Microwave Assisted Extraction of Phenolic Compound From Pineapple (Ananas Comosus) Peel and Core

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***Abstract*— Nowadays, microwave-assisted extraction has caught tremendous interest by researcher for extracting bioactive compound. The reason MAE being so popular are because extraction by using MAE can enhance extraction yield and it is also cost efficient because it can reduce extraction time and usage of extraction solvent compared to traditional extraction process. This experiment was used to study the effect of irradiation power on pineapple peel and core, and to obtain the best solvent for extract phenolic compound. Phenolic compound from a fruit has being proved for the effective management of such chronic disease because in phenolic compound it contain positive intervention in the pathological pathway. The parameters used are different type of solvents and different microwave power (100W, 300W and 600W). The method use for the extraction is by microwave assisted by using ethanol and distilled water as a solvent for 5 minutes exposure time. Then total phenolic compound analysis is being made by using Folin-Ciocalteu spectrophotometric method. After the reaction with saturated sodium carbonate, the extraction sample was measure 765nm by using UV-spectrophotometer for 30 minutes. The determination by the Folin-Ciocalteu reagent using gallic acid solution as a calibration curve. Furthermore, it can be conclude that distilled water is more efficient if used at higher temperature but the extraction yield is quite low and ethanol is more efficient if used at low microwave power because at low power it already enough to break the cell wall without having any bioactive compound degradation.**

***Keywords*— Microwave-assisted extraction, pineapple peel, pineapple core and phenolic compound.**

I. INTRODUCTION

The introduction should include clear statements about the research question or problem that you studied. The introduction should also provide the reader with a synopsis of the key research studies that were used as the foundation for your study or those studies that provide the theoretical grounding for your study. The introduction should then end with a clear statement the purpose of your research study.

The scientific of pineapple is *Ananas comosus* and in the food application industry, pineapple is one of the most appreciated tropical and subtropical fruit crop in the processing industry. In the industry, there are variety of application that can be obtained from a pineapple. Peeled skin, core and crown of the pineapple is an industrial waste that made up from 75% of the whole processed fruit. Pineapple has been investigated from the previous researcher as an interesting source for phenolic compounds, bromelian enzyme extraction, antioxidants, fiber, vinegar, organic acids and biogas. All of this extraction from the pineapple can give a lot of benefit to it consumer. Another benefit of pineapple industrial waste is a, in the bioethanol production, the pineapple can give as a potential of raw material because in the pineapple content it contain a high quantity of potentially hydrolyzable cellulose and hemicellulose and it is also fermentable.

Phenolic compounds are a group of aromatic secondary plant kingdom with a lot of advantages in reducing the risk of onset of many chronic diseases like cancer, cardiovascular and neurodegenerative problem. Phenolic compound from a fruit has being proved for the effective management of such chronic disease because in phenolic compound it contain positive intervention in the pathological pathway.

According to Kim, et al., 2003, phenolic acids and flavonoids are the most important groups of bioactive compounds in plants and secondary metabolites like pineapple. Phenolic is a compound that contain one or more hydroxyl groups with one or more aromatic rings. Phenolic compounds is a natural products and it also an antioxidant element that capable of hunting free superoxide radicals, reducing the risk of cancer and anti-aging. Phenolic compounds can produce unique flavor, taste and health promoting properties found in fruits and vegetables, (Tomas B., et al., 2001). From previous research by Valentine et al., 2003, it state that phenolic compounds are vital for plant growth and reproduction and it produced as a response to environmental factors, such as chilling, light and pollution that can defend injured from happen to the plants.

With today’s emphasis on speed and efficiency in the analytical chemistry laboratory, any technique that will improve the speed and efficiency of the solvent extractions is an important one because other than it can save a lots of time, it also can save a lot of money. Among the latest techniques for extractions such as supercritical fluids extraction (SFE), microwave assisted extraction (MAE) and pressurized liquids extraction (PLE) has been the main focus centers which has drawn considerations research attention for the last period. The latest technique of extraction operation it not only can speed up the process of extraction but it also adds to the precision and accuracy when it comes to sample preparation compatible with any operation of chromatographic in order for determination the quality of the finished products.

Compared to conventional extraction techniques, the microwave assisted extraction can give advantage for reducing duration of extraction time and the volume of solvent used is also can be reduced. This extraction technique has being proved to extract small molecule such as antioxidant like phenolic compounds. During microwave assisted extraction, the temperature used is quite high due to microwave power and gallic acid compounds at temperature up to 100ºC for 20 minutes are quite stable under microwave assisted heating conditions. MAE is the extraction process begin with solvent like ethanol is heating at various level of power and that solvent must have contact with a sample. With the energy from microwave to partition compounds of analytical interest from the pineapple sample matrix into the solvent.

By Andressa Blainski (2013), determination of phenolic compound by using technique of UV/Vis spectrophotometry are proved as a method to be linear, precise, specific, accurate, robust, easy to perform and reproducible. Phenolic compounds is a second metabolites in the plant kingdom and it acting mainly as contributors to protective agents against UV light, antioxidant and plant pigmentation among the others. Calorimetric reaction that are commonly used in the UV/Vis spectrophotometric technique is an easy way to perform, quick and acceptable routine in the laboratory use and the analysis cost is quite low compare to other analysis. Yet, the calorimetric need to use a reference substance then it need to measure the total concentration of phenolic content from the plant extract.

The objectives of these studies are to extract phenolics compound from Ananas comosus peels and cores by using microwave-assisted extraction. The second objectives is to investigate the effectiveness of microwave-assisted extraction with the effect of different microwave power and two different types of solvent on the phenolics compound of pineapple peel and core. This studies can be proved when the total phenolic compound analysis by UV-spectophotometer is done.

II. METHODOLOGY

A. *Raw materials and reagent*

The pineapple peel and core that will be used in this study were bought from the market nearest UiTM Shah Alam, Selangor. The type of pineapple is from Sarawakian. The Ananas comosus was prepared in the laboratory following the procedure explained in the next section. Folin-Ciocalteu's phenol reagent, sodium carbonate, ethanol 95% and sodium hypochlorite were purchased from Merck KGaA and HmbG Chemicals.

B. *Preparation of Ananas Comosus peel and core*

The peel and core of the pineapple that will used in this study were purchased from a local market nearest UiTM Shah Alam, Selangor. Pineapple or *Ananas comosus* were washed in 0.1% sodium hypochlorite for 5 minutes. The sodium hypochlorite were removed by washing the *Ananas comosus* with distilled water. After completed the washing, the peels and core were removed from the fruit, finely cut with a sharp knife. Then the finely cut peels and core were placed on the stainless tray before dried it in a hot air oven at 50oC until it obtained constant weight. In order to produce a fine powder and particle with an average diameter, the dried peels and core will sieved at 40 mesh by using a suitable sieve from the laboratory. The sample of peels and core must separate in different bag and placed both of the sample in a freezer until it used for the extraction.

C. *Microwave-assisted extraction*

Microwave-assisted extraction was performed with the dried peels powder was poured into the solvent ethanol solution with a ratio 20 to 1, solvent to sample. The sample of peel, pineapple was soaked in the two different types of solvent ethanol and distilled water separately for 2 hours in a beaker. The extraction of phenolic compounds from the *Ananas comosus* were measured are affected by microwave power, solvent types and solvent to sample ratio, pH, and extraction time. Then both of the beaker that contains the mixture of solvent and sample were adjusted to the desired the pH value to 2.5. The extracts of microwave assisted were performed at three different levels of microwave power which are 100W, 300W and 600W for 5 minute extraction time. After the extraction had finished completely, the mixture of extraction is allowed to be cool down to room temperature around $25^{o}C$. After the extraction mixture already cooled to room temperature, the mixture is then filtered by using the filter paper from Whatman Paper No 1 Filter Paper before centrifuging (10000×g for 15 minutes) and the supernatant was carefully collected at the top of the centrifuged bottle for further analysis. The extract that obtained from *Ananas comosus* peels and core was stored in the freezer at temperature $4^{o}C$ until its use in total phenolic compound analysis. All of the steps in the above were repeated for the sample of *Ananas comosus* core.

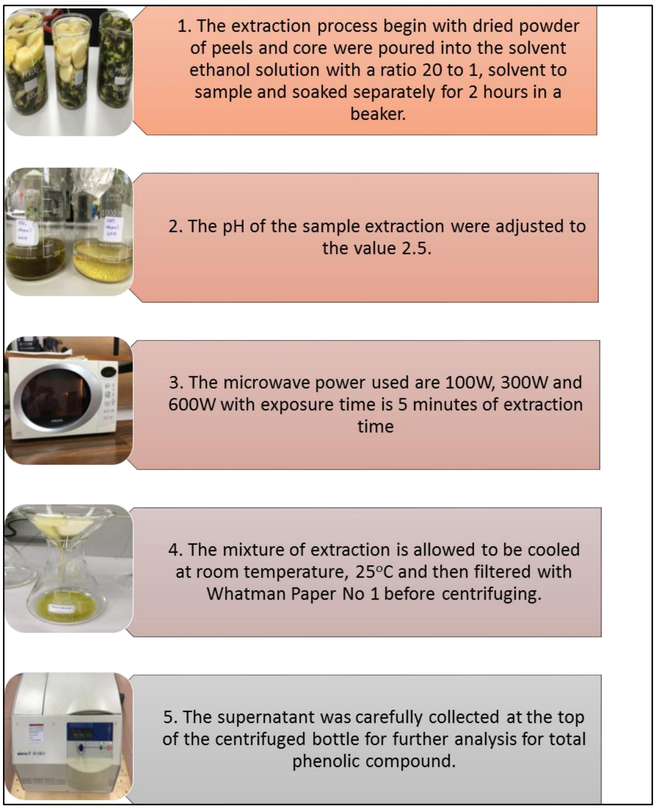

Figure 1: Summary for microwave-assisted extraction.

D. *Total phenolic compound analysis studies*

Measurement of the total phenolic content of the *Ananas comosus* peels and core was determined by means of the Folin - Ciocalteu method. In these method, the technique used are based on the oxidation or reduction reactions of phenols and Gallic acid as a standard. This method was chosen because it is a simple and quick analysis method that's based on the correlation between the measured reactant reagent reduction and color change that caused by the phenolic compounds from the Ananas comosus material sample, (Valentina M. S., et al., 2016). For measuring the total phenolic content in an extract, 0.2ml of the extract of peels and core was transferred into 10ml of volumetric flask and then the extract as mixed with 0.5ml of Folin-Ciocalteu reagent. The mixture of the extract and the reagent was left to stand in the dark for 8 minutes and then 1.0ml of sodium carbonate solution was added into the same 10ml volumetric flask. The sodium carbonate solution was made from 35g of anhydrous sodium carbonate that dissolved with 100ml of distilled water. After added to the flask, the mixture need to add with distilled water until the total volume become 10ml and all of the mixture was shaken and kept for 30 minutes in a dark place. After 30 minutes of reaction time at room temperature, the observance of all the samples was measured at 760nm by using the equipment UV-vis spectrophotometer. The final results that obtained later were expressed as mg Gallic acid equivalents (GAE) /g defatted *Ananas comosus* peels and core.

III. RESULTS AND DISCUSSION

The phenolic compound solubility is governed by their chemical nature in the plant that may be vary from simple to very highly polymerized substances, (Naczk & Shahidi, 2006). During the extraction process, there is a possibility of phenolic compound having interaction with other plant components like proteins and carbohydrates. These kind of interactions may be quite insoluble that may lead to the formation of complexes. Another parameter that also affected the solubility of phenolic is the polarity of solvent used. According to Naczk and Shahidi (2006), the researchers also stated that quantification of phenolic compounds in plant materials is influenced by their chemical nature, the extraction method conducted, particle size of sample, storage time and conditions, as well as assay method, standard selection and presence of interfering substances such as waxes, fats and chlorophylls. In the case of particle size, the smallest the size of particle the efficiency of extraction will increase because particle size are related to an increase in superficial area that can promotes better contact of sample with solvent and penetration of microwaves, (Veggi et al., 2012).

The effect of different microwave power, 100, 300 and 600 watts on the extraction yield of pineapple peel and core was presented in the table 1, when the other factors were fixed such as irradiation time of 5 minutes, solvent and sample at the ratio of 20:1, and pH of 2.5. As can be seen in the Table 1, the extraction yield and total phenolic content in the pineapple peel and core extracts that obtained by microwave assisted extraction are strongly depends on the extraction conditions. By increasing the microwave power as well as the uses of two different type of solvent in the extraction system, the yield of the extraction give varied type of absorbance value. Total phenolic content (TPC) that extracted in this research finding is determine by the Folin-Ciocalteu reagent and it also using gallic acid as a standard. This analysis choose because it is a simple and quick method that based on correlation between reactant reagent reduction and the measured color change that caused by the compound of phenolic from the plant material sample, (Singleton V. L., et al., 2012).

Table 1: Final result of absorbance value

Microwave Power (Watt)	**Peel**		**Core**	
	Ethanol	**Distilled Water**	**Ethanol**	**Distilled Water**
100	2.912	0.730	2.850	0.308
300	2.638	0.434	2.161	0.290
600	2.773	0.899	2.181	0.935

Based on the table 1, it showed result of microwave assisted extraction of phenolic compound from pineapple peel and core at different microwave power and different type of solvent. The experiment is conducted at the same irradiation time that is 5 minutes at each sample.

A. The effects of TPC from pineapple peel

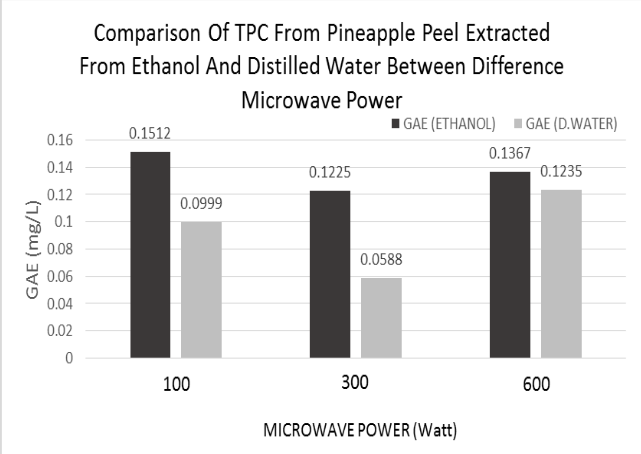

Figure 2: Comparison of total phenolic content (TPC) from pineapple peel from ethanol and distilled water between differences of microwave power.

According to the Figure 2, it showed the result of comparison of total phenolic content of pineapple peel that extracted from ethanol and distilled water. The total phenolic content value is obtained from the analysis of standard curve of gallic acid (mg/L). Extraction from ethanol seems to show more phenolic content compare to extraction from distilled water with highest value of 0.1512mg/L and 0.1235mg/L respectively. The bar chart for extraction from ethanol for total phenolic content seems to decrease it value as the microwave power increasing. The highest value of total phenolic content extracted at microwave power 100 watt with value 0.1512mg/L and at power 300 watt it show the lowest value of phenolic content from extraction sample with value 0.1225mg/L. But the phenolic content increase from 0.1225mg/L to 0.1367mg/L at microwave power 600 watt. Total phenolic content that extracted from distilled water show different result compare to ethanol because according to the bar chart in Figure 2, the highest microwave power used the more yield of phenolic produced. From UV-spectroscopy, the absorbance value of extraction sample is obtained and by using standard curve of gallic acid it show that TPC at 100watt, 300watt and 600watt are 0.0999mg/L, 0.0588mg/L and 0.1235mg/L respectively.

B. The effects of TPC from pineapple core

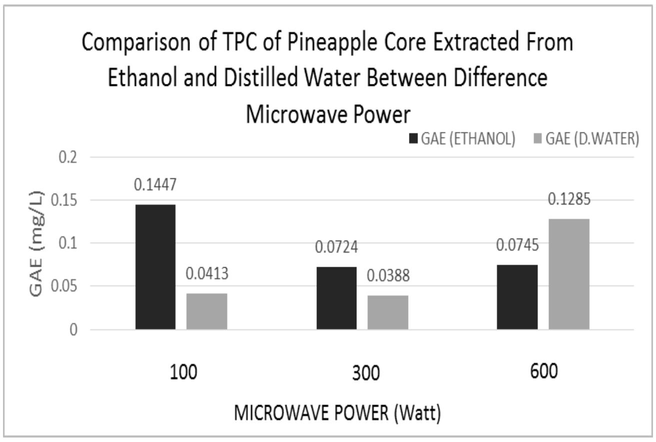

Figure 3: Comparison of total phenolic content (TPC) from pineapple core from ethanol and distilled water between differences of microwave power

In the Figure 3, it showed the result of comparison of total phenolic compound from pineapple core that extracted from ethanol (black) and distilled water (gray) at difference microwave power. Total phenolic compound that extracted from ethanol show the highest phenolic content at microwave power 100 watt with 0.1447 mg/L. While at microwave power at 300 watt and 600 watt, the TPC obtained are 0.0724 mg/L and 0.0745 mg/L. The phenolic content that extracted from distilled water showed vice versa result because the highest yield obtained at high microwave power compared to extract from ethanol which more prefer lower microwave power. At 600 watt, the total phenolic content that are obtained from pineapple core are 0.1285 mg/L and at lowest microwave power 100 watt and 300 watt the TPC that obtained are 0.0413 mg/L and 0.0388 mg/L respectively. The conclusion that can be made in the Figure 3 are, solvent from ethanol seem to give more production yield of phenolic compound from pineapple core compared to solvent distilled water.

C. The effects of TPC on different solvent

As it can be seen from Figure 2 and 3, the yields of extraction phenolic that obtained by pineapple peel and core strongly depends on the extraction conditions. By increasing the microwave power in 95% ethanol concentration, the extraction yields show varied results. The higher the ethanol concentration used as a extraction solvent will increase the acceleration damage on the cell membranes of the plant matrix but after a certain point of ethanol concentration and change of solvent polarity, the extraction of impurities can become faster and the diffusion becomes more difficult probably due to protein coagulation, (Tsao R., et al., 2012). According to journal that written by Hayat K., et al., (2009) said that solvents that are usually recommended for the extraction of bioactive compounds with antioxidant activity are methanol, ethanol, ethyl acetate, water and their aqueous mixtures. Water as extraction solvent offer some advantages such as inexpensive, highly polar solvent and nontoxic for the bioactive compound extraction but for disadvantages are water as a solvent for extraction are the difficulty of determining the proper concentration and the and the high content of water-soluble impurities which can interfere with the identification and quantification of the target compounds, (Rafiee Z., et al., 2011). Ethanol and other solvents are on the other hand because it can promote a moderately polar medium and that solvents are widely used for pharmaceutical and food production industry because of their high extraction yield and low toxicity with a minimal impact on human health and the environment, (Tsao R., et al., (2012).

Microwave assisted extraction need that solvents in order to be able to absorb the power that irradiated from microwave energy and convert this energy into heat but it depends on the solvent dielectric properties. The result in the graph 2 and 3, for peel and core that extracted from ethanol indicate that the extraction yield increased with the decreasing microwave power. By prolonging the microwave power, the impact of power input reduces which suggesting that after sufficient exposure to low microwave power it already enough to break the cell wall and to promote the diffusion of extractive substances to the extraction system. While during exposure to higher microwave power, it could lead to the degradation of natural polyphenol compounds because polyphenolic compounds have different properties and stability from various plant matrices in relation to microwave power and higher temperature, (Velentina M. S., et al., 2016).

The peel and core that extracted by distilled water seem to prefer high microwave power in order to produce higher yield of phenolic compound. The wet matrix or high moisture will improves the extraction recovery in the most cases. This is due to microwave interacting selectively with the free water molecules that present in the vascular system and gland that lead to rapid heating and increase of temperature which enhances the extraction rate, followed by cell walls rupture and release of the phenolic compound into the solvent, (Letellier & Budzinski, 1999). Which is why distilled water more prefer high microwave power in order to obtain high production yield of extraction and the result for the extraction yield agree with the recent report which demonstrated in the Figure 2 and 3 for peel and core that extracted from distilled water. However, the effect in the time needed to obtain maximum yield of extraction it would seem that the use of high microwave power might reduce the process time since higher power promote a faster temperature rise but it will lead to higher cost of processes.

The using of low pH in the extraction procedure is also one of the key factors that influenced the extraction yield of pineapple peel and core. Which is why it is necessary to select a proper pH in order to assure the maximum extraction of total phenolic compound. According to some journal, it stated that the extraction solvent with high acidity had the ability to mix properly with the insoluble pectin and favor the hydrolysis of the insoluble phenolic compound constituents into soluble phenolic compound and then it will increasing the extraction of total phenolic compound from plant materials, (Ma et al., 2013; Maran et al., 2013).

D. The overall comparison of TPC from pineapple peel and core

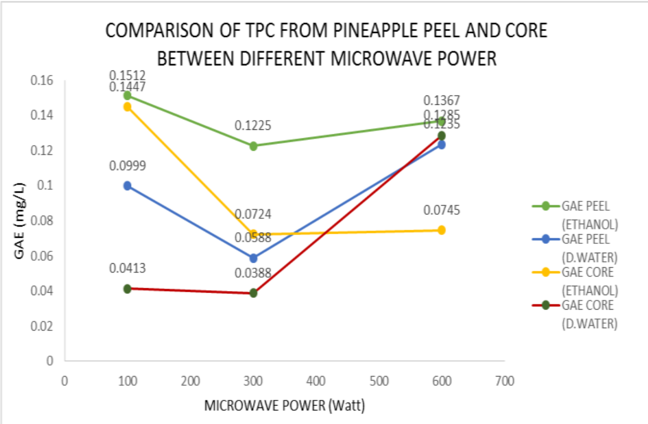

Figure 4: Overall comparison of total phenolic content (TPC) from pineapple peel and core from ethanol and distilled water between differences of microwave power

The graph in the Figure 4 shows that some of the yield of total phenolic content increase and some shows decrease value of phenolic content with the increase of microwave power from 100watt to 600 watt. According to the Figure 4, for phenolic compound that extracted from solvent ethanol show the highest phenolic content compared to distilled water. The highest of phenolic compound that can be extracted is from pineapple peel that extracted from ethanol and follow by pineapple core that extracted from ethanol with differences of 0.1512 mg/L and 0.1447 mg/L respectively at microwave power 100 watt. The third highest of TPC that is extracted from pineapple peel that extracted from ethanol with 0.1367 mg/L and fourth highest is from pineapple core that extracted from distilled water with total phenolic content 0.1285 mg/L at microwave power 600 watt. Extracted of pineapple core from distilled water at microwave power 300 watt show the lowest phenolic content, 0.0388 mg/L but according to the Figure 4, at power 300 watt, phenolic content seems to show low extraction yield. That assumption is made based on the result above because during extraction using solvent ethanol, the highest yield is produce at power 100 watt and the second highest is produce at 600 watt but at 300 watt the production yield is lower than power 600 watt. Same goes to the extraction from distilled water because the highest yield is produce at 600 watt and the second at power 100 watt but at 300 watt the extraction yield obtain is low. According to others journal, for bioactive compound that extracted from ethanol at 300 watt the phenolic compound must be higher compared to power 600watt and same goes to phenolic compound that extracted from distilled water, at 300 watt the extraction yield must be higher than extraction at at power 100 watt.

In the microwave assisted extraction overall result, distilled water extraction was found to give lower amounts of extractable phenolic compounds when compared with ethanol extraction. According to Charalampos P. and Michael K., (2006), water has the highest dielectric constant of common solvents but the dissipation factor of water is significantly lower than other solvents. During extraction process, the rate of distilled water absorbs microwave power is higher than the rate at which the system can dissipate the heat. These phenomena is called as superheating that occurs when water is used as a solvent and these intense heating may cause degradation of the analyte. That is why ethanol solvent give highest yield of extraction because it has a high dielectric constant as well as a high dissipation factor that can facilitate heat distribution through the matrix.

IV. Conclusion

Microwave-assisted extraction has been considered as the potential alternative to traditional like reflux extraction of solid-liquid extraction for the isolation of metabolites such as phenolic compound from plant materials. MAE has been chosen to extract such compound for several reasons like reduced solvent used, reduced extraction time and promote better extraction yield. In this study, microwaves found that pineapple peel and core that extracted from distilled water show lower phenolic yield compared to sample plant that extracted from ethanol. This possible due to localized superheating effect. While for solvent ethanol is proved to be the best solvent choice compared to distilled water to extract phenolic compounds from pineapple plant tissues when it exposed to microwave power. Solvent ethanol have better absorption of microwave energy which cause temperature build up inside the plant cells and cause the cell wall start to break and then release the phenolic compound into the surrounding solvent.

Other than extraction technique, in order to obtain bioactive compound such as phenolic compound from plant materials it depends on many other factors such as the nature of the plant material, temperature, solvent properties, pressure, the plant material to solvent ratio, duration of the process and other parameter which can affect the extraction process. Furthermore, it can be conclude that based on the result in the Figure 2, 3 and 4, distilled water is more efficient if used at higher temperature but the extraction yield is quite low and ethanol is more efficient if used at low microwave power because at low power it already enough to break the cell wall without having any bioactive compound degradation.

Last but not least, microwave assisted extraction are offering more advantages compared to disadvantages. So, MAE is generally applicable over a wide variety of extraction parameters and this type of extraction should be explored more in the future for better extraction of bioactive compound.

Acknowledgment

First and foremost, I would like to express my gratitude to Most Gracious and Most Merciful Allah SWT for giving me the strength to overcome my weakness and determination to complete the research for final year project. This success of this research required the help of various generous of individuals. Without them, I might not meet my objectives in doing this study so I would like to take these opportunities to show my appreciation for their efforts and kindness. Foremost, I would like to express my sincere gratitude to my supervisor Madam Nurul Asyikin Md Zaki for the continuous support of my study and research, for her patience, motivation, enthusiasm, and immense knowledge. Her guidance helps me in all the time of research and writing of this thesis.

Besides my supervisor, I would like to thank our lab assistants, Mr. Irwan, Mr. Ridhwan and Ms. Suhaila for giving me the permission to use their facilities and helping me in conduct the apparatus and materials. Your kindness means a lot to me. Thank you very much.

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