

Therapeutic Bioactives from *Pandanus Amaryllifolius sp* for Functional Food

Nur Nadia Bt Mohd Zakaria, and Nur Asyikin Binti Md Zaki,

Faculty of Chemical Engineering, Universiti Teknologi Mara

Abstract—The benefits and the nutraceutical values contained in the bioactive compounds are proven can be used as the therapeutic agents that have the potentials for prevention and cure human diseases. In this research, the extraction of bioactive compounds by using *Pandanus Amaryllifolius sp* leaves has been carried out to study the effect of microwave power level on extraction of bioactive compounds and also to determine the flavonoid compound and the antimicrobial properties of *Pandanus Amaryllifolius sp*. Traditional method which is soaking extraction is also conducted as comparison to microwave extraction which is the modern method. The HPLC analysis has been conducted for 30 minutes to detect the present of Catechin compound which is in flavonoid group and antimicrobial analysis for *Pandanus Amaryllifolius sp* has been investigated by using *E.coli sp*. From this research, it showed that there is present of Catechin compounds in the extract which is affected by the percentage concentration of solvent used and also the microwave power level used. Increase in microwave power level will enhance the percentage yield of Catechin compound. However, higher microwave power level and longer time of exposure to microwave radiation caused decrease in percentage yield of Catechin compound. This study also showed that the microbial activity of *E.coli sp* is retarded by the highly concentrated extract of *Pandanus Amaryllifolius sp*. Thus, *Pandanus Amaryllifolius sp* leaves contained Catechin compound and also showed antimicrobial property to *E.coli sp*

Keywords—*Pandanus Amaryllifolius sp*, bioactives, Catechin, microwave-assisted extraction, HPLC.

I. INTRODUCTION

Nowadays, the rate of patients having the chronic diseases such as diabetes mellitus, cancer and heart attack around the globe showed a high percentage among others. In Malaysia, at least one over six other people is facing obesity which increases the awareness among the citizens to start a healthy diet and lifestyles. The benefits and the nutraceutical values contained in the bioactives compounds are proven can be used as the therapeutic agents that have the potentials for prevention and cure human diseases causing higher demand for the bioactive compounds.

Bioactive compounds can also be referred as the secondary metabolites that showed pharmacological or toxicological effects in living organisms (Azmir, Zaidul et al. 2013). They are normally can be used as the natural therapeutic agents because of their properties that can interact well with proteins, DNA and other biological molecules. However, problems arise due to the extraction process of the bioactive compounds which is time consuming and higher volume of solvents is needed in order to extract higher yield of natural bioactives. Furthermore, the techniques do not produce the maximum percent of yield and require high cost. The chosen techniques for the extraction of bioactive compounds should be affordable, effective in percentage yield of bioactive compounds,

lower energy consumption, environmental and safety friendly and lastly is favorable for industrial applications (Nastić, Švarc-Gajić et al. 2018). Thus, the improvisations of the traditional techniques are needed in order to gain the maximum percent of yield of the bioactive compounds.

In this modern era, improvements have been made where the limitation of these traditional methods can be solved by using the modern technique such as microwave-assisted extraction. This method applies water vaporization technique in order to minimize the moisture content of the product desired by enhancing simultaneously an etiolate effect (Ferreira, Passos, Cardoso, Ferreira Wessel, & Coimbra, 2017). The beneficial uses of MAE are the time of extraction is decreases, reduction in solvent consumption, and the high purity of crude extracts can be gained (Jila et al., 2011). However, some of these techniques might have several limitations such as the use of toxic solvents and the degradation of bioactive compounds due to the duration of extraction (Garcia-Castello, Rodriguez-Lopez et al. 2015).

The important parameters during the extraction process are the types and concentrations of the solvents which are the main concern for the extraction process. In order to promote green method in extraction of bioactive compounds, green solvent is the most frequently that has been chosen. The two types of green solvent that has been investigated is firstly the ‘bio-solvents’ that can be gained from renewable resources which can replace the conventional used of petro-chemically fabricated solvents and secondly is the solvent that showed better environmental, health and safety properties to replace hazardous solvent (Khandelwal, Tailor et al. 2016)

Besides, the other crucial parameters that affecting the extraction of bioactive molecules are the sampling time, temperature, and desorption time (Jila et al., 2011) In this research, the natural bioactive compounds are extracted from the plant which is from *Pandanus Amaryllifolius*. This species is normally used as the food flavouring, food colouring and also traditionally used as food wrapping. It can also be applied as the aromatherapy for someone who enjoys natural fragrant scent. It is also showed several bioactivities such as antiviral, antioxidant, and antihyperglycemic activities (Cheng et al., 2017). Based on past research, the leaves of *Pandanus Amaryllifolius* are applied in medicinal field to reduce fever, refresh the body and soothe indigestion (Cheeptham & Towers, 2002).

Microwave-assisted extraction is used to investigate the effect of microwave power level on extraction of bioactive compounds and also to determine the flavonoids and antimicrobial properties of *Pandanus Amaryllifolius sp*. Besides, the traditional extraction method which is soaking extraction method is also conducted as the comparison.

II. METHODOLOGY

A. Preparation of *Pandanus Amaryllifolius sp* leaves

1 kg of *Pandanus Amaryllifolius sp* leaves are collected from the garden and each leave has been wash to prevent contamination.

Then, the leaves were cut into smaller pieces to provide high surface area for extraction process.

B. The Extraction of Bioactive Compounds by using Soaking Method

The *Pandanus Amaryllifolius* leaves that has been cut into small pieces undergoes soaking method where the solvent used is ethanol with different percentage concentration of solvent which are, 50%, 75% and 100%. For each of the solvent concentration used, the temperature for the extraction process will be 40°C, 60 °C, 50°C and 70 °C. The solvent and *Pandanus Amaryllifolius* leaves were heat up on top of the hot plate by using beaker for 10mins and the total volume of solvent per sample is 100ml.

C. The Extraction of Bioactive Compounds by using Microwave

50g of the *Pandanus Amaryllifolius sp* leaves were extracted by using 100ml of 50, 75, and 100 percentage concentration of ethanol as the solvent for 10 min. The microwave power that has been used is 100,150,300, 450,600 and 850 watt.

D. Determination of the Flavonoid Compounds by using High Performance Liquid Chromatography (HPLC)

In order to determine the type of the bioactive compounds, HPLC has been used. The content of Catechin compound in *Pandanus Amaryllifolius sp* leaves which is in the group of flavonoid is to be determined based on its retention time. The retention time for Catechin compound is in between 24 to 27 minutes. Catechin compounds is analyzed by using a mobile phase which is acetonitrile and water containing 0.1% acetic acid at pH of 3.0 for the ratio of 30 to 70 (Colombo et al., 2017). The injection volume was 20 µL and the HPLC system was operated at an isocratic flow of 1.0 mL/min, with detection at 368 nm and temperature of 30 °C.

E. The Determination of Antimicrobial Property of *Pandanus Amaryllifolius sp* Leaves

i. The preparation of *Lauria Bertani* agar

500ml of LB liquid which consist of 13g broth powder per 500ml in a 1000mL Erlenmeyer flask. Then, mix it up by swirling. Next, add a fresh piece of aluminum foil to cover the top of Erlenmeyer flask. Ensure that to autoclave the mixture at 121°C for 15 minutes. After that, cool the mixture for a while before pour out the media into petri dishes then replace the lid in the laminar flow. In order to prevent contamination, seal each petri dish by using parafilm tape. Lastly, the petri dishes should be stored upside down in the cold room.

ii. Inoculation of *E.coli sp* on nutrient agar plate

The antimicrobial property of *Pandanus Amaryllifolius sp* leaves can be determined by using microorganism which is *E.coli sp*. *Lauria Bertani* agar is used to provide nutrient in order to inoculate the *E.coli sp*. In order to determine the antimicrobial property of *Pandanus Amaryllifolius* leaves, the extract gained is spread evenly on the top of LB agar. Then, inoculate the *E.coli sp*. culture on the same LB agar and leave it in incubator for 24 hours. Ensure that the process is held in the laminar hood and aseptic techniques are applied to prevent contamination. Repeat the same technique for each of the extracts gained from the soaking method and microwave extraction method. Next, colony counter is used to determine the cell count of *E.coli sp*. The results obtains are tabulated and inhibition of *E.coli sp* for each petri dish is compared.

III. RESULTS AND DISCUSSION

A. HPLC analysis on Catechin compound in *Pandanus Amaryllifolius sp* leaves

High performance liquid chromatography is used to determine the present of catechin *Pandanus Amaryllifolius sp* leaves which is in the group of flavonoid. Catechin is detected based on its retention time which is between 24 to 27 minutes.

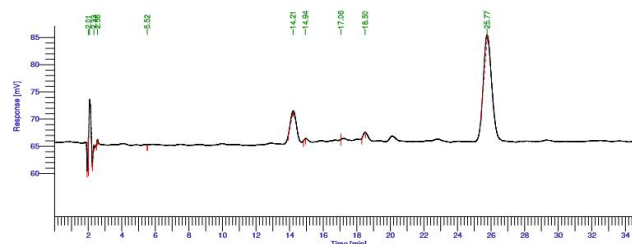


Fig 1: HPLC chromatograms of Catechin compound which can be detected in between 24 to 27 minutes

Table 1: Percentage concentration of Catechin compound extracted from soaking extraction method

Percentage concentration of ethanol	Temperature (°C)	Percentage concentration of catechin (%)
50%	40	4.54
	50	5.36
	60	20.31
	70	35.25
75%	40	0.05
	50	1.17
	60	2.76
	70	2.95
99%	40	0.01
	50	0.01
	60	0.51
	70	0.52

Table 2: Percentage concentration of Catechin compound extracted from microwave-assisted extraction method

Percentage concentration of solvent	Microwave power (watt)	Percentage concentration of Catechin (%)
50%	100	4.28
	180	6.27
	300	19.56
	450	18.39
	600	5.6
	850	6.7
75%	100	0.9
	180	11.16
	300	15.89
	450	13.59
	650	11.53
	850	8.98
99%	100	2.93
	180	0.56
	300	3.37
	450	2.76
	650	2.55
	850	2.33

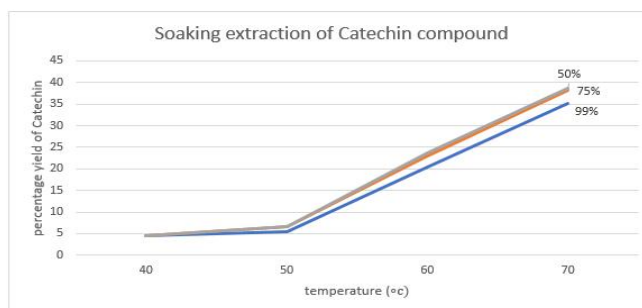


Fig 1: The graph of percentage yield of Catechin compound from soaking extraction method

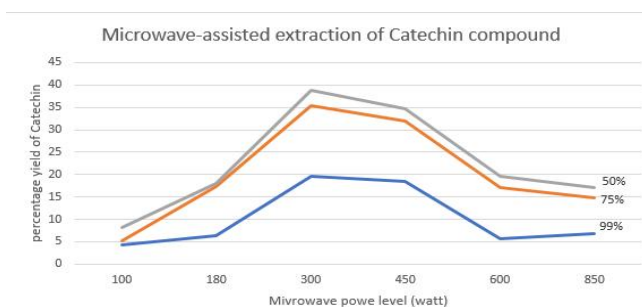


Fig 2: The graph of percentage yield of Catechin compound from microwave-assisted extraction method

The analysis is conducted for 30 minutes and the result shown that for soaking extraction method, the highest percentage concentration of Catechin based on peak area is 35.25% is detected at temperature of 70°C and percentage concentration of ethanol is at 50%. The highest polarity of the solvent which is at 50% is the factor of the highest percentage yield of Catechin. As for 75% concentration of ethanol, it showed better extraction yield of Catechin as compared to 99% concentration of ethanol. However, the percentage yield of Catechin is not much as compared to 50% concentration of ethanol. This is might due to the extraction of more polar compound by polar solvents (Kim and Godber, 2014). As the temperature increases, the percentage yield of Catechin also increases. However, when the temperature above 70 °C, the solvent will evaporated due to the boiling point of ethanol which is at 70°C. As for 75% and 99% concentration of ethanol, also showed that as the temperature increases, the percentage yield of Catechin is also increases. At elevated temperature, it will enhance the solubility due to solute vapour pressure enhancement. Besides, the surface tension of water will decrease which permit enhanced water wetting of the extracting material and rapid dissolution of the desired compounds (Cvjetko Bubalo, Vidović et al. 2018).

For microwave assisted extraction samples, the analysis result shown that at 300 watt of microwave power and at 50% concentration of ethanol showed the highest yield of Catechin compound which is the highest polarity of solvent. Microwave-assited extraction require high polarity solvent in order to extract the bioactives compounds. Higher polarity solvent is capable to absorb microwave energy efficiently thus, diffusivity rate of the compounds will increases from the stagnant layer to the solvent (Vinatoru, Mason et al. 2017). Other reason could be the dielectric loss, the plant material can reach a higher temperature than the solvent when the dielectric loss of the tangent solid is higher than the solvent. It will caused the cell's membrane to rupture and release of the compounds from inside the cell due to the increase in cell pressure affected from the dielectric loss (C.S.Lee et.al, 2016). As the microwave power increases, the percentage yield of Catechin is also increases. However, when the microwave power is too high, it could degrade the bioactive compounds. Thus, decrease the percentage yield of Catechin. As for 75% and 99% concentration of

ethanol, also showed increases in percentage yield of Catechin as the microwave power increases up until 300 watt. As for 450 watt and above showed a decrease in percentage yield of Catechin. The duration of extraction also give impact to the yield of Catechin because longer time of exposure to the microwave radiation will degrade the chemical structures of the bioactive compounds. For 100 watt until 300 watt, the duration of extraction is about 10 minutes while 450 watt and above is only for 5 minutes due to the volatilization of the solvent.

From the result, soaking extraction method showed no degradation of compound as compare to microwave-assisted extraction method and the method require the temperature to approach by at least 50°C to be able to extract the bioactive compounds. Increase in power level of microwave caused the degradation of bioactive compounds to occur as compared to soaking extraction method which used temperature up to 70°C only. The degradation of bioactive compounds occur at 400watt and caused the percentage yield of bioactive compounds depleted. The microwave power used and the duration of extraction effects the yield of bioactive compounds significantly because when the microwave energy and the time of extraction increased, the concentration of free phenolic compounds extracted or other bioactive compounds will increases while the bound bioactive compounds will depleted (Wijngaard, Hossain et al. 2012). However, increasing the power of the microwave and the time of extraction might damage the structure of the compounds (Xiong, Chen et al. 2016).

B. Effect of bioactives compound in *Pandanus Amaryllifolius* sp leaves on microbial activity of *E.coli*

Pandanus Amaryllifolius leaves showed an antimicrobial characteristic by retarding the microbial activity of *E.coli* inoculated on the agar plate for 24 hours. From the result, highly concentrated mixture of grinded *Pandanus Amaryllifolius* leaves and solvent inhibit the microbial activity while aqueous extract of *Pandanus Amaryllifolius* leaves from microwave-assisted and soaking extraction method showed less inhibitory effect to the microbial activity of *E.coli*. Highly concentrated mixture of grinded *Pandanus Amaryllifolius* leaves contain more bioactive compounds as compared to the aqueous extract of *Pandanus Amaryllifolius* leaves such as the present of alkaloids, terpenes, flavonoids, and other types of glycosides that inhibit the microbial activity (Andriani, Ramli et al. 2015).



Fig 1: The agar plate showed inhibitory effect of highly concentrated *Pandanus Amaryllifolius* sp extract towards microbial activity of *E.coli*

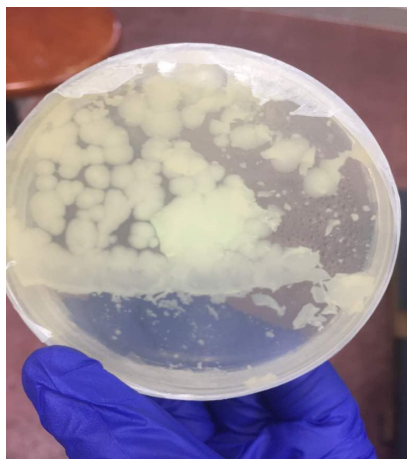


Fig 2: The agar plate showed less inhibitory effect of aqueous extract from microwave-assisted extraction toward microbial activity of *E.coli sp*



Fig 3: The agar plate showed less inhibitory effect of aqueous extract from soaking extraction method toward microbial activity of *E.coli sp*

IV. CONCLUSION

In conclusion, the microwave power level significantly affect the percentage yield of bioactive compound. The result showed that as the microwave power level increases, it will enhance the percentage yield of bioactive compounds. The present of flavonoid compound which is Catechin is also detected based on retention time from HPLC chromatogram. The percentage yield of Catechin is significantly affected by the microwave power level and the percentage concentration of solvent used. Besides, highly concentrated extract *Pandanus Amaryllifolius sp* portrays antimicrobial property by retarding the microbial activity of *E.coli sp*. Thus, microwave-assisted extraction offered a reliable and efficient method for extraction of bioactive compounds and the benefits and nutraceutical values contained in the *Pandanus Amaryllifolius sp* is proven due to the present of bioactives compounds which can be used as the therapeutic agents that have the potentials for prevention and cure human diseases. Future application of bioactive compounds as the therapeutic agent must be investigated for better understanding.

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