Effect of Mass of Solid and Soaking Effect on Chemical Component of Essential Oil of *Physalis minima* Linn by Solvent-free Microwave Extraction (SFME)

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Abstract*—Essential oils extraction from *Physalis minima* L. plant was performed using the solvent-free microwave extraction (SFME) method. The aim of this research is to study the effect of mass of solid and soaking effect on chemical component of essential oil of *Physalis minima* Linn were studied. The mass of solid and soaking days applied in this research are 4,6,8,10g and 1,2,3 days, respectively. GC-MS was used in determination of chemical components in the essential oils. It is found that the main constituents identified in relative higher abundance is Lanosta-8,24-dien-3-ol, acetate or known as Lanosteryl acetate. Therefore, it is demonstrated that by increasing the mass of solid, it will isolate more chemical component in essential oil and extending the soaking time reduces the availability of chemical compound in essential oil from *P. minima

Keywords— Essential oils, Physalis minima Linn, solvent-free microwave extraction (SFME), GC-MS

I. INTRODUCTION

Medical plants have high potential which comprises of complex structure and are capable in giving interaction for pharmacological and biological activities [1]. The search for remedies originates from medical plants have been broaden due to their presumed roles in regulating health and economic component of biodiversity [2]. Fruits, leaves, branches as well as roots of different plants contain bioactive potentials which are well-being advantages and other functional components are a universal propensity to application of phytochemical [3].

Physalis minima Linn, a species belonged to Solanaceae family, is a valuable plant that can be found at warm temperate and regions of subtropical throughout the world specifically in India, Baluchistan, Afghanistan, Tropical Africa, Singapore, Australia, Indonesia and Malaysia [4].

Table 1 Local name for *Physalis minima* Linn according to countries

Scientific Name	Country	Local Name
Physalis minima Linn	Malaysia	Pokok letup kelambu [5]
	English	Sunberry, ground-cherry, wild cape gooseberry [6]
	Indonesia	Cheplukan, Chichiplukan [7]
	Sudan	Chencendet, chenchendetan [8]
	Thailand	Thong theng [9]

Physalis minima has its own local name according to the country where the plant has been found as shown in Table 1. *Physalis minima* has been used traditionally in treatment of various ailments. Extraction of *P. minima* specifically from China and India showed that the plant comprises a lot of anti-inflammatory, analgesic and antipyretic activities [1]. According to researches, most of medical plants originated from the Solanaceae family have the properties of being the natural analgesic and antipyretic for many types of diseases. *Physalis peruvania* [10], *Physalis alkekengi* L. var *franchetti* (Mast) Makino (Physalis Calyx seu Fructus) [11] and *Physalis angulata calyces* [12] having the characteristics of anti-inflammatory, analgesic and antipyretic properties. Contemporaneously, numerous methods of extraction were studied in obtaining these bioactive or chemical compounds from the medical plants either for food uses which are mostly relevant for molecules with functional properties such as texture, color,
antioxidant, aroma or taste as food supplement.

Traditionally, extraction of essential oil is done by using microwave hydrodistillation method which uses solvent and the division of energy to heat the solvent and break down the plant cells containing essential oil makes the extraction run less effective [13]. The prevailing method for extraction is solvent-free microwave extraction (SFME) which is a combination technique of microwave heating and dry distillation conducted at atmospheric pressure without the presence of added solvent or water. Hence, it has been proposed as a green technology in extracting essential oil from aromatic herbs which are widely used in food industry [14]. Chemat et al., 2017 proffer that “green” extraction employs 6 principles (well-reasoned sourcing; minimizing the usage of organic solvents; lowered energy consumption; production of by-product with high added value instead of waste; reduced extraction time and recovery of natural and safe extract). Numerous findings on SFME process are as shown in Table 2.

The fundamental parameters in extraction are type of extraction solvent, pH, temperature, extraction time, solvent-to-liquid ratio and other factors related to the raw material. For reasons of space, mass of solid and soaking effect are the parameters considered in this paper. Pretreatment process in extraction of essential oil is a vital step. The other type of pretreat plant sample is by chemical treatment, sonication and microwaves treatment [16]. In a major advance in 2015, Jok et al.[17] have found that the oil yield at different soaking time produced a bell-shaped graph where the maximum oil yield is at 14 days of soaking.

The effect of sample mass is proven influencing the composition of constituents of oil. Increasing sample weight has no further effect on the mass transfer into extracting solvent and might also be related to solubility of volatile compounds in water [18].

Table 2 Comparison of optimum parameter in SFME researches

Material	Extraction method	Optimum parameters			Findings	References
		Extraction time (min)	Irradiation power (W)	Humidity		
Pigeon pea (*Cajanus cajan* (L.) Millsp.)	SFME	44	660	68% humidity	Maximum yield 0.330 (%)	[19]
Oregano (*Origanum vulgare* L.)	SFME	35	622 W	50 g of dried *Origanum vulgare* L. was soaked in 700 mL distilled water	Yield: • Oxygenated compound (80-85%) • Monoterpene compound hydrocarbons (13-16%) • Sesquiterpenes (1-1.6%)	[20]
Thymus vulgaris L. and *Melissa officinalis* L.	SFME	42	700W	Soak with appropriate proportion of distilled water	In thyme: • Thymol (32.17%), Carvacrol (29.25%) Cymene (9.51%) γ-Terpinene (3.62%). Melissa oils: • Caryophyllene oxide (24.95%), E-Citral (24.19%), Z-Citral (18.45%) α-Muurolene (12.50%)	[21]
Dried patchouli (*Pogostemon cablin* Benth) leaves	SFME	90	450	Solid solvent ratio (0.06mg/L)	16 compounds are found. Major component is patchouli alcohol (53.68%)	[13]
Cinnamomum camphora leaves	SFME	23	580	60% moisture content	• Extraction efficiency (3.51 ± 0.12%) • Initiate extraction rate (3.3772) • Extraction rate constant (0.3002) • Extraction capacity (3.67%) • Oxygenated compound content (83.93%) • Energy consumption (0.22 kW h) • Environmental impact (177.87 g CO_2)	[22]
Schisandra chinensis (Turcz.) Baill	SFME	30	385	68% moisture content	Yield of EO 11mL/kg	[23]
Schisandra chinensis fruit	SFME	45	800	Water pretreatment 30%	Yield extraction 1.75% • ylangene (50.11%) • b-himachalene (10.76%) • a-bergamotene (9.52%) • b-Chamigrene (5.41%) • Total up 75.80% of the oil	[24]

However, the academic community has extensively explored the extraction of *Physalis minima* Linn, hence the optimum parameter for this type of extraction method were studied based on previous researches on medical plants, herbs and also similar type of herbaceous of the plant itself. Therefore, this work discusses the selected parameter which comprises of effect of mass of solid and soaking effect.

I. METHODOLOGY

A. Materials

Fresh *Physalis minima* Linn plant was collected from Kedah, Malaysia. It was cleaned and rinsed with distilled water, then be dried at ±30 ºC for a few days. The dried plant was cut within the size of 1.5 ± 0.5 cm and stored in room temperature. Distilled water was used for all soaking purpose in both parameter experiments.

B. Extraction by solvent-free microwave extraction (SFME)

In conducting solvent-free microwave extraction, a microwave oven (R352ZS, SHARP, maximum delivery power 900 W) with wave frequency of 2450 MHz was used. The capacity of the microwave oven is 25 L and it was modified by making a hole on top of the microwave oven. A sample flask with the capacity of 1000 mL was placed inside the center of the microwave oven and was connected to glass apparatus through the hole. Thus, the hole was closed with aluminium foil on top and bottom part of the hole to avoid any pressure drop and heat loss inside the microwave oven as illustrated in Figure 1. The experimental set up bears a close resemblance to the one proposed by Kusuma et. al., 2018.

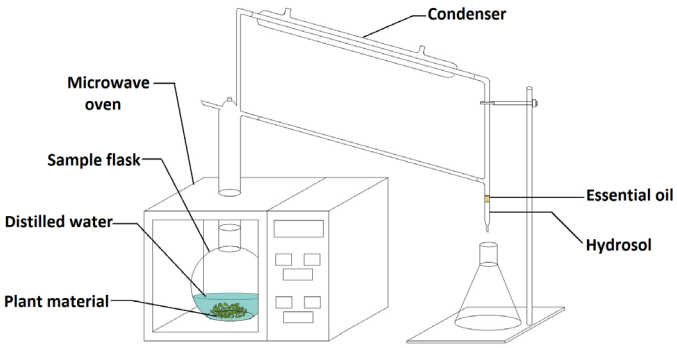


Fig 1 Set up of SFME apparatus

All solvent-free microwave extraction was done at atmospheric pressure by varying the mass of solid sample (4, 6, 8 and 10 g) and the soaking days (2, 4 and 6 days). For experiment of mass of solid, all solid sample were wetted before conducting the experiment, the power and liquid/solid (L/S) ratio are kept constant at 360W and 12:1 respectively. For soaking effect, constant mass solid sample were applied (4 g) and soaked with distilled water with a liquid/solid (L/S) ratio of 12:1. The soaked samples were stored in refrigerator below 5 ºC to prevent any microbial growth until the day of experiment. Basically, each parameter was repeated trice to obtain more accurate reading and it was summarized in Table 3. The extraction was done until all moisture in the round flask dried up. The extracted *P. minima* were then let dried over

filter paper at room temperature for a few days until it is completely dried.

Table 3 Summarization of mass of solid experiment

Mass of solid experiment	
Run of exp.	Mass (g)
1	4
2	4
3	4
4	6
5	6
6	6
7	8
8	8
9	8
10	10
11	10
12	10

Table 4 Summarization of soaking effect experiment

Soaking effect experiment	
Run of exp.	Soaking day
1	2
2	2
3	2
4	4
5	4
6	4
7	6
8	6
9	6

C. Rotavap

Heidolph, Laborota 4000 efficient rotavap was utilized in removing hydrosol from end product of SFME extraction. Hydrosol was made up of distilled water and other moisture which can affect the reading of GCMS later. Acetone act as solvent used in vaporizing hydrosol into another flask.

Pure essential oil was obtained in the large round flask and eventually 5 mL of ethanol was added into the flask before transferring the solution into smaller sample bottle.

D. Chemical analysis of chemical component in Physalis minima Linn

The phytochemical composition of *Physalis minima L.* was analyzed on a Varian 450 gas chromatograph (Varian Inc, Palo Alto, CA, USA) equipped with Agilent CP8944 capillary column, VF-5ms (30m x 0.25mm, 0.25um) and coupled to Varian 240-MS Ion Trap Mass Spectrometer working at 70eV of ionization voltage. Carrier gas which is Helium is injected at 1.0 mL/min in splitless mode. The oven temperature was programmed as follows: temperature held for 3 min is at 110 ◦C, next 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. 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 is used in identifying the component by comparing their mass spectra.

II. RESULTS AND DISCUSSION

Biologically active compounds usually occur in low concentration in plants. The results of GCMS analysis of the essential oil and mass of residue are as tabulated in Table 3 and Table 4:

A. The effect of mass of solid towards chemical component of Physalis minima Linn

The total chemical compound identified by GC-MS varies by the mass of solid. GC-MS result at 6g shows significant peaks and number of identified chemical compound compared to the other analysis of mass of solid. The result is as shown in **Figure 2** whereas the analysis of chemical compound/constituents identified is as in **Table 4**.

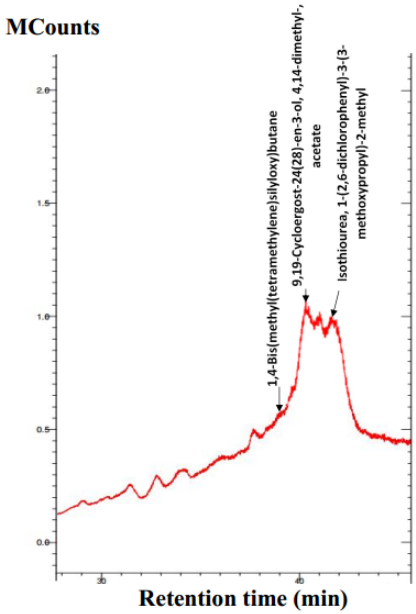


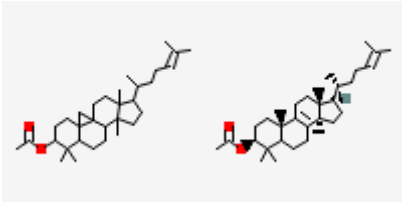
Fig 2 GC-MS result at 6g

Table 4 GC-MS analysis at 6g

No.	Retention time (min)	Amount (%)	Compound
1.	38.7440	0.0278	1,4-Bis[methyl(tetramethylene)silyloxy]butane
2.	38.7614	0.0316	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate
3.	40.0500	0.0884	
4.	40.1059	0.0567	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde
5.	40.1964	0.0226	Cyclohexane, 1,3,5-trimethyl-2-octadecyl
6.	41.0322	0.0211	Isothiourea, 1-(2,6-dichlorophenyl)-3-(3-methoxypropyl)-2-methyl
7.	41.0495	0.0201	1H-Indene, 5,5'-(1,10-decanediyl)bis[octahydro
8.	41.1458	0.0287	Butanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester
9.	41.3866	0.0365	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate
10.	41.6466	0.0711	
11.	42.0125	0.0333	
12.	42.0211	0.0249	Diphenoxylate

At the mass of 4g, the only abundance of constituent found is Lanosta-8,24-dien-3-ol, acetate (0.3153% by total). Surprisingly, at 8g, the only chemical compound isolated is 9,19-Cyclolanost-24-en-3-ol, acetate (0.0679% by total). Increasing the mass of solid up to 10g, the significant compounds found are 9,19-Cyclolanost-24-en-3-ol, acetate which can also be named as Cycloartenol acetate ($C_{32}H_{52}O_2$) and the other chemical component identified is Lanosta-8,24-dien-3-ol, acetate known as Lanosteryl acetate ($C_{32}H_{52}O_2$) and both of the constituent are having the same

chemical compound (Figure 3). Lanosteryl acetate is a family of tetracyclic terpenols which acts as anti-inflammatory, analgesic agents and anticancerigenous thru the inhibition of enzymes which activity is linked to the poliferation of certain types of cancer.



A B

Fig 3 Structure of constituent. (A) Cycloartenol acetate; (B) Lanosteryl acetate

As a result, the higher the amount of solid used in extraction, it will give more chemical compound during analysis process.

B. The effect of soaking towards chemical component of Physalis minima Linn

GC-MS result at 2 days of soaking was chosen because of the significant peak rather than the other analysis of mass of solid. The result is as shown in **Figure 2** whereas the analysis of chemical compound/constituents identified is as in **Table 4**.

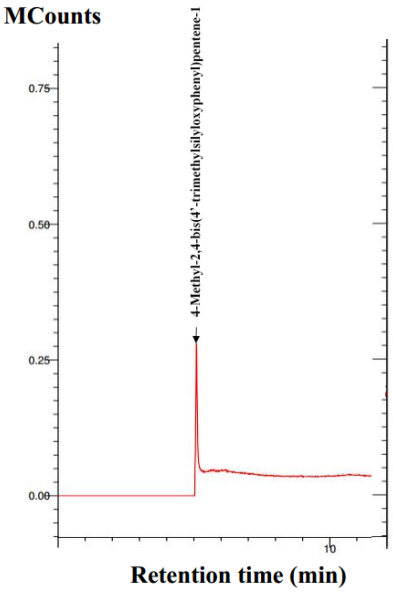


Fig 4: GC-MS result at 2 days of soaking

Table 5: GC-MS analysis at 2 days of soaking

No.	Retention time (min)	Amount (%)	Compound
1.	5.0486	0.00774	4-Methyl-2,4-bis(4'-trimethylsilyloxyphenyl)pentene-1
2.	5.0948	0.595	Ethane, 1,1-diethoxy

From Table 5, Ethane, 1,1-diethoxy is the most abundant constituent found throughout the analysis. It is also known as Diethyl acetal ($C_6H_{14}O_2$) which is a clear colorless liquid with a pleasant odor. Diethyl acetal is also found in garden onion and the other purpose of chemical compound is it acts as flavoring ingredient that is used in fruit, rum and whisky flavor.

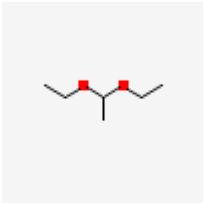


Fig 4: Structure of diethyl acetal

After 1 day of soaking, the chemical constituents isolated are Phthalic acid, diisobutyl ester (0.00481%), Dodecanoic acid, methyl ester (0.199%), Estragole (0.00313%) and Hexadecanoic acid, methyl ester (0.00925%). On the other hand, Dodecanoic acid, methyl ester (0.206%) is the only constituent found by GC-MS.

The experiment of soaking effect revealed that the longer the time taken in soaking the sample, the lesser amount of chemical constituent in the essential oil. The reducing trend of number of chemical compound identified along with soaking time corroborate with the finding of Jok et al., where he proved that over time, the morphology of the plant sample changed. From previos study, excessive of water also may cause some of the VOC compounds to hydrolyze [25].

The cell walls of the plant damaged and being released to the soaking water. This is because soaking water turns more acidic and corrodes the cell well. This explain on the reducing number of chemical constituent along time.

C. *Dried residue*

The results on residue obtained after the extraction of essential oil of *Physalis minima* Linn for both experiment are as shown in Figure 6 and Figure 7. The correlation of mass of residue with both experiment is quite interesting as it shows the effect of water intake during the whole process.

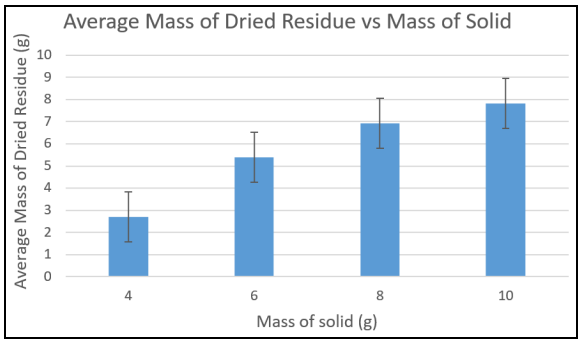


Fig 6: Graph of dried residue for mass of solid experiment

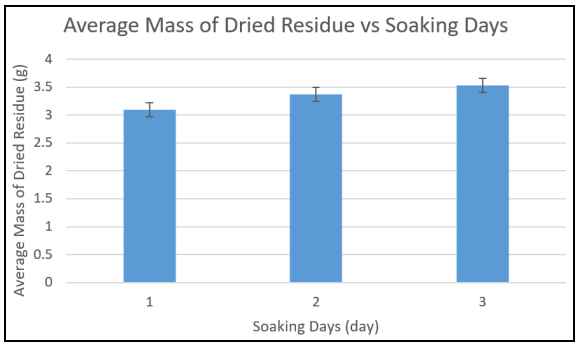


Fig 7: Graph of dried residue for soaking effect experiment

From the graph in Figure 6, the mass of dried residue reduces in the range of 1 to 2g for every parameter of mass of solid. It can be seen almost constant for all of the mass to have the same range of value. Soaking of the plant sample that occur by diffusion makes the cell wall of the plant to expand and simultaneously increasing the turgor pressure. As a result, more water intake causes the dried

residue in soaking effect experiment to have lower reduction in mass of residue contradict with the mass of residue in experiment of mass of solid.

III. Conclusion

This paper has investigated that by varying the parameter of extraction, it will give different type of chemical components which have their own significant health benefits. It has been confimed that *Physalis minima* Linn constraint health benefits properties from the experiment. Lanosteryl acetate is found abundantly in most of the analysis, this proved that *Physalis minima* Linn has the characteristic of analgesic agents, anti-inflammatory and anti-cancerigenous.

The residues remaining after extraction of essential oil are currently disposed as waste. But actually it could be extracted to obtain natural extracts which is rich in phenolic compounds and with high antioxidant compounds. It has been approved that according to Commision Regulation (EEC) No 2568/91, in a specific group of extracts from *Rosmarinus officinalis*, it is wel-grounded as a natural food oxidant in the European Union [26]. It is s recommended that further research should be undertaken in the extraction of residue of plant sample which is collected after essential oil extraction process.

Acknowledgment

Thank you to my supervisor and Universiti Teknologi Mara.