Development of *Piper betle* Nanoemulgel Formulation for Effective Droplet Size Reduction and Excellent Stability

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Abstract. The goal of this research is to formulate and evaluate the current potential of the nanoemulgel which incorporate the natural plant essential oil as central ingredient for effective topical drug delivery system. In general, the nanoemulgel comprise of two different manners which the nanoemulsion contains the active ingredient then embody into the gel base system. In this research, *Piper betle* essential oil had been chosen to be as the active element in the formulation and the steam distillation had been carried out to extract the essential oil. The nanoemulsion consists of two immiscible phases such as soy bean oil as oil phase, distilled water as aqueous phase and tween 80 as surfactant with Glycerol as co-surfactant. Carbopol 940 is employed as gelling agent to increase the viscosity of the formulation of the nanoemulgel. In this research, different ratio of the nanoemulsion and nanoemulgel had been formulated and investigated for few characteristics such as droplet size, viscosity, pH and spreadability. The droplet size of nanoemulsion was determined with Dynamic light scattering (DLS) and in the range of 35.91 nm to 747.27 nm. Besides, pH of the nanoemulsions and nanoemulgels were in the range of 5.5 to 6.9, which is nearly neutral. Plant derived nanoemulgel has been becoming the increasing trend applied in field applications because of their few unique characteristics such as non-toxic, better spreadability, non-greasy, and so on. In short, *P. betle* nanoemulgel presented as a potential and promising manner in topical drug delivery in the future.

INTRODUCTION

Nanoemulsion is one of the promising approaches and growing technology in a wide range of fields such as cosmetic, food, pharmaceutical, chemical industry and others [1]. The products of nanoemulsion are recently becoming one of the most demand product especially in the skin care products, oral drugs, topical drugs and so on [2]. Special characteristic properties of nanoemulsion such as optical clarity, small droplet size with high interfacial area, thermodynamic stability, high solubilization capacity, ease of preparation and others makes nanoemulsion had been given great attention in the research fields [3, 4]. However, the nanoemulsion contains low viscosity which can constrains its application in topical drug delivery and lead to the inconvenient use [5]. Hence, the modification of the nanoemulsion incorporated into hydrogel termed nanoemulgel can solve the limitation of hydrophilicity of hydrogel in delivery of hydrophobic drugs and hence can possess both advantages of the nanoemulsions and gels [6]. The nanoemulgel can enhance better penetration ability, and then it is also easily spreadable, greaseless, improve the stability of the active ingredient and others [4].

Recently, the researchers had been paid a great intention in development of the natural products to reduce the problem of allergic and safety issues. There had been increase intense usage of the natural bioactive materials from plants in order to reduce the use of the harsh chemicals in the formulation of the products. In this research, the *P. betle* essential oil had been chosen to be the active ingredient in the formulation of nanoemulsion and nanoemulgel. *P. betle* essential oil is extracted from the leaves of the *P. betle* and the leaves contain of substantial amount of the

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vitamins and minerals such as Vitamin C, Vitamin A, thiamine, riboflavin and others [7]. Besides, *P. betle* essential oil also contain the properties of antibacterial, antifungal, antioxidant, antiepileptic and so on [8]. These properties make *P. betle* essential oil become a great potential to be applied in the chemical industries and products such as natural care products, natural based food additives and others [9].

In this study, the *P. betle* essential oil had been extract through steam distillation and act as active ingredient in the formulation of nanoemulsion and nanoemulgel. Different ratio of the *P. betle* essential oil in the formulation of nanoemulsion and nanoemulgel were investigate and evaluate on the characterization of the samples.

MATERIALS AND METHOD

Material

P. belte leaves obtained from the area of Jeli, Kelantan. The soybean oil, Tween 80, Glycerol, N-hexane, Carbopol 940 and triethanolamine were purchased from Sigma Aldrich while the distilled water was obtained from laboratory of Universiti Malaysia Kelantan.

Extraction of Piper Betle Essential Oil

The *P. betle* essential oil was extracted from the leaves of *P. betle*. The leaves were washed around three time to remove the dirt and then cut into the size of 7cm X 7cm. Next, the leaves were dried in the oven at temperature of 70◦C and used to undergo steam distillation to extract the essential oil.

Nanoemulsion Formulation

The formulation of the nanoemulsion was produced by using low energy method. The formulation was based on the pseudo-ternary diagram by Wang (2017) with slightly modification on the ratio of the oil phase and essential oil [10]. An optimum ratio of the formulation nanoemulsion had been chosen to formulate the *P. betle* nanoemulsion with different ratio of the *P. betle* essential oil to soybean oil. Different ratios of essential oil to soybean oil were used, which were 2:8, 4:6, and 5:5.

Nanoemulgel Formulation

The formulation of the nanoemulgel was prepared by mixing the optimized *P. betle* nanoemulsion with the Carbopol 940 gel in the ratio of 1:1 [11] [3].In this research, the Carbopol 940 was selected as gelling agent and dispersed it to form the hydrogel in the distilled water. To neutralize the dispersion, the triethanolamine used act as pH adjuster and the mixture was stirred until the viscous gel base formed. The *P. betle* nanoemulsion was slowly added to the gel based under continuous stirring until the nanoemulgel formed [12]. Different concentration of the nanoemulgel were prepared which were 0.5wt% and 1.0wt%.

Testing on Droplet Size and Stability

The droplet size of the nanoemulsion and nanoemulgel with different ratio of essential oils were measured by using Dynamic Light Scattering (DLS) and the stability test was observed by visual inspection. The droplet size and stability are one of the essential measurements for the characterizations of the nanoemulsion and nanoemulgel. This is because the droplet size greatly influences the stability of the performance of the samples.

Testing on pH

The determination of pH is very vital since the formulation of the samples needed to be applied to the skin. Hence, the pH of the formulation should be no cause any skin irritates problems and ensure safety issues. The pH of the prepared samples can be analyzed by using digital pH meter at the ambient temperature.

Testing on Viscosity Test

Determination of viscosity of the samples is very important to evaluate and determine the flow properties of the nanoemulgel to secure the quality and effectiveness of the samples. The viscosity of the nanoemulgels were determined by using viscometer. 0.5 wt% and 0.1 wt% of the Carbopol 940 gel with different ratio of nanoemulgels were measured in this study.

Testing on Spreadability

Spreadability of the nanoemulgels were measured using "Slip and Drag" method. 0.5g of the sample was placed between two horizontal glass plates and recorded the reading of spreadability of the sample after one minute. 5g weight was then applied to the upper plate. The readings of the spreading gels were recorded and the test was repeated three time to obtain an accuracy and consistency of the results.

RESULTS AND DISCUSSION

P. betle nanoemulsion were prepared in different percentage of the oil phase (soybean oil), aqueous phase (Distilled water) with same ratio (1:1) of surfactant (Tween 80: Glycerol) and the *P. betle* essential oil as an active ingredient in the formulation. Table1 showed the formulation, droplet size and pH of the samples.

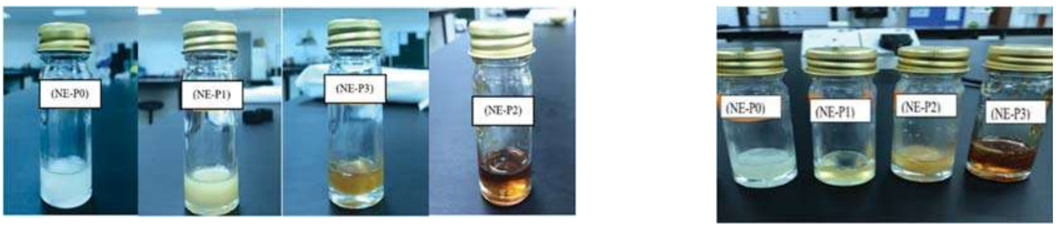
TABLE 1. Formulation, droplet size and pH of the nanoemulsions

Sample	Aqueous phase/%	Oil phase/%		Surfactant: Co-surfactant (1:1)/%	Total/%	Droplet size/nm	pH
		Soybean oil/%	*P. betle* essential oil/%				
NE-P0	30	10	-	60	100	35.91	6.3
NE-P1	30	6	4	60	100	55.98	6.3
NE-P2	30	5	5	60	100	62.63	6.5
NE-P3	30	8	2	60	100	747.27	6.7

*Tween 80: Glycerol in 1:1 – The ratio 1:1 was taken in the mixture of surfactant and co-surfactant.

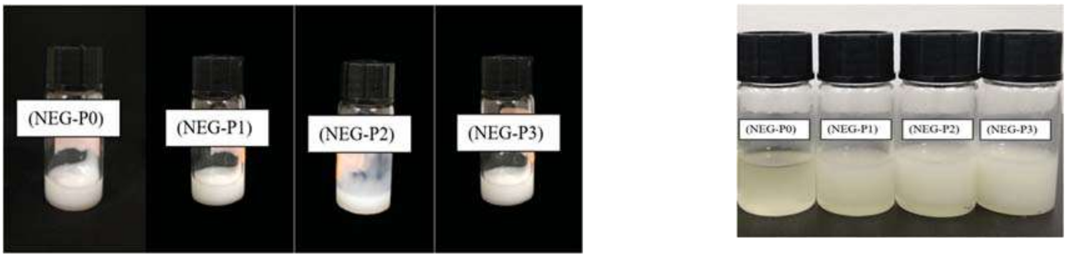
Testing on Droplet Size and Stability

The droplet size of the blank nanoemulsion and different ratio of *P. betle* nanoemulsion are given in the Table 1. Based on the result showed NE-P0, NE-P1 and NE-P2 represent droplet size ranged from 35.91 nm to 62.63 nm, whereas NE-P3 indicate the droplet size of 747.27 nm respectively. The NE-P0, NE-P1 and NE-P2 showed the in nano-sized which in the range of 20nm to 200nm while NE-P3 is not consider as nanoemulsion because its droplet sizes are more than 500nm [4].



(a) (b)

FIGURE 1. (a) represents the nanoemulsion samples at the first day while (b) indicates the condition of nanoemulsion samples after a week



(a) **(b)**

FIGURE 2. (a) represents the 0.5 wt% of nanoemulgel samples at the first day while (b) indicates the condition of 0.5 wt% nanoemulgel samples after a week

The stability of the samples performed by visual observing the samples at room temperature. NE-P1, NE-P2 and NE-P3 indicated slightly yellowish solution because of the presence of *P. betle* essential oil in the formulation. This is because the color of the essential oil in dark yellow and slightly effect on the colour of the formulations. NE-P3 was the most yellowish solution among the samples because the content of the *P. betle* essential oil in formulation NE-P3 was the highest among the others samples. Figure 1(a) and 1(b) showed the physical appearance of the nanoemulsion samples and the stability of the samples produced while figure 2 (a) and 2(b) indicated the samples of nanoemulgels.

Based on the figure 1, all the samples of nanoemulsions and nanoemulgel did not have any phase separation on the first day. After 1 week, all the samples of the nanoemulgels remain stable state and did not have any phase separation while the nanoemulsions samples all showed slightly phase separation. It showed that nanoemulgels retain better stability than nanoemulsions. This is justified to the presence of the gelling agent in the formulation of nanoemulgel. The incorporation of the nanoemulsion into gel can increase the stability of the samples. The gel-sols-gel properties of the nanoemulgel improve its stability and also with the bioavailability of the samples [13].

Testing on pH

Determination of pH of nanoemulsion and nanoemulgel were measured by using a pH meter. Based on the table 1.1, the pH of the nanoemulsions were in the range of 6.3 to 6.7 pH. This result indicated that the formulation of nanemulsion is nearly neutral which can be permitting the use of the formulation on the skin and will not result any skin irritation problem. The range for acceptable pH of topical products is in the range between 4.5 to 7.0 which can avoid the issue of skin irritation[14]. For the nanoemulgel, the pH of the samples was in the range of 6.3 to 6.9. From the result, the 1wt% of blank hydrogel was more acidic than 0.5 wt%. Besides, the pH of nanoemugel which contain the 1wt% of Carbopol were higher than the pH of the 0.5 wt% samples. This is because the concentration of the Carbopol 940 had the effect on the pH on the sample. The higher of concentration of the Carbopol, the more acidic the sample produced. This is due to the Carbopol 940 is acidic in nature [15] . Table 2 represents the average pH reading of the nanoemulgel samples.

TABLE 2. The average pH reading of hydrogel and nanoemulgels

Sample	**pH measurement**	
	0.5 wt% of Carbopol 940	**1.0 wt% of Carbopol 940**
Blank Hydrogel (Before added pH adjuster)	3.4	2.8
Blank Hydrogel (After added pH adjuster)	6.3	5.7
NEG-P0	6.3	6.6
NEG-P1	6.5	6.9
NEG-P2	6.4	6.8
NEG-P3	6.6	6.9

Testing on Viscosity of Nanoemulgel

The viscosity measurement is very vital to liquid and semisolid pharmaceutical products. This is because the viscosity can appraise and control the flow properties of the samples to assure the quality and effectiveness of the formulations [12]. Viscosity is not only influencing the spreadability of the samples, skin feel and also the penetration of the active ingredient into skin. The findings stated applying moderate viscosity in formulations is suggested. This is because high viscosity will arouse minor impairment of drug when its diffuse into the skin. Moreover, the moderate or certain viscosity level can inhibit the drain out of the formulation and promote the residence time on the skin [16].

The viscosity of the Carbopol depends very weakly on temperature [17]. Hence, the tests were performed on room temperature. Besides, the viscosity of the Carbopol also depend on the pH. The viscosity increased when the pH of the Carbopol increased. Table 3 showed the average viscosity reading of the samples.

TABLE 3. The average viscosity reading of nanoemulgels

Sample	Viscosity/ Pa.s	
	0.5 wt% of Carbopol 940	**1.0 wt% of Carbopol 940**
Blank Hydrogel (After added pH adjuster)	1.03	1.57
NEG-P0	1.54	1.97
NEG-P1	1.60	2.04
NEG-P2	1.59	2,02
NEG-P3	1.61	2.01

Testing on Spreadability of Nanoemulgel

Spreadability is one of the subordinate factors that can affect the capability to cover the surface of the skin. The spreadability of the sample is depend on the viscosity and structural behavior of the sample [18]. The spreadability of the sample increase when the viscosity of the samples decreases. High viscosity fluids cause the fluids move more slowly because of the resistance to flow. The sample showed thicker when flowing due to the its viscosity which contain the internal friction of fluids. The sample which consists of low viscosity is more easily to spread on the surface of skin and absorbed faster into the skin due to the low surface tension [18].

Based on the Table 4, NEG-P3 indicated the highest reading of the spreadability among the samples which was 2.60cm. The spreadability of the sample increase when the amount of the soybean oil decrease in the formulation. This is due to the viscosity index of Soybean oil is quite higher amount the vegetables oils and thus lead to increase in surface tension which make the samples more greasy, tacky and difficult to spread on the surface of skin [19].

TABLE 4. The average diameter of nanoemulgel spreadability after one minute

Formulation	Spreadability (cm)
NEG-P0	2.30
NEG-P1	2.20
NEG-P2	2.30
NEG-P3	2.60

CONCLUSION

The formulation of the *P. betle* nanoemulsion and nanoemulgel were successfully formulated. In this study, P. *betle* essential oil was successfully extracted by using steam distillation and acts as active ingredient in the formulation of nanoemulsion and nanoemulgel. The formulations of Piper betle nanoemulsion were achieved with the nanoemulsion state with the droplets size of 35.91nm, 55.98nm, 62.63nm respectively. The nanoemulgel was produced when adding the gelling agent into concentrated nanoemulsion. The nanoemulgel produced were nearly neutral moderate viscosity, which suitable for topical application and moderate spreadibility make the formulation can spread more easily on the skin. Hence, *P. betle* nanoemulgel represents a promising formulation for topical drug delivery.

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