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

Muhammad Faez Bin Mohd Kamaruzaman, Nurhaslina Binti Che Radzi

Faculty of Chemical Engineering, Universiti Teknologi Mara

Abstract— **Essential oil is a kind of remedy that is originated from various part of plants. One of them is *Physalis Minima L.* that is beneficial to human health yet been ignored by Malaysian. There are few research related to the extraction of essential oil from *Physalis Minima L.* However, most of these research use soxhlet extraction method. This method is associated with issues related to hazard when using petroleum-based solvent. Thus, microwave assisted extraction is proposed to be used as it should be a greener extraction technique. The objective of this research is to study the effect of extraction time, liquid-to-solid ratio and microwave power to the extraction yield of oil from *Physalis minima L.* using microwave assisted extraction (MAE). Three parameters were investigated in this study namely extraction time, liquid-to-solid ratio and microwave power. The optimum condition of MAE of *Physalis minima L.* are 12 minutes for duration of extraction time, 12 mL/g for liquid-to-solid ratio and 630 for microwave power. The maximum yield of essential oil at this optimum condition are 21 %. The essential oil at this optimum condition were analyzed by using GC-MS. The highest constituent was 4,5-Dichloro-ortho-phenylenediamine (24.0657), Diethyl Phthalate (24.0864) and Phthalic acid, ethyl 2-pentyl ester (24.0914) with percentage of 4.0970 %. Based on research, it can be concluded that *Physalis minima L.* has some health beneficial as the isolated component is confirmed to exhibits beneficial health like antimicrobial, anticancer and anti-toxic agent.**

Keywords— Physalis minima L., MAE, GC-MS

I. INTRODUCTION

Physalis Minima L., is a kind of an annual herb that is traditionally used in India and China as remedy [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 will be competitor to other agricultural plant for sunlight, nutrient, water and space [2]. Some of the *Physalis minima L.* that is found in Hulu Bernam, Selangor and Teluk Intan, Perak had also been poisoned. This proves that Malaysian do not know about the benefits of this plant to their health. Some potential medical

health of *Physalis minima L.* is it can be used as antimalarial, anti-inflammatory, analgesic and antipyretic, antibacterial, anti-ulcer, cytotoxic, antifertility and antidiabetic agent [3]. Thus, there is a need to harness the benefit of this plant by using appropriate extraction technique.

Some research had been done by previous researchers on the benefits of *Physlis minima L.* to human health. However, most of the researcher had used soxhlet extraction [4,5,6] to extract essential oil from *Physalis minima L.* The use of organic solvent as extracting medium especially the petroleum-based one like methanol and hexane is hazardous to health and environment [7]. Thus, Microwave Assisted Extraction (MAE) is used in this research to extract essential oil from *Physalis minima L.*

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. Microwaves will propagate electromagnetic energy due to the presence of electric and magnetic field [7]. This energy will 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 will create heat at the material surface. This will cause disruption of hydrogen bonding that will aid the movement of dissolved ions. Finally, this process will support the penetration of solvent into the solid matrix [8]. The solvent will then dissolve in solute for extraction process to happen. In this research, distilled water was to overcome the hazardous impact when using organic solvent.

Therefore, while using MAE, this research is aimed to study the effect of extraction time, liquid-to-solid ratio and microwave power to the extraction yield of oil from *Physalis minima L.* The experiment was conducted by manipulating one parameter at a time to obtain highest yield at each parameter. This means while studying the effect of one parameter, the other parameter was kept constant.

At the end of this research, it was expected to have increasing yield when increasing these 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. Once optimum parameters were found, the product will be analyzed for phytochemical content using gas chromatography-mass spectrometry (GC-MS).

II. METHODOLOGY

A. Materials and Plant

The plant used in this study was *Physalis Minima L* (stem, root and leaves) collected in Hulu Bernam, Selangor. 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.

B. *Microwave Assisted Extraction Procedure*

2 g of *Physalis minima L.* sample were measured using analytical balance and placed in 100 mL round bottom flask. Distilled water was then added to the flask as solvent in quantity that follows the liquid-to-solid ratio. The flask containing the sample and solvent was placed in the microwave oven (Panasonic, 900 W, Malaysia). The upper part of the round flask was fit with glass column. The open system was used where the glass column was fit to a hole at top of the microwave. Note that the microwave had been modified with a hole at upper part. The glass column is fitted with condenser connected to a water circulation system to allow the vapor formed to be condensed to liquid. The condenser was fitted with another glass that connect the condenser with Florentine vase. Afterward, the hydro distillation system was insulated with cotton wool to prevent heat loss that will disturb the distillation process. Finally, after the whole extraction system had been set up, the experimental design was followed. Figure 1 shows the full set up of microwave assisted extraction.

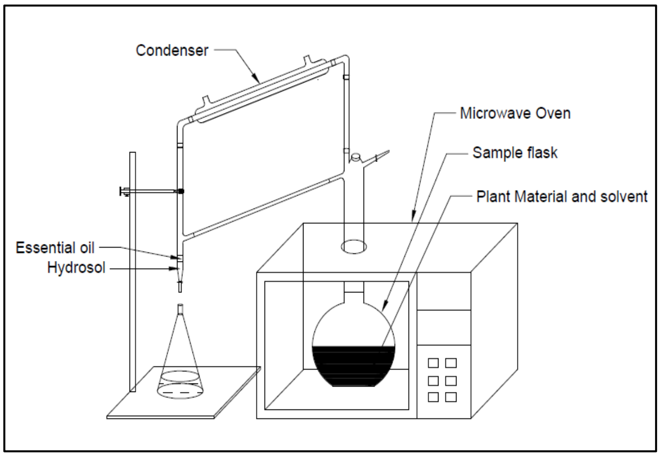

Fig. 1 Full Set-up of MAE

C. *Experimental Design*

Experiment was 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. Essentially, there are total of 15 runs for this experiment. The summary of experimental design can be shown in Table 1.

Table 1 Experimental Design of MAE

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

D. *GC-MS Analysis*

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. 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 3min, 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 National Institute of Standards and Technology 05 MS (NIST) mass spectral library data.

III. RESULTS AND DISCUSSION

A. *The effect of extraction time*

The effect 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 Equation 1.

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

Where W_1 and W_2 are initial weight of sample and final weight of dried sample, respectively.

The graph of oil yield against extraction time were presented in Figure 2.

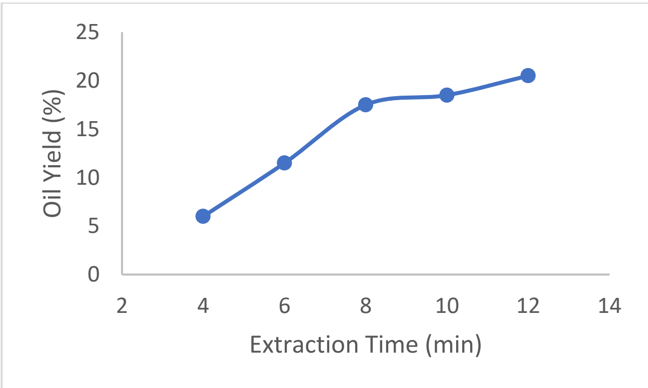

Fig. 2 Effect of Extraction Time against Extraction Oil Yield

Figure 2 shows that the oil yield was increase gradually from 4 minutes to 12 minutes. This due to an increasing of extraction time increased the thermal accumulation from microwave energy within the solvent. The presence of electric and magnetic field from microwave will produce this electromagnetic energy [7]. This energy will produce heat that will disrupt the will hydrogen bonding at material surface. Apparently, this process will support 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.

Based on Figure 2, it is noticed that there is no turning point between extraction time and essential oil yield. This means the essential oil yield kept increasing as extraction time increase without decrement. This oppose the initial hypothesis as the oil yield is expected to decrease when reach maximum point. This contradiction happened due to the sample that is in cut between 1-2 cm instead of grind into powder. The larger size of sample means the smaller total surface area. This requires longer time of extraction for the solvent to penetrate through the sample surface.

After all, the highest yield was found to be 20.5% at 12 minutes of extraction time. This is the optimum extraction time for microwave assisted extraction for *Physalis minima L.*

B. *The 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 essential oil yield at different liquid-to-solid ratio was measured by using Equation 1. The graph of oil yield against liquid-to-solid ratio were presented in Figure 3.

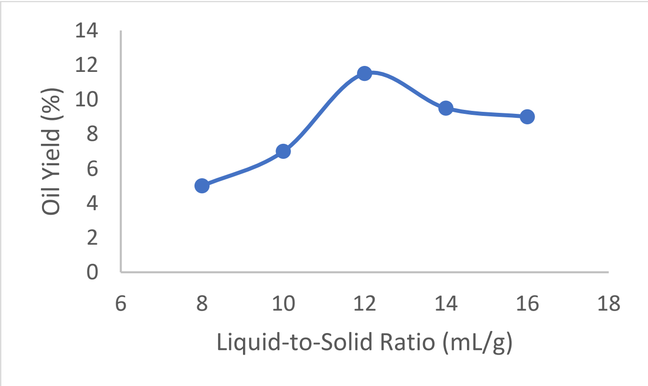

Fig. 3 Effect of Liquid-To-Solid Ratio against Extraction Oil Yield

Based on Figure 3, it is found 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 will result in 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 decrease from 12 to 16 mL/g. This happened because the concentration gradient had reached equilibrium point. This cause the raw material to refuse to take up more solvent. As a result, mass transfer and diffusion rate will decrease followed by decreasing in oil yield [11]. The highest yield is 11.5 % found at liquid-to-solid ratio of 12 mL/g. This is the optimum parameter of liquid-to-solid ratio for this microwave assisted extraction for *Physalis minima L.*

C. *The 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 oil yield at different liquid-to-solid ratio was measured by using Equation 1. The graph of oil yield against liquid-to-solid ratio were presented in Figure 4.

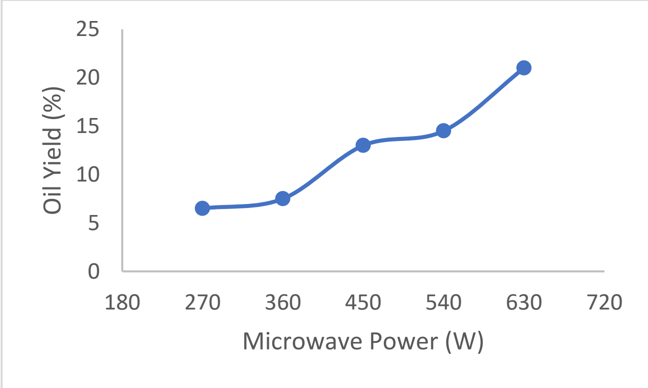

Fig. 4 Effect of Microwave Power against Extraction Oil Yield

From Figure 4, it can be found that the oil yield increases gradually as microwave power increases. This happened because rapid generation of heat were produced at higher microwave power. This will cause ehigher pressure gradient inside the *Physalis minima L* and solvent. As a result, extraction process will be enhanced at higher microwave power [12]. 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 [13]. This contradiction can be explained with same explanation as in extraction time against oil yield trend; this happened due to the use of cut sample instead of 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. As a result, the trend of oil yield keeps increasing as microwave power increase. The highest oil yield is 21 % at 630 W and this will be the optimum parameter for microwave power in MAE for *Physalis minima L.*

D. *GC-MS Analysis*

From the first objective of this research, optimum parameters of MAE of *Physalis minima L.* were obtained at 12 min for extraction time, 12 mL/g for liquid-to-solid ratio and 630 W microwave power. This produces highest yield of 21 % among all experimental parameters. The oil yield at this optimum parameter is used to be analysed using GC-MS. Figure 5 shows GC-MS results at optimum parameters for essential oil extract from *Physalis minima L.* using MAE.

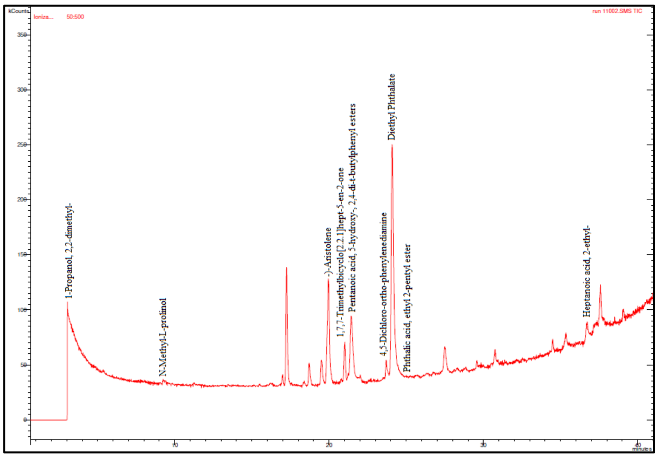

Fig. 5 GC-MS results at optimum parameters

By referring to Figure 5, it can be found that there are some peaks been identified at retention time in between 15 to 40 minutes. These bioactive compound is clearly listed in Table 2.

Table 2 GC-MS analysis of *Physalis minima L.* extract using MAE

No.	R. Time	Amount (%)	Compound
1	3.0482	0.4570	1-Propanol, 2,2-dimethyl-
2	9.2668	0.0960	N-Methyl-L-prolinol
3	19.9534	0.9820	-)-Aristolene
4	21.0167	0.0600	1,7,7-Trimethylbicyclo [2.2.1] hept-5-en-2-one
5	21.4418	0.0290	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters
6	24.0657	2.3220	4,5-Dichloro-ortho-phenylenediamine
7	24.0864	2.3220	Diethyl Phthalate
8	24.0914	2.3220	Phthalic acid, ethyl 2-pentyl ester
9	37.5837	0.4900	Heptanoic acid, 2-ethyl-

Based on Table 2, it can be found that there were 9 components had been identified in *Physalis minima L.* extract using MAE. The highest constituent was 4,5-Dichloro-ortho-phenylenediamine (24.0657), Diethyl Phthalate (24.0864) and Phthalic acid, ethyl 2-pentyl ester (24.0914) with percentage of 4.0970 %. Karpagasundari and Kulothungan [14] also found two phthalic acid in *Physalis minima L*, namely phthalic acid, butyl ester, ester with b and Bis(2-ethylhexyl) phthalate. Research by Srivinasan et al. [15] found that phthalic acid ester as major components in essential oil of *Leea indica* (Burm. F.) Merr. flowers. Diethyl phthalate was also found in essential oil from water caltrop skin. At the same time, research by Xuan et al. [16] also isolate diethyl phthalate from root of exudatres of barnyard grass *Echinochloa crusgalli Beauv*. Phtalates had been reported by previous research to have antimicrobial and other pharmacological activities. For example, study by Al-Bari et al. [17] found that bis-(ethykehexyl) phthalate isolated from *Streotomyces bangladeshiensis* have microbial activity against Gram positive bacteria and some athogenic fungi. Rameshtangam and Ramasamy [18] 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. Despite benefit of phthalate to be used in cytotoxic activity for virus and fungi, there is possibility for this component to also kill good cell. Thus, for commercial use, it is important to separate the phthalate from the essential oil.

Besides, based on Table 2, it can be found that the second largest components isolated was Aristolene (19.9534) with percentage of 0.9820 %. 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, Study by Radzi et al. [19] had found Aristolene in agarwood essential oil. Research by Ferrari et al. [20] also found that aristolene was the one of main constituent of *Ferula communis* essential oil from Corsica. Aristolene had been reported by previous research to be one of component in anti-inflammatory, cytotoxic and antioxidant activities. For example, research by Sun et al. [21] found that aristolene is one of 51 components isolated from that has leaves of *Mentha piperita* anti-inflammatory, cytotoxic and antioxidant activities. Durán-Peña et al. [22] also reported that aristolenes can exhibited antimicrobial activities and cytotoxic activities. This also proves the health beneficial of *Physalis minima L.* by previous research due to the presence of aristolene.

Other than that, Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters also foud in *Physalis minima L.* extract with amount of 0.0290 %. This is a type of unsaturated fatty acid that is believed to contribute in health beneficial. For example, Rahman et al. [23] isolated Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters in *Trillium govanianum* using GC-MS. Rahman et al. [23] found that this unsaturated fatty acid is responsible as anticancer agent has β-glucuronidase inhibitory activities of *Trillium govanianum* rhizome. Research by Yue et al. [24] also found this component in essential oils from sea buckthorn (*Hippophae rhamnoides L.*). This essential oil is reported to has antibacterial activity against food-borne bacteria. As *Physalis minima L.* also contained this unsaturated fatty acid, it has a high potential for it to be an anticancer agent.

1,7,7-Trimethylbicyclo [2.2.1] hept-5-en-2-one (21.0167) was also found in *Physalis minima L.* extract with percentage of 0.06 %. Research by Huang et al. [25] also found 1,7,7-Trimethylbicyclo [2.2.1] hept-5-en-2-one as one of the main component in essential oil from *Artemisia lavandulaefolia* (Asteraceae). In Huang et al. [25] research, the essential oil that contains 1,7,7-Trimethylbicyclo [2.2.1] hept-5-en-2-one exhibited fumigant toxicity against *P. xylostella* adults. Due to the presence of same compounds, *Physalis minima L.* will also have potential to be use anti-toxic agent.

Furthermore, Heptanoic acid, 2-ethyl- (46.9489) was also found in *Physalis minima L.* extract with 0.4900 %. N-Methyl-L-prolinol (9.2668) was also found with percentage of 0.0960 %. 1-Propanol, 2,2-dimethyl- (3.0482) was found with percentage of 0.4750 %. These compound is minor compound that cannot be found in other medicinal plant. However, the beneficial health value of it can still be determined with further research.

In short, it can be concluded that there is some beneficial health of *Physalis minima L.* based on GC-MS analysis as shown in Table 2. This proved by comparison of these components with previous research.

IV. Conclusion

Based on the conducted experiment, it can be concluded that oil yield increased as extraction time increase due to the increase in thermal accumulation from microwave energy within the solvent. Besides, oil yield also increased as liquid-to-solid ratio increase due to the increasing concentration gradient during mass transfer. However, the increment stop at liquid-to solid ratio of 12 mL/g before undergoes decrement in oil yield from 12 to 16 mL/g. This happened because concentration gradient had reached equilibrium point. Apart from that, oil yield also increased as microwave power increase due to the higher-pressure gradient inside the *Physalis minima L* and solvent at higher power. No turning point of essential oil yield for parameters of extraction time and microwave power is due to the form of sample that is been cut (1-2 cm) instead of grind it to powder. This produced lower surface area of the sample that required higher extraction time and microwave energy for extraction process to happen.

The optimum condition of MAE of *Physalis minima L.* are 12 minutes for extraction time, 12 mL/g for liquid-to-solid ratio and 630 for microwave power. The maximum yield at this optimum condition are 21 %. The essential oil at this optimum condition were analyzed by using GC-MS. The highest constituent was 4,5-Dichloro-ortho-phenylenediamine (24.0657), Diethyl Phthalate (24.0864) and Phthalic acid, ethyl 2-pentyl ester (24.0914) with percentage of 4.0970 %. Based on research, it can be concluded that *Physalis minima L.* has some health beneficial as the isolated component is confirmed to exhibits beneficial health like antimicrobial, anticancer and anti-toxic agent.

Despite having a successful result, the overall efficiency of this research can still be considered as lo due to new approach for this plant. Based on conducted experiment, it can be found that there are lots of essential oil loss due to the vaporization to the surrounding due to very high-pressure system inside the hydro distillation apparatus. Thus, it is recommended that the apparatus is equipped with vacuum pump after the condenser to reduce the pressure inside the hydro distillation apparatus. This will enhance the extraction process.

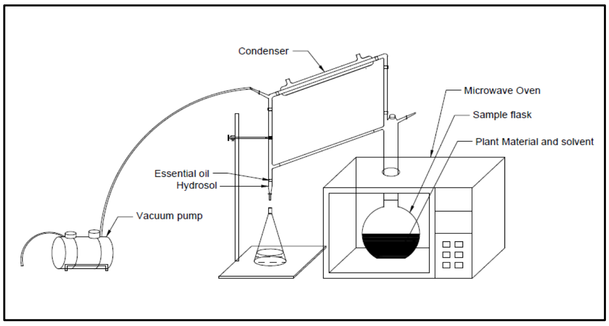

Fig. 6 Proposed new set up of MAE

ACKNOWLEDGMENT

Thank you to my supervisor and Universiti Teknologi Mara.

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