

Evaluation and Characteristics of Nano-emulsion containing Myanma Thanakha

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Abstract

Cosmetic products based on traditional Burmese beauty known as “Myanmar Thanakha” are recently very popular among Asian-wide cosmetic market. In this study, nanoemulsion was formulated from Thanakha (*Hesperethusacrenulata*) extract, olive oil and nonionic surfactant Tween 80 by low-pressure homogenizer and ultrasonicator techniques. The resulting nanoemulsion was creamy colour in appearance and its morphology was identified by transmission electron microscopy (TEM). Transparent nanoemulsion with mean droplet diameter of 58.23 nm was identified by dynamic light scattering (DLS) method. The resulted pH value of Thanakha nanoemulsion was 5.3 and it was compatible to skin pH (4.5–6.0); a poly dispersion index (PDI) was 0.28 and zeta potential value was –28.9. Antibacterial activity of formulated nanoemulsion was tested by agar-well diffusion test against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia.coli* bacteria and the inhibition zone area against skin infective *Staphylococcus aureus* bacteria was the widest among other. The sun protection factor (SPF) of formulated nanoemulsion was also tested by UV spectrophotometer and the result showed that it has low UV protective activity.

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Key words: Thanakha, nanoemulsion, TEM, droplet diameter, DLS, antibacterial activity, SPF;

1. Introduction

Emulsions with droplet size in the nanometer scale (typically in the range of 20–200 nm) referred as nano-emulsions are colloidal nano-dispersions of oil and water being stabilized by interfacial layer of surfactant used [1, 2]. The instability problems of emulsion such as formation of phase separation, creaming, sedimentation, flocculation and phase inversion during storage time can be reduced by the very small droplet size of nanoemulsion [3]. Nowadays, there have been developed many applied researchs that are aiming to study bioactive molecules containing nanoemulsion that can increase the bioavailability of drugs, cosmetics and nutrient [4]. Nanoemulsions are particularly useful systems for cosmetics due to good spread ability, long term stability and high penetration capability of actives into the skin [5,6]. With the help of the small-sized droplet with its high surface area, nanoemulsion is definitely effective transport of the active ingredients by improving the skin layer penetration, thus enhancing efficacy.

Products derived from plant source as well as herbal cosmetics are very popular nowadays [7]. Thanakha (*Hesperethusa crenulata* (Roxb.) Roem) is Myanmar traditionally used cosmetic and it has started to receive increasing attention as many south east Asia cosmetic companies have now developed many of their herbal cosmetic products derived from Thanakha stem bark powder as an ingredient [8]. Research studies on bioactive chemical constituents of Thanakha have revealed that it includes 2-quinolone and 2-hydroxyquinoline (N-acetyl-N-methyltryptamine, tanakine, tanakamine, sitosterol, suberosin, suberenol, 7-methoxy-6-(2,3-epoxy-6-methylbutyl) coumarin, 4-methoxy-1-methyl-2-quinolone and marmesin [4]. The various biological activities of Thanakha were anti-antioxidant activity, prevention of UV skin damage, and tyrosinase inhibition. In this study, we made an effort to prepare nanoemulsion from Thanakha extract.

The aim this research was to prepare and examine the characteristics of nanoemulsion formulated with Thanakha extract and to investigate its antimicrobial activity and its UV protection efficacy.

2. Experiment

2.1. Materials

The surfactant used in this study was Tween 80 (non ionic surfactant) and it was obtained from local chemical supplier. The oil phase olive oil and raw materials Thanakha (Shwe-bo brand) were purchased from local market. Raw material Thanakha were analyzed for trace element composition by *X-ray fluorescence (XRF)*, photochemical constituents, moisture content and biomass component.

2.2. Preparation of Thanakha water extract

Thanakha stem blocks were washed with hot water and dried in oven. Then, these blocks were cutting into small pieces and crushed into powder form. These Thanakha powder were sieved by 180 μ m mesh size sieve shaker to get fine size powder. Phytochemical analysis for solvent extracts of these Thanakha powder was performed to reveal the presence of alkaloids, glycosides, saponin glycosides, steroids, phenolic compounds, flavonoids and tannin. These fine powder (100 g) were then put into 500 ml Erlenmeyer flask by adding de-ionized water. Then these flasks were shaken at 250 rpm for 18 hours. After filtration, Thanakha water extract was obtained.

2.3. Preparation of Thanakha nanoemulsion

Nanoemulsion was prepared based on olive oil, Tween 80 and Thanakha water extract. Coarse emulsion was prepared by mixing oil, Tween 80 and water extract in (6: 10: 84) ratio. This mixture was emulsified by high speed homogenizer at 12,000 rpm for 1 minute with 10 times interval. Then the resulted coarse emulsion was sonicated by using a Sonicator (Ultrasonics, USA) at a high frequency of 20 kHz and power output of 750 W. Energy input obtained through sonicator probe with a probe diameter of 13 mm for 30 minutes generate strong disruptive forces that reduce droplet size of emulsion. The formulated nanoemulsions were then characterized.

2.4 Stability Test

The as prepared Thanakha nanoemulsion was tested for kinetic stability by centrifugation test at 3000 rpm for 30 min. The homogenous appearance was evaluated by macroscopically for resistance to centrifugation. Thermodynamic stability was tested by storing the formulated emulsion at both refrigerator temperature (4°C) and room temperature (26°C). Kinetic stability was also tested whether phase separation or creaming or cracking were occurred during prolonged storage time at room temperature.

2.5 Characterization of nanoemulsions

The emulsion droplet size, size distribution and zeta potential were measured by Dynamic Light Scattering (DLS) technique by using Zeta sizer (Malvern, Nano ZS). Firstly, the formulated nanoemulsion was diluted with 1 ml of deionized water to eliminate the multiple scattering effects. The droplet size and the poly dispersity index (PDI) of the formulated nanoemulsion were obtained as the average of three measurements at 25 °C. The globules of formulated nanoemulsion was observed by Transmission electron microscopy (TEM).

2.6 Antimicrobial Activity of Nanoemulsion

Agar-well diffusion method was used to test antimicrobial activity of Thanakha nanoemulsion. The

following microorganisms were used: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans*. The 1 ml of 1.5×10^8 CFU/ml of each microorganisms were mixed with 19 ml of Mueller Hinton culture broth solution. Muller Hinton agar (MHA) solution was prepared and sterilized in the autoclave at 121°C and 20 min. After cooling, the solution was poured into sterile petri dishes. 10 μl of bacterial broth solution were spread on the whole medium surface. Agar plate was punched with a sterile cork borer of 8 mm size and 25 μl of nanoemulsion sample was poured with micropipette in the bore. The plates were allowed to stable for a few minutes and then placed in the incubator at 37°C for 24 h. After incubation period, antimicrobial activity of nanoemulsion was investigated depending on the diameter of the growth inhibition zones.

2.7 Sun Protection Factor (SPF) of Thanakha Nanoemulsion

Determination of sun protection factor for Thanakha nanoemulsion was tested by using Ultraviolet Spectrophotometry. 1 g of Thanakha nanoemulsion and extract samples was weighed, transferred to a 100 mL volumetric flask, diluted with ethanol, followed by ultrasonication for 5 min and then filtered, discarding the upper ten ml solution. A 5ml solution was then transferred to 50 mL volumetric flask and it was diluted to volume with ethanol. The absorption spectra of samples in solution was examined by putting the sample into 1 cm quartz cell and using ethanol as a blank. Finally, Sun protection factor (SPF) data was calculated by the application of Mansur equation.

$$SPF_{\text{spectrophotometric}} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where: $EE(\lambda)$ – erythemal effect spectrum; $I(\lambda)$ – solar intensity spectrum; $Abs(\lambda)$ – absorbance of sunscreen product ; CF – correction factor (= 10) [9].

3. Result and Discussion

3.1 Raw Material Characteristics

As traditional knowledge, Myanma people used Thanakha not only as cosmetics but also as indigenous medicine. It is important to prove that Thanakha can be used safely as cosmetic because it does not include any toxic trace elements. According to these facts, the Thanakha powder were analyzed for trace elements content using Energy Dispersive X-ray Fluorescence Technique. According to the XRF result data, a total of six elements (K, Ca, Fe, Cu, Zn and Sr) were determined in the powdered Thanakha plant samples. Potassium(K) participates actively in the maintenance of the cardiac rhythm and in constipation. Calcium(Ca) is the main constituent of the skeleton and is important for regulating many vital cellular activities such as nerve and muscle function, hormonal actions, blood clotting and cellular mortality. Iron(Fe) is an essential element for human beings and animals and is an essential component of hemoglobin. Copper(Cu) is an essential nutrient that plays an important role in the production of hemoglobin, myelin, collagen and melanin. Zinc (Zn) is an essential trace element because very small amount of zinc are necessary for human health. Besides, Zn is responsible for sperm manufacture, fetus development and proper function of immune response [10]. It is found that the contents of calcium, iron and potassium were relatively higher than those of the other elements. The presence of toxic heavy metals like mercury and arsenic were not found in Thanakha. In view of the above facts, Thanakha is a source of biologically important elements, which may play part in the observed therapeutic properties and may not constitute a health hazard for consumers.

The moisture content plays an important role for every medicinal plant because if the moisture content is not significantly reduced, it enables maturation of harmful biological processes. Because of this, the moisture content of Thanakha powder was tested and the result showed 15.7 %.

According to phytochemical analysis of Thanakha powder samples, medicinal phytochemicals such as terpenoids, reducing sugar, flavonoids and alkaloids were investigated. The result of the phytochemical analysis was shown in Table-1. Phytochemical analysis revealed the presence of constituents such as alkaloids,

glycosides, reducing sugar, tannins, steroids, saponin, phenols, tannins and flavonoids. Alkaloids possess anaesthetic activity and are found in most of the medicinal plants. According to scientific reports, glycosides are well known constituents that can reduce the level of blood pressure. Tannins bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids. The plant metabolites, phenolic compounds possess biological properties such as antiaging, anticarcinogen and antiinflammation activities. Plant derived natural antioxidant are mostly in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. Flavonoids, hydroxylated phenolic substances have been found as antimicrobial substances that can inhibit a wide range of microorganisms. [11] The results obtained from this analysis suggest that these Thanakha powder contained bioactive phytochemical constituents.

Table 1 Preliminary phytochemical analysis of Thanakha powder.

No	Test	Reagent	Result
1	Alkaloids	Mayer's reagent	Present
2	Glycosides	10% lead acetate solution	Present
3.	Reducing sugars	Fehling's solution	Present
4.	Tannins	1% Ferric chloride solution	Present
5.	Steroids	Acetic anhydrite and Concentrated sulphuric acid	Present
6.	Saponins	Deionized water	Present
7.	Flavonoids	Ribbon and concentrated hydrochloric acid HCl	Present
8.	Phenolic gp	Ferric chloride solution	Present
9.	Cyanogenic glycoside	Conc H ₂ SO ₄	Absent
10.	Acid or base	Bromocresol green solution	Neutral
11	Amino acid	Ninhydrin reagent	Present
12	Carbohydrate	10% nephathanol and conc H ₂ SO ₄	Present

3.2 Stability Test of prepared Nanoemulsion

Stability of nanoemulsions refers to the physical and chemical integrity and protection against microbial contamination.[12] For this reason, stability of prepared Thanakha nanoemulsion was tested to identify its predictive capacity. According to the stability test, results have a good probability of success. Thanakha nanoemulsion remained homogenous and no phase separation occurred after 3000 rpm centrifugation, thermal stress and kinetic stability test, with a white color and macroscopic homogeneity, as shown in figure 1.

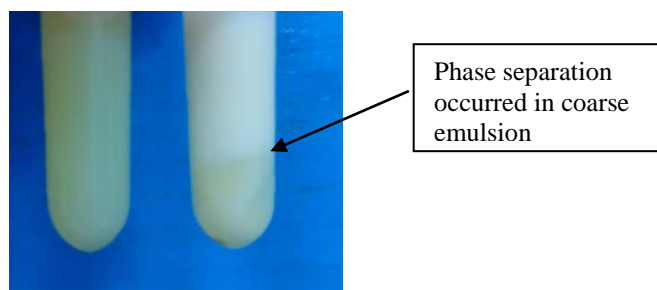


Fig. 1(a) Kinetic Stability of Thanakha nanoemulsion in comparison with coarse emulsion instability.



Fig. 1(b) Thermal stress test of Thanakha nanoemulsion in the refrigerator.

3.3 Characterization of Thanakha Nanoemulsion

Most of the nanoemulsion research reveals that the emulsion stability is higher if it has smaller the droplet size. The average droplet size of the O/W nanoemulsions typically falls within the range of 20–500 nm.[13] The most common identification methods for the stability of nanoemulsions are determination of nanoemulsion droplet size and zeta potential value, because droplet size interferes with flocculation and coalescence phenomena caused by Brownian motion.[14] Mean droplet diameter of the nanoemulsion was calculated to be 58.23 nm with poly dispersity index (PDI) of 0.280.(Fig. 2) A lower PDI value (near zero) indicates emulsion droplets are monodisperse, whereas a PDI value closer to 1 (one) reveals the size of emulsion droplets exist in a wide range[15]. A zeta potential value between $[-30 \text{ mV}]$ and $[30 \text{ mV}]$ provides good stability of disperse droplet solution that can avoid coalescence[16]. The resulting zeta potential value of Thanakha nanoemulsion is -28.9 mV and so the formulation was considered stable enough.(Fig.3) Thanakha nanoemulsion presented pH value about 5.3 ± 0.05 , where skin care products pH should be between 4.5–6.0 that was compatible to the skin[17], and it indicates that Thanakha nanoemulsion will be considered as suitable cosmetic ingredient to be use.

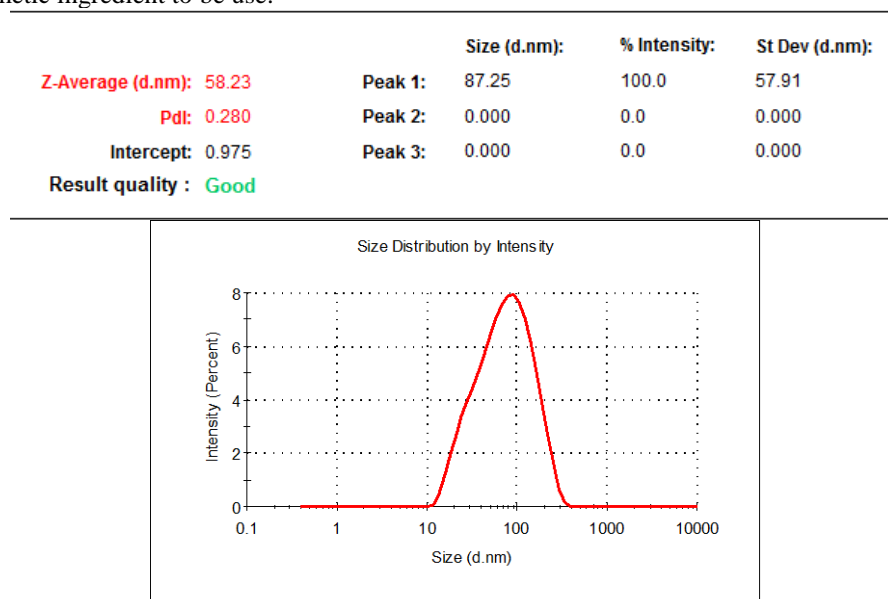


Fig-2 Droplet size of Thanakha nanoemulsions by a dynamic light scattering.

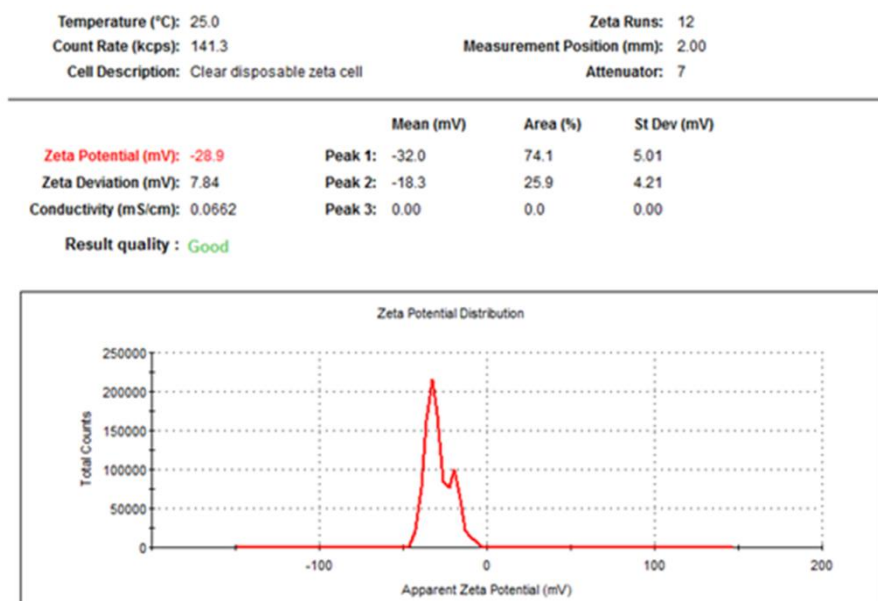


Fig-3 Zeta potential value of Thanakha nanoemulsion.

Transmission electron microscopy is the most important technique for the study of nanoemulsion droplet microstructures. The form and size of the nanoemulsion was revealed by using the combination of bright field imaging at increasing magnification and of diffraction modes [1]. According to TEM micrograph, the dark nanoemulsion droplets are surrounded by the bright appearance. Fig-4

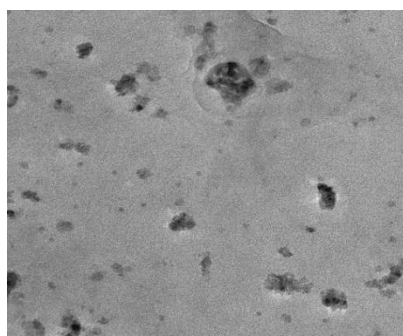


Fig-4 TEM image of Thanakha nanoemulsion.

3.4 Antimicrobial Activity of Thanakha Nanoemulsion

Thanakha nanoemulsion exhibited a slight antibacterial activity against both the Gram-positive *Staphylococcus aureus* and the Gram-negative *Escherichia coli*. [Fig-5] As researcher recommended that Thanaka extract can be produced as new medicines or being constituted in cosmetics of which may decrease costs and enhance quality of antibiotics during infectious therapy because it has strong antimicrobial activity [18], this antimicrobial test result of nanoemulsion derived from Thanakha is in good agreement.

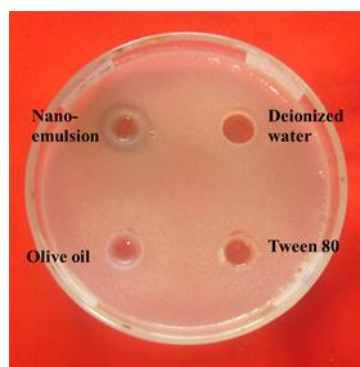


Fig-5 Antibacterial Activity of Thanakha Nanoemulsion against *Staphylococcus aureus*.

3.5 Sun Protection Factor (SPF) of Thanakha Nanoemulsion

In this research, Sun protection factor (SPF) of prepared Thanakha nanoemulsion was evaluated by UV spectrophotometry applying Mansur mathematical equation. The calculated result SPF value of Thanakha nanoemulsion was 2.338. Although this value is relatively lower than that of commercial sunscreen product, this investigation can reveal that this emulsion is derived from Myanmar traditional sunscreen Thanakha (Fig-6)

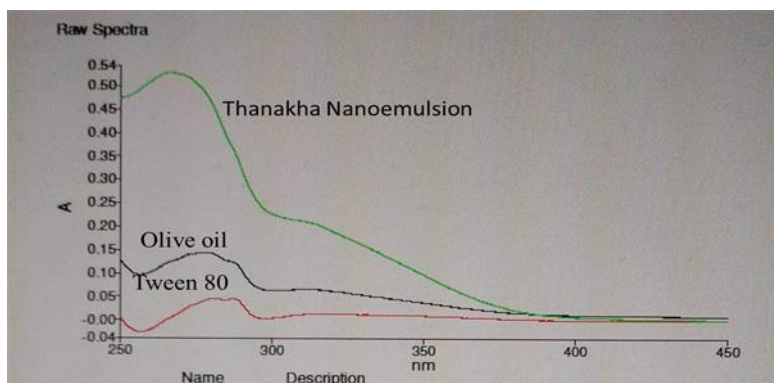


Fig-6 UV Absorption spectra of Thanakha Nanoemulsion in comparison with olive oil and Tween 80.

4. Conclusion

The combination of advanced nanotechnology and traditional knowledge was investigated in this study for the interest and idea of new cosmetic development. Myanmar traditional cosmetic Thanakha was used as raw material for nanoemulsion preparation and its physicochemical characteristics, trace element and phytochemical constituents were investigated. The nanoemulsion formulation containing olive oil, Tween 80 and thanakha water extract was formulated with droplet size of 58.23 nm with poly dispersity index (PDI) of 0.280 by combination of high speed homogenization and ultrasonic emulsification method. Due to its nano droplet size and zeta potential value, Thanakha nanoemulsion prepared in this study was proven to be stable against breakdown processes, such as creaming, sedimentation, flocculation and coalescence phase separation

and Ostwald ripening during centrifugation test, thermal stress test and kinetic stability test. This results emphasizes that the nanoemulsion based on Thanakha has antimicrobial and sun protecting efficacy.

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