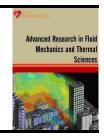


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Extraction of Essential Oil from Indigenous Herb Physalis angulata Linn using Microwave Assisted Extraction



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ARTICLE INFO	ABSTRACT
Article history: Received 28 January 2020 Received in revised form 3 April 2020 Accepted 9 April 2020 Available online 8 June 2020	Essential oil is a kind of remedy that is originated from various part of plants. One of them is <i>Physalis angulata Linn</i> that is beneficial to human health yet very scarce explored by researchers. In this work, extraction of this herbal essential oils using microwave-assisted extraction (MAE) is proposed as an alternative greener extraction technique. The objective of this study is to determine the effect of extraction time (4, 6, 8, 10 and 12 min), liquid-to-solid ratio (ranging from 8 to 16 ml/g) and microwave power (270, 360, 450, 540 and 630 W) on the extraction yield of oil from <i>Physalis angulata Linn</i> . Results showed that the optimum operating condition of MAE of this herb that gave the maximum 21 wt% yield of essential oil was found when extracted in 12 minutes for duration of extraction time using 12 ml/g for liquid-to-solid ratio and 630 W of microwave power. GC-MS results presented the highest constituent of 4,5-Dichloro-ortho-phenylenediamine (24.07 min), Diethyl Phthalate (24.09 min) and Phthalic acid, ethyl 2-pentyl ester (24.09 min) with percentage of 4.10 %. These results demonstrated that <i>Physalis angulata Linn</i> essential oil possess of health beneficial phytocompounds that exhibits as antimicrobial, anticancer and anti-toxic agent.
<i>Keywords:</i> <i>Physalis angulata Linn;</i> Microwave assisted extraction; essential oil;	
phytocompounds	Copyright $ extbf{©}$ 2020 PENERBIT AKADEMIA BARU - All rights reserved

1. Introduction

Physalis angulata Linn, is a kind of a common plant exist in tropical regions especially in Vietnam. It is used to treat some disease such as skin diseases, infection, fever and hepatitis [1]. In Malaysia, this herb is known as "Letup kelambu". "Letup" is Malay words that describe "pop" sound that can be formed when the fruit is popped on hand. This plant can usually be found at roadsides, near grass, on the field or besides drain. In Malaysia, the herb is usually treated as plant that need to be cut down due to believing that this plant would become as competitor to other agricultural plant for

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sunlight, nutrient, water and space [2]. Currently, some of the *Physalis angulata Linn* that is found in Hulu Bernam, Selangor and Teluk Intan, Perak had also been poisoned. This indicates that most Malaysian did not aware of the benefits of the plant to their health. Some potential medical health of *Physalis angulata Linn* reported that is it can be used to treat some disease such as skin diseases, infection, fever, hepatitis, asthma, urinary problems, rheumatism and tumor [1,3]. Thus, there is a need to harness the benefit of this plant by using appropriate extraction technique.

Previous researchers had claimed the benefits of *Physlis angulata Linn* to human health. However, these works employed solvent extraction that use organic solvent [3-6] to extract essential oil from *Physalis angulata Linn*. The use of organic solvent as extracting medium especially the petroleum-based such as methanol and hexane is hazardous to health and environment [7]. Thus, microwave-assisted extraction (MAE) is used in this research to extract *Physalis angulata Linn* essential oil.

Microwave assisted extraction (MAE) is an extraction method that uses heating energy to aid the extraction of oil by means of solvent. The frequency of microwave is in between 100 MHz to 3 GHz. In principle, microwaves propagate electromagnetic energy due to the presence of electric and magnetic field [7]. This energy cause rotation of dipoles due to the molecular motion of ions. Despite the presence of nonionizing radiation, the molecular structure is still intact. The rotation of dipoles of sample (polar material) and solvent (polarizable material) due to the presence of microwave radiation create heat at the material surface. This will cause disruption of hydrogen bonding or rupture of plant material cell walls and thus facilitate the movement of dissolved ions. This process enhances the penetration of solvent into the solid matrix [8] and dissolve the solutes for extraction process to happen. In this research, distilled water was used as extracting medium to replace the hazardous impact when using organic solvent. Water has a higher dielectric constant that leads to a better absorption of microwave energy by the system than it can dissipate. The cell ruptured will happen due to the strong absorption and temperature rise by the in-situ water. As a result, extraction of oil will happen from cell ruptured phenomena [9].

Therefore, the aim of this research is to determine the effect of extraction time, liquid-to-solid ratio and microwave power to the extraction yield of oil from *Physalis angulata Linn*. The experiments were conducted by manipulating one parameter at a time while kept constant other parameters to obtain highest yield at each parameter. The optimum operating parameters that gave the highest essential oil were determined and the oil was analyzed for phytochemical content using gas chromatography-mass spectrometry (GC-MS). It was hypothetically expected that the oil yield is increasing when three parameters namely extraction time, liquid-to-solid ratio and microwave power. However, the increment is expected to reach maximum point before declining. This hypothesis was made based on previous research that extract essential oil from *Physalis alkekengi* [9]. As the plant was from same *Physalis* family, the trend of extraction condition was expected to be similar.

2. Methodology

2.1 Materials and Plant

The *Physalis angulata Linn* (stem, root and leaves) was collected in Hulu Bernam, Selangor. The plant had been identified by Herbarium from Forest Research Institute Malaysia (FRIM) with the specimen number 054/19. The plant sample had been deposited at FRIM. The samples were dried at room temperature for 3 days. Afterward, the dried plant was cut into small pieces (1-2 cm) using scissor. The sample was then stored in sealed hermetically bags and under darkness until use in the extraction process.



2.2 Microwave Assisted Extraction Procedure

Microwave-assisted extraction of *Physalis angulata Linn* were performed in modified microwave oven (Panasonic, 900 W, Malaysia). The arrangement of the experimental setup is showed in Figure 1. A 2 g of *Physalis angulata Linn* sample were measured using analytical balance and placed in 100 ml round bottom flask. Distilled water was then added to the flask as solvent according to the specified liquid-to-solid ratio. The flask containing the sample and solvent was placed in the microwave oven. The upper part of the round flask was fit with glass column while the glass column was fit to a hole at top of the microwave. The glass column is fitted with condenser connected to a water circulation system to allow the vapor formed to be condensed to liquid. Afterward, the hydro distillation system was insulated with cotton wool to prevent heat loss that could disturb the distillation process.

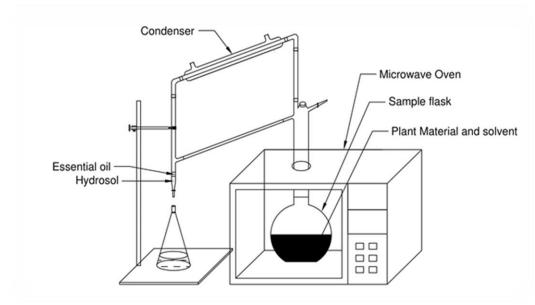


Fig. 1. Full Set-up of MAE

2.3 Experimental Design

Experiments were carried out to determine the effect of duration of extraction time (4, 6, 8, 10, 12 min), liquid-to-solid ratio (8, 10, 12, 14 and 16 mL/g) and microwave power (270, 360, 450, 540 and 630 W) to essential oil yield. The summary of experimental parameters is presented in Table 1.

2.4 GC-MS Analysis

The phytocompounds composition of *Physalis angulata Linn* was analyzed on a Varian 450 gas chromatograph (Varian Inc, Palo Alto, CA, USA) equipped with Agilent CP8944 capillary column, VF-5 ms (30 m x 0.25 mm, 0.25 um) and coupled to Varian 240-MS Ion Trap Mass Spectrometer working at 70eV of ionization voltage. Helium was used as a carrier gas at 1.0 ml/min with injection in splitless mode. The oven temperature was programmed as follows: 110°C held during 3 min, followed by increased to 200°C at rate of 5°C/min, 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.



Table 1					
Experimental setup parameter of MAE for extraction of Physalis angulata Linn					
Run Time	Extraction Time (min)	Liquid-to-solid ratio (mL/g)	Microwave power (W)		
1	4	12	450		
2	6	12	450		
3	8	12	450		
4	10	12	450		
5	12	12	450		
6	12	8	450		
7	12	10	450		
8	12	12	450		
9	12	14	450		
10	12	16	450		
11	12	12	270		
12	12	12	360		
13	12	12	450		
14	12	12	540		
15	12	12	630		

3. Results and Discussion

Effect of extraction time, liquid-to-solid ratio and microwave power on the yield of *Physalis* angulata Linn essential oil were discussed. The optimum extraction time, liquid-to-solid ratio and microwave power to obtain the highest oil yield with significant phytocompounds were presented.

3.1 Effect of Extraction Time

Variation of oil yield in different of extraction time at constant microwave power and liquid-tosolid ratio were presented in Figure 2. The effects of extraction time against oil yield were measured by manipulating the extraction time from 4, 6, 8, 10 and 12 min; liquid-to-solid ratio and microwave power were kept constant at 12 mL/g and 450 W, respectively for each run. The oil yield at each run were measured by using Eq. (1).

$$Oil Yield (\%) = \frac{W_1 - W_2}{W_1} \times 100\%$$
(1)

where w_1 and w_2 are initial weight of sample and final weight of dried sample, respectively.

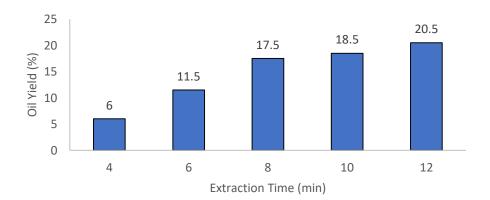


Fig. 2. Effect of extraction time on extraction oil yield



Figure 2 shows that the oil yield was increase gradually from 4 to 12 minutes. This is due to the increasing of extraction time had increased the thermal accumulation from microwave energy within the solvent. The presence of electric and magnetic field from microwave produce this electromagnetic energy which in turn lead to heating process that caused disruption on the hydrogen bonding at material surface or cell wall ruptured [7]. Apparently, this process facilitated the penetration of solvent into the solid matrix and cause enhancement of essential oil-solvent dissolution process [8]. As a result, oil yield was then increased due to the increment in extraction time.

From the Figure 2, it can be seen that the essential oil yield was constantly increased as extraction time increased to 12 minutes. This oppose the initial hypothesis as the oil yield is expected to decrease when reach maximum point. The contradictions happened possibly due to the different sample shapes exposed to the microwave energy. In this work, the samples were cut between 1-2 cm whilst the Liu *et al.*, (2015) [9] grind the samples into finely crushed powder. The larger size of sample means the smaller total surface area and thus requires longer time of extraction for the solvent to penetrate through the sample surface. Therefore, the highest yield was found to be 20.5 wt% at 12 minutes which considered as the optimum in the range of extraction time studied.

3.2 Effect of Liquid-to-Solid Ratio

A range of liquid-to-solid ratio (8, 10, 12, 14 and 16 mL/g) were selected for this experiment while other were fixed as follows: 12 minutes of extraction time and 450 W of microwave power. The effect of liquid-to-solid ratio on oil yield were presented in Figure 3.

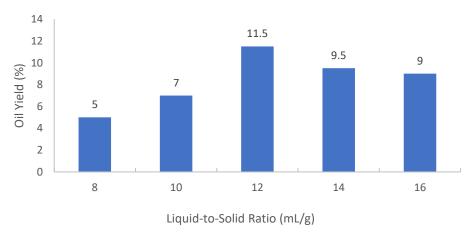


Fig. 3. Effect of liquid-to-solid ratio against extraction oil yield

According to Figure 3, it is showed that oil yield increases gradually from 8 to 10 ml/g, then increase sharply from 10 to 12 ml/g. This happened because, as liquid-to-solid ratio increase, the concentration gradient during mass transfer within solid also increase [10]. This resulted to the increasing of diffusion rate followed by increasing in oil until maximum point at 12 mL/g of liquid-to-solid ratio. After the maximum point, essential oil yield was found to decreased from 12 to 16 ml/g. This possibly due to the concentration gradient had reached equilibrium point and cause the solvent refuse to take up more solutes from the plant samples. As a result, mass transfer and diffusion rate may decrease followed by decreasing in oil yield [11]. At this condition, the highest oil yield was found to be 11.5 wt% at optimum liquid-to-solid ratio of 12 ml/g at constant 12 min and 450 W of extraction time and microwave power, respectively.



The same trend was noticed as compared to previous research; for example, research by Najmuldeen *et al.*, (2017) in extracting essential oil from *Eurycoma longifoliar* fond that the percentage yield increased as liquid to solid ratio increase from 6 to 10 ml/g due to the increasing in diffusion rate [12]. According to Najmuldeen *et al.*, (2017), the yield starts to decrease at 12 ml/g due to the low mass transfer and diffusivity resulting from raw material that could not take up more solvent. Research by Liu *et al.*, (2015) that extracted essential oil from *Physalis alkekengi* also found same trend as in this research; essential oil yield increases as liquid-to-solid ratio increased from 8 to 12 ml/g [9]. However, the extraction yields were markedly declined beyond 12 ml/g. According to Liu *et al.*, (2015), this happened because at very high liquid to solid ratio, dissipation of microwave energy will occur by the shaking mixture and excessive swelling of plant materials [9].

3.3. Effect of Microwave Power

The microwave power was manipulated at 270 W, 360 W, 450 W, 540 W and 630 W while maintaining extraction time and liquid-to-solid ratio at 12 minutes and 12mL/g, respectively. The effect of microwave power on the oil yield were presented in Figure 4.

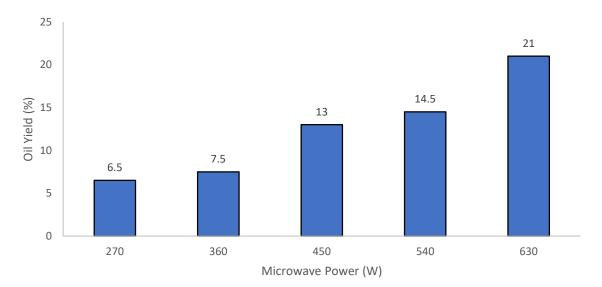


Fig. 4. Effect of Microwave Power against Extraction Oil Yield

Figure 4 showed that the oil yield increased gradually as microwave power increased. This happened due to rapid generation of heat produced at higher microwave power that caused higher pressure gradient inside the *Physalis angulata Linn* and solvent. As a result, extraction process was enhanced at higher microwave power [13]. However, the trends keep increasing at higher microwave power without depletion. This contradicts from usual trends of microwave extraction oil yield in which is usually decrease due to compound breakdown at high power [14]. The contradiction can be explained with same explanation as in extraction time against oil yield trend; due to the shape of the samples that were cut rather than in powder form that been used in most MAE research. The sample that is been cut instead of powdered have less total surface area that required much higher energy to heat up. Chemat *et al.*, (2013) also mentioned that the penetration of microwave was enhanced by using higher surface area of sample [15]. Thus, as the sample used is in this research is quite low, the extraction efficiency is reduced. As a result, more microwave power is required to produce higher



essential oil yield. At this condition, the highest oil yield was found as 21 wt% at 630 W in 12 min with 12 ml/g of liquid-to-solid ratio.

3.4 GC-MS Analysis

Phytocompounds characterization was performed for the highest oil yield obtained from the microwave-assisted extraction. Results demonstrated that the highest yield of 21wt% was obtained at 12 min of extraction time at 630 W of pawer and 12 ml/g of liquid-to-solid ratio. The oil yield at this optimum parameter is used to be analysed using GC-MS. Figure 5 shows the GC-MS results at optimum parameters for essential oil extract from *Physalis angulata Linn* using MAE. According to Figure 5, it is observed that there are some peaks been identified at retention time in between 15 to 40 minutes. These bioactive compounds are listed in Table 2.

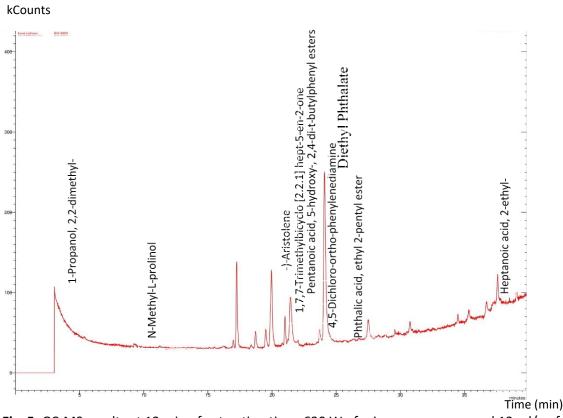


Fig. 5. GC-MS results at 12 min of extraction time, 630 W of microwave power and 12 ml/g of liquid-to-solid ratio

GC-MS analysis of <i>Physalis angulata Linn</i> extract using MAE				
No.	Retention Time (min)	Amount (%)	Compound	
1 .	3.05	0.45	1-Propanol, 2,2-dimethyl-	
2	9.27	0.09	N-Methyl-L-prolinol	
3	19.95	0.98	-)-Aristolene	
4	21.02	0.06	1,7,7-Trimethylbicyclo [2.2.1] hept-5-en-2-one	
5	21.44	0.02	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	
6	24.07	2.32	4,5-Dichloro-ortho-phenylenediamine	
7	24.09	2.32	Diethyl Phthalate	
8	24.09	2.32	Phthalic acid, ethyl 2-pentyl ester	
9 :	37.58	0.49	Heptanoic acid, 2-ethyl-	



From the Table 2, it is showed that there were 9 components had been identified in *Physalis* angulata Linn extracts using MAE. The highest constituent was comprised of 4,5-Dichloro-orthophenylenediamine at retention time 24.07 min, Diethyl Phthalate at 24.09 min and Phthalic acid, ethyl 2-pentyl ester at 24.09 min with percentage of 2.32%. Karpagasundari and Kulothungan (2014) also found two phthalic acids in *Physalis minima L*, name phthalic acid, butyl ester, ester with b and Bis(2-ethylhexyl) phthalate [16]. A report by Srivinasan *et al.*, (2009) found that phthalic acid ester as major components in essential oil of *Leea indica* (Burm. F.) Merr. flowers [17]. Phtalates had been reported by previous research to have antimicrobial and other pharmacological activities. For example, study by Al-Bari *et al.*, (2006) found that bis-(ethykehexyl) phthalate isolated from *Streotomyces bangladeshiensis* have microbial activity against Gram positive bacteria and some athogenic fungi [18]. Rameshtangam and Ramasamy (2006) also found that bis (2-methylheptyl) phthalate have antiviral activities against white spot syndrome virus of *Penaeus monodon Fabrius* due to the presence of bis (2-methylheptyl) phthalate [19].

Furthermore, it can be found that the second largest components isolated was Aristolene (19.95) with percentage of 0.98 %. Aristolene is a type of sesquiterpenes that was found in plant material and had been reported to give health benefits by previous research. For example, Nurhaslina et al., (2018) had found Aristolene in agarwood essential oil [20] whilst Ferrari et al., (2005) found the same class of Aristolene as the one of main constituent of Ferula communis essential oil from Corsica [21]. Aristolene had been reported by previous research to be one of component in anti-inflammatory, cytotoxic and antioxidant activities. Sun et al., (2014) classified that Aristolene as one of 51 components isolated from that has leaves of *Mentha piperita* anti-inflammatory, cytotoxic and antioxidant activities [22]. Durán-Peña et al., (2012) also reported that Aristolenes can exhibited antimicrobial activities and cytotoxic activities [23]. This also proves the health beneficial of Physalis angulate Linn by previous research due to the presence of Aristolene. On the other hand, Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters also was found in Physalis minima L. extract with amount of 0.03 %. This compound is known as type of unsaturated fatty acid that is believed to contribute in health beneficial. Rahman et al., (2015) in their work had isolated Pentanoic acid, 5-hydroxy-, 2,4-dit-butylphenyl esters in Trillium govanianum using GC-MS [24] and claimed that this unsaturated fatty acid is responsible as anticancer agent has β -glucuronidase inhibitory activities of Trillium govanianum rhizome [24]. This component also has been found in essential oils from sea buckthorn (Hippophae rhamnoides L.) [25] and reported to possess as antibacterial activity against food-borne bacteria.

Moreover, 1,7,7-Trimethylbicyclo [2.2.1] hept-5-en-2-one (21.0167) was also found in *Physalis* angulate Linn. extract with percentage of 0.06 %. Huang *et al.*, (2018) found the compound as one of the main constituents in essential oil from *Artemisia lavandulaefolia* (Asteraceae) and exhibited as fumigant toxicity against *P. xylostella* adults [26].

4. Conclusions

The optimum condition of MAE of *Physalis angulata Linn* are 12 minutes for extraction time, 12 mL/g for liquid-to-solid ratio and 630 W for microwave power to obtain the highest oil yield i.e. 21 wt%. The essential oil was analysed by using GC-MS and revealed that it was comprised of important beneficial phytocompounds mainly 4,5-Dichloro-ortho-phenylenediamine, Diethyl Phthalate and Phthalic acid, ethyl 2-pentyl ester. The bioactivity of these compounds as antimicrobial, anticancer and anti-toxic agent show that the *Physalis angulata Linn* has large potential as medicinal plant for human health benefits. The safer, greener and simple extraction method of microwave-assisted



extraction (MAE) for *Physalis angulata Linn* essential oils also makes it suitable for pharmaceutical and food applications.

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