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Development and Characterization of Nanoemulgel Containing *Piper betle* Essential Oil as Active Ingredient

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Abstract. This study aims to formulate and characterize a stable nanoemulgel containing *Piper betle* essential oil as the active ingredient for topical drug delivery. Nanoemulgel is a mixture of nanoemulsion and a gelling agent. Nanoemulsion was prepared by mixing the oil phase that containing soybean oil and *Piper betle* essential oil at different ratios, tween 80 as the surfactant and glycerol as the co-surfactant with the aqueous phase (distilled water). The essential oil of *Piper betle* was extracted by using a steam distillation method. The droplet size of nanoemulsion was in the range of 28 to 161 nm. The sizes were confirmed by dynamic light scattering method. Carbopol 940 was used as the gelling agent to increase the viscosity of the nanoemulsion and turn it into nanoemulgel. Nanoemulsion and nanoemulgel were characterised by using the Tyndall effect, spreadability test, and pH test. The pH of nanoemulgel with essential oil was in the range of 7 to 6, which is safe to be used on the skin. Nanoemulgel can be a suitable carrier for the active ingredient (essential oil) to be penetrated with ease into the skin because of its small droplets size. In conclusion, *Piper betle* nanoemulgel has shown great potential in helping the transdermal delivery.

1. Introduction

Nanotechnology is one of the growing technological applications that had been increasingly applied in various demands, especially in cosmetics, biopharmaceutical, and food industries. Nanotechnology containing products show a potential market because of the superior characteristics' properties such as small droplet size with the high interfacial area, enhance the delivery of the active ingredients, and excellent solubilization capacity [1].

Nanoemulsions are currently gaining much interest in biopharmaceutical and cosmetics industries due to their versatility in delivering both hydrophilic and lipophilic drugs. They can act as a drug delivery system that can be applied through several systemic routes such as topical, oral, and others. Nanoemulsion containing products can be in the semisolid form such as creams, balm, or in a fluidic state such as lotions, liniments, and others. Nanoemulsions are the colloidal dispersions that consist of oil, water, and emulsifier, with the range of the droplets size between 20 nm to 500 nm [2]. The emulsifier plays a vital role in creating small-sized droplets as it decreases the interfacial tension between the water phases and the oil phase of the nanoemulsion [3]. Water in oil (W/O) nanoemulsion offers a superior emollient property, but the costumers do not accept them well because of the high content of oil and greasy texture. On the other hand, oil in water nanoemulsion (O/W) is more favored

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as it enhances the absorption speed, and less oil content in the formulation caused it to be easily washed from the skin [4].

Nanoemulgel is considered as one of the appropriate candidates for drug delivery for skin because of its dual characters which are nanoemulsion and gel base. The benefits from both nanoemulsion and gel have caused nanoemulgel to achieve high patient acceptability [5]. Nanoemulgel has been widely applied in the field of pharmaceutical. Numerous studies and investigations have been done on the formulations and development of nanoemulgel for the vast delivery systems such as transdermal, vaginal, ocular, dental, and nose to brain for the treatment of diverse locals as well as systemic ailments [6]. Currently, there has been an increase interest in the development of natural and eco-friendly products with several beneficial bioactivities. The combination of nanoemulgel and plant-based oils may be a great solution for the researchers to improve the formulation of the application to fulfill the market needs.

Piper betle L. is colloquially well known as betel vine that commonly grows in Southeast Asia and belongs to the same family as the pepper [7]. *Piper betle* is mostly consumed in Asia as a propitious plant by the Indians, Nepali, Singhalese, Thai, and others [8]. The most commonly used plant part of the *Piper betle* plant is the leaves. The leaves are pungent in smell and possess aromatic flavor. They contain the betle oil, a volatile liquid, which contains several phenols, including eugenol, hydroxychavicol, chavicol, and betel phenol [9]. These compounds may, to some extent, be protective, sharing some of the antioxidant properties of many plant polyphenols [5]. *Piper betle* leaves are a non-toxic natural antioxidant and widely applied as the traditional medicinal uses. Several reports showed that the *Piper betel* leaves comprise many beneficial bioactivities. The leaves extraction performs an enormous potential in developing commercial applications such as nanoemulsion and cream [10][11].

In this study, the *Piper betle* leaves were used to extract the essential oil. The combination of *Piper betle* essential oil and nanoemulgel was further investigated, focusing on the formulation and characterization of *Piper betle* nanoemulgel.

2. Materials and Methods

The collection of the *Piper betle* leaves were from the area of Jeli, Kelantan. The fresh leaves were washed with distilled water three times to remove all the dirt. N-hexane, tween 80, glycerol, carbopol 940, triethanolamine and soybean oil were purchased from Sigma Aldrich while the distilled water was produced from the laboratory.

2.1. Extraction of essential oil of Piper betle leaves

The extraction of essential oil was conducted through steam distillation. The *Piper betle* leaves were oven-dried at the temperature of 70°C with 4 hours to ensure the moisture content below 10% to prevent the fungal infection to the samples [12]. The dried *Piper betle* leaves were cut into small pieces and undergo the steam distillation process for six hours to isolate the essential oil. The essential oil was collected and stored to be further used in the nanoemulsion formulation.

2.2. Preparation of Piper betle nanoemulsion

A stable concentrated nanoemulsion was formulated based on the pseudo-ternary phase diagram constructed by Ziheng Wang (2009) with minor modifications to the amount of the oil phase [13]. The phase diagram consists of soybean oil, tween 80, glycerol, and water. The soybean oil was used as the base oil phase, while *Piper betle* essential oil was the active ingredient added into the soybean oil. The nanoemulsion was stabilized with the help of glycerol as the co-surfactant. Different ratios of essential oil to soybean oil were used, which were 1:4 and 4:1.

2.3. Preparation of Piper betle nanoemulgel

The nanoemulgel was prepared by mixing the optimized *Piper betle* nanoemulsion with the Carbopol gel. The Carbopol 940 was used as a gelling agent and dispersed to form the hydrogel in the distilled water [14]. The triethanolamine was used to neutralize the dispersion and the mixture was stirred until the viscous gel base formed. The *Piper betle* nanoemulsion was slowly added into the gel under continuous stirring until the nanoemulgel was formed.

2.4. Droplet Size Analysis of the Piper betle Nanoemulsions
The droplet sizes of nanoemulsion with and without essential oils were analysed by using dynamic light scattering (DLS), one of the common measurement approaches for the size particle analysis in the nanometre range.

2.5. Stability and Physical Appearance
Different samples of nanoemulsion and nanoemulgel were prepared. Their stability and physical appearance were observed based on the colour, transparency and the ability for the sample to stay unseparated.

2.6. Spreadability Test
The spreadability of various nanoemulgel formulations were determined by measuring the spreading diameter of 0.5 g of the sample between two horizontal glass plates after one minute. 5g weight was then applied to the upper plate. Each formulation was determined three times for the accuracy and consistency of the results.

2.7. pH Measurement
The pH of the nanoemulgel was measured by using digital pH meter. pH of the samples should be about 6-7 to match the skin condition.

3. Results and Discussion

3.1. Formulation, Stability and Physical Properties of Nanoemulsion and Piper betle Nanoemulsion
The formulation of the *Piper betle* nanoemulsion involved soybean oil as the oil phase, tween 80 as the surfactant, glycerol as the co-surfactant, and distilled water as the aqueous phase. 1:1 ratio of tween 80 and glycerol was applied in this study. The ratio of soybean oil to *Piper betle* oil used was 1:4 and 4:1, respectively. These two ratios were intended to investigate the appearance of different amounts of essential oil have on the nanoemulsion and nanoemulgel. Concentrated nanoemulsions were labeled as N0 to N1. N0 was found to be the optimum formulation of nanoemulsions which consist the base oil phase, soybean oil. N1 and N2 were the same formulation with the addition of *Piper betle* oil to the base oil. Table 3.1 shows the composition of each formulations. All three samples of concentrated nanoemulsions in Figure 3.1 show different appearances. N0 shows a transparent and clear solution, N1 has the yellowish colour solution while N2 has a lighter yellowish color than N1. *Piper betle* oil produced was yellow to brown and contained a strong and spicy odour. The higher content of *Piper betle* oil would possibly be the reason for N1 becoming more yellowish than the other two formulations. All three samples were stable for more than 30 days.

Table 3.1. The formulation of *Piper betle* nanoemulsions

Ingredients	N0 (w/w%)	N1 (w/w%)	N2 (w/w%)
Soybean oil	20	4	16
Piper betle oil	-	16	4
Surfactant (tween 80 + glycerol)	75	75	75
Distilled water	5	5	5

N0 represents the formulation with only soybean oil

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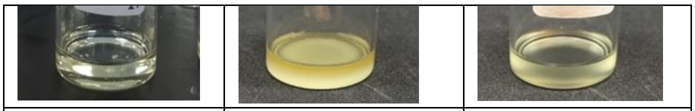
N1 represents the formulation with 1:4 ratio of soybean oil to *Piper betle* oil
N2 represents the formulation with 4:1 ratio of soybean oil to *Piper betle* oil



N0	N1	N2

Figure 3.1. Different formulations of concentrated *Piper betle* nanoemulsion.

Concentrated nanoemulsions were diluted prior to the light scattering measurement. 1.5ml of the concentrated formulations were diluted in 50mL of distilled water. The dilution process did not change the droplet size distributions. Figure 3.2 shows the diluted version of N0, N1 and N2 and labeled as DN0, DN1 and DN2. The bluish coloration of the diluted nanoemulsions was due to the nanosize droplets that exist in the colloidal solution. As the droplet size of the nanoemulsion was significantly smaller than the wavelength of the visible light, the physical appearance of the nanoemulsion is transparent and bluish in look [8][15]. This was supported by the DLS result. Droplet size of DN0 was found to be 41.00 nm, while DN1 and DN2 were 240.49 nm and 27.67 nm, respectively. All of the formulations are proven to be nanoemulsion, as the sizes were within the range of 20 to 500nm [16].

A light beam was pointed through the diluted nanoemulsions and it was visible through all the solutions. The reason was nanoemulsions are the submicron-sized colloidal particulate system. When a light beam pass through a colloidal solution, it will be scattered by the small particles and created a visible light ray, also called as Tyndall effect. The amount of the scattering was depending on the frequency of the light and the density of the particles [17].

DN0	DN1	DN2

Figure 3.2. Diluted nanoemulsions show different colours of solutions (DN0 represents the formulation with only soybean oil, DN1 represents the formulation with 1:4 ratio of soybean oil to *Piper betle* oil and DN2 represents the formulation with 4:1 ratio of soybean oil to *Piper betle* oil).

3.2. Formulation of and Physical Properties of Piper betle Nanoemulgel

Nanoemulgel was produced by mixing the concentrated nanoemulsion and Carbopol 940 (0.5%), triethanolamine and distilled water. In this study, 0.5% w/v of Carbopol 940 was applied as gelling agent. Carbopol 940 has superior properties in thick formulation, good in clarity, and produce low viscosity gel. The nanoemulgel formulations were labelled as NG0, NG1 and NG2.

Physical appearance of the nanoemulgel with different ratio of *Piper betle* oil shows similar result as nanoemulsions but they were all represented cloudier due to the addition of the gelling agent. The nanoemulgel produced usually was white viscous creamy accompanied by a smooth and homogeneous appearance that leads to the nanoemulgel being more easily spreadable with tolerable bio-adhesion and fair mechanical characteristics. However, different ratio of the *Piper betle* oil causes the difference in the colour of the samples produced. High amount of the *Piper betle* oil in the formulation causes the yellowish solution of NG1 as shown in Figure 3.3.

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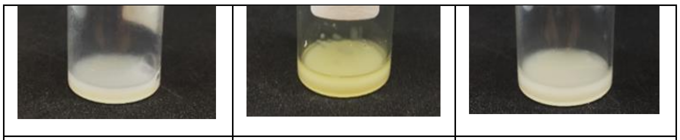

NG0	NG1	NG2

Figure 3.3. Nanoemulgel produced by mixing nanoemulsion and gelling agent (NG0 represents the formulation with only soybean oil, NG1 represents the formulation with 1:4 ratio of soybean oil to *Piper betle* oil and NG2 represents the formulation with 4:1 ratio of soybean oil to *Piper betle* oil).

3.3. Spreadability Test of Piper betle Nanoemulgel

The desirable spreadability of the nanoemulgel is one of the essential criteria for the selection of a topical delivery system [18]. The spreadability of different nanoemulgel formulations was determined, and the result was tabulated in Table 3.2. The values obtained were found to be satisfactory and comply with the standard range. Larger value shows better spreadability that indicates the contentment of the formulation when applied to the skin, spreads easily, and displays the maximum slip and drag [19]. From the result, NG2 recorded the largest value of spreadability.

Table 3.2. The average diameter of nanoemulgel spreadability after one minute.

Formulation	Spreadability (cm)
NG0	2.77 ± 0.208
NG1	2.83 ± 0.058
NG2	2.90 ± 0.173

*Values are expressed as mean ± SD (n=3)

3.4. pH of Piper betle Nanoemulgel

The pH of nanoemulgel were determined by using a calibrated pH meter. The readings were obtained for average of three times. The pH achieved was nearly neutral, which in the range of 6.0 to 7.07. It shows that the nanoemulgel formulations were safe to be used on the skin and will not cause any irritation when applied to the skin [19]. The pH value of the nanoemulgel was gradually decreasing with the addition of *Piper betle* oil and more acidic compare to the blank hydrogel. The addition of the *Piper betle* oil slightly influences the acidic condition of the formulation but still in the acceptable range to be applied for topical delivery purposes. Table 3.3 represents the average pH of the different nanoemulgel formulations.

Table 3.3. The average pH reading of samples.

Samples	pH Measurement
Hydrogel	7.07 ± 0.21
NG0	6.73 ± 0.25
NG1	6.80 ± 0.30
NG2	6.60 ± 0.20

*Values are expressed as mean ± SD (n=3).

4. Conclusion

The stable nanoemulgel containing *Piper betle* oil as active ingredient was successfully formulated. *Piper betle* essential oil was extracted from the leaves by steam distillation and used as active ingredient in the nanoemulsion. The optimized formulation of concentrated nanoemulsion was achieved and different ratios of *Piper betle* essential oil were added into the formulation. The nanoemulgel was produced by addition of carbopol 940 to the concentrated nanoemulsion. The nanoemulgel appeared to be translucent with slightly yellowish in colour with no phase separation occurred for 30 days. The droplet sizes for all formulations are in nanosize which confirms the success of producing nanoemulgel

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in this study. The pH of *Piper betle* nanoemulgels were nearly neutral, and they are also easily spread on the skin. *Piper betle* nanoemulgel shows a very high potential to be used as an effective carrier for the active ingredients.

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