Microwave-Assisted Hydrodistillation of *Clinacanthus nutans*

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***Abstract*—Microwave Assisted Hydrodistillation (MAHD) was used to extract *Clinacanthus nutans (C.nutans)* in order to take advantage on this novel extraction to evaluate the potential of MAHD. Different power of microwave such as 300W, 400W and 600W were investigated on extraction yield produced and compared with conventional hydrodistillation. Also, the effect of MAHD and HD extraction on chemical components of *C.nutans* extract were evaluated using GC-MS chromatography and to evaluate the mechanism of MAHD by verifying through first order and second order kinetic model. The condition of extraction was optimized at 6 g of plant sample, ratio of plant sample to solvent was kept at 1:14 (w/v) and extraction time of 80 min for MAHD and 120 min for HD. Higher yield, 3.33% was able to obtain through MAHD method for extraction time of 80 min at power of 300 W compared to conventional hydrodistillation (HD) which only produced 1.667% of oil yield. Second order kinetic model was proven to be a suitable model to stimulate on basis of MAHD and HD experiment results. High coefficients of correlation (R^2) were stimulated by the second order model which satisfactorily applied to the process. MAHD is proven to produce more components compared to HD when analysed by GC-MS.**

Keywords*— *Microwave assisted hydrodistillation, Clinacanthus nutans, Hydrodistillation, microwave power, kinetic model

I. INTRODUCTION

Clinacanthus nutans (Burm. f.) Lindau (*C.nutans*) is well known in Southeast Asia primarily in Malaysia, Indonesia and Thailand as a useful traditional medicinal plant. It has several name based on native languages of countries. “Belalai Gajah” or Sabah snake grass is locally named in Malaysia whereas in Thailand, the plant is known as Saled Pangpon Tua Mea (saliva of female mongoose), Phaya-Yor, or Phaya Plong Thong (Yahaya et al., 2015). Nowadays, this plant is not only can be found in Sabah but all around Malaysia since it has been widely cultivated in all states of Malaysia due to its abundance of beneficial values. Public awareness in using medicinal herbs in treatment, which are found to be safe in consumption, has increased the demands for herbal remedies. At the moment, many researchers attracted to make research on this plant for its medical potency in treatment of skin rashes, wounds, burns and virus herpes simplex. Several studies such as from Pongmuangmul et al., (2016) have been done on the this plant to study monogalactosyl diglyceride (MGDG) and digalactosyl diglyc- eride (DGDG) for their in vitro antiviral activities against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). Furthermore, diseases such as gout, diabetes, hypertension, liver cancer, kidney syndrome and uterine fibroid are treated using *C.nutans* as traditional medicine in Malaysia (Sekar & Rashid, 2016).

According to the previous study, this plant possesses anti-hepatitis, anti-herpes, anti-inflammatory, anti-venom, analgesic, antiviral and antioxidant properties due to its phytochemical compound content in plant. Based on medical perspective, the effectiveness of the medicinal plant is depending on their phytochemical contents since different compound of phytochemical can inhibit different of activity such as phenolic acids and flavonoids contribute to antioxidant activity (Kong & Abdullah Sani, 2017).

Before new developed method was introduced, conventional extractions have been used to extract many plant materials such as hydrodistillation, steam distillation, steam and water distillation and maceration. Hydrodistillation (HD) is the common approach to be used in extraction of essential oil from medicinal herbs and plants because it can provide good quality of essential oil and most importantly, it is operated in a simple, safe manner and also environmentally friendly (Jeyaratnam et al., 2016; Milojevi et al., 2013). The inevitable trend in natural products research has introduced the green extraction concept where its technology is defined as by reducing solvent, energy, wastes and environmental pollution while obtains the addition of yields. Driven by the green extraction mission, various novel methodologies in extracting plant material have been developed as alternative methods to the conventional extractions. Those newest method are including ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, and microwave-assisted hydrodistillation (Jeyaratnam et al., 2016; Wen et al., 2018).

Recently, much attention has been focused on microwave-assisted hydrodistillation (MAHD). An advanced hydrodistillation method, MAHD is developed to extract the essential oil from the solid plant matrix efficiently associates with its abundant advantages including selective heating, increased production, effective heating, elimination of process steps, reduced thermal gradients, reduced equipment size and faster start-up time (Jeyaratnam, Nour, & Akindoyo, 2016). Based on the use of microwave oven and connected to Clevenger apparatus, the plant material is heated and irradiated through the heat produced by the microwave energy (Moradalizadeh et al., 2013).

Therefore, to take the advantage of this novel extraction method, this research is to evaluate the potential of MAHD on extraction yield of C. nutans. In addition, kinetic model is identified base on MAHD and HD experiment results.

II. METHODOLOGY

A. Materials

C.nutans fresh leaves are obtained from Taman Angsamas, N.Sembilan, Malaysia. Ethanol (95%) is purchased from R&M Chemicals (Malaysia) and anhydrous sodium sulfate is purchased from JT Baker (Merck, Germany).

B. Sample preparation

The fresh leaves were washed with water to remove any impurities. They were then further dried in an oven at 100°C for 5 hours. Prior to the extraction, the dried leaves were blended into smaller pieces. The prepared samples were then stored in an airtight container and kept in a refrigerator (4°C).

C. Microwave assisted hydrodistillation

A domestic microwave oven (NN-ST651M, Panasonic, 32 L, 1000W, 2.45 GHz) with some modifications in order to allow the access of the neck of the round bottom flask to the Clevenger apparatus to collect the extracted essential oils. 6.00 ± 0.02 g of sample was mixed with the distilled water with the ratio of water to the *C.nutans* sample of 14:1 (v/w). The sample solution was transferred into the 1 liter sized reactor (round bottom flask). The microwave power level was operated at different level which are 300, 400 and 600 W for a period of 80 min and oil extracted are noted as EO3, EO4 and EO6 respectively. Four extraction cycles of 20 min to 80 min was carried out. After the extraction, the essential oil was collected, the moisture was removed by using sodium sulfate and stored in a refrigerator until further used.

D. Hydrodistillation

Based on (Jeyaratnam, Nour, & Akindoyo, 2016), HD method for the extraction of C. nutans oil was performed using a Clevenger apparatus according to the European Pharmacopoeia. The same size reactor, mass of plant material as well as the ratio of the water to C. nutans sample as per MAHD was maintained in this HD extraction. The extraction was extended until 120 min (extraction cycle of 20 min) since the conventional method requires a longer extraction time to obtain maximum oil recovery. The essential oil was collected and known as EOHD, the moisture was removed by using sodium sulfate and stored in a refrigerator until further used. The yield of the extracted essential oil was obtained using following Eq.:

$$\textbf{Yield (\%)} = \frac{\textbf{Amount of essential oil (g) obtained}}{\textbf{Amount of raw material (g) used}} \textbf{ x 100\%}$$

E. Extraction kinetic model

First-order model

According to Ho et al. (2005), first order kinetic equation can be written in a differential form as follows:

$$\frac{dC_t}{dt} = k_1 (C_s - C_t) \quad (1)$$

where; k_1 = extraction rate constant (min^{-1})

Next, the Eq. (1) is integrated with the boundary condition $C_t = 0$ at $t = 0$ and $C_t = C_t$ at $t = t$;

$$\ln\left(\frac{C_s}{C_s - C_t}\right) = k_1 t \quad (2)$$

Then, the Eq. (2) can be arranged into a linear form as follows;

$$\log (C_s - C_t) = \log (C_s) - \frac{k_1}{2.303} t \quad (3)$$

The extraction capacity C_S, and the extraction rate constant for the first-order k_1 can be determined experimentally from the slope and the intercept by making a plot between $\log (C_s - C_t)$ with t.

Second-order model

The second-order kinetics Eq. for the rate of extraction by Ho et al. (2005) is given in Eq. (4):

$$\frac{dC_t}{dt} = k_2 (C_s - C_t)^2 \quad (4)$$

where; k_2 = extraction rate constant for the second-order (L g^{-1} min^{-1})

By grouping variables in Eq. (4), the Eq. (5) is obtained:

$$\frac{dC_t}{(C_s - C_t)^2} = k_2 \, dt \quad (5)$$

Using the boundary conditions of $C_t = 0$ at $t = 0$ and $C_t = C_t$ at $t = t$, the Eq. (5) can be integrated and by performing rearrangement, the new Eq. can be obtained as follows:

$$\frac{1}{(C_s - C_t)} - \frac{1}{C_s} = k_2 t \quad (6)$$

$$C_t = C_s - \frac{C_s}{1 + C_s k_2 t} \quad (7)$$

$$C_t = \frac{C_s^2 k_2 t}{1 + C_s k_2 t} \quad (8)$$

The Eq. of (8) can be converted into a linear form as follows:

$$\frac{t}{C_t} = \frac{1}{C_s^2 k_2} + \frac{t}{C_s} \quad (9)$$

Thus, the extraction rate $\frac{C_t}{t}$ can be obtained:

$$\frac{C_t}{t} = \frac{1}{\left(\frac{1}{C_s^2 k_2}\right) + \left(\frac{t}{C_s}\right)} \quad (10)$$

The initial extraction rate h with $C_t = t$ when t approaches 0 can be defined as follows:

$$h = C_s^2 k_2 \quad (11)$$

By substituting Eq. (11) into Eq. (9) gives:

$$\frac{t}{C_t} = \frac{1}{h} + \frac{t}{C_s} \quad (12)$$

The initial extraction rate h, the extraction capacity C_S, and the extraction rate constant for the second-order k_2 can be determined experimentally from the slope and intercept by making a plot between *t/Ct* with *t*.

F. The chemical analysis of C.nutans extracts by using GC-MS analysis

Essential oil composition was determined by gas chromatography coupled with mass spectrometry (GC–MS) analysis on GC-MS Model; QP 2010 series which has been equipped with a VF-5 ms fused silica capillary column of 80 m length, 0.25 mm diameter and 0.25 µm film thickness. For the GC-MS detection, an electron ionization system, and ionization energy of 70 eV was used. Helium gas (99.99 %) was used as a carrier gas at a constant flow rate of 1 mL/min. The oven temperature was set 110 ºC held during 3min, then increased to 200 ºC at rate of 5 ºC/min, then increased to 250 ºC at rate of 10 ◦C/min and maintained at 250 ºC for 10 min. The peaks from GC-MS were identified using the National Institute of Standards and Technology (NIST) mass spectral library.

III. RESULTS AND DISCUSSION

A. Yield of C.nutans oil

The extraction yield of both MAHD and HD are shown in Figure 4.1. Various power level were used such as 300, 400 and 600 W (EO3,EO4,EO6) as parameters for the microwave hydrodistillation extraction to compare with the conventional extraction yield. Based on the Figure 4.1, it can be seen that the yield percent of extracted oil declining from the EO3 to EO6. The highest yield of 3.33% was obtained when the microwave power is at 300 W.

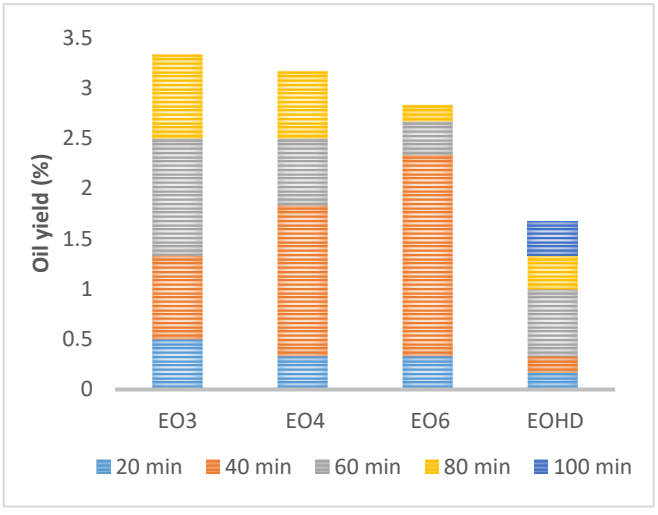

Figure 4.1: The oil yield produced at different extraction power and method

At optimum power level, the molecular interaction of the electromagnetic field can be enhanced by the irradiation energy which aiding fast penetration of solvent into plant cell and recovery of the solutes. When increasing the microwave power, it overheats the plant sample and lead to the evaporation of volatile components in the extracts which caused the degradation of the components. Thus, it can reduce yield due to higher wattage. This can be proven when at the EO6, the oil yield, 2.83% shown the lowest among three of power levels. Similar observation was also reported in a previous research done by Nazarni Che Isa et al., when the yield of vitexin and isovitexin were increased from 100 W to 500 but decreased as the power increased from 500 W to 700 W. They claimed that, it might cause by the different behaviour of differences chemical structure with other molecule when exposed to the power of irradiation though the use of high power associated with the risk of compound degradation.

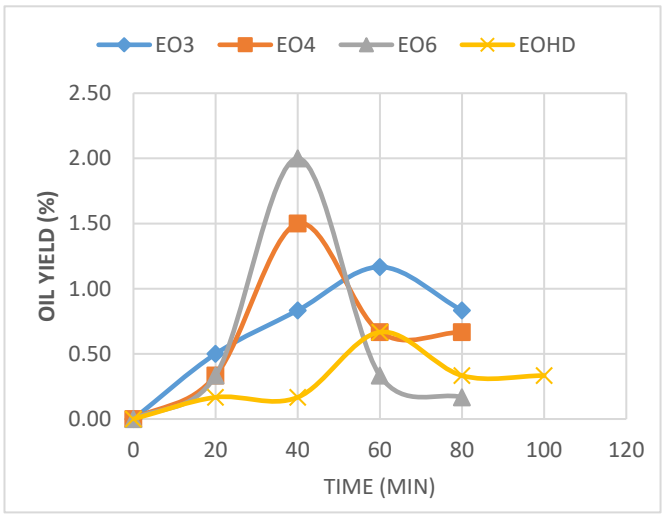

Figure 4.2: The oil yield produced at different extraction power and method

Nevertheless, the extraction yield from the MAHD was considerably high compared to the conventional extraction (HD). For HD, the oil yield produced only for 1.67% for a period of 120 minutes. In other study by (Jeyaratnam et al., 2016), it is reported that oil yield produced by the MAHD is the higher compared to conventional HD. MAHD could extracts more yield due to the speedy generation of heat occurred inside the cell of the plant matrix with the absorption of microwave energy and made the subsequent formation of a higher-pressure gradient inside the plant material. Also, microwave is more efficient in terms of heats flow where it can heat the entire samples simultaneously at higher rate. Furthermore, degradation of thermolability of constituents and partial hydrolysis of water sensitive compounds may occur when applying HD method since high temperature is used and long extraction applied, thus caused the lowest yield produced.

Based on Figure 4.2, it shows that the initial yield of extracts by both method increased steadily and decreased gradually by time. Nazarni Che Isa et al., suggested that initial rise in the yield were caused by the break up of cell wall which resulting to the release of compounds from the cellulosic cell wall. However, initial rise of yield by HD was not as high as MAHD because power induces in MAHD method can accelerate ionic conduction and dipole rotation better than HD. Prolong the extraction time in MAHD can reduced the yield of extracts caused by the overheat supplied to the plant

Table 4.1: Linearization of second order kinetic model from the extraction of *C.nutans*

Sample	Slope	k_1 (min^{-1})	Intercept	Cs (gL^{-1})	R^2
EO3	-0.0179	0.0412	-0.3790	0.4178	0.2471
EO4	-0.0300	0.0691	0.0460	1.1117	0.6530
EO6	-0.0210	0.0484	0.0517	1.1264	0.2569
EOHD	-0.0214	0.0493	-0.2449	0.5690	0.4957

sample lead to the evaporation of volatile components. This can be observed from the Figure 4.2, *C.nutans* yields by microwave were reduced as it reached after 40-60 minutes and contributed to high yield at the same time compared to HD method. HD needs extra extraction time to have fully extracts the plant material to achieve plateau since the yield was increased in between 80-100 minutes.

B. Kinetic modelling on C.nutans oil yield extracts by Microwave assisted hydrodistillation and hydrodistillation.

In this experiment, both extraction methods shown the yield of the *C.nutans* oil increased at the beginning and declined as the length of the extraction time was increased. The data obtained can be demonstrated to study the kinetic model of *C.nutans* oil extraction using both methods. First order and second order kinetic model were used by plotting a graph to study the extraction of *C.nutans* oil. First order kinetic model was used by making a plot between log (Cs-Ct) versus time. Linearization of the plot able to obtain the slope and the intercept which represent the value of k_1 and Cs respectively as shown in Table 4.1. The coefficient of determination, R^2 obtain from the linear graph showed generally low value. This low R^2 implies that this kinetic model is not satisfactorily represented by the experimental data of the extraction. This is also can be supported by H. S. Kusuma & Mahfud (2017) which the first order kinetic model in extracting sandalwood by microwave hydrodistillation and microwave air-hydrodistillation is less able to represent well the experimental result due to the low coefficient of determination. This model is not able to demonstrate all the processes because it can represents well only one mechanism.

From the Table 4.1, the extraction rate constant (k_1 = 0.0691 min^{-1}) and extraction capacity (Cs = 1.1117 gL^{-1}) on *C.nutans* extraction by MAHD by EO4 shown the highest value of k_1 and Cs compared to HD method. This is can be used to explain that MAHD method can run the extraction process faster compared to HD. However, k_1 and Cs by EO3 shown the lowest value among the microwave power. Since the first order model has low value of R^2, the model is not suitable to represent well the data. The same study also can be seen from the H. Kusuma & Mahfud (2017) where the linear graph is showed better at the beginning of the process than at a later stage because the extraction process model does not follow the evolution of this model. Generally, in extraction process there are two mechanisms which are change of the structure of cell of matrix and the absorption of microwave energy by the organic compounds (H. S. Kusuma & Mahfud, 2017). Thus, second order kinetic model is selected to represent the extraction since it has the highest coefficient of determination, R^2.

From the Table 4.2, we able to obtain the value of extraction rate constant for the second-order kinetic model. Generally, rate constant gives a direct measure of the relative reaction rate. If the k value is small means that the reaction is slow. It shown that from the experiment, for the three different power which are EO3, EO4 and EO6 the value of extraction rate constant, k_2 by EO4 is the highest compared to others. However, for the extraction capacity (Cs) value, EO3 shown the highest (0.7022 g L-1). Although the extraction of *C.nutans* oil by EO3 has the extraction rate constant lower than EO4, but with the value higher on extraction capacity (Cs), EO3 has faster reaction and extraction compared with extraction done at EO4. This can be proven as the Eq. 13 shown the extraction capacity (Cs) is more influential when compared with the value of the extraction rate constant for second order (k_2). Second-order kinetic model proved that EO3 is the suitable power to be used as the rapid generation of heat inside the plant matrix and it can lower the possible degradation on the sample so the cell can withstand until its maximum extraction capacity.

$$\frac{C_t}{t} = \frac{1}{\left(\frac{1}{C_s^2 k_2}\right) + \left(\frac{t}{C_s}\right)} \qquad (13)$$

In the meantime, the extraction rate constant on *C.nutans* oil extraction by MAHD is higher compared to HD. The highest extraction rate constant by MAHD is from EO4 (0.48 L g-1 min-1). Thus, extraction by MAHD is a rapid reaction than HD because the k value of HD only 0.19 L g-1 min-1. In addition, the extraction capacity (Cs) by EO3 also shown the highest (2.4 times) compared to extraction done by EOHD. Hence, this concluded the extraction by MAHD method is far better since the value of extraction rate constant and extraction capacity are both higher. Second-order kinetic model can be used to explain that radiation from the microwave can enhance and improve the process of extraction of the *C.nutans* leaves. The electromagnetic waves released from

Table 4.2: Linearization of second order kinetic model from the extraction of C.nutans

Sample	Slope	Cs	Intercept	h	k_2	R^2
EO3	1.4240	0.7022	8.9600	0.1116	0.2263	0.8843
EO4	1.8900	0.5291	7.4667	0.1339	0.4784	0.7916
EO6	7.5600	0.1323	-95.2000	-0.0105	-0.6004	0.7416
EOHD	3.42	3.4200	0.2924	60.0000	0.1949	0.6492

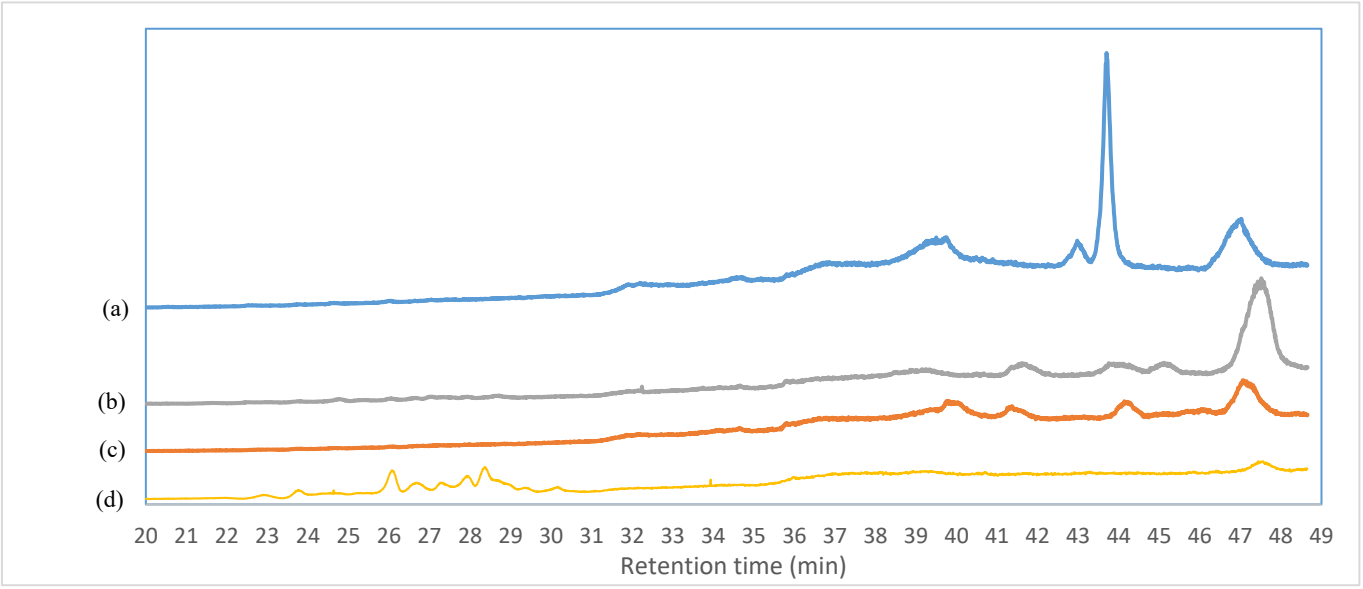

Figure 4.3: Chromatogram of GC-MS analysis of extracts obtained by the a) EO3 b) EO6 c) EO4 d) EOHD

microwave is different with the conventional method because it can change the structure of cell and resulting to give fast reaction and higher yield. According to Kusuma & Mahfud (2017), the process acceleration and high extraction yield occur in MAHD is probably resulting from the synergistic combination of two transport phenomena which are heat and mass gradients working in the same direction. Also, in MAHD method gave the shorter time to boil water at its boiling point since it consumed 6 minutes to boil rather than 7 minutes by HD method.

C. Phytocompound identification of C.nutans extracts by using GC-MS chromatography

The extracts from MAHD and HD were analyzed using GC-MS. GC-MS of the MAHD and HD extract of *C.nutans* showed four to six peaks. The constituents were shown and tabulated in the Table 4.3 and Figure 4.3. For MAHD, five compounds are identified at sample EO3 and EO4 whilst EO6, six compounds are identified. The components are mostly detected at the range of 39 to 47 retention time. EO3 shown the highest peak of 6-Chlorohexanoic acid, 4-nitrophenyl ester at retention time of 43.69, which represents 17.55% of peak area. Meanwhile for the rest of the power level in between the retention time of 43 to 44, only 2.91% of peak area on EO4 and 1.13% of peak area on EO6 which are identified as 3-Oxo-androsta-1,4-dien-17á-spiro-2'-3'-oxo-oxetane and 1H-Imidazole-4-methanol, à-heptadecyl- respectively. Also, three to four higher peaks are obviously seen in the chromatogram by the EO3 compared to EO4 and EO6. Higher peak area by EO3 compared to other extracts could be associated with possible degradation of compound due to higher wattage that are not suitable for the volatile compounds of *C.nutans*. In fact, higher power directly proportional to the higher temperature which resulting the rapid rupture of the cell wall and lead impurities to release into the solvent together with the desired solute (Chemat & Cravotto, 2012).

On the other hand, MAHD produced more components than HD. There are only four components in the *C.nutans* oil were extracted by HD such as 22,23-Dibromostigmasterol acetate, 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-9-(phenylsulfonyl)-, (E,E)-, dimethylthexylsilyl chloride and digitoxin which are not present in any extracts oil by MAHD. According to the Figure 5, the chromatogram by HD are mostly having peak in between 20 to 30 of retention time whilst the MAHD, they are mostly abundant at 40 to 48 minutes. At retention time of 47 to 48 minutes, HD shows the lowest peak which represent only traces. Furthermore, MAHD components are such as 1,4-dimethyl-2-octadecyl-cyclohexane, bamipine and 2,6,9,12,16-Pentamethylheptadeca-2,6,11,15-tetraene-9-carboxylic acid are found to have therapeutic effect as anti-cancer, anti-microbial and anti-bacterial.

The reason behind for this could be the nature and composition of essential oils are varied depending on the extraction used. Other than accelerating the extraction process, microwave irradiation also can extract better than HD without causing changes in the volatile oil composition (Moradalizadeh et al., 2013). Also, differences in compound found in the HD and MAHD are might due to the storage duration of plant sample which decreased with the prolonged storage (Raya et al., 2015).

IV. CONCLUSION

MAHD is able to extract *C.nutans* with higher yield compared to conventional HD. The highest yield of extraction, 3.33% was found at 300 W of microwave power for extraction time of 80 minutes. The kinetic modelling of experimental result is represent well on second order kinetic model by MAHD and HD. Meanwhile, MAHD extracts contain more components found with high peak compared to HD with therapeutic effect. Thus, MAHD can be used to produce more quantity of oil and quality of *C.nutans* oil.

Table 4.3: The chemical composition of *C.nutans* oil by MAHD and HD methods

Compound	Retention time	Area (%)
EO3		
1,4-dimethyl-2-octadecyl-cyclohexane	39.24	1.77
2,6,9,12,16-Pentamethylheptadeca-2,6,11,15-tetraene-9-carboxylic acid	39.71	5.33
Bamipine	42.97	4.70
6-Chlorohexanoic acid, 4-nitrophenyl ester	43.69	17.55
1-Allyl-5-(3,4-dimethoxy-benzyl)-pyrimidine-2,4,6-trione	46.97	6.62
EO4		
3-Methyl-4-(methoxycarbonyl)hexa-2,4-dienoic acid	40.02	4.94
exo-2,10-Bornanediol	41.33	2.57
1H-Indene, 5,5'-(1,10-decanediyl)bis[octahydro-	41.46	2.13
3-Oxo-androsta-1,4-dien-17á-spiro-2'-3'-oxo-oxetane	44.23	2.91
2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl	47.21	5.09
EO6		
1-cyclopentyl-2,2-dimethyl-1-propanol	41.35	1.33
2,6,10-Trimethyl-3-oxo-12-(tetrahydropyran-2-yloxy)-dodeca-6,10-dienoic acid, methyl ester	41.65	3.27
1H-Imidazole-4-methanol, à-heptadecyl-	43.77	1.13
Cyclopropyl)trivinylsilane	45.18	3.15
Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane], 1-methyl-4-isopropyl-7,8-dihydroxy-, (8S)-	47.34	12.22
Isoquinoline, 1-[(3,5-dihydroxy)benzyl]-N-formyl-1,2,3,4,5,6-hexahydro-	47.50	16.27
EOHD		
22,23-Dibromostigmasterol acetate	26.08	46.26
2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-9-(phenylsulfonyl)-, (E,E)-	28.36	4.41
Dimethylthexylsilyl chloride	28.64	1.61
Digitoxin	47.51	3.25

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