EXTRACTION OF VALUABLE PHYTOCOMPOUNDS FROM MEDICINAL PLANTS OF *CLINACANTHUS NUTANTS LINDAU* BY SOLVENT-FREE MICROWAVE TECHNIQUE

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***Abstract*: Clinacanthus Nutants Lindau(C.nutants) belonging to the Acanthaceae family also known as Sabah Snake Grass, mostly found in South East region. The solvents used for this research was distilled water. Process parameter was pursuing out under three different type power (160 W, 240 W and 360 W) and three different solvent-to-feed ratio which is (1:10, 1:20 and 1:30). These three ratios acted differently as the time needed is different between ratio 1:10, 1:20 and 1:30. The increase of solvent requires longer time for the heat to be supplied to the samples. Oher than that, the data shown that the extraction yield increase with as the power of the microwave increase. The highest yield observed was 28.91% (Power =360 W, S/F=1:30).**

***Keywords*— Phytocompounds, Clinacanthus Nutants Lindau, Solvent-Free Microwave Technique**

1.0 INTRODUCTION

Medicinal plant is one of the ancient ways used in traditional medicine practices[10]. It is known by the medicine field to contain a valuable phytocompounds such as alkaloids, terpenoids, carotenoids and many more[1]. One of the medicinal plants that being used widely in South-East Asia is Clinacanthus Nutants Lindau (C nutants) also known as Sabah Snake Grass[7].

In addition, it is also being used in many fields such as the regional medicinal uses, pharmacological activities, toxicology, clinical application, and phytochemicals content[2]. Previous research found that C. nutants can be used in anti-oxidant, anti-cancer, anti-inflammation and analgesic, immune response activity, anti-bacterial activity, anti-venom activity and last but not least, cosmetic uses[8]. Different region in Asian has their own used[12]. In China, C. nutants is being used in Chinese traditional medicine to treat various inflammatory conditions such as strains, gout, rheumatism[6]. Malaysia used C. nutants as complement or as an alternative to current medicine after diagnose with a disease[7]. It can be used to lower the high level of blood cholesterol, glucose level, blood level and blood pressure[4]. Other than that, Malaysians used C. nutants as herbal tea to detoxify the body and clean the kidney[8].

In addition, the chemical composition of Clinacanthus Nutants Lindau essential oil can be described as a complex compound. The plants contain phytocompounds in the leaves, stem and roots. It is because it contains variation of phytocompounds[5]. Different solvents produced different core phytocompounds such as steroid and triterpenoids (petroleum), phenolics acids and flavonoids(methanol), and also C-glycosyl flavones, and sulphur-containing glucosides (methanol, ethanol)[1].

There are techniques requires the solvents and does not require the solvent to extract the phytocompounds from the plant. The extraction of valuable phytocompounds from Sabah Snake Grass used solvent such as n-hexane, dichloromethane, ethyl acetate and ethanol which actually a high performances liquid chromatography[1]. For example, the method known as Soxhlet extraction include ethanol as solvent to be added to snake grass plant. The procedure of using the solvent will produce a byproduct that can affect the environment and classified as chemical waste after being used as solvents. In addition, the solvents such as ethanol can cause cancer to the user and it is not suitable for all industrial branch[2]. Thus, it is important to find a better technique that reduces the production of hazardous substance to the environment[11]. In addition, different solvent has different boiling point which the experiment cannot be execute to high. The heating of highly flammable solvents required extra counter measure to be taken[13]. Other than that, high temperature can cause the denatured of chemical compounds.

The parameter for experiment design allows information needed to understand the influence factors on extraction process and the optimal conditions for extraction. The parameter that significant with microwave-assisted extraction (MAE) is solvent composition, solvent-to-feed ratio, power applied, extraction temperature, extraction time, size and moisture of plants materials[13]. In this study, the focus was the three different solvent to feed ratio (1:10, 1:20 and 1:30) and three types of power (160 W, 240 W and 360 W). Other than that, the extraction time is every 20 minutes and extraction temperature is at 40°C.

2.0 METHODOLOGY

2.1 PLANT MATERIALS

The Clinacanthus Nutants Lindau was collected from the nursery at Sungai Buloh, Selangor. The leaves parts were separated and weighted. The leaves then dried by using air drying method in oven at 105°c for 24 hours. The weight of C. nutants after the air-drying process was recorded. The formula to calculate the moisture content is: $[(Mass_{old} - Mass_{new})/Mass_{old}]$ x 100%. The leaves dried in oven at 60°C for 2 hours to reduce the moisture content within the samples (prior to extraction). After that, the sample was grounded and sieved to a particle sized in the range of 500-1000µm. The sample was stored in airtight bags, swept with nitrogen gas and kept in refrigerator (-8°C)[3].

2.2 Preliminary experiments

The objective of preliminary experiment is to investigate the relationship between the temperature of the microwave with time and solvent to feed ratio for microwave (160W, 240W, 320W). The solvent-to-feed ratio is to decide by the mass of C.nutants and volume of solvent (water). The experiment was started at the power for the microwave of 160 W. The microwave switched on and the time was clocked at the same time. The temperature is taken for every 30 seconds and the temperature should not exceed 80°C. The experiment was repeated for the power of the microwave for (240W and 360W).

2.3 Extraction of valuable phytocompounds

The C.nutants leaves weighted at 1.0000±0.0005g and the amount of solvent was decided from preliminary experiments. The samples and solvents were insert in a 100mL round-bottom flask. The mixture was keeping under magnetic stirring for 3 minutes to allow the sample to soaked in the solvent. The time and power needed for microwave was decided in preliminary experiment. The flask should be covered with aluminium foil to avoid UV-ray denature the chemical composition that present in the oil. After that, the sample was cooled down by using ice bath to 40°c. At the same time, 6mL of cold solvent(water) is added. The experiment was proceeded with a conventional extraction in warm water at 40°C for 80 minutes. For every 20 minutes, use droplet to remove solution for the conventional extraction. The mixture/extracted essential oil is filtered by using 0.2 µm PTFE to remove solid residues before storage.

The solution was dried by using rotary evaporator with unit operation of RE-52. The extract then was covered with aluminium foil and stored in the refrigerator (-4°C) upon analysis.

2.4 Gas Chromatography-Mass Spectrometer (GC-MS)

The sample of essential oil extracted was being solubilized with ethanol before performing a GC-MS analysis. The equipment for analysis of GC-MS was Agilent gas chromatograph model 6890 Technologies which offered by University Technology MARA Selangor (Shah Alam). Helium was used as a carrier gas at 1.0 mL/min with injection and the oven temperature was programmed as 110°C held during 3 min, then increased to 200°C at rate of 5 °C/min, then increased to 250°C at rate of 10 °C/min and finally maintained at 250°C for 10 min. Components identification was made based on comparison of their mass spectra with those in Wiley Registry of Mass Spectral Data, 7th edition (Agilent Technologies, Inc.) and National Institute of Standards and Technology 05 MS (NIST) mass spectral library data.

3.0 RESULTS AND DISCUSSION

3.1 Effect of power of the microwave and temperature with time

The purpose of this preliminary experiments is to find the time taken when the temperature reaches 80°C. The temperature of 80°C is chosen because it is to avoid the oxidation and degradation of phytocompounds within the samples. Other than that, the boiling point of water(solvent) is 100%. The first power for this experiment was 160 W. The time taken for each of the solvent-to-feed ratio is different. According to **Figure 1**, at ratio 1:10, the time for the temperature exceeding 80°C was 8 minutes(81.42°C), ratio 1:20 at 7 minutes (80.2°C) and 1:30 at 6 minutes (80.6°C). The time taken was longer because the supply heat through the power of the microwave (160 W) is low heat emission.

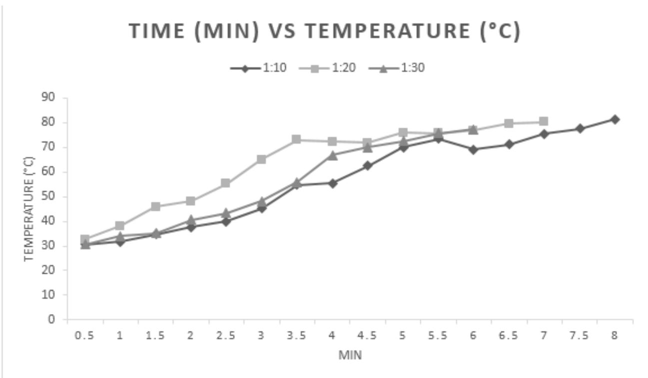

Figure 1: Graph of time versus temperature profile for Power = 160 W

The medium power was 240 W. Based on **Figure 2**, the solvent-to-feed ratio for 1:20 and 1:30 shared the same time needed for the temperature exceeding 80°C which was 3 minutes, 1:20 (88.0°C) and 1:30 (89.0°C). The ratio 1:10 needed 2.5 minutes to achieve 83.8°C. Other than that, the data shown a less time needed compared to power of 160 W for all the ratio of 1:10, 1:20, and 1:30.

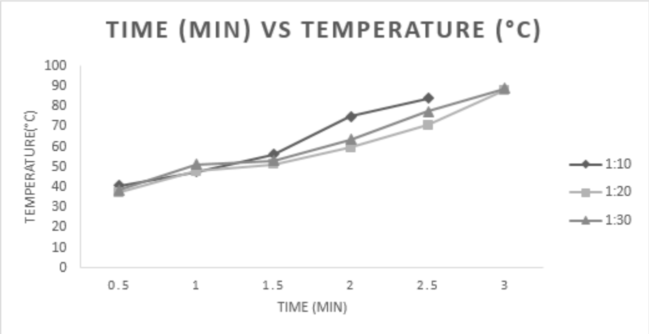

Figure 2: Graph of time versus temperature profile Power = 240 W

The highest power for this experiment was 360 W. **Figure 3** shown that the temperature increased rapidly by time. Ratio 1:10 shown that it reaches 82.1°C in 1.5 minutes. Ratio 1:20 shared the same time taken (1.5 minutes) to reached 81.6°C. Only ratio 1:30 (85.6°C) needed more time to exceed 80°C.

Furthermore, there are three types of solvent-to-feed ratio. These three ratios acted differently as the time needed is different between ratio 1:10, 1:20 and 1:30. The increase of solvent requires longer time for the heat to be supplied to the samples[13].

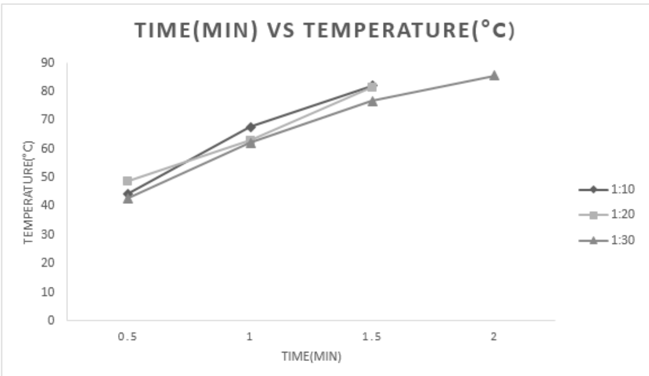

Figure 3: Graph of time versus temperature profile for Power = 360 W

3.2 Yield (%)

The yield for the extraction has shown an increase pattern. According to **Table 1**, the highest yield was a ratio 1:30 for every power involved in this experiment. Other than that, as the time is increased, more essential oil was extracted from the C. nutants. It is also because of the heat emission supplied during microwave extraction caused the access towards the surface of leaves during the heating by microwave. The stem cells at the leaves part. In addition, the solvents became a medium for the compounds to form an essential oil. This study obeyed the research by Che Sulaiman et al(2017) which shown an increase of yield with high solvent to feed ratio[9]. Oher than that, the data shown that the extraction yield increase with as the power of the microwave increase. A higher solvent to feed ratio (S/F) in extraction techniques can increase the recovery. Similar trend is shown based on the research conducted by Emilie Destandau, T. M. (2013) with different materials and solvents[13].

Table 1: Yield (%) at 80 minutes

Power (W)	Ratio	Yield (%)
160	1:10	5.13
	1:20	7.08
	1:30	10.03
240	1:10	13.01
	1:20	16.39
	1:30	19.63
360	1:10	21.34
	1:20	26.17
	1:30	28.91

3.3 Analysis by GC-MS

4.0 Conclusion

This study shown that solvent free microwave-assisted extraction is an effective tool to extract valuable phytocompounds from C nutants. Under optimum condition which is below 80°C, all the responses which indicated that all extraction parameters employed in this study were important in the optimization process. Furthermore, 360 W shown the highest yield produced compared to 160 W and 240 W. As the solvent-to-feed ratio is increase, the yield produced is also high. The highest yield produced was 28.91% (Power = 360 W, S/F = 1:30). Other than that, in order to reduce the costs of actual production, it is reasonable to estimate the economic conditions that are required in order to allow minimum energy and solvent consumption but at the same time, achieving the desired output.

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