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Contributed Paper

Preparation and Characterization of Ylang-Ylang (*Cananga odorata*) Essential Oil and Ascorbic Acid Loaded Olive Oil-in-Water Emulsion

Vicit Rizal Eh Suk* [a], Khalisanni Khalid [b] and Misni Misran [a]
[a] Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[b] Biotechnology and Nanotechnology Research Center, Malaysian Agricultural Research and Development Institute (MARDI), MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
*Author for correspondence; e-mail: konsrangfun@yahoo.com

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ABSTRACT

Essential oils are widely used for cosmetic and medicinal purpose. However, their viscosity, volatility, and oily physical appearance became obstacles in their usage, especially for dermal application. In this study, ylang-ylang (*Cananga odorata*) essential oil was produced by using steam distillation technique. Amount of sucrose stearate in the formulation was manipulated with the aim to form the emulsion that can optimize the delivery of ylang-ylang essential oil and ascorbic acid. Optical polarizing micrograph of olive oil-in-water emulsion showed a polydispersed size up to more than 10 μm. Average hydrodynamic particle size analysis showed the increase in average particle size within 28 days of incubation at room temperature, 8, and 45 °C. Zeta potential measurement for 28 days revealed the shift in the magnitude of to less negative in all incubation condition. Accelerated stability test showed the prediction of sedimentation, flocculation, and coalescence mechanism on the shelf at room temperature. The data showed a promising alternative for delivering essential oils with the use of ascorbic acid or other agents to the body through dermal delivery by employing the emulsion system.

Keywords: oil-in-water, emulsion, essential oil, ylang-ylang, accelerated stability test

1. INTRODUCTION

Odoriferous essential oils have been used since ancient Egypt and Greek for cosmetic and medicinal applications [1, 2]. They contain aldehyde, terpenes, alcohols, phenols, and ketones [3]. It was reported that out of 60 botanical families, 2000 species contained essential oils [5], and can be extracted using various techniques such as steam distillation, solvent extraction, effleurage, or maceration. They can be extracted from resins, roots, stalks, leaves, beans, seeds, fruits, and flowers.

Flowers from *Cananga odorata* (*C. odorata*) or ylang-ylang which is native to the islands of Indian Ocean and tropical Asia such as Philippines, Indonesia, and Malaysia [6], is one of the most popular essential oils [6]

that being widely used due to their sweetly fragrant flowers. The oil comprised of *p*-methyl anisole, linalool, methyl benzoate, benzyl acetate, geranyl acetate, (E)-caryophyllene, germacrene-D, benzyl benzoate, and benzyl salicylate [7]. It is also used as food additive, flavoring [7], antibacterial, and antifungal agents[5]. The research by Phasomkusolsil and Soonwera [8] showed that formulation with *C. odorata* can protect up to 98% of mosquitoes bite in human volunteers. Various reports had successfully proved the anxiolytic effect of ylang-ylang oil when tested to both animal and human models [6, 9]. In aromatherapy, ylang-ylang oil is known to have an ability to improve the blood circulation [2] and significantly reduce the blood pressure in both healthy and hypertension patients [10, 11]. The most effective way to use essential oil is through inhalation of transdermal [2].

In this study, ylang-ylang oil was combined with ascorbic acid to be used in topical delivery in the aim to achieve a novel synergistic effect. This is due to partitioning of ylang-ylang oil at the oil phase, while the water phase will be occupied by ascorbic acid. Ascorbic acid is known for their effectiveness in overcome poor wound healing (associated with collagen formation), thickening of the stratum corneum, and subcutaneous bleeding (due to fragility and loss of connective tissue morphology) [12]. In 2015, Crisan and coworkers reported that ascorbic acid was significantly improved skin structure and reconstitutes the loss of interstitial collagen [13], which is a sign of a good anti-aging agent.

Hence, one of the best alternatives to deliver ylang-ylang oil and ascorbic acid is by delivering these two active ingredients in an emulsion system [14], which can enhance penetration of active ingredients into the skin [15]. An emulsion is a unique form of the colloidal system consisting at least two immiscible phase dispersed as spherical droplets in either phase [16]. Oil-in-water emulsion is a very common carrier used in the food, pharmaceutical, and cosmetic industries. In this study, olive oil was used to prepare the emulsion. However, the high interfacial tension between olive oil and water produced a thermodynamically unstable emulsion. Incorporation of surfactant, sucrose stearate, will adsorb at the olive oil-water interface which later reduces the interfacial tension of olive oil-water and prevent the droplets from coalescence, hence forming a stable emulsion.

2. MATERIALS AND METHODS

2.1 Materials

Laleli extra virgin cold pressed olive oil (99% purity) was purchased from Thye Huat Chan Sdn. Bhd., Malaysia while glycolipid surfactant sucrose stearate (98% purity) was purchased from Mitsubishi-Kagaku Food Corporation, Japan. Ascorbic acid (Merck-99% purity) was provided by Malaysian Agricultural Research and Development Institute (MARDI).

2.2 Preparation of Ylang-Ylang Essential Oil

Ten kg of ylang-ylang flowers (*C. odorata*) were harvested and extracted by using steam distillation process in MARDI Kuala Linggi, Melaka, Malaysia. The steam distillation technique was used due to its robustness and efficiency compared to hydro distillation process. The extracted ylang-ylang products containing essential oil, emulsion, and water (H_2O) were separated by the use of separating funnel. Two tablespoon of sodium sulphate (Na_2SO_4) were soaked into the ylang-ylang essential oil within overnight for the removal of water residues. 159 g of ylang-ylang

essential oil was obtained after the removal of water residues.

2.3 Preparation of Olive Oil Emulsion

The composition and preparation techniques of emulsions are very important in engineering the physical properties of emulsion [14]. The aqueous phase containing sucrose stearate and ascorbic acid was heated to 60 °C using a water bath until the formation of clear gel solution. A homogenous oil phase solution which comprised of olive oil, ylang-ylang essential oil, and sucrose stearate, was then added dropwise into the aqueous phase followed by homogenization using Silent Crusher (Heidolph, Germany) at 13000 rpm for five minutes to produce oil-in-water emulsion [16]. The samples were then stored in 8±1°C LCF402-30 freezer (Linden, USA), room temperature, and 45±1°C incubator (Memmert, Germany). Table 1 shows the composition of sucrose stearate that being tested and formulation 5 was further used for the whole experiment.

Table 1. Composition of sucrose stearate in aqueous and oil phase.

Formulation	Composition of Sucrose Stearate (% w/w)			Observation after 1 hour
	Aqueous Phase	Oil Phase	Total	
1	0.0	0.0	0.0	Immediate phase separation
2	0.5	0.5	1.0	Phase separation was observed
3	1.0	1.0	2.0	Phase separation was observed
4	1.5	1.5	3.0	Phase separation was observed
5	3.0	3.0	6.0	No separation was observed
6	5.0	5.0	10.0	No separation was observed

2.4 Optical Polarizing Microscope (OPM)

The size, distribution, and morphology of the emulsion were observed using Leica Optical Polarizing Microscope (Leica, Germany) in Colloid Laboratory, Department of Chemistry, University of Malaya. Fifty μL of the emulsion solution was directly transferred onto the glass slide followed with a coverslip. An immersion oil (Merck, Germany) was carefully dropped onto the coverslip. The micrograph was taken via 20×objective lens at 200 times magnification at room temperature.

2.5 Particle Size

The average particle sizes of the emulsion were evaluated by employing a backscattered dynamic light scattering (DLS) method using Zetasizer NanoZS (Malvern Instruments Ltd., United Kingdom) equipped with a 4 mW He-Ne laser at 633 nm. Fifty μL of emulsion was transferred into 5 mL of deionized water of 18.2 MΩ cm resistivity collected from Barnstead NANO pure®Diamond™ ultrapure water system. The emulsion was sonicated using bath ultrasonicator (JEIO, Japan) for 5 minutes to make sure that the emulsion was homogeneously dispersed in the deionized water. An appropriate amount of dispersion was then transferred into four-sided clear fluorescent quartz cuvette and analyzed using the pre-set Standard Operation Procedure (SOP) for particle size analysis. All measurements were carried out in triplicates at 30±1 °C within a period of 28 days for the stability test.

2.6 Zeta Potential Analysis

The zeta potential values of the emulsion were measured using Zetasizer NanoZS (Malvern Instruments Ltd., United Kingdom) by determining the electrophoretic mobility followed with Henry equation. Fifty µL of emulsion was dispersed in 5 mL of deionized water as mentioned previously. The dispersion was then transferred into a U-shape polycarbonate cell with gold plated electrodes and analyzed using the pre-set Standard Operation Procedure (SOP) for zeta potential analysis. All measurements were carried out in triplicates at 30±1 °C within a period of 28 days for the stability test.

2.7 Accelerated Stability Test

One mL of olive oil emulsion was slowly transferred into 7 mL glass vial. A pre-set incubator (Memmert, Germany) was used to incubate the emulsion at 45±1 °C for the period of 1 month. The separation of oil, water, and emulsion were observed and measured using measurement ruler at day 1, 3, 7, 14, 21, and 28.

3. RESULTS AND DISCUSSION

Morphological, particle size, and stability tests have been carried out to determine the structural changes and the ability of the oil-in-water emulsion to withstand the effect of heat. The morphological analysis study revealed the structure of vesicles or droplet of the emulsion. Particle size analysis determine the morphological changes in term of average droplet size for the olive oil emulsion at different temperatures in order to study the effects of time and heat for the oil-in-water olive oil emulsion.

3.1 Optical Polarizing Microscope (OPM)

Information on size, shape, and dynamic properties of the emulsion was gained through polarizing microscopy technique that can achieve submicron scale. Figure 1 exhibits the oil-in-water emulsion with the dense network of small spherical droplets [18]. The droplets are polydispersed, ranging from 1 to 30 µm. This is due to the coalescence of small emulsion droplet through flocculation, collision, or fusion of many small droplets [19]. The emulsion droplets of more than 30 µm (large emulsion droplets) were sporadically present. The large emulsion droplets contained small emulsion droplets of less than 1 µm, can be known as multivesicular vesicles [20]. The presence of ylang-ylang oil and ascorbic acid entrapped in vesicle hydrophobically formed in the olive oil emulsion. The dynamic of the formation of vesicles such as giant unilamellar vesicles can be seen through the polydispersity of the droplets size. The small emulsion droplets of less than 1 µm encapsulated in vesicles of

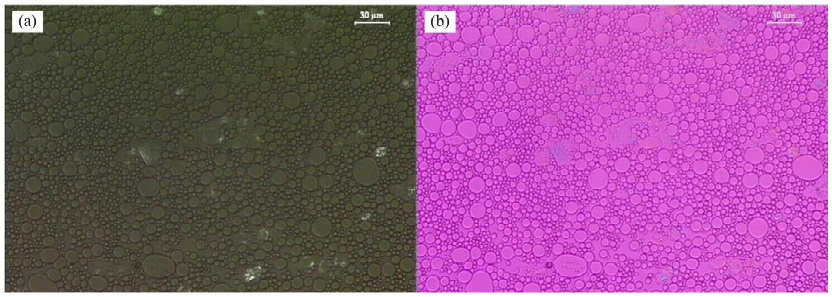

Figure 1. Micrograph of olive oil-in-water emulsion viewed using Optical Polarizing Microscope (OPM) under dark phase (a) and light phase (b) at room temperature. The scale is 30 µm.

oil-in-water emulsion can be seen under the light phase of OPM. However, it was difficult to be seen under the dark phase of OPM as oil droplets do not possess optically active properties in the dark phase [21].

3.2 Particle Size Analysis

Average hydrodynamic size of olive oil-in-water emulsion encapsulating ylang-ylang essential oil and ascorbic acid was agreeable with the size observed via OPM. The average size was increased within the incubation period as displays in Figure 2. Over the incubation period, the average particle size shows the significant increase from day 1 to day 28. The surfactant in the formulation reduced the interfacial tension of oil-water interface, resulting in the emulsion droplet to aggregate causing the sudden collapse of the film [22]. In addition, the Brownian motion cause emulsion droplets to collide, coalescence, and form a larger emulsion droplet [23]. Emulsion incubated at 45 °C showed the reduction in the droplets size from day 1 to day 15 due to the reduction of the water in the emulsion by evaporation [24]. After day 15, the average particle size was increased dramatically due to the fusion of the droplets. Based on the literature, this is compatible for dermal delivery as the pore size of the skin is within 0.05 to 0.37 mm^2 [25].

3.3 Zeta Potential Analysis

Zeta potential is measured using Laser Doppler Electrophoresis technique by applying the Henry equation, $U_E = \frac{2\varepsilon\zeta f(ka)}{3\eta}$, where U_E is electrophoretic mobility, ε is dielectric constant, ζ is zeta potential, *f(ka)* is Henry's function, while η is viscosity [25]. Zeta Potential values were calculated by Zetasizer NanoZS. Figure 3 exhibits the zeta potential of the olive oil-in-water emulsion encapsulating ylang-ylang essential oil and ascorbic acid for the period of 28 days. Zeta potential values of the emulsion incubated at room temperature, 8, and 45 °C became less negative with incubation up to day 15. This phenomenon were observed may be due to the adsorption of the surface-active compounds at the olive oil-water interface [1] and fusion of emulsion droplets that separated out the oil layer from the emulsion

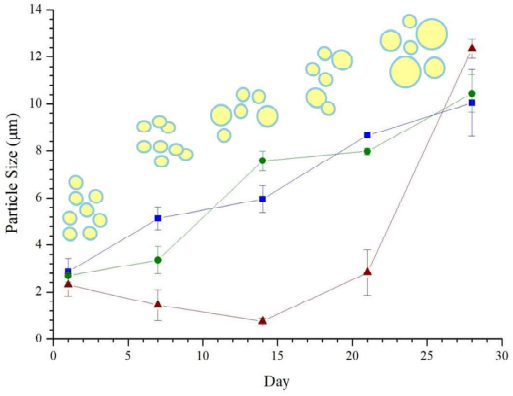

Figure 2. Average particle size of olive oil-in-water emulsion encapsulating ylang-ylang essential oil and ascorbic acid as incubated at room temperature (●) , 8 (■), and 45 (▲) °C.

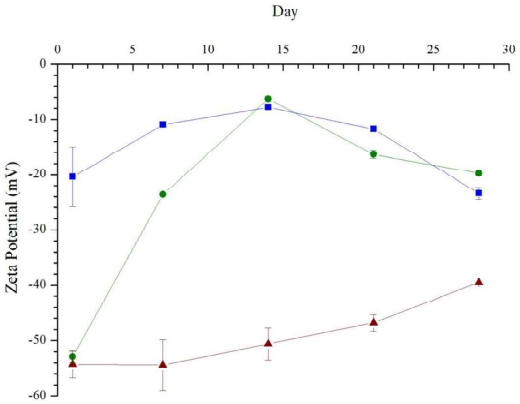

Figure 3. Zeta potential of olive oil-in-water emulsion encapsulating ylang-ylang essential oil and ascorbic acid as incubated at room temperature (●) , 8 (■), and 45 (▲) °C.

solution. However, at room temperature and 8 °C, the magnitude of zeta potential became more negative after day 15. This is attributed to the spontaneous emulsification as the effect of surface activity to achieve the thermodynamic equilibrium [22, 24].

3.4 Accelerated Stability Test

The stability of emulsions was first evaluated by visual inspection of the prepared samples. Aqueous separation ratio, creaming, clarification, and sedimentation of emulsions indicated the stability of emulsions [27]. The accelerated stability test is the normal routine in emulsion industry aiming to determine the shelf-life of cosmetic and pharmaceutical products by using empirical rules. A sample stored at 45 °C for 4 weeks is equivalent to 2 months on the shelf at room temperature [28]. Olive oil-in-water emulsion encapsulating ylang-ylang essential oil and ascorbic acid shows a stable emulsion system up to day 7, suggesting that it will stable for a half month on the shelf at room temperature, as shown in Figure 4. This agrees with the zeta potential data where the readings were approaching the measurement on the day 1 even after incubating for 28 days. On the day 7, less than 20% of water layer was formed at the bottom of the vials and remain stables to more than 14 days and a slight oil layer was observed on the day 21. Since then, the formation of oil layer was increasing up to 8% on the day 35. Through

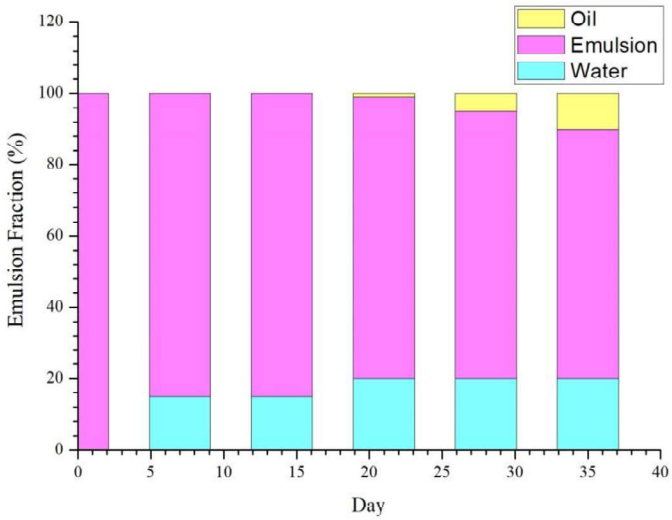

Figure 4. Accelerated stability of olive oil-in-water emulsion encapsulating ylang-ylang essential oil and ascorbic acid.

this incubation period, all mechanisms which are sedimentation of the water layer, flocculation of the emulsion, and coalescence [23] of oil droplets that formed an oil layer was observed [20, 22]. Incorporation of co-surfactant will improve the stability of emulsion.

4. CONCLUSIONS

A stable emulsion was successfully prepared by using olive oil and water with the presence of sucrose stearate as a surfactant. Ylang-ylang essential oil was prepared using steam distillation technique that yields 1.59 % of 10 kg ylang-ylang flowers and ascorbic acid were incorporated in the emulsion. The small average particle size of olive oil-in-water

emulsion encapsulating ylang-ylang essential oil and ascorbic acid was measured to have 2.8 μm and increased to 13 μm after day 28 due to sedimentation, coalescence, and flocculation. Zeta potential analysis displayed the change in the magnitude of zeta potential over the incubation period. The acceleration stability test postulated a stable emulsion system up to three months on the shelf at room temperature. This showed that the olive oil-in-water emulsion has a wide potential to be manipulated as a carrier for various essential oils together with ascorbic acid or other agents to the body through dermal delivery. Further physical characterization such as changes in the density and viscosity of emulsion droplets over time, demulsification [29], as well as *in vitro* and *in vivo* tests such as toxicology test, skin irritancy test, and skin efficacy test could further done to justify the potential application in dermal delivery. This formulation can be potentially used for many poorly soluble active ingredients to enhance their solubility, *ex vivo* skin permeation, and therapeutic efficacy [30].

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