THERAPEUTIC BIOACTIVE OF PANDANUS AMARYLLIFOLIUS ROXB LEAVES FOR FUNCTIONAL FOOD APPLICATION

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***Abstract* — Over the years, many researchers and the people in food industry have become increasingly interested in phenolic compounds in natural food source. The conventional or traditional way of extraction have a few downside in terms of extraction time and the extraction yield. Microwave assisted extraction (MAE) is one of the advance technology developed for the purpose of extraction. The objective of this research is to study the effect of microwave power level on extraction of Pandanus Amaryllifolius Roxb leaves and to compare the total phenolic content (TPC) and antioxidant activity of traditional and microwave-extracted phenolic sample. The parameters that were varied during this experiment is the solvent concentration, soaking temperature and power level of the microwave. From the results of the experiment, MAE produce higher yield of TPC compared than that of soaking method with value 1.557 mg/g GAE and 0.979 mg/g GAE respectively. The result also established a linear correlation between total phenolic content (TPC) and antioxidant scavenging activity with R^2 value is 0.915.**

Keywords— Pandanus Amaryllifolius Roxb, phenolic content, antioxidant activity, microwave-assisted extraction (MAE), soaking method.

1.0 INTRODUCTION

Pandan leaves is a tropical plant with aromatic scent. It is a vertical, green plant with fan-shaped sprays of long, narrow, blade-like leaves and woody aerial roots. The plant is rare in the wild but it is widely cultivated in Asian countries. It is a source of natural flavoring that is widely used in various parts of South East Asia including Thailand, India, Indonesia and also Malaysia (Andriani et al., 2015). They are commonly used in culinary area such as food coloring, flavor enhancing and aromatic flavor for the dishes.

Bioactive compounds are nutritional elements that is naturally present in small quantities of foods. It is a type of chemical compound found in plants and certain foods such as fruits, vegetables, nuts, oils, and whole occur naturally in plants or fruit but it can also be produced in a certain environment, such as controlled fermentation of vegetable for alcohol production (Liu, He, Valiente, & Montserrat, 2017).

Therapeutic bioactive field are currently experiencing a rising interest to be used in wide range of applications not just in food industry, but in geo-medicine, plant science, modern pharmacology, and cosmetics. The conventional extraction techniques such as Soxhlet, solid–liquid extraction (SLE), or liquid–liquid extraction (LLE) have a certain drawbacks. They often require long extraction time and produce low extraction yields of bioactive components (Angiolillo, Nobile, & Conte, 2015). It is also extracted compounds will be damaged during this type of extraction. Thus, it is crucial to come out with other technique of extraction that will reduce the period of extraction while simultaneously increase the quality of the extracted bioactive materials.

The traditional method of soaking pandan leaves are widely applied in the Asian culture. It is used in many culinary application as food coloring, flavor enhancing and also to add an aromatic smell to the dish. There is no known result whether the method of soaking pandan leaves could extract the bioactive compounds. The great medicinal of pharmaceutical benefits of the bioactive compounds may or may not be present in the soaked sample.

That is why the new and advanced technique such as microwave assisted extraction (MAE) have been developed to overcome the problem. This extraction techniques have been used to extract bioactive compounds from various plant materials and have been proven to yield better extraction results. With the help of MAE, the extraction can be completed within minutes with better extraction results compared than that of conventional techniques.

2.0 METHODOLOGY

In this research, the plant that will be used for the extraction is Pandanus Amaryllifolius Roxb leaves. The bioactive compounds of these pandan leaves will be extracted to test the total phenolic content (TPC) and the antioxidants activity of the extracts. For this research purpose, traditional and microwave assisted extraction (MAE) is used to extract the bioactive compounds from the Pandanus Amaryllifolius Roxb leaves.

The solvent concentration for the soaking of pandan leaves are 50%, 75% and 100%. The range of power level of the microwave are 100W, 180W, 300W, 450W, 600W and 850W. For the traditional extraction method, it involves soaking the leaves in hot or boiling water. The temperature for soaking process is 40°C, 50°C, 60°C and 70°C.

A. Sample Preparation

Pandan Amaryllifolius Roxb leaves were purchased on a local market. The leaves were washed under running water to remove the all the dirt or impurities on the leaf. The washed leaf was then dried and cut into smaller pieces. 1.0 L of the extraction medium were prepared for each 50%, 75% and 100% of ethanol-water concentration.

B. Soaking Method

For each ethanol-water concentration, 100 mL of the prepared solvent was transferred in a transparent beaker and placed on top of a heating plate. The ethanol-water solution is heated up until desired temperature. After the solvent reached the desired temperature, 3 gram of shredded pandan leaves were added into the heated mixture. The soaking process was continued for 10 minutes. After 10 minutes, the solutions were remove from the heating plate and left aside to be cooled. The cooled sample were then subjected to Folin-Ciocalteu and DPPH assay to test the phenolic content and the antioxidant capacity. This step is repeated for each different temperature and concentration.

C. Microwave-Assisted Extraction (MAE)

For MAE extraction method, the power level of the microwave were varied in 6 different level. 100 mL of each ethanol-water sample was prepared in transparent glass beaker. 3 gram of shredded pandan leaves were added into the sample and then placed at the center of microwave oven. The oven was then set accordingly to the desired power level. The MAE extraction process was carried out for 10 minutes. The extracted sample were then subjected to the Folin-Ciocalteu assay and DPPH assay to test the phenolic content and the antioxidant activity.

D. Total Phenolic Content

0.5 mL of the sample was pipetted into volumetric flask and 0.5 mL of Folin-Ciocalteu reagent were added into the solution. 1.5 mL with 20% Na2CO3 were added and the volume was made up with 5 mL distilled water. The sample was then incubated for 2 hours at room temperature in the dark. After 2 hours, the sample were transferred into a cuvette and the absorbance was measured using spectrophotometer at 760nm against the blank sample. The measurement was compared to a standard curve of prepared gallic acid solutions.

E. Antioxidant Activity

The free radical scavenging activity of antioxidant extracted from Pandan Amaryllifolius Roxb leaves were determined using spectrophotometer, using a stable free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. 50 µL of extract were added to 200 µL DPPH solution. The mixture was vortexed and incubated for 30 min at room temperature. After 30 minutes, the incubated sample was taken out and the absorbance was then measured at 517 nm using spectrophotometer. All test analysis were run in triplicate and averaged. The scavenging activity was calculated using the formula below:

$$(\%\ inhibition) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Abs_{sample} = Absorbance of sample
$Abs_{control}$ = Absorbance of DPPH solution

3.0 RESULTS AND DISCUSSION

The gallic acid calibration curve was prepared and plotted. From the curve, the linear equation line, y = 0.2512x – 0.1994 and R2, 0.994 were generated. From the equation, the total phenolic content (TPC) were calculated and tabulated in Table 3.1 below.

Table 3.1: Total phenolic content (mg/g GAE) of extracted sample from soaking and MAE method.

	Solvent Concentration (%)		
Temperature (°C)	**50**	**75**	**100**
40	0.484	0.546	0.428
50	0.704	0.718	0.472
60	**0.892**	**0.979**	0.492
70	0.699	0.874	**0.562**
Power Level (W)			
100	0.494	0.675	0.594
180	0.603	1.030	0.630
300	0.982	1.310	0.744
450	1.018	**1.557**	0.799
600	**1.317**	1.448	**1.029**
850	1.172	1.203	0.971

A. TPC from soaking method

From the result in Table 3.1, we can see that for each concentration, the total phenolic compound increase gradually as the temperature increase. The highest total phenolic content (TPC) of 0.979 mg/g GAE at ethanol-water concentration of 75% and temperature 60ºC. After 60ºC, the TPC decreases. It proves that prolonged heat subjected to the sample will degrade all the existing TPC inside the extracted sample of pandan leaves. According to previous studies, the extent of degradation of bioactive depends on the genotype, cooking method and heating time. Most studies on vegetables reported less than 50% loss of bioactive after prolonged heat treatment (Beta & Hwang, 2018).

When comparing the result in terms of the solvent concentration, it shows that in overall, 75% ethanol-water concentration yields greater number of total phenolic compound. At 60ºC, it records the highest TPC value for 50% and 75% solvent concentration. But 75% solvent concentration shows the higher yield with 0.979 mg/g GAE while for 50%, the TPC is 0.892. For 100% concentration, the phenolic compound started to degrade and the highest results it produce is 0.562 mg/g GAE. The lowest TPC value of 0.428 mg/g GAE resulted from 100% ethanol-water concentration and 40ºC soaking temperature.

B. TPC from MAE method

In Table 3.1, for each concentration, we can see the result of TPC increasing progressively as the power level (W) of the microwave increase. But after certain power level, the result of TPC extracted from the pandan leaves decreases. The highest TPC extracted is at solvent concentration 75% and power level of 450W with value of 1.557 mg/g GAE. But at the same solvent concentration, at 600W and 850W, the yield of TPC decreases gradually. The lowest number of TPC recorded from this method is during 100% ethanol-water concentration and at 600W with the TPC value of 1.029%.

C. Comparing TPC from both soaking method and MAE method

For soaking method, the time taken for the extraction for all sample is 10 minutes. But for the microwave assisted extraction, the extraction time is around 5 to 10 minutes. This is because for the higher power level, the sample could not be extracted for 10 minutes due to the evaporation of the solvent resulted from the long exposure to the microwave power. The pandan leaves that were extracted using lower power level of microwave, 100W, 180W and 300W are extracted for 10 minutes. Figure below shows the result from the 10 minutes extraction between soaking and MAE method.

From the trend on the graph below, we can conclude that for all three solvent concentration, the total phenolic content extracted using microwave assisted extraction, MAE produce higher number of TPC compared to the results from soaking method. The graph shows that for 10 minutes extraction, the highest yield of TPC is 1.31 mg/g GAE for MAE extraction at 75% solvent concentration.

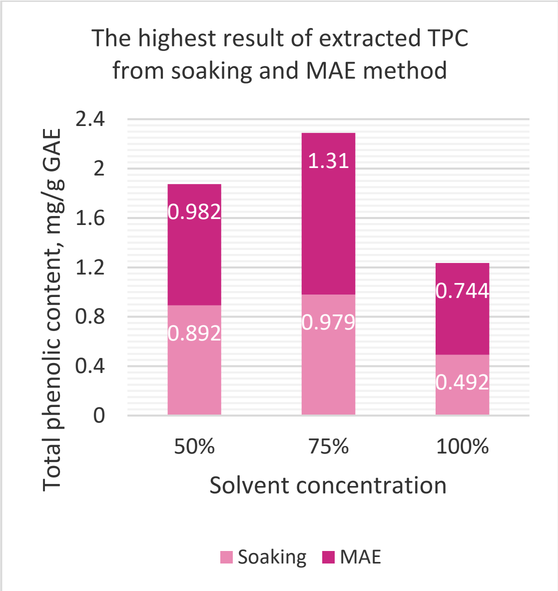

Figure 3.1: The highest result of extracted TPC for soaking and MAE method.

This proves that the usage of advance technology of MAE as the means of extraction is much more effective and efficient compared to the conventional way of extraction (Flamini et al., 2007). The MAE method provide more efficiency in extracting the phenolic compound because it induced a faster and more thorough heat to the sample since each molecule exposed to the microwave field is directly affected by the microwave energy or irradiation (Lavric, Alupului, & Calinescu, 2012).

In case of microwave irradiation on biological material, electromagnetic waves were absorbed selectively by media possessing a high dielectric constant resulting in more effective heating. During absorption, the microwaves' energy is converted into kinetic energy, thus enabling the selective heating of the microwave-absorbent parts of the plant material. This will make the volume increases and eventually will resulted in the explosion of the cells presence in the plant, releasing their content into the liquid phase. When the liquid phase absorbs the microwaves energy, the kinetic energy of its molecules increases, therefore, the diffusion rate will increases and it will lead to faster mass transfer of the desired bioactive into the extraction medium (Lavric et al., 2012).

D. Antioxidant activity of extracted sample

The antioxidant activity of the *Pandanus Amaryllifolius Roxb* extract was assessed bases on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The changes in color (deep violet to a pale yellow) were measured at 517 nm on a spectrophotometer. Table 3.2 shows the result of the absorbance reading for the antioxidant activity.

Table 3.2: Absorbance value of antioxidant from soaking and MAE method.

	Solvent Concentration (%)		
Temperature (°C)	**50**	**75**	**100**
40	0.784	0.670	0.721
50	0.672	0.593	0.598
60	**0.563**	**0.494**	**0.521**
70	0.578	0.506	0.613
Power Level (W)			
100	0.768	0.704	0.833
180	0.677	0.637	0.767
300	0.469	0.425	0.543
450	0.333	**0.204**	0.424
600	**0.214**	0.220	**0.310**
850	0.397	0.375	0.517

The lower absorbance value indicated strong antioxidant activity (Abas, Lajis, Israf, Khozirah, & Umi Kalsom, 2006). If we look at the overall result, it shows that the phenolic compound extracted from MAE method provides stronger antioxidant activity compared to the phenolic compound extracted from soaking method. From the result, we can clearly see that the lowest absorbance reading is 0.204 from MAE extracted sample at solvent concentration 75% and 450W power level. For phenolic compound extracted from soaking method, the highest antioxidant activity is 0.494 at 75% solvent concentration and soaking temperature of 60°C.

E. Antioxidant scavenging activity of extracted sample

Table 3.3: DPPH scavenging activity (% inhibition) from soaking and MAE method.

	Solvent Concentration (%)		
Temperature (°C)	**50**	**75**	**100**
40	14.32	26.78	21.20
50	26.56	35.19	34.64
60	**38.47**	**46.01**	**43.06**
70	36.83	44.70	33.01
Power Level (W)			
100	16.07	23.06	8.96
180	26.01	30.38	16.17
300	48.74	53.55	40.66
450	63.61	**77.70**	53.66
600	**76.61**	75.96	**66.12**
850	56.61	59.02	43.50

The DPPH radical scavenging activity is known to be related to the nature of phenolic contributing to their electron transfer or hydrogen donating ability (Loganayaki, Siddhuraju, & Sellamuthu, 2013). Based on the results in Table 4.5, the highest scavenging activity for the phenolic extracted from Pandanus Amaryllifolius Roxb is from MAE extracted method which is 77.70% for power level 450W at solvent concentration 75%. There is also an antioxidant scavenging activity from soaking method but it is not as high as the MAE method. The highest scavenging activity from soaking method from phenolic extracted using solvent concentration 75% at soaking temperature of 60°C with value of 46.01%.

The antioxidant presence in phenolic compounds found in herbs act as reducing agents to these free radicals. The high scavenging activity shows that the antioxidant presence inside the phenolic compound are able to scavenge the free radicals or oxidants and terminate the chain reaction before vital molecules are damage.

F. Correlation between TPC and DPPH antioxidant scavenging activity

As an important category of phytochemicals, phenolic compounds have been recognized as the main source of antioxidant capacity in plants (Han et al., 2017). Usually, phenolic that possess antioxidant activity are known to be mainly flavonoids and phenolic acids. Based on other research, the antioxidant activity is said to be proportional to the concentration of phenolic compound extracted (Álvarez et al., 2017).

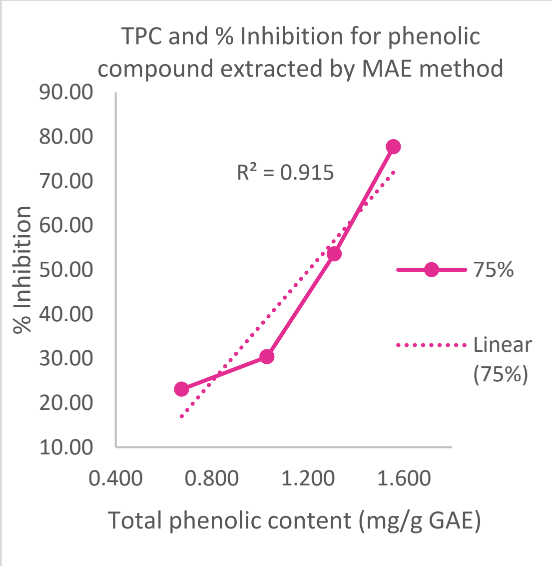

Figure 3.2: Relationship between DPPH scavenging activity and the TPC.

From the graph, it can be seen that there is a linear correlation between total phenolic content (TPC) and antioxidant scavenging activity. From the linear graph, the R^2 value is 0.915. R^2 is a statistical measure of how close the data are to the fitted regression line. In this case, the higher the R^2 value means the antioxidant activity increase as the total phenolic content increase (Abdel-Hameed, 2009). These result are also supported by other research done previously in 2007 by Paixao et al regarding the relationship between TPC and the antioxidant capacity of wines.

4.0 Conclusion

It has been proven that the bioactive compound or the phenolic content presence in Pandanus Amaryllifolius Roxb can be extracted by using soaking method and microwave assisted extraction method. In terms of process efficiency, the use of microwave prove to have a higher extraction yield and phenolic richness compared to the soaking method. The acceleration effect of microwaves on extraction kinetics was more pronounced on polyphenols than on other compounds such as sugar and fibers. This result led to an optimal extraction operation time when all the phenolic compound had already been fully extracted.

The highest TPC extracted is at solvent concentration 75% and power level of 450W with value of 1.557 mg/g GAE. The lowest absorbance reading is 0.204 from MAE extracted sample at solvent concentration 75% and 450W power level. That makes the optimal extraction condition is 450W microwave power and 75% ethanol-water concentration. This also proves that the antioxidant activity is proportional to the concentration of phenolic compound extracted (Álvarez et al., 2017).

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