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Research Article

Development and characterization of clove oil microemulsion

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Abstract

Clove oil is one type of essential oil from clove flower buds (*Syzygium aromaticum*) which contains eugenol compounds. Clove oil is volatile in nature and highly affected by heat, thus incorporating it in a microemulsion system can increase its shelf life of the oils. This study aimed to formulate and characterize microemulsion preparations with clove flower essential oil. The microemulsion existence region was determined by constructing a pseudo-ternary phase diagrams and prepared with four components, i.e. isopropyl myristate, tween-80 as a surfactant, polyethylene glycol 400 (PEG 400) as a co-surfactant, and water as the aqueous phase. The optimized clove oil microemulsion formulation was subjected to an evaluation of various parameters, such as organoleptic properties, %transmittance, pH, viscosity, stability, particle size, zeta potential, and polydispersity index (IP). Based on the results of the study, the highest compound component in clove oil was eugenol with a % area of 63.79%. The results revealed that the construction of a phase diagram and the use of the phase titration method constituted a suitable technique for the preparation of microemulsions as most of the formulations were transparent. It was found that the tween-80:PEG 400 ratio of 2:1 with an oil:S-mix ratio of 1:9 generated an optimum results. The clove oil microemulsion had a globule size of 17.69±0.025nm, a polydispersity index value of 0.057±0.0043, zeta potential of-5.36±0.23mV, and a pH value of 7.3±0.1, a viscosity value of 466.7±9.06., and %transmittance of 99.9±0.1. According to these findings, the microemulsion formulation might serve as a suitable drug delivery system.

Keywords

microemulsion, clove oil, formulation, characterization

Introduction

The clove (*Syzygium aromaticum*) is included in the Myrtaceae family, which is widely produced in Indonesia. The Clove is primarily produced in Indonesia, accounting for roughly 70% of total global clove production yearly (Amelia et al. 2017). Clove plants are also used to obtain, among other things, clove oil, a type of essential oil, from clove flower buds (*Syzygium aromaticum*), which contains eugenol compounds. Clove oil has antibacterial properties and is commonly used to treat many disorders, such as toothache, GI disturbances, respiratory diseases, and inflammation (Cui et al. 2015). It shows anti-ulcer and gastroprotective activity in rat models with indomethacin and ethanol-induced ulcers (Tanzeem et al. 2019). The essential oil content in clove flowers reaches 21.3%, with a eugenol content of 78–95% (Hadi 2013).

The potency of clove leaf essential oil still needs to be improved. It is immiscible with water, which minimizes contact with polar ingredients (Van de Vel et al. 2019).

Clove essential oil has also been widely used in the food and perfume industries, but it has weaknesses like being a very volatile compound, unstable to heat, light, and air (Cui et al. 2015). Hence, to improve clove essential oil's stability, solubility, and effectiveness, it may be prepared in microemulsions (Saini et al. 2019).

Microemulsions are thermodynamically stable, transparent, and homogeneous (Sharma et al. 2016). They can increase drug solubility, have a long shelf life, be easily prepared, and increase the bioavailability of poorly soluble drugs (Hasrawati et al. 2016). They can also be used as a drug delivery system by multiple; routes and makes microemulsions is an promising dermal delivery route through an efficient drug delivery route (Muzaffar et al. 2013). Microemulsions may be used for enhanced oil recovery and for formulations for drugs, food, and cosmetics that are edible using oral or transdermal administration methods as delivery methods vehicles for dosages to be released under control (Callender et al. 2017).

A microemulsion is a bicontinuous system containing water and oil, separated by a surfactant and a cosurfactant. Microemulsions have low interfacial tension. It will be challenging to achieve the required interface area if only a single surfactant is used; thus, a co-surfactabt is needed (Deepak and Vedha Hari 2013). The surfactants often used in microemulsion preparations are non-ionic surfactants, such as tween-80 (Hidayat et al. 2020). The co-surfactants are usually short- to medium-chain alcohols (C3–C8), such as PEG 400. Polyethylene glycol (PEG) is a polymer with different molecular weights that exhibits excellent properties, such as biocompatibility, minimal toxicity, and good solubility (Fan et al. 2020). The combination of the surfactant tween-80 and the co-surfactant PEG 400 in microemulsion has been employed in previous studies with the use of various oils, such as oleic acid oil (Sisak et al. 2017), citronella oil (Hasrawati et al. 2016), and limonene (Ramli et al. 2019).

This study aimed to make a microemulsion preparation containing clove flower essential oil with various concentrations of tween 80 as a surfactant and PEG 400 as a co-surfactant. The results of the formulation of the microemulsion preparation will be characterized to obtain a stable and high-quality clove flower essential oil microemulsion.

Materials and methods

Materials

The main ingredients used in this research was clove flower essential oil, which was obtained from the Center of Essential Oil Studies (CEOS) of the Indonesian Islamic University, Yogyakarta. The additives used in the formulation of microemulsion gel preparations, including isopropyl myristate, tween-80, PEG 400, and aquadest, were of pharmaceutical grade and obtained from CV Nurul Jaya Medicallabsains, Banyumas.

Instruments in this study were: Iwaki Pyrex glassware, GC-MS instrument, magnetic stirrer, digital pH meter (Pico+ Labindia, Mumbai, India), V-Visible spectrophotometer (UV, 1700, Shimadzu, Japan), Brookfield viscometer (DV- II+Pro Brookfield, USA), and Zetasizer (Malvern instrument ltd ZEN3600, UK).

Methods

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS analysis was performed using an Agilent 7890 gas chromatograph connected to an Agilent 5975C Mass Spectrometry detector. The column used was HP-5MS UI (cross-linked 5% methyl phenyl silox) capillary column (30 m × 0.25 mm, film thickness 0.25 m). The oven temperature is raised from 40 °C to 200 °C at a rate of 6 °C/min, and further from 200 °C to 280 °C at a rate of 30 °C/min. Post-run was then conducted for 10 minutes at 280 °C. The carrier gas was Helium with a flow rate of 1 mL/min. The injector and detector temperatures was 250 °C (Amelia et al. 2017).

Pseudo-ternary phase diagram study

The pseudo ternary phase diagrams of surfactant and co-surfactant mixture (S-mix), oil and doubled distilled water were plotted by the water titration method. The surfactant used was tween-80, and the co-surfactant used was PEG 400. The ratio of surfactant (S) to co-surfactant (CoS) was fixed at different ratios of 1:1, 2:1, and 1:2 on the weight basis for each phase diagram. The oil phase was mixed with the surfactant and co-surfactant mixture at the ratios (volume basis) of 1:9, 1:8, 1:7, 2:12, 2:10, 2:8, 2:7, 2:6, 3:7, 3:6, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1(w/w) (Laothaweerungsawat et al. 2020). The oil and S-mix phases at each ratio were weighed separately, mixed, and stirred using a magnetic stirrer at a speed of 500 rpm until homogenous mixtures were achieved.

The oil-S-mix mixture at each ratio was slowly titrated with distilled water. The addition of distilled water was carried out with a constant stirring speed, and the final mixture was stirred with a constant stirring speed for 15 minutes at room temperature until a microemulsion (ME) was formed, i.e., a transparent liquid with low viscosity (Laothaweerungsawat et al. 2020). The system was visually and carefully inspected after each addition of aquadest. The ME and emulsion (EM) regions were clearly visible in the phase diagrams using the SIGMA plot software. Various batches of microemulsions were prepared by the water titration method, and the formulation was optimized in terms of organoleptic properties, pH, viscosity, stability test, percentage of transmittance, particle size, polydispersity index, and zeta potential.

Clove essential oil microemulsion formulation

The most stable microemulsion base was achieved. The manufacture of clove oil microemulsions was carried out by weighing all ingredients using a 10% clove oil concentration. The surfactant and co-surfactants were mixed in a beaker glass, to produce a surfactant mixture by stirring at 500 rpm using magnetic stirrer for 5 minutes

until homogeneity was achieved. Oil was added by continuously stirring with the same stirring speed using a magnetic stirrer for 5 minutes until homogeneity was achieved. Weighed clove oil was added to the mixture by continuously stirring with the same stirring speed using a magnetic stirrer for 5 minutes until homogeneity was achieved. Distilled water was also added little by little to the mixture until all the distilled water was added. Stirring was carried out continuously using a magnetic stirrer for 15 minutes until homogeneity was achieved.

Characterization of the clove oil loaded Microemulsion

Organoleptic measurement

An organoleptic test was carried out by observing the shape, color, and smell of the clove oil microemulsion. Observations were made visually and using the five senses (Fitriani et al. 2016).

pH measurement

The apparent pH of the prepared formulations was measured (in triplicate) by using a calibrated digital pH meter (Pico+ Labindia, Mumbai, India) at ambient temperature with a glass electrode at 25±1 °C.

Percentage of transmittance

Transparency of the microemulsion was determined by measuring the percentage of transmittance at 650 nm against distilled water as the blank using a UV-Visible spectrophotometer (UV, 1700, Shimadzu, Japan).

Viscosity measurement

The viscosity of the samples was measured at 25 °C with a Brookfield viscometer (DV-II+Pro Brookfield, USA) using a spindle no 5 with a shear rate 6 rpm. Each measurement was performed in triplicate.

Measurement of globules and Zeta potential

Particle size measurements

The average droplet size of the samples was measured at 25 °C by SCATTER SCOPE 1 QUIDIX (South Korea), and their polydispersity index (IP) was also calculated.

Zeta potential determination

The zeta potential of the samples was measured by Zetasizer (Malvern instrument ltd ZEN3600, UK). The samples were placed in clear disposable zeta cells and the results were recorded.

Physical stability studies

Centrifugation

Formulations passing from the heating and cooling cycle were centrifuged at 3500 rpm for 30 min. All formulations that did not show any phase separation were taken for the heating and cooling stress test.

Heating and cooling test

Six cycles between refrigerator temperatures of 4 °C and 45 °C with storage at each temperature for no less than 48 h were studied. Those formulations which were stable at these temperatures were subjected to a centrifugation test.

Statistical method

All the experiments were repeated three times, and data were expressed as the mean values ± SDs. Statistical data were analyzed by a one-way analysis of variance (ANOVA), and $P<0.05$ at a 95% confidence interval was considered to be significant.

Results and discuccion

Analysis of clove essential oils compounds

In this study, the analysis of the chemical components of clove flower essential oil was carried out using gas chromatography (GC-MS). Gas chromatography is able to read compounds with the lowest concentrations; thus, secondary metabolites in plants can be identified with results in the form of chromatograms and mass spectra (Al-Rubaye et al. 2017). The mass spectrometry data from the GC-MS showed the molecular mass of each compound and its fragmentation pattern. The compounds making up the essential

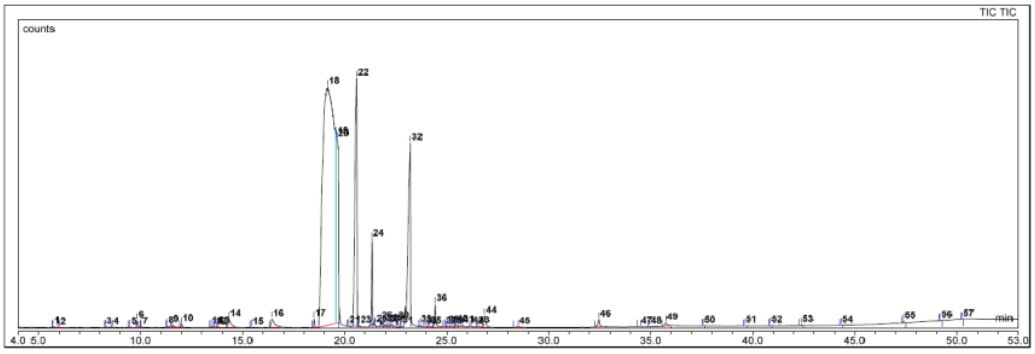

Figure 1. The chromatogram profile of clove essential oil.

oil were interpreted based on the fragmentation pattern of the percent similarity to the Data Base (>90%).

The results of the analysis showed that there were 57 peaks can be seen in Fig. 1 and 171 possible compounds contained in clove flower essential oil with different similarity indices (SI). The most common compound components in clove oil are listed in Table 1. The most common compound components are located at peak 18 are shown in Fig. 1. There may be eugenol compounds with a % area of 63.79%. The second largest content is Caryophyllene. Besides, clove oil also contains Phenol compounds, 2-methoxy-4-(2-propenyl)-, acetate, which appears at peak 35 under another name according to the PubChem website and the NIST webbook (National Institute of Standards and Technology). The compound is synonymous with eugenol acetate. The results of the clove oil analysis used in this study are the same as those produced by Amelia et al. (2017), which showed that clove oil from Java and Manado contains eugenol at 55.61% and 74.65%, respectively, as well as caryophyllene at 14.85% and 12.80%, respectively, and eugenol acetate 20.55% and 8.71%, respectively.

Table 1. The chemical profile and % area of clove essential oils components.

No	Peak	Name of Component	Molecular structure	Weight	Rt(minutes)	% area
1.	18	Eugenol	$C_{10}H_{12}O_2$	164	19.17	63.79
2.	22	Caryophyllene	$C_{15}H_{24}$	204	20.58	10.43
3.	20	Phenol, 2-methoxy-3-(2-propenyl)-	$C_{10}H_{12}O_2$	164	19.62	8.57
4.	32	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	$C_{12}H_{14}O_3$	206	23.21	8.48
5.	19	Eugenol	$C_{10}H_{12}O_2$	164	19.60	2.08

Pseudo ternary phase diagram study

For the microemulsion base, this study used the surfactant tween-80 and the co-surfactant PEG 400 because previous studies have proven that the combination of both is the right choice to produce microemulsion preparations with good physical characteristics and stability. The ratio of oil, surfactant, and co-surfactant in the microemulsion region was determined using cosurfactant using a pseudo-ternary phase diagram. Pseudo-ternary diagrams were created to obtain the maximum ratio that would precisely describe the phase formation limit (Nurfauziah and Rusdiana 2018). The results of co-surfactant screening can be seen in Fig. 2.

If the ratio of surfactant to co-surfactant changes, where the former continuously increases, the interfacial

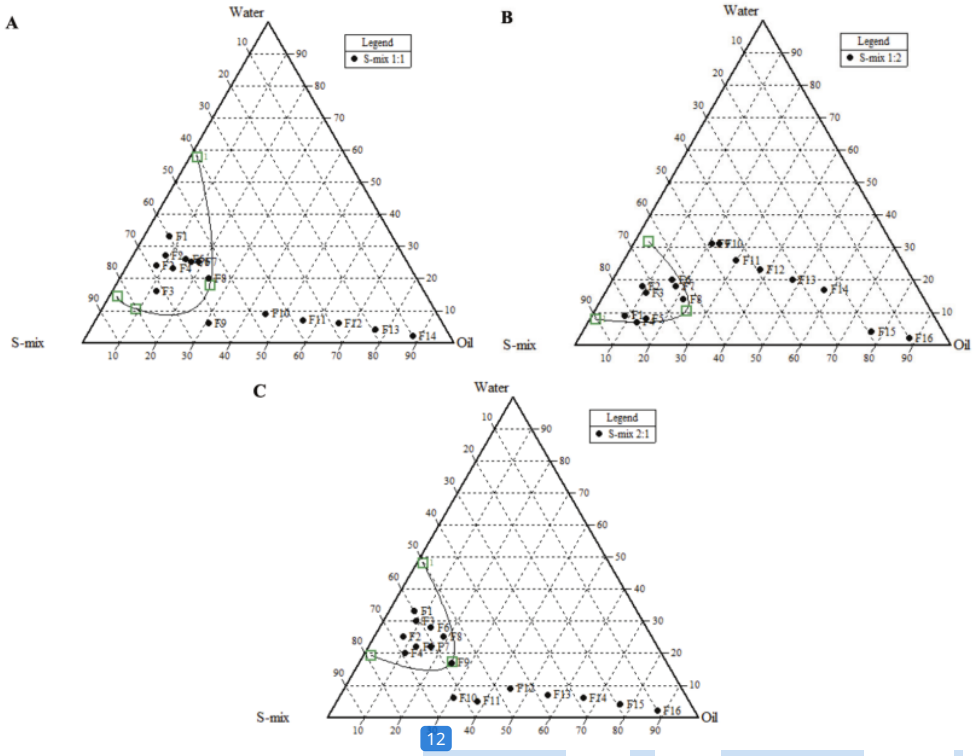

Figure 2. The pseudo-ternary phase diagram for (**a**) the tween 80:PEG 400 ratio of 1:1, (**b**) the tween 80:PEG 400 ration of 1:2, and (**c**) the tween-80:PEG 400 ratio of 2:1.

tension will become better and more optimal, producing a good microemulsion. However, if the amount of co-surfactant is elevated beyond the surfactant, a reduction in emulsification will occur, therefore, it is better to use more surfactant than co-surfactant (Sharma et al. 2016). The results of this study mirror those of Syafitri et al. (2020), who made microemulsion preparations with precise characteristics and good stability for seven days with a tween 80:PEG 400 ratio of 2:1.

Fig. 2 shows the S-mix of the 2:1 ratio had a wider microemulsion area. To form a microemulsion, water should be added to a rate of no more than 33%. The larger the microemulsion formation area, the greater the efficiency of the micro emulsification system. A decrease in the oil level can lead to an increase in the microemulsion formation area. Non formation of microemulsion may be attributed to the surfactant concentration. The lower the surfactant concentration, the less micellar formation that plays a role in bringing together the oil and water phases in the microemulsion. Surfactants and co-surfactants are adsorbed at the interface, reducing the interfacial energy, and providing a mechanical barrier to prevent coalescence. The added cosurfactant can create and fill the gaps in the surfactant molecule. The co-surfactant addition can cause greater penetration of the oil phase in the hydrophobic region of the surfactant monomer, thereby lowering the interfacial tension (Nirmalayanti 2021). The S-mix of the ratio of 2:1, showed a greater area than that S-mixes of other rations.

Organoleptic test

The composition of the selected microemulsion base is shown in Table 2 which is then evaluated and compared with its physical characteristics and stability.

The first evaluation carried out was the organoleptic evaluation. This evaluation was carried out by storing the microemulsions at room temperature (25 °C) for 30 days. In all formulas, the microemulsion was clear, transparent in appearance, and with no precipitate. The reason for this was the presence of both hydrophilic and hydrophobic chains in eugenol, which resembled the structure of a surfactant and, therefore, could align themselves along with the original surfactant, i.e., tween-80, at the interfacial film. The results of the organoleptic evaluation can be seen in Fig. 3.

Table 2. Microemulsions base optimized formulas.

Formulation	S/C	%Oil	%(S+C)	%water
ME-1	1:9	7	60	33
ME-2	1:8	8	68	24
ME-3	1:7	9	64	27
M3-4	2:10	13	64	23
ME-5	2:8	15	59	26

Note: ME = microemulsion. S= surfactant, C= Co-surfactant.

pH measurement

A pH measurement is important to determine the suitability of the pH of the preparation with the pH of the skin. The results of the pH measurements in this study are shown in Fig. 4, according to which shows that the pH of the preparations fell within the recommended pH range for topical preparations, namely 4.5–8.0 (Jafar et al. 2018). However, during 4 weeks of storage there was a decrease in pH. According to Jafar et al. (2018), this could be due to the preparations experiencing hydrolysis and the release of ions, which caused the pH to be acidic. The decrease in pH could also be caused by the influence of CO_2 on the preparations, where CO_2 from the air reacted with the water phase of the microemulsion, leading to the formation of an acid. In addition, the decrease in pH was also caused by the hydrolysis of tween-80 in the preparations which released the fatty acid sorbitone, and by environmental conditions such as light and air humidity. Based on the statistical test, all the base microemulsions formulations had a significant difference of $p>0.05$ in pH at 30 days of storage.

The results showed that the base microemulsion formulations were stable under acidic conditions. This was due to the use of nonionic surfactants in the microemulsion manufacture. Non-ionic surfactants are uncharged emulsifiers; thus, they are not affected by the concentration of H^+ , which makes microemulsions stable under acid conditions (Zheng et al. 2022). Non-ionic surfactants have better stability at low pH (acidic) than at high pH (Esmaeili et al. 2019).

Percentage of transmittance

The percentage of transmittance was measured using a UV-Vis spectrophotometer with distilled water as the blank. The transmittance percentage can be used to indicate the level of clarity of the microemulsion preparations.

Figure 3. The organoleptic profile of the base microemulsion. **Note:** (**a**) Oil:S-mix ratio of 1:9, (**b**) Oil:S-Mix ratio of 1:8, (**c**) Oil:S-mix ratio of 1:7, (**d**) Oil:S-mix ratio of 2:10, (**e**) Oil:S-mix ratio of 2:8.

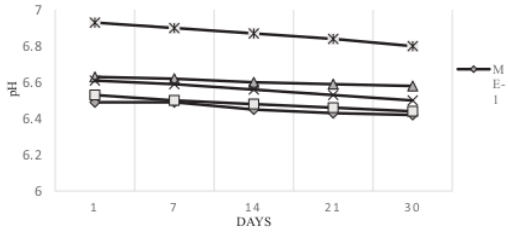

Figure 4. Graph of base microemulsion pH values.

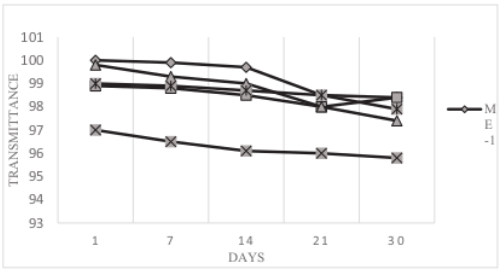

Figure 5. Graph of base microemulsion percentage of transmittance values.

If a microemulsion preparation has a % transmittance value close to 100%, it can be concluded that the microemulsion preparation is optically clear (Pathan et al. 2012).

In Fig. 5, we see high percentages of transmittance ranging from 98% to 99%. This result shows that the five base microemulsion formulas had transparent and clear characteristics because they had percent transmittance values close to 100%. The difference in percent transmittance of the five base microemulsion formulations was not statistically significant ($p>0.05$). This shows that the percent transmittance of the base microemulsion formulations was not affected by the ratio of the surfactant tween-80 to the co-surfactant PEG 400.

Viscosity measurement

Viscosity serves to see the flow properties of a preparation, which is an important parameter in examining the physical properties and stability of a preparation (Sharma et al. 2016). The results of the observation of the viscosity of the base microemulsion formulations can be seen in Fig. 6.

The results of measuring base was measured for 30 days using a Brookfield viscometer at a temperature of 27 °C. The results showed that all the base microemulsion formulas tended to increase viscosity in the first week and decreased viscosity from day 7 to day 30.

The resulting viscosity was not too large, this indicates that the base microemulsion formulations contained well-dispersed particles, which results in a good flow rate (300–1000 Cps). In addition, a statistical analysis was also carried out on the storage time and its relationship with the viscosity of the base microemulsion formulations. Statistical results showed that there was a significant difference between the formulas, with $p<0.05$. This means that that the storage time had an effect on the viscosity value. This increase, according to the statistical analysis data of the viscosity of each base microemulsion formulations, showed a significant difference ($p<0.05$). This indicates that the viscosity of the base microemulsion formulations was influenced by the ratio of surfactants to co-surfactants.

Measurements of globules and Zeta potential

The results of the globule measurements of all base microemulsion formulas (Table 3) fell within the optimum

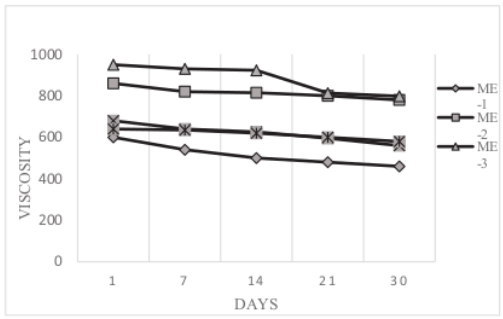

Figure 6. Graph of base microemulsion viscosity values.

Table 3. Optimized base microemulsion formulas.

Formulation	Particle size (nm)	Polydispersity	Zeta potential (mV)
ME-1	19.02±0.01	0.222±0.004	5.70±0.36
ME-2	34.24±0.94	0.645±0.093	-6.43±0.72
ME-3	59.85±0.80	1.0±0.00	-8.68±0.25
ME-4	138.07±2.17	0.243±0.005	-7.47±0.18
M3-5	128.30±0.71	0.168±0.009	-5.49±0.11

Note: ME= microemulsion.

range of 10–200 nm, which indicates that a microemulsion system was formed. This was proven by tween-80 as a surfactant being adsorbed on the droplet surface of the oily phase and forming micelles. The micelles formed could reduce the interfacial tension. Therefore, a good microemulsion is formed with a small particle size. In addition, PEG 400 as a co-surfactant helped to prevent phase separation from recombining. In addition to the particle size, surfactants and co-surfactants can also affect the polydispersity index (IP), which describes the particle size distribution of a microemulsion base. IP values of 0.01–0.7, belong to the monodisperse range, while the polydipsia index values are >0.7. The IP measurement results in this study showed that all base microemulsion formulas were monodisperse; thus, the microemulsions formed were more homogeneous and could prevent creaming or cracking (Urmaliya et al. 2016). The microemulsion base system showed a uniform particle size distribution and tended to be more stable (Kamaria et al. 2015).

A zeta potential measurements was carried out to measure the surface charge of the globules, which functioned to maintain optimum distance and prevent coalescence (Kale and Deore 2017). The optimum zeta potential value of the base microemulsion formulas ranged from +30mV to -30mV, with the most optimum value being close to 0 (Syafitri et al. 2020). In the five formulas, the zeta potential values obtained met the required characteristics of a microemulsion preparation. This indicates that the base microemulsion formulas made were thermodynamically stable. The resulting zeta potential values were negative because the non-ionic surfactants used could cover the microemulsion droplets, which caused the particle mobility to be reduced and caused no two particles ti combine to form aggregates (Silva et al. 2013).

Physical stability test

The stability test consisted of two tests: the centrifugation test and the heating and cooling test, which tested the physical stability of the microemulsion base. A centrifugation test was performed to determine whether or not there was a phase separation caused by gravitational force. The principle of centrifugation was to separate particles based on their molecular density, with centrifugal force causing particles with smaller thicknesses to be on top and particles with larger densities to go down (Braja et al. 2021). Based on the research data, all formulations were stable against centrifugation force because they had a transparent appearance and demonstrated no separation (see Fig. 7). Heating and cooling test results showed neither separation and nor color change after testing for 6 cycles at 4 °C and 45 °C (see Fig. 8).

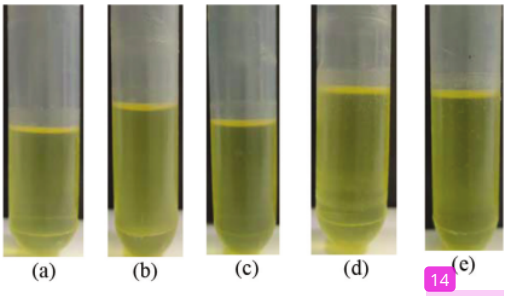

Figure 7. Results of the centrifugation test. **Note:** (**a**) Oil:S-mix ratio of 1:9, (**b**) Oil:S-Mix ratio of 1:8, (**c**) Oil:S-mix ratio of 1:7, (**d**) Oil:S-mix ratio of 2:10, (e) Oil:S-mix ratio of 2:8.

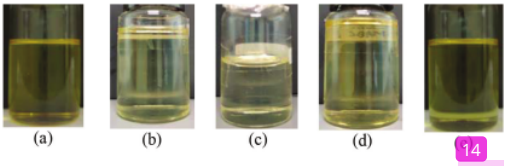

Figure 8. Results of the heating cooling test. **Note:** (**a**) Oil:S-mix ratio of 1:9, (**b**) Oil:S-Mix ratio of 1:8, (**c**) Oil:S-mix ratio of 1:7, (**d**) Oil:S-mix ratio of 2:10, (**e**) Oil:S-mix ratio of 2:8.

Clove essential oil formulation

Based on the optimization results and observations made on each base microemulsion formula, it was determined that the 1:9 formula was chosen. After obtaining the optimal microemulsion base, the clove flower essential oil microemulsion was made at a concentration of 10%. Clove oil microemulsion preparations and evaluations were carried out. These included observations of organoleptic properties, p[illegible]ent transmittance, pH, viscosity, determination of globule size, polydispersity index, and zeta potential. The results can be seen in Table 4. In addition, thermodynamic stability tests and accelerated stability tests were also carried out.

The resulting transmittance percentage met the requirements where the microemulsion system was clear with a transmittance percentage close to 100%. Likewise, the pH measurements results fell within the expected range of 4.5–8.0. Afterward, cha[illegible]terization of the clove oil microemulsion preparation was carried out using a PSA (Particle Size Analyzer) to determine the size of the globules. The globule size distribution is a very important factor to determine the stability of a microemulsion pre[illegible]ation. The stability of the microemulsion depends on the droplet size in the dispersed phase. The results of the observation of the globule size in Table 4 show that the clove oil microemulsion preparation meet the required microemulsion globule size. The results of this study agree with those of Gandhi et al. (2021) that the clove oil microemulsion using tween-80 and isopropyl alcohol (as a surfactant and a co-surfactant, respectively) has a globule size of 14.41 nm. Since the droplet size and the rate of incorporation were small, the microemulsion was not easy to be creaming. In addition, the small particle size allowed the microemulsion to be stored longer, is not easily damaged, and is easily absorbed by the body.

The particle size distribution is an important characteristic of microemulsion systems as it can affect drug release and the stability of a microemulsion. It is expressed in terms of polydispersity index. The polydispersity index is categorized into two, namely, monodispersity (unimodal) and polydispersity (bimodal). The formulation of the clove oil microemulsion in this study was monodisperse (Table 4). According to Rahmawaty et al. (2014), belonging to the monodispersity category are particles with size

Table 4. Evaluation of clove essential oil.

Evaluation	Results	
	Day 0	Day 30
Organoleptic properties	Color: yellow, clear Odor: specific Form: one phase	Color: yellow, clear Odor: specific Form: one phase
%Transmittance	99.9±0.1	98±0.0
pH	6.32±0.1	6.57±0.1
Viscosity	269±3.77	222.2±15.7
Globule size (nm)	17.69±0.025	
Polydispersity index	0.057±0.0043	
Zeta potential (mV)	-5.36±0.23	

distribution that tends to be narrow. This indicates that the clove oil microemulsion had a good level of uniformity, causing it to have inclination to be more stable than microemulsions in the polydispersity category.

The zeta potential predicted the stability of the clove oil microemulsion preparation. The interaction between particles has an important role in the stability of a colloid, while the zeta potential is a measure of the repulsive strength between the particles (Shah et al. 2014). The zeta potential result obtained was negative because it was influenced by the high pH value of the preparation, namely, 6.57±0.1. Additionally, this study used non-ionic surfactant, which tended to reduce the zeta potential value (Handayani et al. 2018). The zeta potential is not the main parameter determining the stability of a microemulsion. It is also influenced by other characterization results such as particle size and distribution (Shah et al. 2014).

A thermodynamic stability test was carried out to see the physical stability of the microemulsion, which included centrifugation and a heating and cooling cycle. Centrifugation is a mixture separation method used to separate insoluble liquids and solids based on differences in the particle size of the mixed substances. In the centrifugation test, the phase separation that occurred in the clove oil microemulsion preparation was observed. The results showed that the clove oil microemulsion did not undergo phase separation ; thus, the clove oil microemulsion was declared stable.

The heating and cooling test refers to an accelerated condition of temperature fluctuations to determine the stability of the preparation during storage. Heating and cooling were performed to see if crystallization, phase separation, loss of viscosity, aggregation and precipitation occurred and if the changes that occurred were reversible or not. This test was carried out by testing the stability of the microemulsion alternately at low and high temperatures, each for 24 hours. The test was carried out for 6 cycles. The results showed that the preparation could pass 6 cycles well. The preparation remained clear, homogeneous, and with no separation. This shows that the changes in properties that occurred when the preparation was stored at a high temperature of 40 °C or a low temperature of 4 °C were reversible.

Conclusions

Pseudo-ternary phase diagrams of mixtures of oil phase, surfactant, co-surfactant, and water were created. The microemulsion prepared with 7 w/t% of oil phase, 60 w/t% of surfactant, and co-surfactant mixture (S-mix ratio of 2:1), 33 w/t% of water, and 10 w/t% of clove oil was found to have the most favorable characteristic in terms organoleptic properties, % transmittance, pH, viscosity, physical stability, globule size, polydispersity index, and zeta potential. The use of this novel approach to delivering pharmacologically active natural oils in this study can indeed prepare a base research for the upcoming survey so we can see a simultaneous commercial formulation shortly.

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