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FORMULATION AND INVESTIGATION OF ANTIOXIDANT POTENTIAL OF O/W LOTIONS CONTAINING *Tamarindus indica* L. FRUIT PULP EXTRACT

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Abstract

The fruit pulp extracts of Tamarindus indica have been reported to possess several biological activities, especially antioxidant property which is suitable for cosmetic application. Therefore, the aims of this study were to formulate the tamarind fruit pulp extract loaded lotions (o/w emulsions), and to assess the antioxidant activity of tamarind fruit pulp extract loaded lotions. Initially, tamarind fruit pulp extracts were prepared by a solvent extraction method. The solvents used were water or a mixture of water and ethanol (water: ethanol= 1:1). Afterwards, the obtained tamarind fruit pulp extracts were subjected to lyophilization process. The tamarind fruit pulp extracts were tested for antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay to determine suitable concentrations of the extracts to be incorporated into the lotions, which were 2 and 4%w/w in the current study. Apart from the basic ingredients of the oil phase and water phase, ViscOptima SE (2%w/w) was selected as an emulsifier and a thickener of the formulations. The tamarind fruit pulp extract loaded lotions were characterized for physicochemical property and antioxidant potential. The freshly prepared tamarind fruit pulp extract loaded lotions were light brown with homogeneity and no phase separation was observed after centrifugation at 3,000 rpm for 30 min. They had weak acidic pH (4.4-5.1), considered acceptable for skin application. The loaded formulations (F1, 2%w/w) and F2, 4%w/w) exhibited significantly higher conductivity values than that of the unloaded formulation (F0) ($p < 0.05$). The formulations behaved as pseudoplastic flows with low viscosity. The DPPH measurement revealed that the formulations F1 and F2 had potential antioxidant activity. In conclusion, topical o/w lotions containing tamarind fruit pulp extract were successfully prepared. They had substantial antioxidant activities. As a result, tamarind fruit pulp extract loaded lotions displayed a potential use in cosmetic formulations, especially antiaging products.

Keywords

Tamarind Fruit Pulp Extracts, Antioxidant, Lotions, Topical Delivery

1. Introduction

Nowadays, a variety of plant extracts has been applied in cosmetic field. Among them is the extract of *Tamarindus indica* (or tamarind) due to its remarkably useful biological activities. The fruit pulp extract of tamarind is of interest since it possess antioxidant property which is promising for antiaging products. Several signs of aging such as wrinkles, hyperpigmentation and age spots are associated with oxidative stress (Saliou, Weber, Lodge, & Packer, 2014). Therefore, tamarind fruit pulp extract would have high potential in reducing or preventing these signs of aging. In the current study, tamarind fruit pulp extract was prepared from ripe tamarind fruit pulp (sour type) using water or a mixture of water and ethanol as a solvent, followed by lyophilization process. Lotions (o/w emulsion type) were selected as vehicles for the obtained tamarind fruit pulp extract owing to aesthetic appearance, good skin feeling, ease of application and suitability for acne prone skin. The two immiscible oil and water phases were emulsified by an appropriate emulsifier, here ViscOptima SE (by Croda) which could also act as a thickener and a stabilizer. Thus, it is beneficial for formulating plant extracts. The INCI name of ViscOptima SE is sodium polyacrylate, ethylhexyl cocoate, PPG-3 benzyl ether myristate and polysorbate 20. Derived from coconut, it is in a liquid state and complied with Inventory of Existing Cosmetic Ingredients in China (IECIC) (Croda datasheet). The beaker method was employed to prepare the lotions. After mixing oil phase and water phase together, suitable amounts of tamarind fruit pulp extract based on the antioxidant activity (EC_{50} , effective concentration of sample required to scavenging DPPH radical by 50%) were incorporated into the cool lotions and evaluated for their physicochemical properties and antioxidant activities.

The aims of the current work were to prepare tamarind fruit pulp extract using solvent extraction method, to formulate o/w lotions containing tamarind fruit pulp extract and to investigate the antioxidant potentials of the lotions containing tamarind fruit pulp extract.

2. Materials and Methods

2.1 Materials

The fresh ripe tamarind fruit pulps (sour type) were purchased from a local market in Na Mom district, Songkhla Province, Thailand and identified by Assoc. Prof. Dr. Juraithip Wungsintaweekul, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. ViscOptima SE was a gift from

Croda (Thailand) Co., Ltd (Bangkok, Thailand). Refined glycerin, cetyl alcohol, and isopropyl palmitate were supplied by Chemipan Corporation Co., Ltd (Bangkok, Thailand). L-ascorbic acid (analytical grade) was purchased from S.M. Chemical Supplies CO., Ltd (Bangkok, Thailand). Distilled water was used in a whole experiment, except HPLC analysis (Milli-Q water was used). All the chemicals, except L-ascorbic acid was pharmaceutical grade.

2.2 Preparation of Tamarind Fruit Pulp Extracts

Tamarind fruit pulps (without seeds) were mixed with water or a mixture of water and ethanol (water: ethanol= 1:1, or 50% ethanol) as solvents (tamarind: solvent =1: 5) and homogenized by a Panasonic MX-GX1462 blender. Then, the samples were macerated for 24-hours, filtered, and lyophilized. The preparation process was based on the published papers (Gupta, Prakash, & Gupta, 2014; Viyoch, Patcharaworakulchai, Songmek, Pimsan, & Wittaya- Areekul, 2003)

2.3 High Performance Liquid Chromatography (HPLC) Analysis

Tartaric acid, a major organic acid in tamarind fruit pulp, which has antioxidant activity (Santosh Singh Bhadoriya, Aditya Ganeshpurkar, Jitendra Narwaria, Gopal Rai, & Alok Pal Jain, 2011) was measured by HPLC method based on the analysis of Kordis-Krapez, Abram, Kac, and Ferjancic (2001). The Shimadzu HPLC system (Shimadzu, Japan) and a reverse-phase Appollo C18 column (5 μ m, 4.6 x 250 mm, Alltech, IL, USA) were used for analysis. The mobile phase was 0.006 M phosphoric acid (pH = 2.1) at a flow rate of 0.8 mL/min and UV detection wavelength was 210 nm.

2.4 Preparation of Formulations

The concentrations of tamarind fruit pulp extract used were 2% w/w (5-times of EC₅₀) and 4% w/w (10-times of EC₅₀) according to antioxidant activity of the tamarind fruit pulp extracts (EC₅₀ = 4.0025 mg/mL). The excipients of lotions consisted of ViscOptima SE as an emulsifier, thickener and stabilizer, cetyl alcohol (stiffening agent) and isopropyl palmitate (emollient) as oil phase, glycerin as humectant, and water as vehicle (Table 1). 1% w/v ethylhexyl glycerin and 0.2% w/v phenoxyethanol were used as preservatives.

Table 1: The Compositions of Tamarind Fruit Pulp Extract Loaded Lotions

Ingredient	%w/w		
	F0	F1	F2
Tamarind fruit pulp extract	-	2.00	4.00
ViscOptima SE	2.00	2.00	2.00
Cetyl alcohol	10.00	10.00	10.00
Isopropyl palmitate	20.00	20.00	20.00
Glycerin	20.00	20.00	20.00
Ethyhexyl glycerin	1.00	1.00	1.00
Phenoxyethanol	0.20	0.20	0.20
Distilled water	48.80	46.80	44.80

Briefly, the lotions (emulsion type) were prepared with a beaker method. Oil phase and water phase were heated until 70°C on a water bath. Then, the oil phase was added into the water phase and stirred continuously until homogenous preparation was obtained. After emulsions were cool to room temperature, tamarind fruit pulp extract was added into the emulsion bases. The formulation without tamarind extract was named as F0, whereas the formulations which contained tamarind fruit pulp extract at 2%w/w or 4%w/w were named as F1 and F2, respectively.

2.5 Physicochemical Properties of Formulations

2.5.1 Centrifugation Test

The stability of freshly prepared formulations were determined with a centrifugation test at 3,000 rpm for 30 min. Then, the appearance and phase separation were observed by macroscopic observation.

2.5.2 Characterization of Emulsion Type

The type of the prepared emulsions were determined by a microscopic observation (light microscope) with Ponceau 4R aqueous solution and a conductivity measurement (conductivity meter, model CM-115, Kyoto Electronics Manufacturing Co., Ltd., Kyoto, Japan) for 1 day-old preparations.

2.5.3 pH Measurement

The pH of the formulations were measured by a digital pH meter at room temperature (30 ± 2°C), (n=3).

2.5.4 Viscosity and Flow Measurement

The viscosity and rheological properties of the formulations were assessed using a bob-cup Brookfield rheometer (model LVDV-III Ultra, Brookfield Engineering Laboratories Inc., MA, USA) and a small adapter. The spindle SC4-31 was used. The measurements were performed at room temperature ($30 \pm 2^\circ\text{C}$) ($n=3$).

2.6 Antioxidant Test

In this study, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to measure the scavenging ability of both tamarind fruit pulp extracts and the lotion formulations. The DPPH test was performed using a 96-well plate and a microplate reader (NS-100 Nano scan, Hercuvan Lab Systems, UK). Firstly, the EC_{50} of tamarind fruit pulp extracts was determined in order to find the suitable concentrations used in the lotions. The test concentrations of tamarind fruit pulp extracts were 0.78 to 40 mg/mL.

The concentrations of all formulations (F0, F1 and F2) were fixed as 25, 50, and 100 $\mu\text{L/mL}$. The positive control was L-ascorbic acid (0.1-50 $\mu\text{g/mL}$ diluted in water), while the samples (weight 2g) were mixed with 2 mL of 50% ethanol, vortexed, and sonicated for 30 min to obtain clear solution. Then, the samples were mixed with DPPH solution. The DPPH solution at concentration of 6×10^{-5} M was prepared in 80% v/v ethanol. The mixtures were incubated in the dark at room temperature for 25 minutes and measured spectrophotometrically at 517 nm. The scavenging activity of formulations was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \frac{1 - \text{absorbance of sample}}{\text{absorbance of control}} \times 100 \quad (1)$$

2.7 Statistical Analysis

Data were expressed as mean \pm standard deviation (SD). Statistical comparisons were made using Student's *t-test* performed by SPSS statistics 20 (SPSS, Cary, NC, USA). Differences at $p < 0.05$ were considered to be significant.

3. Results and Discussion

3.1 Preparation of Tamarind Fruit Pulp Extracts

The percent yield of tamarind fruit pulp 50% ethanol extract was higher than that of tamarind fruit pulp water extract, which were 61.79 and 41.60%, respectively. The two crude

tamarind fruit pulp extracts had brownish-black colored and acidic smell.

3.2 High Performance Liquid Chromatography (HPLC) Analysis

The contents of tartaric acid presented in both tamarind fruit pulp extract samples were similar. In water solvent, the tartaric acid content was 24.857 mg% (w/w), while in a mixture of solvent, the amount of tartaric acid was 23.005 %mg (w/w) ($p > 0.05$).

3.3 Antioxidant Activity of Tamarind Fruit Pulp Extracts

According to S.S. Bhadoriya, A. Ganeshpurkar, J. Narwaria, G. Rai, and A.P. Jain (2011), several parts of tamarind such as fruits, leaves and seeds are natural sources of antioxidants which can be alternative to replacing synthetic antioxidants. Before being loaded into the formulations, screening of antioxidant activity of two tamarind fruit pulp extracts was carried out using DPPH technique. The EC_{50} values of tamarind fruit pulp water extract and tamarind fruit pulp 50% ethanol extract were 11.3154 mg/mL and 4.0025 mg/mL, respectively. Tamarind fruit pulp 50% ethanol extract had significantly lower EC_{50} than another sample ($p < 0.05$). However, its EC_{50} was markedly higher than that of a positive control, ascorbic acid ($EC_{50} = 2.8698 \mu\text{g/mL}$). The low EC_{50} value indicated that tamarind fruit pulp 50% ethanol extract had better antioxidant activity. In a study conducted by Čujić et al. (2016), for dried chokeberry, 50% ethanol was found to be the best solvent to obtain high yields of total phenolics and anthocyanins in comparison with the other mixed ratios between water and ethanol. The antioxidant activities of juices from *Pomegranate cultivars* were due to high yields of total phenolics and anthocyanins (Kalaycioğlu & Erim, 2017). Based on EC_{50} values, tamarind fruit pulp 50% ethanol extract was chosen to be incorporated into the lotion preparation.

3.4 Preparation of Formulations

All lotion formulations were successfully prepared without any phase separation after adding tamarind fruit pulp extract. This was due to the appropriate amount of ViscOptima SE used in the formulations (2%w/w). In a preliminary study, 0.5, 1 and 3%w/w of ViscOptima SE were also employed. However, phase separation happened in the case of low concentrations, 0.5 and 1%w/w, whereas too viscous lotions were obtained for 3%w/w. The macroscopic observation revealed that the formulations F1 and F2 were homogeneous with light yellow color. The prepared lotions had high aqueous proportion (water and glycerin) of about 65-69%w/w and contained the plant extract. Thus, suitable preservatives were added to inhibit bacterial growth.

3.5 Physicochemical Properties of Formulations

3.5.1 Centrifugation Test

All formulations remained homogenous after 3,000 rpm centrifugation (30 min). Thus, the prepared lotions were considered stable under this experimental condition. It has been reported that the separation of two phases in the emulsion system can affect several characteristics, especially solubility of active ingredient(s) (Mhatre et al., 2015), which is not the case for the current work.

3.5.2 Characterization of Emulsion Type

It is generally recognized that lotions are formulations with high water content (Anton, Akram, & Vandamme, 2018). In the prepared lotions, percentages of water content started from 44.80 to 48.80 %w/w. Electrical conductivity is associated with the ability of water to pass an electrical current (Shoghl, Jamali, & Moraveji, 2016). According to conductivity values summarized in Table 2, the high values indicated the o/w type-emulsions. It was observed that the increasing conductivity value from F0 to F2 was related to pH values of the formulations. The negative charge of ions in the formulations can affect the conductivity values (Amani, Amani, Kasaeian, Mahian, & Wongwises, 2017). There were significant differences in the conductivity values of Fo, F1 and F2 ($p < 0.05$).

The results of microscopic observation under a light microscope, which are presented in Figure 1, confirmed that the emulsion system was o/w. Red color from Ponceau 4R aqueous solution was located in the external phase (water). All formulations showed oil droplets (internal phase) with different sizes. A high speed homogenizer is recommended to achieve more homogeneous droplets.

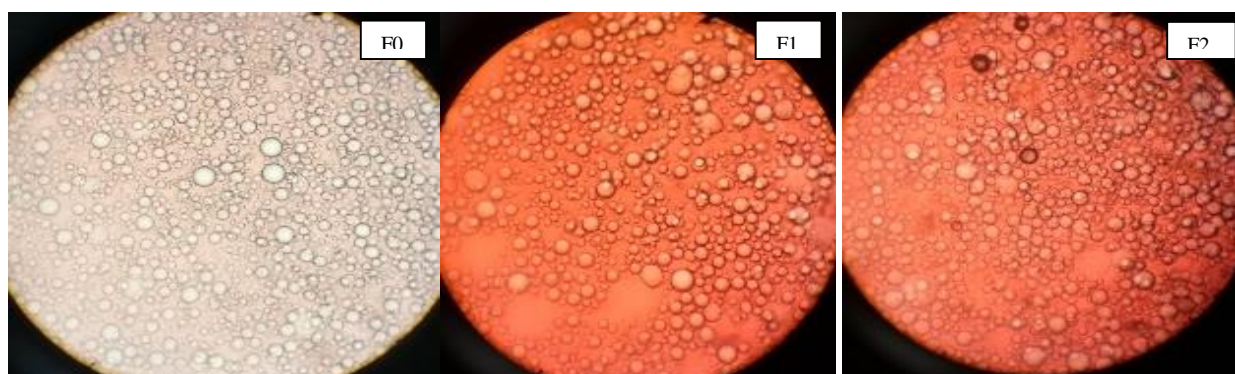


Figure 1: Light Microscopic Images of Formulations F0 (without tamarind extract), F1 (2%w/w tamarind extract), and F2 (4%w/w tamarind extract), magnification 100X.

3.5.3 pH Measurement

The pHs of the formulations were decreased significantly after the addition of tamarind fruit pulp extract ($p < 0.05$). This phenomenon may be related to the content of organic acids in the extract. However, the pH values of the tamarind extract loaded formulations were considered in the range of skin pH values (Ono et al., 2015).

Table 2 : pH Values and Electrical Conductivity Values of Formulations (mean \pm SD, $n=3$, where n is Number of Samples)

Parameters	F0	F1	F2
pH value	5.97 \pm 0.05	5.12 \pm 0.10	4.37 \pm 0.09
Electrical conductivity ($\mu\text{s/cm}$)	85.92 \pm 1.82	141.77 \pm 1.77	190.00 \pm 1.54

3.5.4 Viscosity and Rheological Measurement

The flow characteristic comparison of three formulations are displayed in Figure 2. According to Figure 2, in general, the viscosity of the lotions tended to decrease when the shear rates increased. The prepared lotions appeared to exhibit a shear-thinning system (Non-Newtonian flow). The viscosity of the formulations decreased when the shear rates increased. At speeds of 45, 60 and 90 rpm, there was no statistical difference in the viscosity between F0 and F1 ($p > 0.05$). However, the viscosity of F2 was significantly lower than that of the other two formulations ($p < 0.05$). The decreased viscosity of F2, which contained 4%w/w tamarind extract, was probably caused by high content of organic acids in the extract as reported by Garcia-Olvera, Reilly, Lehmann, and Alvarado (2016). The viscosity values of the tamarind fruit pulp extract loaded formulations were rather low $< 1,000$ cps. Therefore, they are expected to be easily applied on the skin surface.

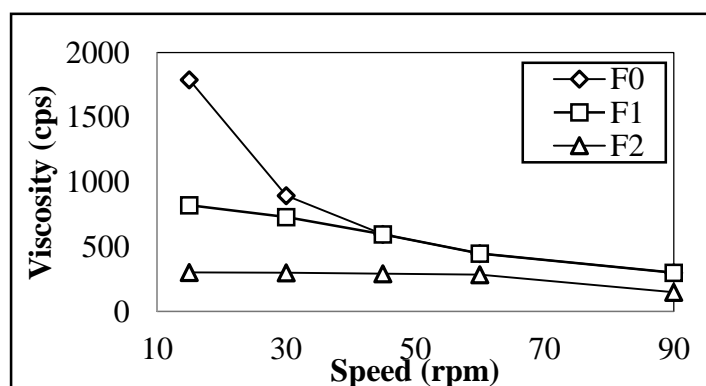


Figure 2: Rheograms of F0 (without tamarind extract), F1 (2%w/w tamarind extract), and F2 (4%w/w tamarind extract) determined at room temperature (30 ± 2 °C), (mean \pm SD, $n=3$, where n is number of samples)

3.5.5 Antioxidant Activity of Tamarind Fruit Pulp Extract Loaded Lotions

For the lotions formulations, the radical scavenging activity was expressed as percentage of reduction of initial radical absorbance by formulations at different concentrations (Figure 3). Ascorbic acid was used as a positive control with EC_{50} of 2.795 $\mu\text{g}/\text{mL}$. The unloaded formulation F0 did not have any antioxidant activity based on %SCV activity values at all concentrations tested. In contrast, both tamarind extract loaded formulations F1 and F2 displayed the existence of antioxidant activity as shown in Figure 4. According to these results, it can be speculated that the antioxidant properties were solely caused by tamarind fruit pulp extract which was incorporated in the lotion bases. It was found that the formulation F2 had markedly higher %SCV compared to the formulation F1. This phenomenon may be related to high content of tamarind fruit pulp extract in the formulation that can give more active ingredient(s) to inhibit the oxidation process.

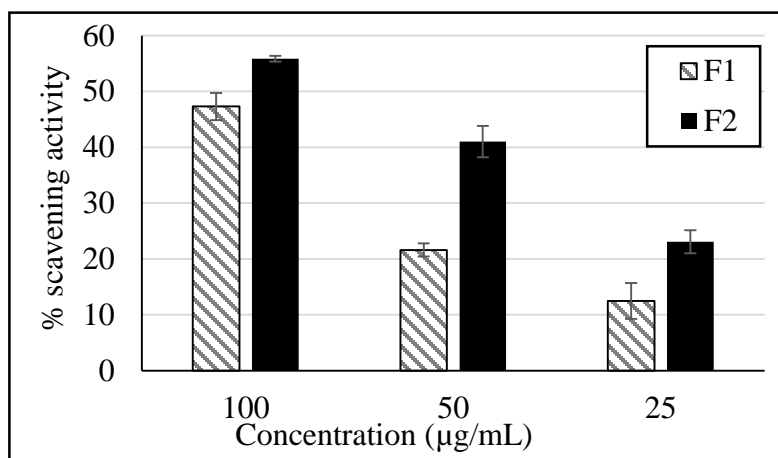


Figure 3: % Scavenging activity of F1 (2%w/w tamarind extract) and F2 (4%w/w tamarind extract), (mean \pm SD, $n = 5$ where n is number of replications)

4. Conclusion

The current results demonstrated that tamarind fruit pulp extract was successfully incorporated into o/w lotions which contained suitable amount of VisOptima SE. The tamarind fruit pulp extract loaded lotions had acceptable physicochemical properties based on macroscopic observation, pH, and viscosity measurements. These loaded lotions also had satisfactory antioxidant activity, which can be regarded as an effective and economical skin care products for topical uses.

4.1 Scope of Future Research

In the future, the stability should be performed for short and long term to know the storage time of the product.

4.2 Research Limitation

In this research, we formulated tamarind fruit pulp extract in lotion which had large droplet size. Large droplet size will be affected to penetration of active compound through the skin. The limitation data of droplet size was the major problem to know the formulation can be easily penetrate through the skin or not.

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