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Microwave- Assisted Extraction and Phytochemical Analysis of Peperomia Pellucida for Treatment of Dengue

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Dengue is a fatal mosquito-borne disease which is spreading at an alarming rate and it is endemic in many tropical and sub-tropical parts of the world. To date, no effective antiviral or vaccine is available for this chronic disease. Peperomia pellucida is a plant that can be found in tropical countries and it has demonstrated positive results against some chronic diseases due to the presence of phenolic compounds. As many herbal for dengue have been proven to possess high phenolic content, this plant is deemed as another alternative for dengue remedy. Solvent extraction can be used as an efficient method to collect the extract from this plant with high bioactivity. Microwave-Assisted Extraction (MAE) is a “green technology” solvent extraction method which is still quite unconventional. In this work, extraction of peperomia pellucida leaves using MAE was carried out to investigate the effect of extraction time (5 – 25 min) and temperature (65–145 °C) on the extraction yield and total phenolic content (TPC). Furthermore, the phytochemical analysis in 10 extracts was performed by spectrophotometer in order to know its total phenolic content. The results show that temperature of 145 °C and extraction time of 15 min were the best extraction conditions. Temperature and extraction time have been shown to be potential factors in affecting MAE for obtaining bioactive compounds from Peperomia pellucida. The identification of the concentration of phenolic compound in the extracts of Peperomia pellucida leaves warrants further pharmacological studies on this species.

1. Introduction

Dengue virus (DENV) is originally a mosquito-borne virus that comes from genus Flavivirus from Flaviviridae family. Dengue viruses propagate to various countries around the world and spread through the bites from female Aedes aegypti mosquitoes. Presently, there are four groups of antigenic which are related to each other. Virus serotypes of DENV-1, DENV- 2, DENV-3 and DENV-4 have been identified to be in the Flavivirus genus of Flaviviridae family (Guzman and Isturiz, 2010). Dengue has spread in many countries in Africa, Eastern Mediterranean, America, Southeast Asia and Western Pacific. Western Pacific and the Southeast Asia are the regions most affected by this virus. Malaysia, with a population of 27.7 mM people and a population density of 84 per square kilometer (Muhammad Azami et al., 2011) is not exempted. In a press statement, the Director General of Health Malaysia, reported the highest increase of dengue cases and deaths were in 2012 with a total of 545 cases and 4 deaths in 5 weeks. This is an increase of 57 cases compared to 488 cases with two deaths in the previous week (Kementerian Kesihatan Malaysia, 2012). In the first three months of 2019, Selangor, with the highest population state resulted in the highest dengue cases of 22,343, followed by Kuala Lumpur (3,084 cases), Johor (3,051 cases), Penang (2,173 cases), Sabah (1,717) and Kelantan (1,164 cases). Federal Territory of Labuan shows the lowest cases with 15 cases and a total of 59 fatalities caused by dengue fever itself (Life Engineering, 2019). As of today, there is still no effective antiviral or vaccine available for this chronic disease.

Peperomia Pellucida (P. Pellucida) or locally known as Sirih Cina and Ketumpang Air in Malaysia is quite a common edible herb which grows widely throughout the tropics including Asian, America and Africa. Its stems are translucent pale green and freely branched while its leaves are blunt and heart shaped as shown in Figure 1 (Majumder et al., 2011). This species develops during rainy periods and thrives in loose, humid soils under the shade of the tree. Local communities around the world have been consuming this leafy vegetable as part

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of their diet and in the treatment of diarrhea, tumor, cancers and some ailments such as abdominal pain, indigestion, gout, and fatigues (Ediriweera, 2010). This species has been extensively investigated as a source of natural pharmacologically active compounds. However, its efficacy as antiviral for dengue virus has not yet been investigated. Extraction is among the processes to recover phenolic compounds from plants and it can be categorized into conventional or unconventional extraction method. Soxhlet extraction is one of the conventional extraction methods used for solvent extraction. This method has a few shortcomings where the extraction time is longer as mentioned by Llompart et al. (2018) where the extraction process needs 12 to 24 h. This is because a period of time is needed to heat the vessel before heat can be transferred to the sample/solvent. The method also requires high solvent volume to complete the process (Llompart et al., 2018). This method also gives a comparatively low yield (Idris and Sulaiman, 2017). Looking at these shortcomings, it is thus necessary to introduce a new unconventional method to improve the process in order to obtain extract of high quality.

Figure 1: Peperomia Pellucida plant

In this research, an unconventional method which is microwave-assisted extraction (MAE) technique was used. As suggested by previous research, the time of extraction can be reduced by using MAE because the microwave heats the solvent/sample inside the vial. Because of the effective heating, lower solvent volume consumption can be expected and hypothetically can give higher yield (Llompart et al., 2018). In this study, *P. Pellucida* was extracted using MAE and the objective was to observe the percentage yield as affected by temperature and extraction time. Meanwhile, as the plant's efficacy towards dengue can be signified through its total phenolic compound (TPC) content, a phytochemical analysis was conducted in order to determine the TPC of the crude extracts. This is due to the fact that higher extraction yield does not guarantee high TPC content, where this quality can be affected by the operation conditions applied.

2. Materials and method

2.1 Preparation of plant material

Peperomia pellucida plants were collected from local wild shrubs in Universiti Teknologi Malaysia, Johor. The leaves were first separated from its stems and then washed under running tap water to clean them from dirt. The washed leaves were then put into a food dehydrator (Septree 6 tiers, China) for 2 d at 53 °C to remove water and ground using an electric blender (Panasonic MX – 337N, Malaysia). The powdered leave was stored in air tight container before the extraction process was conducted.

2.2 Chemical and reagents

95 % Ethanol solution, Folin-Ciocalteu phenol reagent, Na_2CO_3 and standard gallic solution were supplied by QReC (Asia) Sdn.Bhd.

2.3 Microwave-assisted extraction of *P.Pellucida* leaves

The microwave assisted extraction experiments were carried out using a modern microwave reactor/extractor, Monowave 450 (850 W, 600 rpm stirring, Anton Paar, Austria). The microwave extractor was operated based

on 3 heating levels: preheating for 2 min to the desired temperature, irradiation and allowing the machine to cool down for 2 min (the cooling temperature was set to be at 55 °C). Powdered P. Pellucida leaf (3 g) was extracted using 30 mL of 95 % ethanol solution.

The effects of the operating parameters in the MAE including extraction time and temperature were studied using one-factor-at-a-time (OFAT) method. The experiments were carried out by manipulating one operating parameter and keeping other parameters constant. For extraction time, the variations were 5, 10, 15, 20 and 25 min when microwave power, temperature, volume and concentration of ethanol were fixed at 850 W, 100 °C and 30 ml of 95 % ethanol solution. Meanwhile for temperatures the variations were 65 °C,85 °C,105 °C,125 °C and 145 °C and the other parameters were kept constant like in the previous experiment. After each experimental run, the extract was filtered using vacuum filter under 100 psi and evaporated using rotary evaporator under vacuum at 45 °C in order to separate the solvent and the crude extract. Then, percentage of yield was calculated according to Eq(1):

$$Percentage\ yield\ (\%) = \frac{Weight\ of\ extract\ after\ evaporation\ (g)}{Weight\ of\ sample\ (g)} \times 100\% \quad (1)$$

2.4 Determination of Total Phenolic Content (TPC) in the extract

TPC in the *P. Pellucida* leaf extract was measured using the Folin Ciocalteau (FC) method based on methodology in the work by Rosli et al. (2016) with slight modifications. Stock reagent needed was prepared by mixing 10 mL of Folin Ciocalteau reagent with 100 mL distilled water and 7.5 g of Na_2CO_3 with 100 mL of distilled water and sonicated for 5 min. 1 mL of *P. Pellucida* extracts sample was than mixed with 5 mL of Folin Ciocalteau reagent stock and left at room temperature for 10 min.

Later, 4 mL of Na_2CO_3 stock was added to the mixture and left for 30 min. Lastly, absorbance of the mixture was measured at 765 nm using UV-Vis spectrophotometer. Total phenolic content (TPC) result in *P. Pellucida* extracts was calculated from a gallic acid standard curve and expressed as gallic acid equivalent (mg GAE/g extract sample).

3. Results and discussions

3.1 Effect of extraction time

In the investigation of the effect of extraction time and temperature on the yield of extract, a calibration curve of gallic acid standard for total phenolic content graph was plotted to relate the absorbance to the concentration, with an R^2 of 0.9952 (Figure 2).

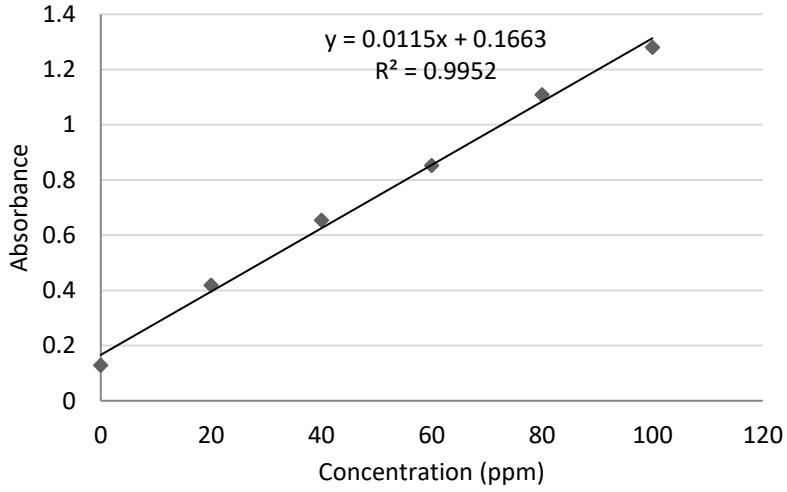

Figure 2: Gallic Acid Standard for Total Phenolic Content

Theoretically, as the extraction time increases, the yield of extract will also increase, but there is always the risk of degradation of the desired bioactive compounds (Bhuyan et al., 2015), in this case the phenolic compound. At certain extraction time, the quality and quantity of the phenolic compounds extracted from this plant will start to decrease. In this study, the effect of extraction time (5 –25 min) on the yield and TPC was

examined at a fixed microwave power of 850 W, feed-to-solvent ratio of 1:10 g/mL, temperature of 100 °C, and ethanol concentration of 95 % v/v. The influence of extraction time on the quantity of yield and TPC is summarized in Table 1 and displayed in Figure 3. From Figure 3, it can be observed that the yield increased with time. As the time exceeded 15 min, the yield dropped. This is probably because there was some loss of the extract to the rapid evaporation occurring through microwave heating. Thus it should be noted that the heating should be done under 15min at this temperature.

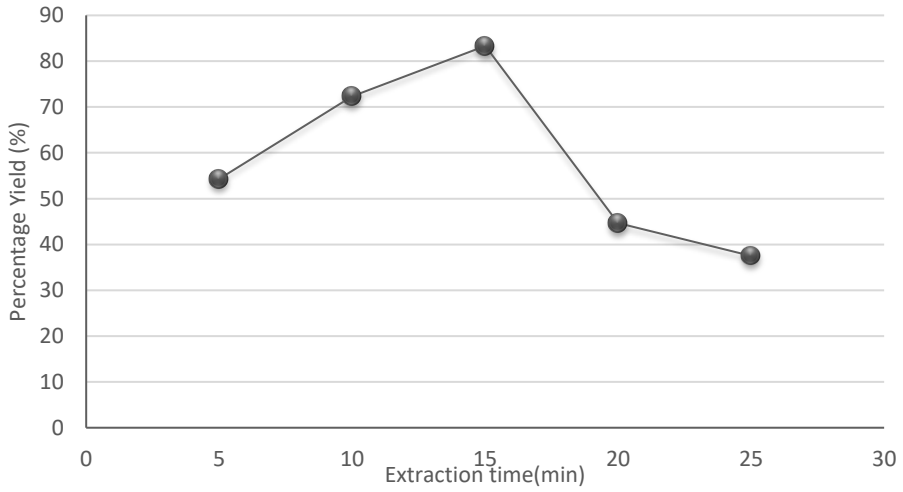

Figure 3: Effect of extraction time towards extraction yield

As can be seen in Table 1, initially in the extraction process, the TPC increased along with the increased extraction time with maximum recovery of 350.46 ppm but later it started to decrease when the time exceeded 10 min. This might due to the degradation of phenolic compound from MAE of P.Pellucida leaf beyond 10 min and the longer time of exposure of the solvent during the extraction process resulted in the evaporation of solvent inside the system itself.

Table 1: Total Phenolic Compound (TPC) content at different extraction times

Extraction Time (Min)	TPC Content (ppm)
5	278.46
10	350.46
15	305.06
20	270.60
25	241.40

This occurrence can be explained by Fick's second law of diffusion, which predicts that there will be a final equilibrium between the solute in the solid matrix (plant sample) and in bulk solution (extraction solvent). Thus, excessive extraction time was no longer useful to extract more phenolic compound from plant material (Chew et al., 2011). Hence, after taking consideration for the percentage yield and total phenolic content on the extract, 15 min was chosen as the optimal extraction time since it has the highest percentage yield with reasonably high TPC in the crude extract.

3.2 Effect of Temperature

From previous research it has been reported that at elevated temperature, higher amount of yield can be obtained (Pimentel-Moral et al., 2018). In this research, the temperature limit was fixed at 200 °C due to the fact that this value is the maximum temperature at which ethanol can be exposed to microwave energy without experimental problems. In this study, the studied range of extraction temperature ranged from 65 °C to 145 °C. The extraction time was fixed at 15 min. The pressure built up according to the temperature, and was not set to a single value. The influence of extraction temperature on the quantity of yield and total phenolic compound is summarized in Figure 4 and Table 2.

Based on the plotted graph in Figure 4, it is observed that the yield increased with temperature and the highest value was observed at 145 °C of extraction temperature.
Previous study reported that the increase of the extraction temperature will decrease the strength of solvent intermolecular forces and thus its viscosity allows better penetration into the matrix particles, and decreases surface tension allowing the solvent to better coat the solid matrix and therefore increases rates of yield of the extraction (Alaraa et al., 2018). Although high yield can be obtained at elevated temperature, the value of phenolic content also needs to be considered for optimal extraction temperature.

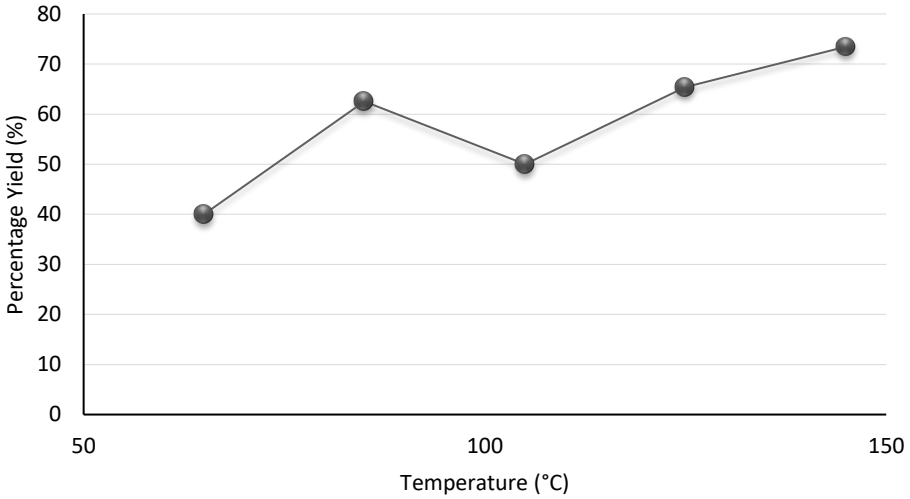

Figure 4: Effect of extraction temperature towards extraction yield

As observed in Table 2, the TPC content increased initially with temperature until 105 °C, achieving the highest TPC content of 154.8 ppm. Increase in extraction temperature would favor extraction by enhancing both solubility of solute and diffusion coefficient.

Table 2: Total Phenolic Compound (TPC) content at different extraction temperatures

Extraction Temperature (°C)	TPC Content (ppm)
65	43.22
85	79.14
105	154.80
125	122.94
145	114.60

Beyond 105 °C, the TPC content started to decrease. This is due to thermal destruction of phenolic compounds which takes place at higher temperature and causes a reduction in the antioxidant capacities of crude extract (Chew et al., 2011). As the extraction temperature increased beyond 105 °C, some phenolic content in the crude extract may be destroyed and caused the reduction of concentration of phenolic content. Thus, from the result provided, it is suggested that the optimal temperature for extraction of *P. Pellucida* leaf is 105 °C since it has the highest TPC content at a reasonably high yield.

4. Conclusion

From this study, it can be concluded that the yield of extraction is affected by temperature and extraction time. For extraction time, the yield of extraction increased with time and decreased significantly when the final equilibrium between plant sample and solvent has been reached. Extraction time at 15 min was the most effective as the extraction yield obtained was the highest which is 83.33 %. For temperature, it can be concluded that the yield of extraction increased with temperature but if the temperature is too high it may cause degradation for phenolic content in the yield. From the investigation, it was found that the best temperature that gave the highest yield was 105 °C. It has to be noted that high yield does not guarantee high TPC content. Thus proper selection of the operating condition is needed in order to obtain extract of satisfying quality. As this study has produced some ground results of the yield and TPC content, further study on the

active compound specific for dengue virus is needed. The mechanism of how the active compound works in controlling dengue disease can also be further investigated.

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