Antioxidant in cosmeceutical products containing *Calophyllum inophyllum* oil☆

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**Abstract** – Every part of *Calophyllum inophyllum* L. has been used in various traditional remedies, especially the oil from its nut was mostly used to treat skin diseases. This study aimed to investigate the composition and antioxidant activity of *C. inophyllum* nut oil and formulate the oil as a cosmeceutical product. The chemical composition and the amount of total phenolic compounds (TPC) were demonstrated by Gas Chromatograph-Mass Spectrometer (GC-MS) and Folin–Ciocalteu method, respectively. Additionally, the antioxidant activity was tested using the DPPH method. Calophyllolide (4.35%) was a major component. Additional components were calanolide A, inophyllum D, and inophyllum B. We found that the TPC contained 25.9 ± 1.2 mg GE/g oil and a free radical scavenging activity approximate to that of the synthetic Trolox. Emulgel formulation consisted of tween 80, span 80, and isopropyl alcohol as a surfactant, and carbopol 940 as a gelling agent. The microemulsion was formulated using distilled water, oil, tween 80 with span 80, as a surfactant, and isopropyl alcohol as a cosurfactant. The mean droplet size for optimized microemulsion formulations was 34.37 ± 1.06 nm. Furthermore, the results of thermodynamic stability tests (freeze-thaw cycle) and long-term stability tests indicated that emulsions and microemulsions remained stable. In conclusion, this nut oil could potentially be used as a cosmeceutical product, and the obtained emulgels and microemulsions exhibited good characteristics in terms of being a potential agent for skin antioxidant.

**Keywords:** *Calophyllum inophyllum* L. / calophyllolide / antioxidant activity / emulgels / microemulsions

**Résumé** – Antioxydant dans les produits cosmétiques contenant de l’huile de *Calophyllum inophyllum*. Toutes les parties de *Calophyllum inophyllum* L. ont été utilisées dans diverses médecines traditionnelles, en particulier l’huile de sa noix qui est particulièrement utilisée pour le traitement des maladies de la peau. Cette étude vise à étudier la composition et l’activité antioxydante de l’huile de noix de *C. inophyllum* et à formuler une huile destinée au domaine de la cosmétique. La composition chimique et la quantité de composés phénoliques totaux (TPC) ont été respectivement évaluées par chromatographie en phase gazeuse couplée à un spectromètre de masse (GC-MS) et par la méthode Folin–Ciocalteu. De plus, l’activité antioxydante a été évaluée par la méthode au DPPH. Le calophyllolide (4,35 %) est un composant majeur. Les autres composants mis en évidence sont : le calanolide A, l’inophyllum D et l’inophyllum B. Nous avons constaté que la teneur en TPC de l’huile est de 25,9 ± 1,2 mg GE/g d’huile et que l’activité de piégeage des radicaux libres est proche de celle du Trolox synthétique. La formulation de l’émulgel est composée de tween 80, de span 80 et d’alcool isopropylique comme agent tensioactif, et de carbopol 940 comme agent gélatin. La microémulsion a été formulée en utilisant de l’eau distillée, de l’huile, du tween 80 avec du span 80 comme tensioactif, et de l’alcool isopropylique comme cosurfactant. La taille moyenne des gouttelettes de la microémulsion optimisée est de 34,37 ± 1,06 nm. En outre, les résultats des tests de stabilité thermodynamique (cycle de gel-dégel) et des tests de stabilité à long terme ont indiqué que

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les émulsions et les microémulsions restaient stables dans le temps. En conclusion, cette huile de noix pourrait potentiellement être utilisée comme un produit cosmétique et les formulations de type émulgels et microémulsions obtenues présentent de bonnes caractéristiques pour une éventuelle utilisation comme agent antioxydant pour la peau.

**Mots clés :** *Calophyllum inophyllum* L. / calophyllolide / activité antioxydante / émulgels / microémulsions

1 Introduction

*Calophyllum inophyllum* L., also known as Alexandrian laurel, Indian laurel, Kamani, Honne, Tamanu, etc., is widespread along the coasts and around areas adjacent to lowland forest. The plant is native to East Africa, southern India to Malaysia, northern Australia, and the Pacific Ocean. It is of medium to large sizes with an average height of 8 to 20 m and a broad spreading crown of irregular branches. The fruit is a round, green drupe of approximately 2–4 cm in diameter, and has a single large seed (Fig. 1). The seeds have a very high content of oil, up to 75%, which is non-edible in nature. This plant has been used for a long time in various ways, including wood, forages, perfume, dye, soaps, biofuels, and medicals. For traditional folk remedies in Asia and the Pacific Islands, the plant is used as a diuretic, an antibiotic, an astringent, and an analgesic (Lim, 2012). The seed oil was extracted and used as a topical application for the treatment of skin injuries and diseases (Léguillier et al., 2015). Calophyllolide is a major constituent isolated from *C. inophyllum* seed. In previous studies, anti-inflammatory, wound healing, anti-coagulant, anti-microbial, and anti-cancer activities of calophyllolide were reported (Arora et al., 1962; Yimdjo et al., 2004; Nguyen et al., 2017).

Antioxidants are a group of substances able to prevent degenerative disorders caused by extreme cellular oxidative stress, such as cardiovascular diseases, hypertension, diabetes, and cancers (Pellegrino, 2016). A major group of phytochemicals that elicit antioxidant activity is phenolic compounds in which the mechanisms are related to the actions of free radical scavengers, hydrogen donators, metal chelators, and singlet oxygen quenchers. Antioxidant activity of *C. inophyllum* in prior studies was found in ethanolic and petroleum extract leaves. (Prasad et al., 2012; Raju and Victoria, 2015).

Currently, several cosmeceutical products have been studied and manufactured for skincare purposes usage. The substances from nature were usually employed as the main ingredients (Dorni et al., 2017). Mostly, topical products that are effective for skincare treatment are creams, lotions, emulsions, foams, gels, ointments, etc (Ueda et al., 2009). The gel is a dosage form that has a higher aqueous component. Gels provide greater dissolution and easy migration of the drugs, compared with the ointment or cream base (Allen and Ansel, 2013). Emulsions are the most widely used dosage forms in the pharmaceutical and cosmetic industries. The combination form of gels and emulsions is referred to as emulgels which provide the advantages of gels and emulsions. It contains less oily components, is less greasy, and has good spreadability which are desirable properties for a topical drug delivery system. Microemulsions, another skin formulation, are clear; thermodynamically stable; isotropic liquid mixtures of oil, water, and surfactant; and frequently combined with a cosurfactant (Kogan and Garti, 2006). The droplet size of the dispersed phase is less than 100 nm. The major advantages of microemulsions are the ease in preparation and scale-up process, improved lipophilic drug solubilization, longer shelf life, modified drug release characteristics, and the reduction of intersubject and intrasubject variation in absorption. Thus, emulgels and microemulsions are dosage forms that overcome limitations of the conventional dosage form such as creams and ointments (Aggarwal et al., 2013; Fouda et al., 2013).

The present study aimed to analyze the chemical composition and the antioxidation potential of the oil extracted from *C. inophyllum* seeds. Moreover, emulgels and microemulsions which were the cosmeceutical formulation with antioxidant activity were developed and assessed.

2 Material and methods

2.1 Material

Folin–Ciocalteu reagent, gallic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma–Aldrich (Darmstadt, Germany). (Saint-Louis, MO, USA). Tween 80 were purchased from P.C. Drug Center Co., Ltd., (Bangkok, Thailand). Span 80 were obtained from CT chemical (Bangkok, Thailand). Other chemicals were of analytical grade and purchased from Merck (NJ, USA).

2.2 Preparation of *C. inophyllum* seed oil

The recently fallen fruits of *C. inophyllum* were collected from Huasai coastal regions, Nakhonsithammarat, Thailand. The fruits were dried under the sunlight until the shell color changed to brown. The seeds inside were then taken out by a small hammer and completely dried at 60°C in a hot-air oven (UF110, Memmert, Germany). *C. inophyllum* seed oil was extracted by cold pressing at Friend Energy Ltd., Part., Chiang Mai, Thailand.

2.3 Degumming of the oil

Any gums were removed to purify the oil using the procedures previously described (Ong et al., 2014). Briefly, the oil was blended with 0.5% v/v of 20% H3PO4, and it was then heated at 60°C for 15 min. After that, the mixture was centrifuged at 1000 rpm for 30 min to separate the gums. The supernatant oil was washed with 15 ml of 60°C of distilled water three times using a separatory funnel. To purify the oil, the saturated gum was removed by passing through cotton, and water droplets were adsorbed by magnesium sulfate.
were mixed with 100 μl of 2% AlCl3 in methanol. Quercetin was used as a calibrator. The absorbance was measured at 415 nm after 10 min incubation at room temperature. Results were expressed as μg quercetin equivalent (QE)/g oil.

2.6 DPPH antioxidant activity

DPPH assay was performed according to the method described by Thaipong et al. (2006) with some modifications. In brief, the stock solution of DPPH (0.11 mM) was prepared by dissolving in ethanol and stored at −20 °C until use. The 40 μl of C. inophyllum seed oil was incubated with 160 μl of DPPH working solution at room temperature for 30 min in the dark. The absorbance of each sample was measured against an equal amount of DPPH and ethanol as a negative control on the UV-Visible spectrophotometer at 515 nm. Trolox, a modified vitamin E derivative by changing the alkane group to the carboxylic group, is water-soluble and was used in a range of 0.0125–0.2 mM as a standard. Results were expressed as percent inhibition.

\[
\%\text{DPPH scavenging} = \left( \frac{A_{\text{sample}} - A_{\text{ctrl}}}{A_{\text{ctrl}}} \right) \times 100, \\
\text{where } A_{\text{sample}} \text{ is the absorbance of Trolox or } C. \text{ inophyllum seed oil and } A_{\text{ctrl}} \text{ is the absorbance of the negative control. The antioxidant activity was expressed as } IC_{50} \text{ (50 percent of inhibition concentrations). The } IC_{50} \text{ values were obtained by linear regression of the plot between the percentage (%) inhibition } \text{versus } \text{various concentrations of sample plot. All measurements were performed in triplicate and the mean values were calculated.}
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2.7 Pharmaceutical dosage form containing C. inophyllum seed oil extract

Emulgels is an emulsion blended with gelling agents. Emulgels can be easily washed and it also shows good penetration through the skin. Microemulsion is isotropic transparent liquid mixtures of oil, water, surfactant, and cosurfactant. Moreover, it enhanced the absorption of drugs across biological membranes as well as bioavailability.

2.7.1 Preparation of emulgels

Different formulations of emulgels were prepared using varying an amount of gelling agent and oil (Boonme et al., 2016). Carbopol 940 was used as a gelling agent and was dispersed in distilled water. Then, the pH was adjusted to 5.6–5.7 using triethanolamine (TEA), resulting in a hydrogel base. The water phase comprised of water, and tween 80 while the oil phase included C. inophyllum extracted oil, span 80, and isopropyl alcohol. The water phase and oil phase were mixed and then added and incubated at room temperature for 90 min. Samples were analyzed spectrophotometrically at 725 nm (SPECTROstar Nano, UV-Vis Spectrophotometer, BMG Labtech, USA). Results were expressed as mg gallic acid equivalent (GE)/g oil.

The total phenolic content (TPC) of the extracts was determined using the Folin–Ciocalteau assay. TPC of the extracted oil was carried out according to the method in a 96-well plate, reported by Azlim Almey et al. (2010). The standard solutions of gallic acid in ethanol were prepared and diluted with water in a concentration range between 0.01 and 0.05 mg/ml. Each sample (10 μl) was mixed with 75 μl of 2 N Folin–Ciocalteau reagent. A 75 μl of 6%w/v Na2CO3 solution was then added and incubated at room temperature for 90 min. Samples were analyzed spectrophotometrically at 725 nm (SPECTROstar Nano, UV-Vis Spectrophotometer, BMG Labtech, USA). Results were expressed as mg gallic acid equivalent (GE)/g oil.

The total flavonoid content (TFC) of the extracts was carried out according to a procedure by Kandouli et al. (2017). The different concentrations of the sample (0.1–1 mg/ml) were prepared in DMSO. One-hundred microliters of each sample
2.7.2 Preparation of microemulsions

To improve the permeability and appearance of the topical formulation, the microemulsion formulations were prepared. The mixture of span 80, IPA, tween 80, and oil was continuously stirred with a magnetic stirrer at 600 rpm for 30 min (LMS-1003, Labtech, Korea) to produce clear microemulsion which was then further equilibrated at room temperature for 24 h. The turbid formulations followed by phase separation were discarded because they were biphasic formulations. The obtained formula which was clear and transparent was marked as microemulsions. The ingredients of microemulsion formulations (M1-M4) are given in Table 3.

2.8 Characteristics of the emulgel and microemulsion

2.8.1 Morphological observation and droplet size

The morphology of the optimized microemulsion was evaluated by using transmission electron microscopy (TEM) (JEOL, JEM-2010, Tokyo, Japan). The microemulsion was directly determined without any dilution. The microemulsion was dropped in a 200-mesh carbon film copper grid. After the microemulsion was dried, it was monitored under 160 kV conditions.

2.8.2 Droplet size, pH value, viscosity, and spreadability measurements

The average droplet size, polydispersity index (PDI), and zeta potential of the microemulsion were determined using a Zetasizer Nano ZS90 (Malvern Instruments, UK), which is capable of measuring sizes in the range of 0.6 to 6000 nm. The pH of emulgel and microemulsion formulation was measured using a pH meter (Mettler Toledo, Switzerland), the viscosity was performed at 25 ± 2 °C through a Brookfield viscometer (Model LVDV III Ultra Brookfield, USA), and the spreadability was evaluated according to the method described by Ahsan et al. (2020). To determine the spreadability, 1 g of each formulation was placed on a glass slide. The second slide was then placed 5 cm over the first slide and the diameter of the circle was measured. The measurement of pH and viscosity was determined before and after the stability test. The spreadability was measured using freshly prepare emulgel (E1-E3). All measurements were performed in triplicate and presented as the average ± standard deviation (SD).

2.8.3 Stability testing

The prepared emulgel and microemulsions were evaluated for physical properties and phase separation to ensure physical stability by centrifugation and thermodynamic stability studies.
2.8.3.1 Centrifugation

Both formulations were centrifuged at 3000 rpm for 10 min (Centrifuge 5922, Kubota, Japan), and inspected visually for their homogeneity.

2.8.3.2 Thermodynamic stability studies

The thermodynamic stability of the prepared emulgel and microemulsions containing *C. inophyllum* seed oil was performed as a short-term and long-term stability test. The freeze-thaw method was used for the short-term stability test (Manee and Kaewsrichan, 2017). The system was completed in six cycles, each cycle was composed of the storage at the temperature of 45 °C for 24 h in the incubator (IN75, Memmert, Germany), and of 4 °C for 24 h in the refrigerator (RT54FB1, Samsung, South Korea). After that, the physical characteristics, viscosity, and pH were examined. For long-term stability, the samples were stored in ambient conditions for two months. Physical appearances, viscosity, and pH were determined periodically after 0, 1, and 2 months.

3 Results and discussions

3.1 Phytochemicals

The *C. inophyllum* seed oil from cold pressing was a green-yellowish liquid (Fig. 2). The process allowed a yield of 45.02 ± 1.44% (w/w), and a density of 0.966 ± 0.02 g/ml of the oil to be produced. The oil was investigated for phytochemicals by using the GC-MS technique. The major components were determined in the positive mode, namely calophyllolide (4.35%; RT 63.1690 min), calanolide A (1.29%; RT 56.2433 min), inophyllum D (0.36%; RT 65.4422 min), and inophyllum B (0.02%; 64.0516 min). The retention time of the nut oils was identified by comparison with the reference mass spectrum of the NIST-2014 database (Tab. 1). The GC chromatogram is shown in Figure 3.

As mentioned before, some constituents were potent against oxidants, bacteria, fungi, viruses, and cancers (Creagh et al., 2001; Itoigawa et al., 2001; Yimdjo et al., 2004; Kostova and Mojzis, 2007; Saravanan et al., 2011; Ginigini et al., 2019). Especially, the activity of Calophyllolide which exhibits antimicrobial activities involved in skin pathogens, and anti-inflammation through reduction of myeloperoxidase (MPO) activity and pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α). Moreover, It has also been reported to possess a wound healing activity in keratinocytes (Nguyen et al., 2017).

3.2 TPC, TFC, and antioxidant activity

TPC of the extracted oil was analyzed and compared with gallic acid. The mechanism of gallic acid, a natural phenolic compound, is similar to vitamin C which is normally used as a reference compound. The TPC was calculated to contain 25.9 ± 1.2 mg GE/g oil. Moreover, quercetin was used as a positive control for TFC which is the one of phenolic compounds. The TFC was found to be 23.4 ± 0.2 μg QE/g oil. The extracted seed oil showed the activity against oxidant by the DPPH method with the IC50 value of 0.057 ± 0.004 μg/ml. Its antioxidant activity might be from phenolic compounds. The phenolics are the second metabolites from plants, they can inhibit free radicals by hydrogen atom transferring from their hydroxyl groups of phenol (Chen et al., 2020). Typically, phenolics were divided into two groups, flavonoids and non-flavonoids. All phenolics in the previous part were coumarins which were non-flavonoids. Therefore, the potent of antioxidant was almost from phenolics.

3.3 Development of cosmeceutical products

In cosmetics, oils are generally not applied to the skin directly because of the oiliness and difficulties in skin penetration. Thus, the emulgel and microemulsions are an alternative dosage form to overcome these limitations (Hardenia et al., 2014; Sharma et al., 2016). In terms of skin penetration, both dosage forms are preferred to conventional dosage forms, and the small droplet size of microemulsions could increase skin penetration also.

3.3.1 Emulgel formulations

Among semisolid dosage forms, such as gel, cream, and ointment, gel normally shows better drug release than cream and ointment. Hydrophobic drugs are difficult to deliver by gels but are easy to deliver by cream or ointment. To overcome this limitation, gel and emulsion were blended to form the emulgel (Yadav et al., 2016).

In this study, the appearance of the emulgel was pale yellow, turbid, thick, and homogeneous (as shown in Fig. 5). The emulgel was prepared by increasing the amount of a gelling agent (Carbopol 940) and oil. When the concentration...
of Carbopol 940 was increased, the formulation was thicker. The acceptable amount of Carbopol 940 was 0.5%. When the amount of oil was increased from 3% to 6%, the prepared formulation seemed oily.

### 3.3.2 Microemulsions formulations

Microemulsions are homogeneous, clear, and thermodynamically stable dispersions. Microemulsions consist of different ratios of oil, surfactant, co-surfactant, and water (Abbasi and Radi, 2016). It has advantages to enhance penetration through the skin. Moreover, the emulsion containing *C. inophyllum* oil in the previous study showed anti-inflammatory and wound healing activities (Ansel et al., 2016). Consequently, we then prepared microemulsions with this oil in this study. To prepare the microemulsion, the concentration of tween 80 in the preparation was increased from 10%w/w to 40%w/w, and the concentration of oil, span 80, and isopropyl alcohol was fixed. The microemulsions formulation (M1 and M2) were turbid and quite yellow, whereas formulation M3 and M4 were clear, transparent, and yellow. Only the formulation M3 presented phase separation after equilibration at room temperature for 24h. The microemulsion (M4) containing *C. inophyllum* extracted oil was successfully prepared at a ratio of span80, tween 80, and IPA 6:20:5 so it was evaluated in further studies.

### 3.4 Characteristics and stability studies of emulgel and microemulsion formulation

The physical properties of all formulations are listed in Table 4. The determination of pH value is important for the permeability barrier of the skin and cutaneous normal flora (Schmid-Wendtner and Korting, 2006). The pH range of all formulations was 5.6–5.9 which was suitable for topical use. The viscosity of emulgel and microemulsions was in a range of 19 000–28 000 and 600–700 cps, respectively. For emulgel formulations, the viscosity was increased when the gelling agent was added to the formulation. Although, decreased viscosity was observed as the *C. inophyllum* oil level increased, as shown in formulation E4.

For the emulgel, the increase in viscosity decreased the spreadability values of the emulgel. Formulation E2 exhibited less spreadability than formulation E1 because formulation E2 contained higher carbopol content, whereas, formulation E3 showed better spreadability than formulation E2 because of the higher oil content (p < 0.05). The more the spreadability of emulgel, the higher the spreadability on the skin.

The appearances of microemulsion formulations M1-M3 were turbid, and their viscosities were slightly increased with the further addition of surfactant. Since the viscosity can be generally modified by adding a surfactant or a cosurfactant to the microemulsion, therefore, the variation of microemulsion viscosity can be changeable in a wide range when the composition was properly adjusted. (Moghimi and Moghimi, 2013; Bera and Mandal, 2015). The microemulsion (M4) was homogeneous, clear, and transparent. The mean droplet sizes of the formulation M4 was 34.37 ± 1.06 nm, with PDI values of 0.169 ± 0.005, indicating a uniform microemulsion with a narrow size distribution. Zeta potential results of the microemulsion have been shown in Table 5 and were found to be 37.48 ± 0.87 mV. The TEM micrographs as shown in Figure 4 revealed separate single droplets with a spherical shape, and its size was considered in the particle size range of microemulsion (10–100 nm) (Burguera and Burguera, 2012).

Furthermore, emulgels (E1-E3) and microemulsions (M4) were examined by centrifugation test and short-term stability test (freeze-thaw for 6 cycles), and long-term stability tests (stored at room temperature for 2 months). Emulgels (E1, E2, and E3) and microemulsions (M4) demonstrated no sign of phase separation when subjected to centrifugation at 3000 rpm for 10 min. These formulations remained homogenous without
any phase separation throughout the centrifugation test, indicating good physical stability.

Thermodynamic stability studies demonstrated satisfactory stability of experimental formulations as the prepared emulgels (E1, E2, and E3) and microemulsion (M4) (as shown in Figs. 5 and 6, respectively). After centrifugation and thermodynamic stability tests, the emulgel E1, E2, and E3 were stable and showed no signs of creaming, cracking, or phase separation. Therefore, the formulation (E3) was the most suitable emulgel because it contained the highest amount of oil.

The physical properties of the microemulsion (M4) under short-term and long-term stability studies are shown in Table 5. The microemulsion M4 remained clear, transparent and no sign of precipitation or phase separation was observed. The pH value and viscosity remained stable. The droplet sizes were still below 100 nm. Zeta potential determination is a significant characterization technique of nanosystems to estimate the surface charge and is often used as an indicator of droplet stability. The values greater than $+30$ mV and less than $-30$ mV indicate no aggregation of the product (Kadu et al., 2011; Joseph and Singhvi, 2019). The zeta potential results were in the range of $+37$ to $+38$ mV and displayed no aggregation takes place, due to the positive charge of the

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Appearance</th>
<th>pH</th>
<th>Viscosity (cPs)</th>
<th>Spreadability (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>turbid, homogeneous</td>
<td>5.62 ± 0.01</td>
<td>19383.74 ± 260.55</td>
<td>8.11 ± 0.41</td>
</tr>
<tr>
<td>E2</td>
<td>turbid, homogeneous</td>
<td>5.63 ± 0.01</td>
<td>28547.37 ± 283.99</td>
<td>7.89 ± 0.66</td>
</tr>
<tr>
<td>E3</td>
<td>turbid, homogeneous</td>
<td>5.65 ± 0.01</td>
<td>26694.20 ± 204.88</td>
<td>8.44 ± 0.61</td>
</tr>
<tr>
<td>E4</td>
<td>turbid, oily</td>
<td>5.66 ± 0.01</td>
<td>24292.25 ± 286.99</td>
<td>–</td>
</tr>
<tr>
<td>M1</td>
<td>turbid</td>
<td>5.90 ± 0.01</td>
<td>571.46 ± 0.77</td>
<td>–</td>
</tr>
<tr>
<td>M2</td>
<td>turbid</td>
<td>5.91 ± 0.01</td>
<td>609.84 ± 0.95</td>
<td>–</td>
</tr>
<tr>
<td>M3</td>
<td>turbid</td>
<td>5.92 ± 0.01</td>
<td>660.61 ± 0.94</td>
<td>–</td>
</tr>
<tr>
<td>M4</td>
<td>clear and transparent</td>
<td>5.92 ± 0.01</td>
<td>693.94 ± 0.70</td>
<td>–</td>
</tr>
</tbody>
</table>

**Fig. 5.** Characteristics of emulgel containing *C. inophyllum* seed oil (E3) after stability test (A) E3 was kept at room temperature for 2 months; (B) freeze-thaw (4 and 45 °C) for 6 cycles.

**Fig. 6.** The comparison of the characteristics of microemulsion containing *C. inophyllum* seed oil (M4) between before (Left) and after (Right) freeze-thaw (4 and 45 °C) stability for 6 cycles.
droplets more positive than +30 mV. Therefore, it is implied that the microemulsion (M4) is an optimized formulation. It should be noted that the results of this work contribute to the development of stable and functional cosmeceutical products of nut oils.

4 Conclusions

Phenolic compounds were the main active ingredient of *C. inophyllum* extracted oil that provided antioxidant activity, especially, calophyllolide. Moreover, this extracted oil was successfully prepared as emulgels and microemulsions. The emulgel could contain 3%w/w of oil while a higher amount of oil (12%w/w) could be incorporated into the microemulsion. The mean droplet size of the optimized microemulsion formulation was 34.37 ± 1.06 nm with PDI values of 0.169 ± 0.005, ranging within the microemulsion size range. Additionally, the optimized emulgel and microemulsion were found to be stable in physical appearance and no phase separation occurred after the stability test. These results demonstrated that the emulgel and microemulsion could fabricate with *C. inophyllum* extracted oil for the skin as an antioxidant cosmeceutical product.

**Declaration of conflict of interest**

The authors declare the absence of any conflicts of interest.

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**References**


**Table 5.** The droplet size, PDI, zeta potential, pH value, and viscosity of the microemulsion (M4) under short-term and long-term stability studies.

<table>
<thead>
<tr>
<th>Day</th>
<th>Droplet size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
<th>pH</th>
<th>Viscosity (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>34.37 ± 1.06</td>
<td>0.169 ± 0.005</td>
<td>37.48 ± 0.87</td>
<td>5.92 ± 0.01</td>
<td>693.94 ± 0.70</td>
</tr>
<tr>
<td>Day 7</td>
<td>34.96 ± 0.97</td>
<td>0.167 ± 0.006</td>
<td>37.70 ± 0.52</td>
<td>5.92 ± 0.01</td>
<td>694.95 ± 0.68</td>
</tr>
<tr>
<td>Day 30</td>
<td>34.87 ± 1.02</td>
<td>0.167 ± 0.005</td>
<td>37.90 ± 0.44</td>
<td>5.92 ± 0.01</td>
<td>695.18 ± 0.68</td>
</tr>
<tr>
<td>Day 60</td>
<td>35.10 ± 1.18</td>
<td>0.170 ± 0.010</td>
<td>38.00 ± 0.46</td>
<td>5.92 ± 0.00</td>
<td>695.32 ± 0.80</td>
</tr>
<tr>
<td>6-cycles</td>
<td>34.60 ± 1.23</td>
<td>0.171 ± 0.005</td>
<td>38.18 ± 0.43</td>
<td>5.92 ± 0.01</td>
<td>694.14 ± 0.68</td>
</tr>
</tbody>
</table>


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