Development and Characterization of Nanoemulsion Containing Essential Oil of *Piper betle* as the Active Ingredient Via Low Energy Emulsification Method

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Abstract. The aim of the study is to develop and characterize the formulation of nanoemulsion containing essential oil of *Piper betle*. Plant based essential oil had been widely applied in cosmetics and aromatherapy, food flavoring or preservative and insect repellent products. However, these essential oils showed high volatility which can reduce the effectiveness of the products compared to the synthetic compounds. Hence, plant -based nanoemulsion is one of the manners that can overcome the disadvantage of the essential oil and improve the products' effectiveness with the application of nanotechnolgy to the formulatoin. In this study, plant based nanoemulsion was prepared using low energy emulsification method. The *Piper betle* essential oil was extracted using steam distillation from the leaves of *Piper betle* and acts as an active ingredient in the formulation.The system that was used in the making of nanoemulsion containing the essential oil of *Piper betle* was water/polysorbate 80/soybean oil/glycerol. The effect of surfactant to oil ratio and surfactant mixture concentration was determined. The stability of nanoemulsions under storage condition was determined, and the validity of the nanoemulsion were characterized for their physical appearances, and droplet size. The particle size obtained from the Dynamic Light Scattering (DLS) test showed four samples were nanoemulsions with droplet size of 68.85 nm, 132.29 nm, 181.85 nm, and 35.91 nm. The results revealed that only four out of nine samples were stable with no separation occurred within 14 days. The making of nanoemulsion containing *Piper Betle* essential oil was successfully prepared via low energy emulsification method. *Piper betle* naoemulsion was successfully formulated and represented high potential as a natural nanoemulsion product.

INTRODUCTION

Piper betle belongs to the family of *Piperaceae* and indigenous in India. It is suitable to grow in tropical country like Malaysia, Thailand, and India. Extracts of *Piper betle* leaves were shown to be effective against several human pathogens, although the mechanism involved has not been elucidated. Its extracts were used for various treatment ailments due to its essential properties like antioxidants and anti-allergic [1]. In this research, *Piper betle* needed to undergo a steam distillation process to obtain the hydrosol. Condensate water co-produced during of the steam distillation process was called hydrosol and most hydrosol produced due to essential oil distillation [2]. Nanotechnology is a multidisciplinary approach which involved creation and utilization of different systems on a nanometric scale [3]. Few types of nano formulations have been reported including nanoemulsions. Nanoemulsions were nano sized emulsions and thermodynamically unstable in which two immiscible liquids were mixed for a single phase by means of an emulsifying agent i.e., surfactant and co-surfactant. It is a colloidal particulate system in the

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submicron size range varies from 10 to 1000 nm [4]. Nanoemulsions were used in food, cosmetic, personal care, and pharmaceutical industries to provide desirable optical, textural, stability, and delivery characteristic. In many industrial applications, it is desirable to formulate nanoemulsions using natural ingredients to develop label friendly products [5]. To study the preparation and characterization of nanoemulsions containing essential oil of *Piper betle* as the active ingredients, low energy emulsification method will be used to prepare the nanoemulsions.

MATERIALS AND METHODS

Material

Piper betle leaves were obtained from the area of Jeli, Kelantan, Malaysia. The soybean oil, Tween 80, Glycerol and N-hexane were purchased from Sigma Aldrich. The distilled water was provided in the laboratory of Universiti Malaysia Kelantan.

Piper Betle Essential Oil Preparation

Piper betle leaves were dried at 40-50°C overnight [6]. The dried leaves were boiled in distilled water using steam distillation method. *Piper betle* oil produced was yellowish dark brown in color.

Nanoemulsions Preparation

Nanoemulsion system that was used in this study was water/polysorbate 80/soybean oil/glycerol. Polysorbate 80 act as surfactant and grycerol act as co-surfactant with the ratio 1:1 on a weight basis [7]. In the making of nanoemulsion concentrates, nine points were plotted on a pseudo-ternary phase diagram [8]. Tween 80 and glycerol were put in a 50 ml beaker. Soybean oil was added and lastly, water was slowly added into the solution. Different ratios of essential oil to soybean oil were used to the optimum nanoemulsion formulation obtained earlier. The ratios used were 4:6, 5:5 and 8:2.

Tyndall Effect

Tyndall effect was conducted to test the validity of nanoemulsion containing *Piper betle* essential oils. The effect test is light scattering by particles to determine whether the solution is colloid or particles. A laser was pointed from one side of the solution. Tyndall effect test was conducted on water, macroemulsion and nanoemulsion to identify the difference of appearances between each liquid.

Dynamic Light Scattering (DLS) Test

The samples of blank nanoemulsion and nanoemulsions containing *Piper Betle* essential oil obtained were tested by using DLS. DLS was used to measure the droplet size of the emulsions. The measurement was performed at 25°C using 90° scattering angle [9].

RESULTS AND DISCUSSION

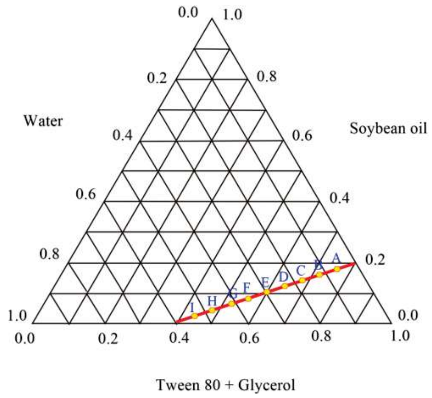
Piper betle Essential Oil

0.96 g of *Piper betle* essential oil was successfully extracted via steam distillation process. The essential oil obtained was dark yellow in color as shown in Fig. 1. The essential oil was kept in clear vial wrapped with aluminum foil because it is best to avert any light exposure to avoid oxidization.

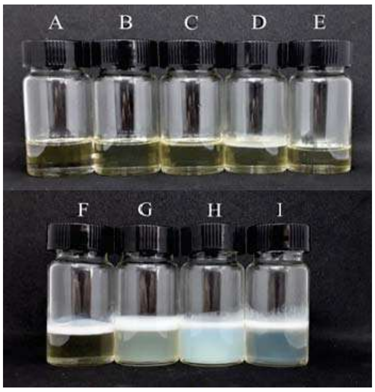
FIGURE 1. ***Piper betle*** essential oil produced through steam distillation

Nanoemulsions

Nine points consist of A, B, C, D, E, F, G, H, and I were plotted on a ternary phase diagram as shown in Fig. 2(a) to find nanoemulsion's configuration point. The formulations of every plotted point were listed in Table 1. The concentrates of each point can be seen in Fig. 2(b).



(a)



(b)

FIGURE 2. Points of nanoemulsions plotted on the ternary phase diagram (a) and nanoemulsion concentrates of each points (b).

Table 1. Quantity of water, surfactant with co surfactant, and soybean oil for point A, B, C, D, E, F, G, H, and I

Point	Quantity of water (g)	Quantity of soybean oil (g)	Quantity of surfactant and co-surfactant (g)
A	0.60	1.80	7.60
B	1.20	1.60	7.20
C	1.80	1.40	6.80
D	2.40	1.20	6.40
E	3.00	1.00	6.00
F	3.60	0.80	5.60
G	4.20	0.60	5.20
H	4.80	0.40	4.80
I	5.40	0.20	4.40

To form dilution, 1.5 ml of each concentrates of nanoemulsion was diluted in 50 ml distilled water and stirred with magnetic stirrer. Nanoemulsions achieved when the dilution turned into a bluish transparent solution. The particle size of each sample had to go through DLS test to affirm nanoemulsion.

Dynamic Light Scattering Test (DLS) Test

The result of samples A, B, C, D, E, F, G, H, and I can be seen in Table 2. Based on the result, sample A, B, D, and E exhibited nano sized particles. Nanoparticle size ranges from 20 nm to 200nm [10]. Particle size of samples C exceeded 200 nm hence, it was not considered as nanoemulsion. Sample F, G, H, and I droplet size outpaced between 2000 nm to 3000 nm.

Table 2. Droplet size of each samples based on DLS test result

Point	Droplet Size (nm)	Point	Droplet Size (nm)
A	68.85	F	3733.49
B	135.29	G	2893.54
C	211.69	H	2680.78
D	181.85	I	3001.14
E	35.91		

Nanoemulsion Formulation

The nanoemulsion formulation was referred from a previous study to determine the ratio of soybean oil and *Piper betle* essential oil [11]. Base on the results obtained, point E was taken as the optimum formulation for the nanoemulsion containing *Piper betle* essential oil. Three oil ratios were chosen and the comparisons of the ratio of nanoemulsion containing *Piper betle* essential oil to soybean oil with droplet size were shown in Table 3. The physical appearances of the formulation can be seen in Fig. 3.

Table 3. Ratio of nanoemulsion containing *Piper betle* essential oil to soybean oil with droplet size (nm).

Ratio of PBLO to SO	Droplet Size (nm)
4:6	55.98
5:5	62.63
8:2	<200

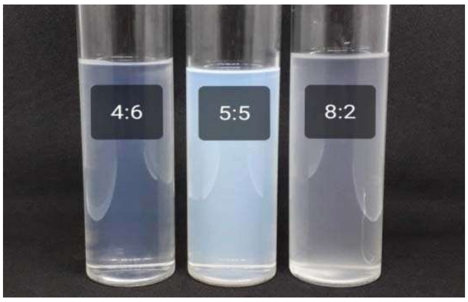


FIGURE 3. Nanoemulsion containing *Piper betle* essential oil and soybean oil with ratio 4:6, 5:5, and 8:2

Validity Test of Nanoemulsion Samples

Physical Appearance Test for Water, Macroemulsion, and Nanoemulsion

The data shown in Table 4 were based on visual observation. All three solutions were different in appearances and hence, it was easy to determine which one is nanoemulsion.

Table 4. Physical appearance differences

Solution	Water	Macroemulsion	Nanoemulsion
Color	Clear	White	Bluish/Cloudy
Transparency	Scattering	Opaque	Transparent

Tyndall Effect

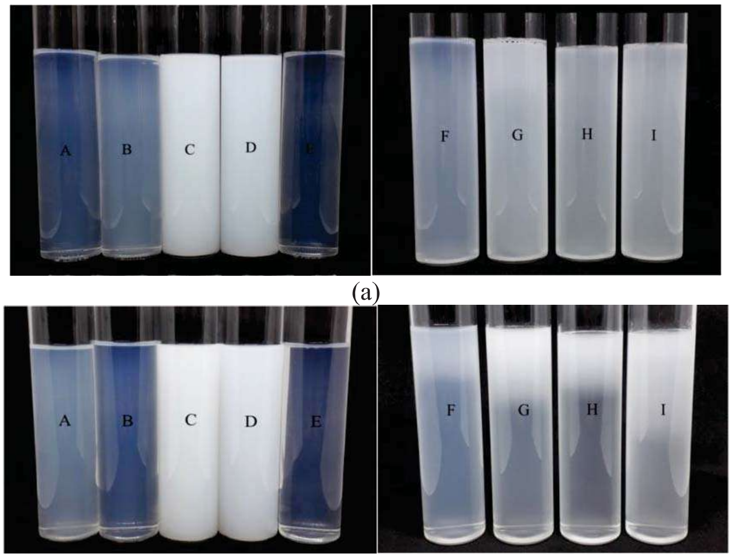
Nanoemulsions are a colloidal particulate system in nano-sized oil droplet [12]. The colloidal particles are able to scatter light as it passes through the solution containing the particles. A source of light was pointed at three different samples which were nanoemulsion from sample E, distilled water and nanoemulsion containing *Piper betle* essential oil as shown in Fig. 4. A light beam was clearly seen in the bottles containing the nanoemulsion samples, thus proved that both of the nanoemulsion formulations were stable and containing nano-sized droplets that illuminate the light path and made it visible to the eyes. The amount of scattering depends on light frequency and density of particles in the solution [7].

(a) (b)

FIGURE 4. Tyndall effect test on nanoemulsion, and distilled water (a) and Tyndall effect on nanoemulsion containing *Piper betle* essential oil (b)

Stability Test

All nanoemulsion samples were left at room temperature for 14 days to test its shelf life. Based on visual observation, there were no separation during the first day but some samples did lose their stability after a period of time as shown in Fig. 5. After 14 days, samples A, B, C, D, and E were stable and no separation occurred. However, samples F, G, H, and I had shown some separation post 14 days. Nanoemulsion was kinetically stable but after a period of time, separation phase can occur [13]. Samples A, B, C, D, and E remained stable due to their small droplet size which made them kinetically stable over a long period of time. The samples of nanoemulsions containing *Piper betle* essential oil were also stay unseparated after 14 days.



(a)

(b)

FIGURE 5. Samples of point A to I at Day 0 (b) and the same samples at Day 14 (b)

CONCLUSION

Piper betle essential oil was successfully extracted via the steam distillation process. Nine samples of blank nanoemulsion were formulated following the ternary phase diagram which consisted of oil, water surfactant, and co-surfactant. The validity of all nanoemulsion samples were proven via physical appearances test and Tyndall effect test. The optimum nanomeulsion formulation was found at point E and nanoemulsion containing *Piper betle* essential oil was produced using point E as the base formulation.

According to the results, the particles size obtained from DLS test, only sample A, B, D, and E achieved nanoemulsion state with droplet size of 68.85 nm, 132.29 nm, 181.85 nm, and 35.91 nm respectively. Droplet size of sample C was 211.69 nm, exceeded 200 nm. Hence, it was not considered as nanoemulsion since nanoparticle size should stay between 20 to 200 nm. Sample F, G, H, and I were unstable and separated post two weeks of sample preparation.

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