and reconditioning the column with 80% A isocratic for 4 min. The column temperature was maintained at room temperature with an injection volume of 5µl and flow rate of 0.2 ml/min. The peak of kaempferol was detected at 368nm and identified by standard substances. The sample was filtered by nylon syringe filter 0.22 µm before injected to the UPLC system.

2.6. Moisture Content Analysis

A moisture analyzer (AND MS-70, Japan) was used to determine the moisture content of the plant powder. The sample (0.1 g) is heated continuously at 100 °C, causing the moisture content to evaporates, and the heating is terminated automatically once the mass of the sample is no longer changing.

3. Result and Discussion

3.1. UPLC Quantification of Kaempferol in *Cassia Alata* Extracts

Kaempferol was identified by comparing the retention time and UV spectrum of the standard and the plant extracts. Retention time and UV spectrum for kaempferol in the extract were found to be well matched with standard compound as shown in Figures 1 and 2. Hence, this proved its presence in the extract. Kaempferol was quantified by comparing the kaempferol peak area in the sample with the results of a calibration series using external standard obtained from Sigma-Aldrich. The calibration curve was constructed by using six different concentrations of standard solution ranging from 0.0025 to 1 mg/ml of kaempferol. The regression lines were constructed and the linearity of calibration curves was determined by calculating the correlation coefficients ($R^2=0.9993$) for kaempferol. LOD and LOQ were determined by setting the signal to noise ratio of 3:1 and 10:1 respectively. The limit of detection (LOD) which defined as the lowest detectable analyte amount in a sample was calculated using the formula $LOD = (3.3\alpha)/S$, where α is the y-intercepts of the regression lines and S is the slope of the calibration curve. The LOD for the kaempferol was found to be 0.04 mg/ml. Apart from that, the limit of quantification (LOQ) which is defined as the lowest quantifiable analyte amount in a sample was calculated using the formula $LOQ = (10\alpha)/S$. The LOQ for the kaempferol was found at 0.12 mg/ml. The developed UPLC separation run time is 17.5 minutes and kaempferol peak was found at 7.79 min. UPLC is a faster analysis compared to HPLC which requires 45-50 minutes of running time [2, 12]. Hence, this method is efficient for an accurate qualitative and quantitative analysis of kaempferol from *C. alata* extracts, and hence similar method were used throughout this work.

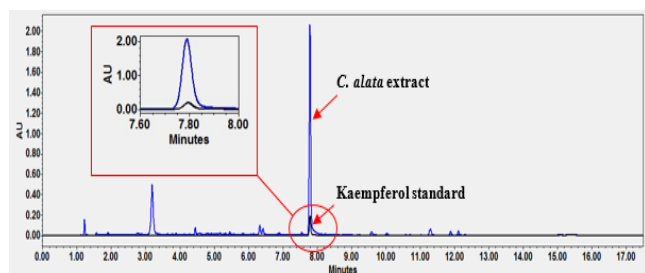


Fig. 1: Overlay chromatogram profile of kaempferol standard and *C. alata* extract at 368nm

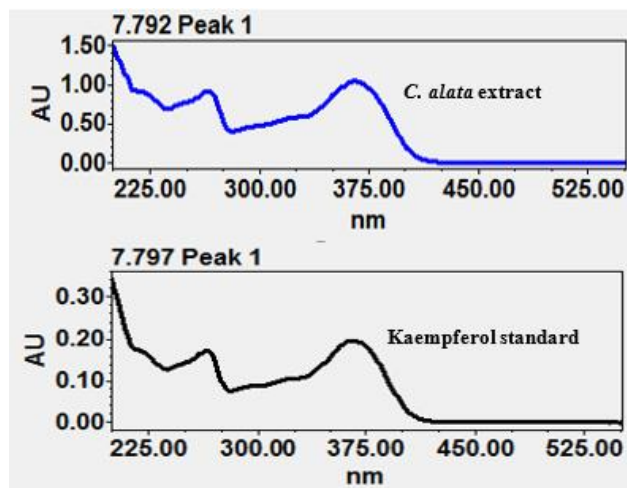


Fig. 2: UPLC spectra of kaempferol standard and *C. alata* extract at 368nm.

3.2. Effect of Solvent Type on Polyphenol Extraction

Two solvents of various polarities i.e. ethanol and methanol were used for extracting kaempferol from *C. alata* by using UAE. Turkmen et al. [13] reported that solvent with different polarity have a significant effect on the extraction yield of polyphenol. The effect of solvent with different types of extraction to the kaempferol yield from *C. alata* is shown in Figure 3. The result shows that ethanol was the best solvent for kaempferol extraction from *C. alata*. The yield of kaempferol in ethanol (6.21 mg/g DW) was over threefold higher than with methanol (1.47 mg/g DW). The polarity of ethanol (0.654) is lower compared to methanol (0.762). The yield of kaempferol extraction from *C. alata* decreased with increasing polarity, which is in agreement with the findings reported by previous researchers, where ethanol was most effective in extracting kaempferol from paprika pepper compared to methanol [14]. The result shows that extraction yield of kaempferol from *C. alata* is greatly affected by the solvent polarity.

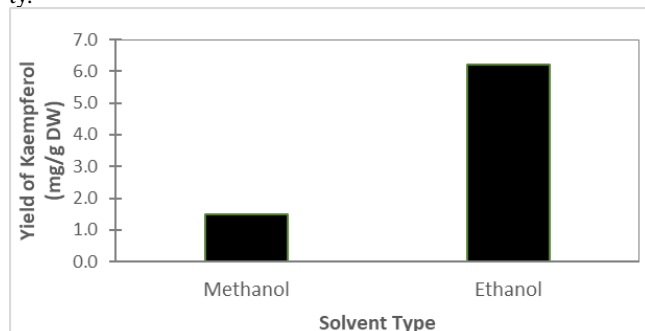


Fig.3: Yield of Kaempferol in Different Solvent Type

3.2. Effect of Particle Size Diameter on Polyphenol Extraction

The yield of kaempferol extraction from plant material is strongly influenced by the particle size diameter (PSD) of plant material used. For instance, the mass transfer area of smaller particles is greater than that of larger particles, which in turn improves the overall mass transfer process. The dried *C. alata* leaves was ground, sieved and classified according to the particle size diameter before the extraction of kaempferol was performed. The *C. alata* powder consisted of particles with size of $\leq 125 \mu\text{m}$, $125 \mu\text{m}$ to $\leq 500 \mu\text{m}$, $500 \mu\text{m}$ to $\leq 800 \mu\text{m}$ and $\geq 800 \mu\text{m}$. The extraction yield of kaempferol showed an increasing trend as the particle size decreases. The *C. alata* powder consisted of particles with size of $\leq 125 \mu\text{m}$ was the highest which is 7.08 mg/g DW compared to other PSD as shown in Figure 4. The smaller particles have higher contact surface that enhanced mass transfer, which consequently lead to reduction of extraction time [15-16]. Since ethanol gave the highest yield of kaempferol extraction, hence it is used for the remainder of this work.

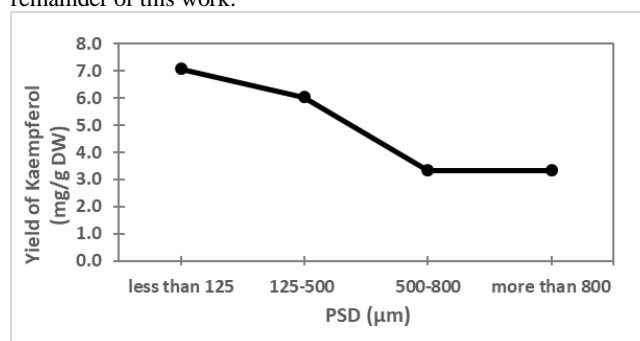


Fig. 4: Yield of Kaempferol in Particle Size Diameter (PSD)

3.4. Effect of Solvent Ratio on Polyphenol Extraction

Aqueous solvent e.g. 70% isopropanol is known to improve polyphenols extraction yield from *O. stamineus* [5]. Therefore, the extraction yield of aqueous ethanol at several concentrations (20%, 50%, 70%) is compared to pure water and pure ethanol 100%. The yield of kaempferol increased with increases in ethanol content from 0% to 100% as shown in Figure 5. This result agrees with previous work by Bae et al. [14] who reported a higher kaempferol yield obtained using pure ethanol compared to aqueous ethanol (80%). This is due to the fact that most of the campfire presence in polymerized form, which is easily soluble in a moderate polar extraction medium such as ethanol. It is known that solvent favour extraction of a similar or closer polyphenols polarity [17]. In this work, kaempferol has the same polarity with extraction solvent (pure ethanol), and hence easier extracted by pure ethanol.

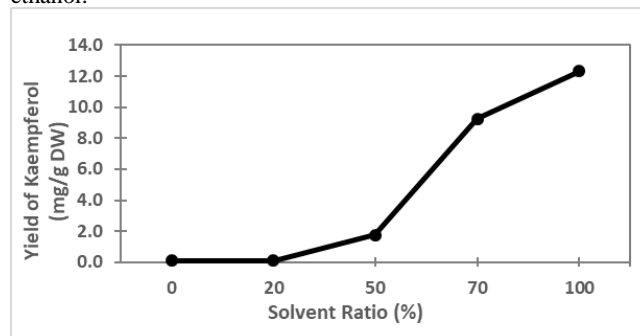


Fig. 5: Yield of Kaempferol in Different Solvent Ratio

3.5. Effect of Solid to Solvent Ratio on Polyphenol Extraction

The phenolic and flavonoid extraction yield is affected by the volume of extraction solvent used [18]. The effect of plant solid to

solvent ratio on the extraction yield of kaempferol from *C. alata* was evaluated by varying the ratio from 1:5 to 1:25. Figure 6 shows that the yield of kaempferol increased gradually from solid to solvent ratio of 1:5 to 1:20 which gave the highest yield (14.93 mg/g DW) but slightly decreased at 1:25. A higher plant solid to solvent ratio is favourable for polyphenols extraction because it offers a high concentration gradient between the plant material and solute, causing in an increase of diffusion rate of polyphenols into the bulk liquid [18]. However, the amount of kaempferol from the plant material is limited, and once the concentration of kaempferol in the plant material is becoming too low, the extraction yield is no longer increasing with the increase of plant to solvent ratio. The remainder of this work on the comparison of UAE, MAE and ME was performed using a PSD $\leq 125 \mu\text{m}$, pure ethanol solvent and plant solid to solvent ratio of 1:20 following the result of the initial screening.

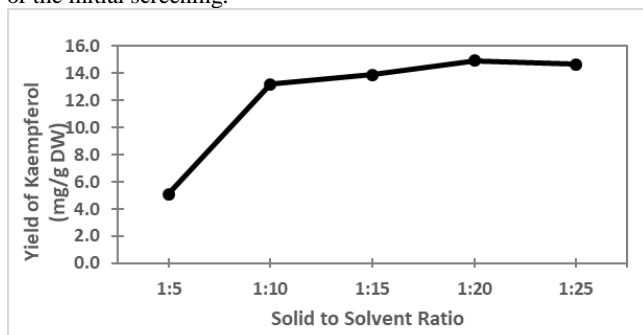


Fig. 6: Yield of Kaempferol in Different Solid to Solvent Ratio

3.6. Effect of UAE Amplitude and Time on Polyphenol Extraction

Figure 7 shows the kaempferol extraction yield from *C. alata* at different amplitudes ranging from 10%, 30%, 50%, 70% and 90% at a constant sonication time of 10 minutes. It is clear that at lower amplitude from 10% to 50%, kaempferol yield increased marginally to the maximum yield of 19.06 mg/g DW. This showed that acoustic cavitation improves extraction efficiency of UAE [19]. However, increases in amplitude beyond 50%, actually adversely affecting the kaempferol extraction yield, which reduced to 17.87 mg/g DW at 90% amplitude. High sonication amplitude creates ultrasonic waves with a more violent bubble collapse [20], which is associated with more hot spots (high local temperature and pressure) during explosion of bubbles that may cause the thermal sensitive compound degradation [21]. In addition, higher temperature causes lower efficiency in cavitation phenomenon [22]. The combined effect of less efficient cavitation and thermal degradation is suspected to cause reduction in kaempferol extraction yield. In this work, a 50% amplitude gave the highest kaempferol yield that generates a sufficient acoustic cavitation to enhance penetration of solvents into the plant matrix by facilitating cell wall disruption. Sonication enhances the external and the internal mass transfer, which promotes an efficient extraction.

The effect of extraction time to the kaempferol yield is shown in Figure 8. The result shows that, the difference is not significant and excessive time was not useful. Therefore, the shortest extraction time of 5 minutes is preferred with the kaempferol extraction yield of 18.60 mg/g DW. The extraction yield is almost plateau when the time is extended up to 25 minutes. The findings in this work is in agreement with other previously reported UAE of polyphenols from *Urtica dioica* [23] and *Lawsonia inermis* [24] who concluded that extraction time is not significantly affecting the extraction yield. Polyphenols are very unstable and highly susceptible to degradation. Thus, the shortest extraction time (5 min) is preferred to mitigate the potential adverse effect of prolonged sonication.

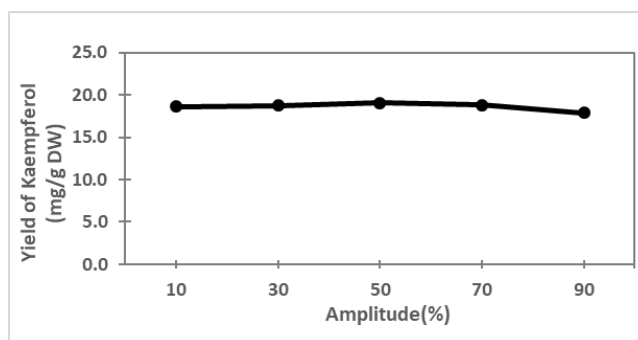


Fig. 7: Yield of Kaempferol in Different Amplitude in UAE

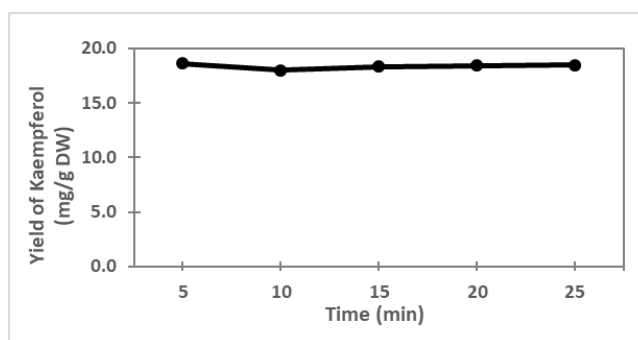


Fig. 8: Yield of Kaempferol in Different Time in UAE.

3.7. Effect of MAE Power and Time on Polyphenol Extraction

Selecting a proper extraction time plays an important factor that not only affects the extraction yield, but also the preservation and stability of polyphenols in plants. In Figure 9, the extraction yield of kaempferol increased with time from 1 to 4 minutes and achieved the optimum yield at 4 minutes with 0.42 mg/g DW. There was a decrease in kaempferol extraction yield at 5 minutes. This can be explained by the Fick's second law of diffusion, which predicted that there would be a final equilibrium between the solute in the sample and the extraction solvent after certain of time for a maximal extraction yield of polyphenols [25]. A longer extraction time is not increasing the yield further. In fact, there is a degradation of kaempferol due to elevated temperature from microwave radiation. The result of this work agrees with Lovric et al. [26] who reported that the overexposure in MAE caused degradation of the polyphenols and hence reduction in yield. Therefore, exposure time of 4 min is sufficient to obtain the highest kaempferol yield from *C. alata* via MAE.

The MAE power affects the irradiation energy supplied to the solvent, which causes rapid direct heating. Figure 10 shows that kaempferol yield increases marginally when power is increased from 50 W to 100 W. However, a marked decrease in kaempferol yield is observed when the power is increased beyond 100 W. Higher power causes a faster molecular movement due to microwave induced dipole rotation and ionic conduction, which in turn cause a rapid increase in temperature. A slight increase in temperature improves the mass transfer and hence facilitates enhancements in extraction yield. However, too much temperature increment at higher MAE power causes a thermal degradation of kaempferol, hence reducing its yield. Li et al. [27] also found that excessive microwave power causes the degradation of some antioxidants, and hence adversely affecting the extraction yield. The highest kaempferol yield (21.55 mg/g DW) from *C. alata* was achieved at microwave power of 100 W.

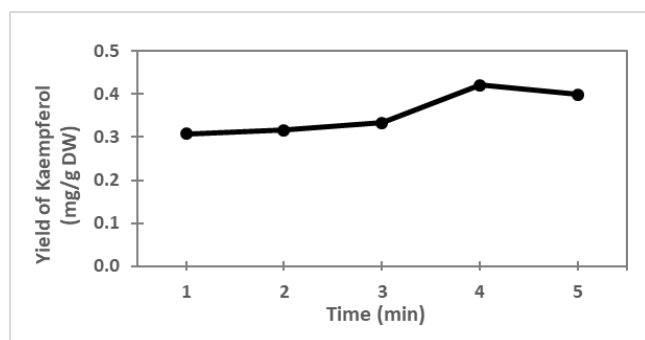


Fig. 9: Yield of Kaempferol in Different Time in MAE

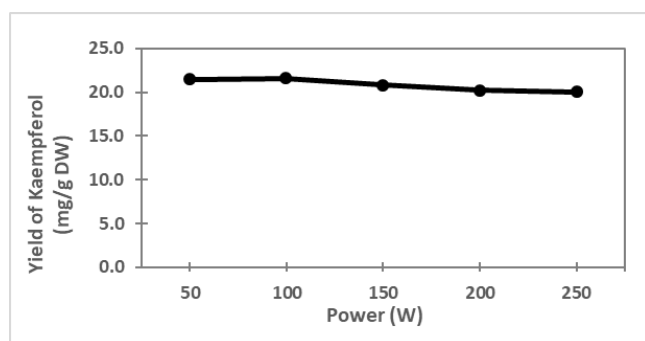


Fig. 10: Yield of Kaempferol at Different Power in MAE

3.8. Comparison of UAE, MAE and ME

Polyphenols from plant materials is often extracted using a conventional extraction technique, which is maceration. However, alternative methods such as MAE and UAE, which can reduce operating time and achieve a better yield, are widely used in recent years. The major difference between the three different extraction methods is the amount of auxiliary energy used, i.e., ME 0.045 W/ml, UAE 0.83 W/ml and MAE 20 W/ml. Maceration is considered as reference method to compare the yield with the newer extraction method. The kaempferol extraction yield from the three different extraction methods is rarely compared, and this was one of the aims in this work. Figure 11 shows that MAE has the highest extraction yield of kaempferol (21.55 mg/g DW) followed by UAE (18.60 mg/g DW) and ME (12.01 mg/g DW). This shows that conventional method cannot perform an effective extraction of kaempferol compared to the UAE and MAE. Apart from that, ME is time consuming, which required 2 hours compared to 5 minutes for UAE and 4 minutes for MAE. As mentioned earlier, sonication induces cavitation that causes plant cell wall disruption and allows solvent penetration to the plant matrix resulting in enhancement of kaempferol extraction by the UAE. Meanwhile, the MAE cause a direct rapid heating due to microwave induces molecule dipole rotation and ionic conduction, which cause the cell to break and facilitating the release of kaempferol to the extracting solvent [28]. In this work, MAE are the most effective techniques of extraction of kaempferol from *C. alata* that gave the highest kaempferol yield with only 4 minutes compared with ME (120 min) and UAE (5 min).

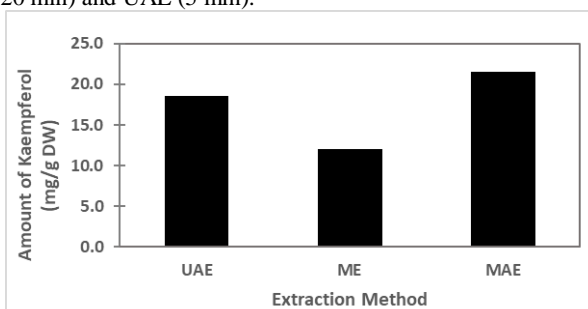


Fig. 11: Comparison of Different Extraction Method on Kaempferol Yield

4. Conclusion

The UPLC separation method for *C. alata* extracts developed in this work is capable to perform a faster analysis of kaempferol compared to other published method. It was found that PSD ≤ 125 μm , pure ethanol solvent and plant solid to solvent ratio of 1:20 gave the highest kaempferol yield from *C. alata*. Among the three extraction methods tested, MAE is the most efficient, i.e. shorter extraction time (4 min) and the highest yield (21.55 mg/g DW) of kaempferol compared to UAE and ME. Extraction of kaempferol from *C. alata* favour higher auxiliary energy such as the MAE.

Acknowledgement

Research funding from the Ministry of Higher Education Malaysia FRGS/1/2016/TK02/UMP/02/4 managed by Research and Innovation Department UMP (RDU160124) and Universiti Malaysia Pahang (PGRS180342). Dr. Pang Sook Fun is the recipient of UMP Post-Doctoral Fellowship in Research.

References

- [1] Rajagopal PL, Majetha R, Sreejith KR, Kiron SS, Aneeshia S & Premaletha K (2014), Phytopharmacological review on *Cassia alata* Linn. *Journal of International Academic Research Multidisciplinary* 2, 280-307.
- [2] Fernand VE, Dinh DT, Washington SJ, Fakayode SO, Losso JN, Ravenswaay ROV & Warner IM (2008), Determination of pharmacologically active compounds in root extracts of *Cassia alata* L. by use of high performance liquid chromatography. *Talanta* 74, 896-902.
- [3] Okwu DE & Nnamdi FU (2011), Cannabinoid dronabinol alkaloid with antimicrobial activity from *Cassia alata* Linn. *Der Chemica Sinica* 2, 247-254.
- [4] Escobar CAL, Capote FP & Castro MDL (2015), Comparative study of the effect of auxiliary energies on the extraction of Citrus fruit components. *Talanta* 144, 522-528.
- [5] Pang SF, Lau MZ, Yusoff MM & Gimfun J (2017), Microwave-irradiation induced fast simultaneous extraction of methoxylated and hydroxylated phenolic compounds from *Orthosiphon stamineus* leaves. *Material Science Forum*, 155-158.
- [6] Nguang SL, Yeong YL, Pang SF & Gimfun J (2017), Ultrasonic assisted extraction on phenolic and flavonoid content from *Phyllanthus niruri* plant. *India Journal of Science Technology* 10.
- [7] Sule WF, Okonko IO, Omo-Ogun S, Nwamze JC, Ojezele MO, Ojezele OJ, Alli JA, Soyemi ET & Olaonipekun TO (2011), Phytochemical properties and in-vitro antifungal activity of *Senna alata* Linn. crude stem bark extract. *Journal of Medicinal Plants Research* 5, 176-183.
- [8] Gritsanapan W & Mangmeesri P (2009), Standardized *Senna alata* leaf extract. *Journal of Health Research* 23, 59-64.
- [9] Aminuddin MF, Basri AM, Taha H, Abidin AM & Ahmad N (2016), Antimicrobial activities of soaps containing *Senna alata* leaf extract. *Scientia Bruneiana Special Issue*.
- [10] Oladeji O, Adelowo F, Odelade K, Aremu S & Adisa M (2016), *Senna alata*: phytochemical components, characterization and medical benefits. *CIBTech. Journal of Pharmaceutical Sciences* 5, 40-51.
- [11] Pandey D & Gupta AK (2015), *New Dimensions in Microbiology*, Lenin Media Private Limited, Delhi, India, 53-67.
- [12] Varghese GK, Bose LV & Habtemariam S (2012), Antidiabetic components of *Cassia alata* leaves: identification through α -glucosidase inhibition studies. *Pharmaceutical Biology* 3, 345-349.
- [13] Turkmen N, Sari F & Velioglu YS (2006), Effect of extraction solvents on concentration and antioxidant activity of black and black mate polyphenols determined by 9ferrous tartrate and Folin-Ciocalteu methods. *Food Chemistry* 99, 838-841.
- [14] Bae H, Jayaprakasha GK, Jifon J & Patil BS (2011), Extraction efficiency and validation of an HPLC method for flavonoid analysis in peppers. *Food Chemistry* 130, 751-758.
- [15] Silva EM, Rogez H & Larondelle Y (2007), Optimization of extraction of phenolics from *Inga edulis* leaves using response surface methodology. *Separation and Purification Technology* 55, 381-387.
- [16] Čujić N, Šavikin K, Jankovic T, Pljevljakušić D, Zdunić G & Ibrić S (2016), Optimization of polyphenols extraction from dried

- chokeberry using maceration as traditional technique. *Food Chemistry* 194, 135–142.
- [17] Yang Y & Zhang F (2008), Ultrasound-assisted extraction of rutin and quercetin from *Euonymus alatus* (Thunb). *Sieb. Ultrasonics Sonochemistry* 64, 3-19.
- [18] Tan PW, Tan CP & Ho CW (2011), Antioxidant properties: effect of solid-to-solvent ratio on antioxidant compounds and capacities of pegaga (*Centella asiatica*). *International Food Research Journal* 18, 553-558.
- [19] Li H, Pordesimo L & Weiss J (2004), High intensity ultrasound-assisted extraction of oil from soybeans. *Food Research International* 37, 731-738.
- [20] Herrera MC & Castro MDL (2005), Ultrasound-assisted extraction of phenolic compounds from strawberries prior to liquid chromatographic separation and photodiode array ultraviolet detection. *Journal of Chromatography* 1100, 1-7.
- [21] Tuulmets A & Salmar S (2001), Effect of ultrasound on esters hydrolysis in aqueous ethanol. *Ultrasonics Sonochemistry* 8, 209-212.
- [22] Boonkird S, Phisalaphong C & Phisalaphong M (2008), Ultrasound-assisted extraction of capsaicinoids from *Capsicum frutescens* on a lab and pilot-plant scale. *Ultrasonics Sonochemistry* 15, 1075-1079.
- [23] Vajić UJ, Grujić-Milanović J, Živković J, Šavikin K, Godevac D, Miloradović Z, Bugarski B & Mihailović-Stanojević N (2015), Optimization of extraction of stinging nettle leaf of phenolic compounds using response surface methodology. *Industrial Crops and Products* 4, 912–917.
- [24] Uma DB, Ho CW & Aida WMW (2010), Optimization of Extraction Parameters of Total Phenolic Compounds from Hena (*Lawsonia intermis*) Leaves. *Sains Malaysiana* 39, 19–128.
- [25] Chew KK, Khoo MZ, Ng SY, Thoo YY, Aida WMW & Ho CW (2011), Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Orthosiphon stamineus* extracts. *International Food Research Journal* 18, 27-35.
- [26] Lovrić V, Putnik P, Kovačević DB, Jukić M & Dragović-uzelac V, Effect of Microwave-Assisted Extraction on the Phenolic Compounds and Antioxidant Capacity of Blackthorn Flowers. *Food Technology and Biotechnology* 55, 243–250.
- [27] Li Y, Li S, Lin SJ, Zhang JJ, Zhao CN & Li HB (2017), Microwave-assisted extraction of natural antioxidants from the exotic *gordonia axillaris* fruit : optimization and identification of phenolic compounds. *Molecules* 22, 1481.
- [28] Yeong YL, Pang SF, & Gimbin J, (2017), The effect of auxiliary energy on rhein, kaempferol and astragalin extraction from *Cassia alata*. *MATEC Web of Conferences* 111, 02009.