Extraction of Polyphenol from Clinacanthus nutans Lindau Medicinal Plant Using Solvent-Free Microwave Extraction (SFME)

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***Abstract*— Clinacanthus Nutans Lindau (C. nutans) is one of the family 'Acanthaceae'. In Malaysia, C. nutans is known as Sabah Snake Grass or 'Belalai Gajah'. C. nutans is well-known with their high polyphenol content and one of the medicinal plants traditionally used to treat various diseases and injuries because of their potential therapeutic benefits. In this work, solvent-free microwave extraction (SFME) method have been used to extract polyphenols from C. nutans. The effect on extraction is analysed: microwave power and extraction time. In order to explain more the efficiency in extraction of the polyphenol content, energy absorbed during the exposure to the microwave radiation has been studied and analysed. Results indicate that the polyphenol content extracted by SFME which used microwave power of 240 W and extraction time of 1 min, 1.4610 mg GAE/g DS is the best result.**

INTRODUCTION

Clinacanthus nutans Lindau is one of the family 'acanthaceae'. It is basically a small shrub that native to tropical asia countries. In malaysia, c. Nutans is known as sabah snake grass or 'belalai gajah' [1]. In malaysia and thailand, this plant has been used as a remedy for bites and stings which is from snake, skin rashes, herpes simplex virus (hsv) and varicella-zoster virus (vzv) lesions [2, 3]. The extensive research is being conducted on the c. Nutans for its medicinal properties [4]. In recent years, the c. Nutans has attracted many people interest due to their properties which is properties for cancer treatment.

In order to extract the polyphenols in the C. nutans, various methods of extraction have been done to study the polyphenols. Moreover, each of the methods have different efficiency to extract. In which, there are parameters that affect the extraction process may differ and must be examined in order to determine the effectiveness of the method. The parameters are such as solvent polarity and concentration, solvent-to-feed ratio, extraction time, thermal degradation, microwave power and temperature of extraction.

A simple Maceration and Soxhlet extraction method are conventional method that being used to extract the phytocompounds. However these method consume a longer time to extract and high amount of solvent. When using high amount of solvent it may poison the photocompounds. Therefore, further study on the C. nutans is continued with Supercritical fluid extraction. This method consume lesser amount of solvent to extract and well known method for producing high quality plant extracts in a safe and clean way. Furthermore, a Microwave assisted extraction (MAE) is developed to extract the phytocompounds. The extraction is less consumption of solvent and higher yield of phytocompounds in a shorter time of extraction. However this method still consume solvent to extract the phytocompounds. Recently, an extraction method being introduced called Solvent-free Microwave extraction (SFME) to extract the phytocompounds from medicinal plant without using any solvent to extract and recognized as a green method.

The main aim of this study is to identify polyphenol content in Clinacanthus Nutans Lindau extracted by SFME. Additionally the effect of SFME parameters such as microwave power, 160W, 240W and 320W, as well as extraction time, 1min, 3min and 5min will be studied. Besides, the C. nutans will extracted by SFME and the extraction will be analysed by using a UV-vis spectrometer to calculate total phenols content. The result is predicted that SFME can extract the phytocompounds in a shorter time extraction and higher yield of extraction than MAE.

I. METHODOLOGY

A. Materials

The fresh leaves of C. nutans (Burm.f.) Lindau sample was purchased from Mr. Azizi from D' Kaduk Herbs Floral, Kajang Selangor, Malaysia. The collected plant materials are air dried and stored at room temperature. Thus, only fresh leaves materials was used in this extraction. The anhydrous sodium sulphate is obtained from the laboratory of Faculty of Chemical Engineering, UiTM Shah Alam.

B. Sample Preparation

The plant materials' stems and leaves was air dried in an oven set at 105°C for 8 hours to determine symmetrically total moisture content. While for extraction used the samples were stored in an airtight bags that filled with nitrogen gas and keep in a refrigerator at -8°C until used in extraction experiments. Then, the ethanol (99.7 %) was diluted with acidified water (pH 1 by hydrochloric acid) into 50% v/v. The distilled water that used for the dilution was boiled to remove dissolved gasses.

C. Solvent-Free Microwave Extraction Method (SFME)

In SFME, a domestic microwave oven (Panasonic, 20l, 800W maximum power) with wave frequency of 2.45 MHz was used to extract the plant samples.

The SFME procedure was performed at atmospheric pressure and there was several extractions. Thus, the leaves C. nutans that placed inside the 250 ml conical flask was 5 g of fresh leaves. In addition, the weight of the samples were constant. Each of the samples was heated by microwave irradiation with different power 160, 240 and 320 W. Each of the powers was tested with different extraction time, 1, 3 and 5 min. Overall of the extraction process was performed without using any solvent or water. Every extraction was performed twice and calculated an average of the standard deviation. After the exposure to microwave extraction, the temperature was measured immediately and recorded as the final temperature. Then, the samples were cooled down to 40°C in an ice water bath rapidly for sudden dropped temperature and 4 ml of ethanol-water solution (50%) was added. Then, the samples were kept under magnetic stirring and placed into water bath (40°C).

Furthermore, the extract was taken after exposed to the microwave extraction and during the water bath process for every 20 min until a total of 100 min of extraction time. Each of the samples was covered with aluminium foil to avoid the phytocompounds exposed to sunlight. The dried samples is stored at 4°C in fridge until further used for analysis.

D. Determination of Total Phenols Content (TPC)

The extracts were analysed by calculated the total phenols content (TPC), which determined by Folin-Ciocalteu colorimetric method. It was expressed as mg gallic acid equivalents (GAE) per gram of extract (mg/GAE/g extract). Firstly, mixed the 40 µL of sample extract with 3 mL of deionized water. Then, added 200 µL of Folin-Ciocalteu reagent. The mixture was stirred for 5 min. Then proceeded with addition of 400 µL of sodium carbonate, $NaCO_3$ to the mixture and immersed into the water bath (40°C) for 30 min before proceeded with UV–vis spectrophotometer analysis at 765 nm. The total phenols content was calculated by used equation (1)[5].

Equation (1):

$$\text{Total phenols content} = \frac{\text{Concentration } ({}^{mg}/_{L}) \times \text{volume of extract } (mL)}{\text{Weight of dry sample } (DS)(g) \times 1000}$$

E. Determination of Energy Absorbed and Energy Emitted

Based on the SFME, it was utilised the absorbed microwave energy to extract the sample. More active compound can be extracted when higher amount of microwave energy was been absorbed. The total amount of microwave energy absorbed in the water moisture content of the plant itself played important part. This is due SFME is a green method which is extraction without solvent and water moisture content plant itself acts as the solvent that extracts the sample. It is acts as the basis this modelling SFME process. Thus, the moisture content of C. nutans is taken into this process as the mass of the material that absorbed the microwave energy because the dielectric constant of C. nutans is not measured. Moreover, the microwave energy emitted will not completely absorbed by the system and converted to thermal energy. Regardless, the maximum nominal power of the microwave not showed the real power emitted by the microwave as reported by manufacturer. In general, the nominal power was estimated from the amount of energy absorbed by a water sample under a specific set of conditions. Since in this research, the microwave powers were used as a parameter and more convenient to indicate the experimental conditions employed. Therefore in discussion and analysis, the absorbed energy and energy emitted are needed [5, 6].

The amount of energy absorbed from the microwave power emitted must be measured. In order to calculate the energy absorbed, the mass of the samples exposed to the microwave radiation and the temperature difference between initial and final state must be taken account. The moisture content of the C. nutans was measured and taken into account as the mass of the material that absorbed the microwave energy. This was applied since the dielectric constant of C. nutans was not determined. This can be shown in equation (2) and (3):

Equation (2):

$$Q_{sensible} = m_s C_{p,s} (T_f - T_i) + m_w C_{p,w} (T_f - T_i)$$

Since the extraction is without used any solvent,

Equation (3):

$$Q_{sensible} = m_w C_{p,w} (T_f - T_i)$$

Where m_w is the weight of water within the samples, which the moisture content of the samples itself was taken into account as the weight. $C_{p,w}$ is heat capacity of water (Cp, w = 4.184 kJ/mol K). While T_i and T_f known as intial temperature and final temperature. Furthermore, latent heat of evaporation of the water within the C. nutans must be considered since in this research was not used any solvent. Thus, the enthalpy of the water due to the vaporization can be calculated as in equation (4).

Equation (4):

$$Q_{latent} = m_{vap} H_{vap}$$

Where m_{vap} is the weight of solvent evaporated, but for this extraction solvent was not used so water within the C. nutans was taken into account. While H_{vap} was calculated as the molar average of the heats of vaporization of water. Thus, the total energy absorbed can be shown in equation (5).

Equation (5):

$$Q_{absorbed} = Q_{sensible} + Q_{latent}$$

II. RESULTS AND DISCUSSION

A. The Total Phenols Content (Microwave Power and Extraction Time)

The result of the total phenols content were illustrated in curved graph to compare with each other. Based on the result, the highest TPC was shown in Extraction 4, 1.4610 mg GAE/g DS.

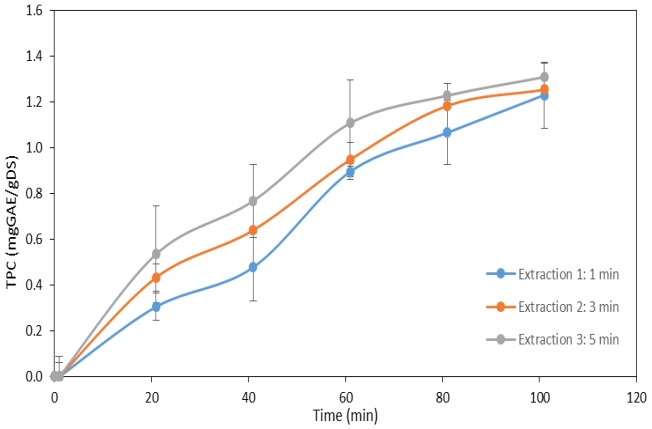

Figure 1: Extraction with Power of 160 W

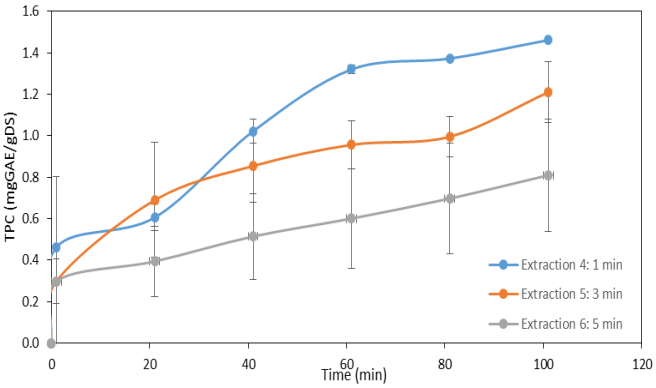

Figure 2: Extraction with Power of 240 W

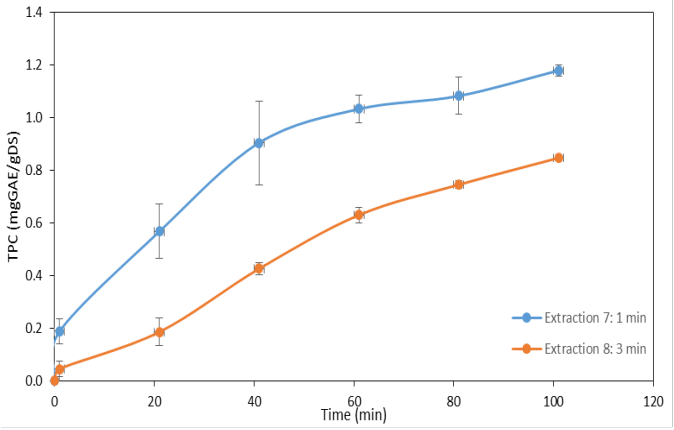

Figure 3: Extraction with Power of 320 W

In extraction 9, the sample is burned due to high microwave power and extraction time. Thus, it is not suitable to proceed to the extraction process. The final temperature recorded in Extraction 4 was 63°C which was performed with microwave power, 240 W and extraction time, 1 min. From Extraction 1 to Extraction 4, it were resulted to the increased of TPC. In general, TPC was increased when microwave power and time extraction were increased. When used higher microwave power and longer extraction time, the final temperature (after exposed to microwave extraction) was increased. Nevertheless, the used of microwave extraction method may obtain higher TPC values. In which, after microwave radiation the microwave energy is absorbed and will convert into heat, thus lead to the evaporation of moisture content within the fresh leaves. From the evaporation of water content, a pressure is created within the cell wall that eventually leads to cell rupture. Then will facilitate the active compounds to free into the surrounding and improved extraction yield [7]. However, when microwave power used was beyond the power of 240 W the TPC was decreased, as well as applied to the extraction time. The longer the samples exposed to the extraction process with the higher microwave lead to low TPC. As for this research, microwave power and time extraction were one of the specific parameters to study. From the precious research, they reported that the consumption of extraction time can be reduced when used higher microwave powers which may increase the TPC respectively [8-10]. Furthermore, the degradation may occur to the phenolic compound in the extraction. Unfortunately, the phenolic compounds are sensitive to degradation. Their unstable structure was relatively can be related to several environmental condition such as pH, oxygen, light, storage temperature and time [11]. As for this studied, the degradation was affected by several factors such as microwave power, extraction time and temperature extraction.

One of the factors is extraction time which affect the phenolic compounds. The extraction time is played important part in extraction industry. Where low consumption of extraction time is preferred due to the shorter time extraction and quality of the product. Additionally, extraction time is preferred because of the cost of the extraction process particularly reduced and essential in economizing energy [12]. Not just that, a longer extraction time may degrade the phenolic compounds due to expose to microwave radiation. This was particularly related with the thermal degradation and oxidation of sensitive compounds to overheating [13, 14]. On the previous studied, the TPC values were increased along with the increased extraction time and followed by a significant decrease after a specific extraction time [15]. However, the risk to degrade the phenolic compounds may present although increased the extraction time can increased the quantity of analytes extracted [16]. Some of the reports on the extraction time, stated that increased the extraction time did not affect the recoveries except for total flavonoid content. Hence, this can be supported by the Fick's second law of diffusion which a final equilibrium between the concentrations of solute in the solid matrix and in the bulk solution can be predicted after a certain time. This proven that higher extraction time is not preferred [12]. In short, the TPC extraction is increased when used higher extraction time till reached the maximum TPC at specific extraction time. When the extraction time used longer without temperature control could have induced thermal degradation of phenolic compounds [17]. Hence, most of the extraction are preferred shorter extraction time which lead to a reduction energy costs.

Furthermore, microwave power also contributed as one of the factor that affected the TPC. In extraction, that applied higher microwave power tends to lead the degradation. In past, they reported TPC increased when used higher microwave power but decreased rapidly when beyond a specific microwave power. In which, this is particularly due to the degradation of compounds [18]. Previous studied reported that a higher microwave power used in a short time can be effective extraction process on the phenolic compounds. Although the usage of higher microwave power may become effective way, it may tends to thermal degradation of the phenols [19]. Ballard et al., 2010; Dahmoune et al., 2015, reported that in the extraction of polyphenols can be positively affected by the microwave power and irradiation time. The degradation can be presented at longer time extraction with higher microwave power [19, 20]. The efficiency of TPC extraction can be reduced by applied higher microwave power [21].

Based on the result of the total phenols content, the TPC was increased along the temperature. The TPC was increased when temperature increased but when involved with higher microwave power and extraction time the TPC decreased. In the **Figure 1**, it was based on the microwave power 160 W with extraction time 1, 3 and 5 min. The temperature in extraction 1 to 3 were increased orderly. The TPC values were increased when applied higher microwave power and longer extraction time. However in **Figure 2** which used microwave power 240 W, shown TPC values were decreased when applied higher temperature and longer extraction time, as well as in the **Figure 3** which used microwave power 320 W. These two parameters, microwave power and extraction time were significant related to the temperature. Regardless, the temperature will increase when applied higher microwave power and longer extraction time. By used microwave power 240 W and 320 W, the temperature reached higher temperature in short time. Thus, higher temperature may resulted to low TPC due to the degradation to the phenolic compounds occurred in the extraction.

When it involved with higher microwave power, the microwave heating increased too. This lead to the elevated of the temperature in rapid which resulted to degrade [22]. One of the previous studied reported the phenol compounds were heat sensitivity and it may lead to deterioration of phenolic compounds when higher temperature is involved [23]. Kim, Kang, Heo, Chun, and Choi (2015) also stated that TPC decreased with increased heating temperature and time [24]. Nevertheless, the higher temperature lead to degrade of phenol compounds, which the plant tissue is soften through the heating and the phenol-protein and phenol-polysaccharide interactions were weaken [25]. From the resulted TPC, it shown that heat improved the TPC extraction efficiency. This can be related to some of the conditions, such as increased phenolic solubility, better mass transfer, faster diffusion rate and reduced solvent viscosity and surface tension [26]. Although application of higher temperature may increase the TPC values, it will reach the maximum TPC values at a certain specific temperature. Beyond the specific temperature it could promoted to the degradation of phenolic compounds. The degradation may occurred on the phenolic compounds which mobilized at lower temperature or it can be the decomposition of residual phenolic compounds that remained in the plant matrix [12]. For this reason, the microwave power and extraction time must be identified and measured to avoid any degradation to thermal sensitive phytocompounds [27].

B. The Total Phenols Content (Energy Absorbed)

Energy emitted and energy absorbed were calculated on each of the extraction, which energy absorbed is more efficient to

conclude the TPC obtained than using microwave power. The energy emitted and energy absorbed were tabulated in **Table 2**. Nevertheless, the **Figure 4** is illustrated based on the result in extraction 4, 7 and 8. The figure is compared the result based on the energy absorbed, which the energy absorbed is increased when applied with higher microwave power and extraction time. This also stated in previous studied which supported this hypothesis [6]. The higher TPC extraction was attained in extraction 4 which energy absorbed is 0.90417 kJ and energy absorbed is more efficient to relate with TPC than microwave power. Furthermore, Li et al. (2012) was stated that the total energy absorbed by the sample was more relevant than the microwave power [28]. Based on previous studied, it reported that extraction with a higher absorbed energy, gained higher extraction yield of TPC [6]. However, in this research the TPC value is decreased when applied higher microwave power and longer extraction time. Even though in the previous paper, it stated that TPC value increased with the higher energy absorbed [6]. This is because higher temperature is attained and resulted to the degradation of polyphenols as discussed as in the section TPC in terms of microwave power and extraction time which related to the temperature.

A series of conventional method extractions were performed after the microwave extraction process which the method was infusion at 40 °C using 50% v/v ethanol. This was performed to further investigate the effect of microwave power pre-treatment on the extraction of polyphenols from C. nutans. The result was illustrated in curved graph, where the TPC extraction was higher at a shorter extraction time and tend to decrease at a longer extraction time. The reduction of TPC values may be due to slow degradation of the dissolved polyphenols. This shows that the microwave power significantly affect the extraction and energy absorbed is determined.

In general, microwave heating on the plant samples will lead the matrix undergoes dramatic swelling and tissue will rupture. Thus, the essential oil can be free flow with the water layer within the samples. One of the properties of the essential oil played part is solubility. In which, ability to dissolve in water is affected the extraction process. Regardless, this ability becomes an essential parameter to SFME method and the solubilisation is the limiting step. In terms of organic compounds in the essential oils, they are particularly absorb microwave energy. Additionally by using microwave extraction, the compounds which have high and low dipolar moments can be extracted. These components can be in various proportions.

Table 2:
Energy Emitted and Energy Absorbed

Extraction	Power (W)	Time Extraction (min)	Initial Temperature (°C)	Final Temperature (°C)	Energy Emitted (kJ)	Energy Absorbed (kJ)
1	**160**	1	28	47.50	9.60	0.76
2		3	28	66.50	28.80	0.94
3		5	28	70.50	48.00	0.99
4	**240**	1	28	63.00	14.40	0.90
5		3	28	70.00	43.20	0.98
6		5	28	76.00	72.00	1.06
7	**320**	1	28	70.00	19.20	0.99
8		3	28	75.00	57.60	1.05
9		5	28	none	none	None

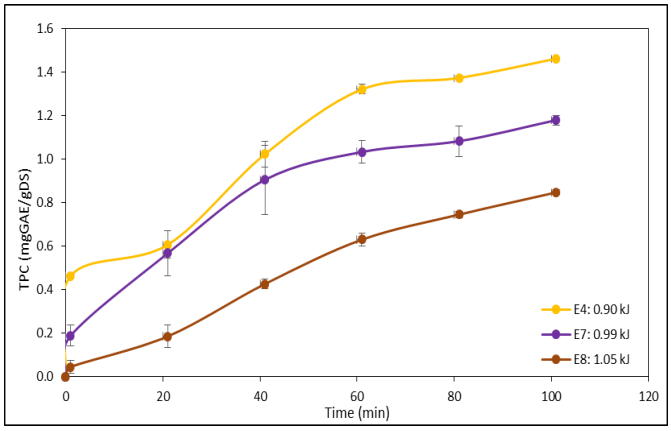

Figure 4: TPC obtained from different temperature and energy absorbed

Which the organic compounds with higher dipolar moment can be interacted more vigorously with microwaves. Thus this is more easily to extract than the compounds with aromatic compounds which have low dipolar moments [27].

When used microwave radiation to extract, basically the cell structure was damaged by microwave heating. This in because of sudden rise of temperature during microwave heating and fast cooling technique was applied to lead to a sudden drop of temperature in ice bath [6]. Thus, internal pressure inside the cells was occurred which helps in releasing the solutes from the samples. This is also stated by Dahmpune et al. 2015, that the reason behind of the microwave extraction of polyphenolic compounds from plant samples was the electromagnetic wave. Nevertheless, it caused the cell wall of plant samples to damage and rupture due to sudden temperature rise [20]. This also supported by the mechanism of heat and mass transfer within the plant samples which occur in the same direction from inside to outside when used microwave heating. Hence, the solubilisation of the solutes was enhanced [12]. Moreover, the extraction of the phenolic compounds was optimized by an additional of solvent (40ml) after exposed with microwave extraction and fast cooling technique. This helped the solubility of the compounds to dissolve into the solvent and minimized the mass transfer limitation of compounds. Hence, this method improved the extraction of polyphenols from the sample matrix [6].

I. Conclusion

The efficiency of the extraction polyphenol from C. nutans by the SFME method were reported. The feasibility of parameters used on the extraction of polyphenols such as microwave power and extraction time were analyzed and reported. Based on the different microwave power and extraction time used on the extraction, it were explained in term of the energy absorption, thus extraction temperature was discussed to show a better understanding on effect of energy absorbed on the extraction process. When using microwave power, 240 W with extraction time, 1 min was resulted the highest total phenols content while energy absorbed is 0.90417 kJ. The polyphenols yield was

depended on the microwave power and extraction time but the energy absorbed in more efficient to evaluate the yield. However, the extraction temperature must be taken consideration although higher energy absorbed may increase TPC value. In which, the yield was decreased when used higher microwave power and extraction time which resulted to high extraction temperature. This is because polyphenol compounds were denatured when extraction temperature increased. SFME method has shown a good result in extracting the polyphenols from the Clinacanthus nutans Lindau, which the extraction can be reach in shorter time without consumed higher microwave power.

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