Journal of Pharmaceutical Research International

31(4): 1-14, 2019; Article no.JPRI.52459
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Formulation Development and Facial Skin Evaluation of Serum Containing Jellose from Tamarind Seeds

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Authors' contributions

This work was carried out in collaboration among all authors. Author TA designed the study, performed the statistical analysis and wrote the protocol. Author SK conducted the experiment and wrote the first draft of the manuscript. Author PK managed the analyses of cell studies. Authors TA and SK managed the clinical study. Author SK managed the literature search. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2019/v31i430306
Editor(s):
(1) Dr. Q. Ping Dou, Professor, Barbara Ann Karmanos Cancer Institute, Departments of Oncology, Pharmacology and Pathology, School of Medicine, Wayne State University, USA.
Reviewers:
(1) Verisa Chowjarean, Rangsit University, Thailand.
(2) Maria Antonietta Toscano, University of Catania, Italy.
(3) Jaqueline Carneiro, University of Paraná, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/52459

Original Research Article

Received 02 September 2019
Accepted 07 November 2019
Published 12 November 2019

ABSTRACT

Jellose is a natural polysaccharide that is extracted from tamarind seed kernel. This natural polymer has many good properties to be a useful excipient for pharmaceutical and cosmeceutical products. It can used as a thickening agent, suspending agent, viscosity enhancer, emulsifying agent and more. Recently, it was reported that jellose can inhibit tyrosinase enzyme and has antioxidant activity which makes this ingredient interesting to be utilized for skin lightening in cosmeceutical products. The aim of this study was to formulate a facial serum product containing jellose as one of active ingredients, evaluate the physicochemical properties and stability, and also obtain a clinical result of product with the help of volunteers. Two types of jellose serums were developed: oil in water formula; and oil-free formula. The stability of serum was studied under

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heating and cooling cycle tests for 6 cycles, and then placed in room temperature (30±1°C) for 3 months. Results showed that both types of serums had good stability. The physicochemical properties such as viscosity, pH, antioxidant activity and tyrosinase enzyme inhibition activity were evaluated to select the optimal formulation. The selected formulation was tested by applying it on the facial area of 35 healthy volunteers aged 35-75 years, and then analyzing with Skin Visual Analyzer at 15, 30 and 60 days. It was found that the jellose serum can reduce skin roughness, has an anti-wrinkle property, and brightened the skin significantly when regularly used for at least 30 days. During this study, none of the volunteers reported any kind of allergies and the overall satisfaction assessment of jellose serum was excellent. It was concluded that jellose can become a novel and potential ingredient in cosmeceuticals.

Keywords: Antioxidant activity; clinical testing; Jellose; serum formulation; stability testing; tamarind gum; tyrosinase inhibition activity.

1. INTRODUCTION

Tamarind (*Tamarindus indica L.)* is a dicotyledon in the Fabaceae family (Leguminosae), and it is found in more than 50 countries around the world, especially in the Southeast Asian region [1]. In Thailand, tamarind is an important economic plant and mostly used in tamarind pulp industry that leaves the seed as a waste. Tamarind seed is usually considered worthless but, on the contrary, it can be extracted for a substance called jellose. Jellose or tamarind gum is a natural polysaccharide which is mainly composed of xyloglucan, glucose, xylose and galactose. It has good physical properties such as high viscosity, broad pH tolerance and adhesive effect [2], these led to jellose being used a stabilizer, thickener, gelling agent and sustaining agent. Jellose was first found and recorded in 1943, when it was used as a replacement for starch in cotton sizing in Indian textile market [3]. After improvements, it was permitted as a food additive in Japan because of its safety, and pharmaceutical and cosmetic industries chose jellose as a carrier for novel drug delivery systems in oral, buccal, colon and ocular systems. It was also used for nanofabrication, wound dressing, and as a suspending agent in cosmetic products [4]. Some studies and experiments had also proved that jellose could be used as a mucoadhesive [2,4]. Furthermore, tamarind seeds could be extracted to obtain jellose with a cost-effective yield. The application of jellose as an excipient in pharmaceutical and cosmetic industries is also increasing because of its multi-functional potential. However, mostly jellose is still used as an additional excipient in formulations. In our previous study, jellose was tested and it was found that it had an antioxidant activity of 87.09±3.89%, which also accorded with the previous reports [5-7]. Moreover, it was found jellose exhibited mushroom tyrosinase inhibition activity and was able to suppress cellular melanogenesis by inhibiting both tyrosinase activity and mRNA expression, which suggests its benefits to be used as a safe and potential agent in skin whitening cosmeceuticals. Besides, jellose showed no cytotoxicity as per the study of Sano, et al. [8], and they found that jellose is not carcinogenic to mice. Due to properties like thickening, gelling, antioxidant and whitening, it was very interesting to develop a cosmeceutical formulation using jellose. Recent cosmeceutical products have focused on making products with antioxidant and whitening effect, which used ingredients from natural sources. In this study, jellose was used as an active ingredient to create a formulation that has antioxidant and whitening properties. Jellose formulations were developed and their physicochemical properties and bioactivities were studied. The optimal formulation was selected and tested in healthy human volunteers for evaluating skin whitening effectiveness and satisfaction.

2. MATERIALS AND METHODS

2.1 Materials

Jellose was kindly given by Pinphet Corporation Co., Ltd., Thailand. Xanthan gum, disodium ethylenediaminetetra acetate (disodium EDTA), butylene glycol, glycerin, phenoxyethanol, tartaric acid, capric triglyceride, Isopropyl myristate, ethanol and triethanolamine were obtained from P.C. Drug Center Co., Ltd., Thailand. Polyacrylamide and C13-14 Isoparaffin and Laureth-7 (sepigel 305) was purchased from ChemicoPlus Corp., Co., Ltd., Thailand. L-DOPA, mushroom tyrosinase, DPPH (2,2-Diphenyl-1-picrylhydrazyl), and other chemical

reagents were purchased from Sigma-Aldrich (Missouri, USA). DMEM, penicillin/streptomycin and trypsin solutions were purchased from Gibco (MD, USA).

2.2 Preparing of Jellose Formulations

Jellose formulations were developed into two types, first was oil in water emulsion base and the other was oil free base formulation. The oil in water emulsion base was prepared by solubilizing hydrophobic ingredients in oil phase, and solubilizing hydrophilic ingredients in water phase separately, and then mixing the oil and water phase. Explaining in detail, first, water phase was prepared by dispersing jellose and xanthan gum in hot water (60-70ºC), and it was let to cool down until 50ºC, and disodium EDTA, tartaric acid, phenoxyethanol, butylene glycol, glycerin was added and mixed until it was homogenous using a homogenizer. Next, the oil phase was prepared by mixing capric triglyceride and isopropyl myristate together. Finally, the water phase was gradually added into the oil phase with constant stirring and sepigel 305 was added to emulsify both phases to form emulsion. Triethanolamine was used to adjust pH of formulation in the range of 4.5 to 5.0 at the end. The oil free base formulation was prepared by solubilizing or dispersing all the excipients as described above, but oil phase and sepigel 305 were not added into the formulation. Triethanolamine was used again for adjusting the pH.

2.3 Physical Properties Characterization of Jellose Formulations

Jellose formulations were assessed for their color, phase separation and appearance by visual assessment. Results were evaluated, recorded and pictures were captured. The pH of each formulation was measured at 25ºC using pH meter, and the test was done in triplicate. The viscosity of formulation was measured at 25ºC using Brookfield DV-III Ultra Rheometer with LV spindle, and the test was done in triplicate.

2.4 Determination of DPPH Radical Scavenging Activity

The antioxidant activity of formulations was measured using 2, 2-diphenyl-1- picrylhydrazyl (DPPH$^{\bullet}$) decolorization assay [9]. DPPH$^{\bullet}$ 70% ethanolic solution was freshly prepared before use. Each sample was diluted 1:5 in 50% ethanol, then centrifuged at 12,000 G for 25 minutes at 25ºC. Then, the supernatants (100 µL) were added to 100 µL of the DPPH$^{\bullet}$ solution. After mixing thoroughly, this solution was kept in the dark for 30 minutes for the reaction to occur, and next, the absorbance was measured at 517 nm by a microplate reader (SPECTROstarNano, BMG LABTECH Gmbh, Germany). Calibration was performed with L-ascorbic acid as a positive standard control. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The % inhibition can be calculated using absorbance with the following equation:

% inhibition = [(A control- A sample)/(A control)] 100

Where A sample is the absorbance of the sample solution and DPPH solution, and A control is the absorbance of 50% ethanol and DPPH solution.

2.5 Determination of Tyrosinase Inhibition Activity

The inhibition of tyrosinase was determined by a modification of the dopachrome method using L-DOPA and L-tyrosine as substrate [10]. The samples were dissolved in 50% ethanol at the ratio of 2:5 then centrifuged at 12,000 G for 25 minutes at 25ºC. Next, 50 µl of samples were diluted in phosphate buffer (pH 6.6) at a concentration of 200 mM, and 50 µl of 2.5 mM L-dopa was added and gently shaken, then it was incubated for 10 minutes at 30ºC. After incubation, 50 µl of L-tyrosine was added into it in a 96-well plate. The microplate was kept in dark for 20 minutes at 30ºC until the reaction completed. Kojic acid was used as positive control. The absorbance was measured at 470 nm, and % inhibition was calculated as:

% Inhibition = [(A-B) – (C-D)]/ (A-B) x 100

Where A is the mixture with tyrosinase but without test sample, B is the mixture without tyrosinase and test sample, C is the mixture with tyrosinase and test sample, and D is the mixture without tyrosinase and with test sample. All measurements were performed in triplicate.

2.6 Stability Study of Jellose Formulations

The stability of the formulations was studied by accelerated freeze-thaw method. They were stored at 4ºC and 45ºC for 6 cycles, each cycle was 24 hours. Each formulation was prepared in

triplicate for the test. The physicochemical properties of jellose serum such as appearance, color, pH, phase separation and viscosity were observed before and after the freeze-thaw testing. Moreover, the antioxidant activity and tyrosinase inhibition activity of jellose serum were determined. Furthermore, serum formulations were kept at a room temperature of 30±2ºC for 3 months, and their physicochemical properties were evaluated.

2.7 Microbiological Determination of Jellose Formulations

For safety and stability of product, the microbiology of jellose serum formulations was tested. The colony-forming unit (cfu) of selected formulation was the criteria, which had to be within the limits prescribed by European Pharmacopoeia (EP) [11].

Total bacterial count: $<10^3$ cfu/g

Samples of 10 g were diluted with diluent of 90 ml, then 1 ml of this mixture was poured into a warm agar, mixed together without forming bubbles. Agar plates were incubated at 35-37ºC for 24 hours and the bacterial colony was counted and reported as cfu/g.

P.aeruginosa/g and *S.aureus*/g: None

Samples of 10 g were diluted with diluent of 90 ml, then 1 ml of this mixture was mixed with Tryptic Soy Broth, shaken well, and incubated at 35-37ºC for 24 hours. This mixture was observed for turbidity, the broth was not turbid, which meant that there was no *P.aeruginosa* and *S.aureus* contamination in formulation. For confirmation, the incubated broth was used to subculture on specific agars: Baird-Parker agar for *P.aeruginosa;* and Brain heart infusion (BHI) broth for *S.aureus,* and then observed for turbidity. Since there was no turbidity, it was confirmed that *P.aeruginosa* and *S.aureus* was not present.

Clostridium spp/g: None

Five milliliters of each of the samples were mixed with 50 ml of thioglycollate medium, heated at 80ºC for one minute, and then incubated at 37ºC for 48 h in a carbon dioxide incubator (model Nu-4500/E, NuAire Inc, USA). The resulting growth was sub-cultured on the surface of reinforced clostridial agar medium plates. *Clostridium spp* only grow in airless condition. It was then observed for turbidity and gas. There was no turbidity, which proved that there was no *Clostridium spp* contamination in the formulation.

2.8 Cytotoxicity Testing of Jellose Formulations

Cytotoxicity of a formulation is the ability of the test substance or formulation to reduce cell survival rate. Human keratinocytes (HaCaT), the most common skin cells in dermis and epidermis was used in this study for determining the cytotoxicity of the jellose formulations. These HaCaT cells were used a control and stained with the Sulforhodamine B assay (SRB assay) colorimetric method to determine the survival rate of untreated cells. The principle of Sulforhodamine B Assay is based on the ability of SRB dye to bind with proteins in living cells. First, 3,000 cells per well were cultivated in a 96-well microplate using a Dubecco-Modified Eagle Medium (DMEM) which was supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Next, these cells were incubated in a cell culture incubator at 37ºC with 5% carbon dioxide for 24 hours. Cells were cultured in the cell culture incubator for 1, 2, 3, and 7 days, and then fixed with 10% Trichloroacetic acid (TCA) at 4ºC for 1 hour. These plates were rinsed with distilled water 3 times and stained with 100 µL SRB solution. After staining with SRB, it was left to remain at room temperature for 30 minutes, then rinsed 3 times with 1% acetic acid. After drying, 100 µL of 10 mM Tris base solution, to solubilize the protein bound dye. Finally, the optical density was measured at a wavelength of 492 nm using a microplate reader. This experiment was repeated 3 times each for different concentrations of jellose formulations and also the control [12]. The % Cell viability was calculated using the equation:

% Cell viability = [OD sample / OD ctrl]x 100

Where, OD sample is the absorbance of the sample, and OD ctrl is the absorbance of the control

2.9 Determination of Melanogenesis Inhibition Activity of Jellose Formulations

For skin whitening efficacy assessment of cosmeceutical product, murine melanoma cells

are commonly used to determine melanogenesis inhibition activity of a substance. B16 melanoma cells (5 × 10^5 cells/dish) in 60 mm dishes were treated with tamarind jellose formulation at 1:100 dilution for 48h. After treatment, the cells were washed twice with ice-cold phosphate-buffered saline, and lysed in 200 µL of 1 N NaOH for 1 h at 95ºC, and then vortexed to solubilize the melanin. Hundred µL lytic solution was withdrawn and added in a 96 well plate. The total amount of melanin was measured at 405 nm. The melanin content was determined as the absorbance divided by the protein concentration in the extract from each cell pellet. The melanin content of each formulation was then calculated and presented as percentage against that of the control cells [13].

2.10 Facial Skin Evaluation of Jellose Formulation in Healthy Volunteers

All authors hereby declare that all experiments have been examined and approved by the Ethics Committee of Faculty of Pharmaceutical Science (Prince of Songkla University, Thailand) (MOE.0521.1.07/2367) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. A formal informed consent was obtained from each subject before the study for publication of this clinical study result and accompanying images. A copy of the written consent is available for review by the Editorial office of this journal. Inclusion criteria were (1) 35 healthy Thai male or female aged between 35 and 75 years (2) not related to the other research studies, (3) willing to comply with the treatment regimen and written consent. (4) They must have no skin disease that might interfere the evaluation of test site reaction and (5) refrain from using lotions, creams, or other products on the face and (6) no pregnancy or taking birth control pills. Volunteers were excluded on the basis of (1) disappearance or discontinuation of volunteers, (2) observing third level of irritation or skin allergy, and (3) having allergic histories from chemicals or other natural ingredients in formulation. However, none of volunteers discontinued in this evaluation study.

Skin irritation test

The serum formulation was applied on the forearm (size 2x2 inches) of volunteers for observing erythema, swelling or rash on the skin for at least 24 hours [14]. If the tested volunteer did not have any irritation on the skin site, they were allowed to go into the process of applying the product on facial skin to evaluate the serum efficiency.

Skin testing by Visia skin health analysis

The volunteers cleaned their facial area before each test. The marked sites were measured the facial skin surface appearances with Visia skin health analysis and set the record as a baseline of volunteers on day zero. This instrument has a scan function called “skin CT” that is an advanced analysis system. It uses digital technologies via RGB and UV spectrum. The system can diagnose problems such as facial acne, spots, pores, color, wrinkles, creases, density and amount of pigment, etc. In this study, volunteers applied 1 ml of serum formulations on facial skin, which was tapped on gently until absorption, and used twice a day on a cleaned face for 2 months. On 0, 15, 30 and 60 days, the result was measured with a Visia skin health analysis and was analyzed to measure the effectiveness of the formulation. In this clinical evaluation, volunteers’ facial skin surface parameters like skin roughness, wrinkle, spot, pore, sensitivity, uv acne and uv spot were measured and shown in Fig. 1.

Satisfaction questionnaire study

At the completion of the 60 days facial skin evaluation study period, volunteers were asked questions related to formulation appearances such as color, texture, spread ability, viscosity and smell. The following question were asked: “Are you satisfied with the improvement of the skin?” and “How about the overall satisfaction of the serum?” The answer choices were as follows: excellent; good; moderate; poor; and bad, the choices then were given a score of 5, 4, 3, 2 and 1 respectively. The scores were collected and averaged, and the shortcomings of the formulation were analyzed, to be resolved.

2.11 Data Analysis

The data of experiment was collected in triplicate. It was expressed as mean± standard deviation (SD). One-way analysis of variance (one-way ANOVA) will be used to analyze or test with the level of significance at 95% (P< 0.05).

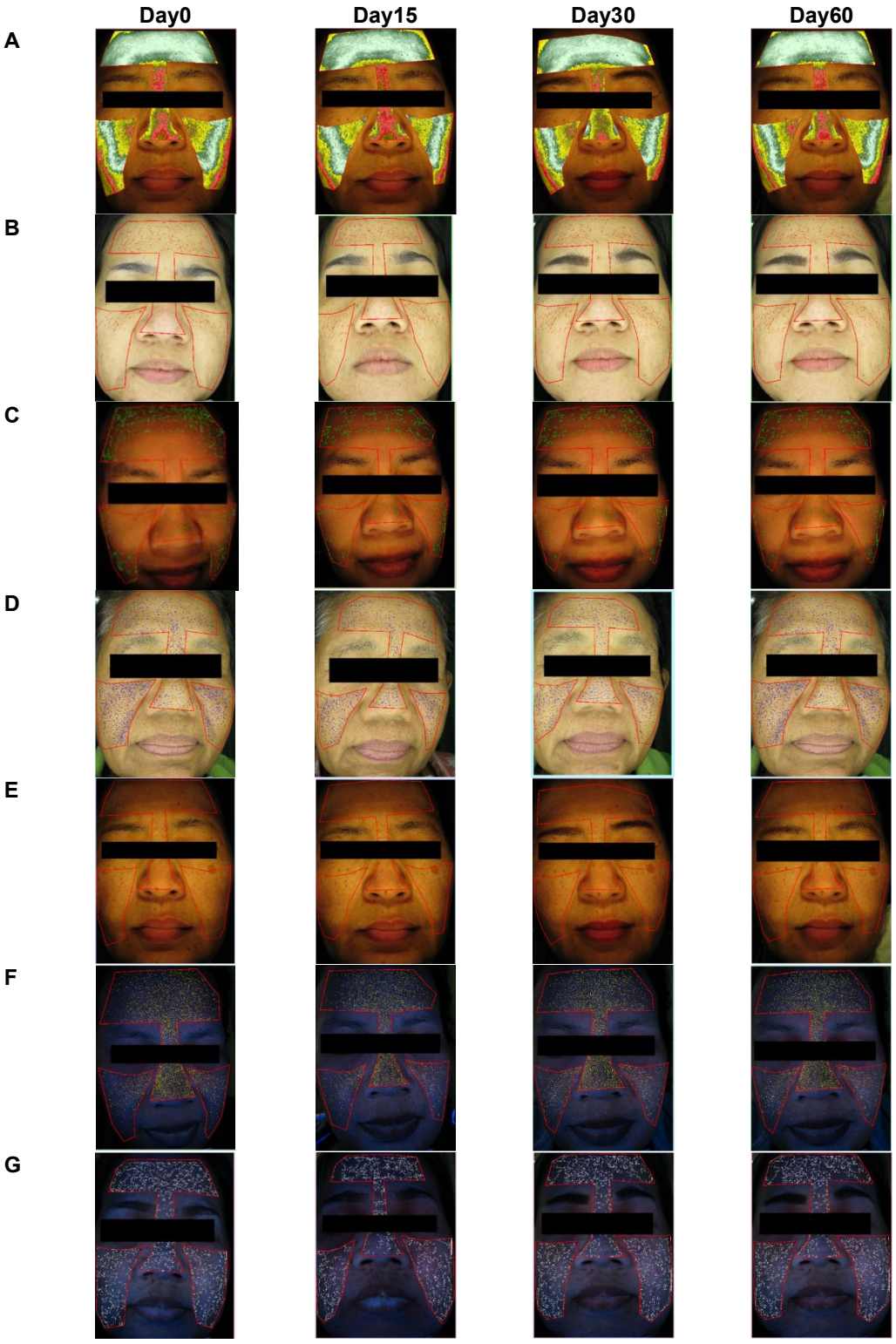

Fig. 1. Illustration of skin roughness (A), wrinkle (B), spot (C), pore (D), sensitivity (E), UV acne (F) and UV spot (G) analysis

3. RESULTS AND DISCUSSION

3.1 Development of Jellose Serum Formulation and Stability Evaluation

In the preliminary study, jellose was tested to find an optimal concentration that gives a suitable gelling viscosity for the formulation, the optimal concentration of jellose was found as 2-3% w/w, which showed a good flow behavior as a pseudoplastic flow with a smooth texture and suitable viscosity which was not higher than 10,000 cPs. Nevertheless, when only jellose was used for formulating the gel, sedimentation was observed in the formulations, so, xanthan gum was added to prevent this sedimentation. The optimal base formulation was a formula that contained 2-3 %w/w of jellose, and 0.5 %w/w xanthan gum as a thickening agent. This optimal formulation was selected to develop the jellose serum due to its unchanged physical properties such as appearance, color, pH, and viscosity before and after freeze thaw cycle testing.

The selected formulation was developed to 2 types: oil in water emulsion; and oil free formulation by adding additive ingredients such as skin conditioning agent, humectant or emollient, preservative and other complementary ingredients. The appearance of two formulations are shown in Fig. 2. Then, physicochemical properties of both oil in water and oil free formulations were determined by freeze thaw cycle testing. Results of DPPH antioxidant activity and tyrosinase inhibition activity were also used for selecting the best effective formulation. Antioxidant activity of ascorbic acid, and tyrosinase inhibition activity of kojic acid were used as positive controls, which were 98.24 ± 0.55% and 92.15 ± 0.26%, respectively. Under freeze thaw condition, both formulations remained unchanged in color with no phase separation. However, slight changes in pH and viscosity were found in all formulations. Whereas, the criteria for selection of the final serum formulation were antioxidant activity and tyrosinase inhibition activity results. Oil in water emulsion gave a higher result (Table 1) in both activities with only slight changes in activities after freeze thaw test.

3.2 Microbiological Content in Jellose Formulations

Microbiological determinations of jellose formulations of O/W and oil free emulsion were evaluated and they were found to be 60 cfu/ml and 65 cfu/ml of total aerobic plate count respectively. *S.aureus, P.aeruginosa*, and *Clostridium spp.* were not found in both formulations. These results confirmed that the jellose formulations were safe to use on facial skin.

Fig. 2. The jellose serum formulations; oil in water emulsion and oil free formulation

Table 1. The physicochemical properties of jellose serum formulations of O/W emulsion and oil free before and after freeze thaw cycles testing

physicochemical properties	O/W		Oil free	
	Before	After	Before	After
Color	Pale yellow	Pale yellow	Clear pale yellow	Clear pale yellow
sediment	No	No	No	No
Viscosity (cps)	2926.87±71.27	3066.85±23.71	6896.02±106.66	7395.68±27.61
pH	4.54±0.01	4.52±0.02	4.51±0.01	4.52±0.01
%inhibition DPPH (100 µg/ml)	68.31±0.94	63.18±0.44	48.10±0.36	38.61±0.69*
% inhibition tyrosinase (100 µg/ml)	88.09±4.43	83.22±3.61	79.78±4.16	78.21±1.37

** indicates a significant difference between the values before, and after freeze thaw cycles (P < 0.05)*

3.3 Cytotoxicity of Jellose Formulations

Both jellose formulations were evaluated for their safety in topical applications before applying on human volunteers. The results in Table 2 shows that o/w formulation had percentage of viability over 90 at all concentrations, whereas in oil free formulation percentage of viability reduced at high concentrations (2.5 µg/ml). According to Banskota, et al. [15] the cytotoxicity of tested product is deemed safe if the value of cell viability is more than 80%. Jellose formulation which was o/w emulsion type showed higher percentage of cell viability than oil free type. Therefore, the o/w emulsion type was chosen for clinical evaluation in healthy human volunteers because it was safe enough to use in humans. Furthermore, cell culture study is the accepted method for safety assessment of cosmetic formulations according to the regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products [16].

3.4 Melanogenesis Inhibition Activity

The Inhibitory effects of tamarind jellose formulation on melanin production in B16 melanoma cells were evaluated in this study. Based on the cytotoxicity study, o/w emulsion type jellose formulation at a dilution of 1:100 was used for the melanogenesis inhibition study. The results showed that this jellose formulation significantly decreased the levels of melanin, approximately 21%, compared to control (untreated) group (Fig. 3). These results suggest that the tamarind jellose formulation could be used as skin-whitening product. However, the clinical evaluation of this formulation in healthy volunteers was required to confirm the effectiveness of this formulation.

3.5 Facial Skin Evaluation

Thirty-five volunteers completed the clinical study, without any adverse event occurring during the entire period of study. The average age of volunteers in this study was 51.03±7.74. Visia skin health analysis was used to capture images and collect the necessary results from all volunteers on day 0 (baseline), and also after the application of jellose formulation on days 15, 30 and 60. The data of all parameters such as skin roughness, wrinkle, spot, pore, sensitivity, UV acne and UV spot were reported as percentage values. Visia skin analysis had a program to calculate skin age of volunteers after processing the overall profile. All parameters which were determined by Visia at various time points were compared to baseline for evaluating the effectiveness of the formulation. The moisture content in skin was determined by skin moisture analyzer. The facial images of volunteers at baseline, and after 15, 30 and 60 days of using the jellose serum are shown in Fig. 4. An improvement in skin brightness and a reduction in wrinkles can be clearly observed at 15, 30 and 60 days, in the shown images. Fig. 5 shows the skin parameters of all volunteers as mean±SD values in a column chart after using tamarind jellose formulation at 0, 15, 30 and 60 days. The results show that there is a significant improvement in several skin parameters such as reduction of roughness, spot, wrinkle and skin age at 15, 30, 60 and 60 days, respectively (Fig 5A, 5C, 5B and 5H) (P<0.05). However other skin parameters such as pore, sensitivity, UV acne, UV spot and moisture content did not change significantly from the baseline, even after treating with jellose serum for 60 days. This result might be because, most volunteers had said that they did not apply any sunscreen product, which might have caused the ineffectiveness of the jellose formulation in reducing UV acne and UV Spots. The reduction of skin roughness, spots and wrinkles increased in a time dependent manner, after treatment with jellose serum. Skin roughness decreased from 82.16±8.96% at baseline to 78.63±8.63 %, 77.15±7.25 %, and 72.80±9.4 % at 15, 30 and 60 days respectively. The skin spot count decreased from 65.55±15.37% at baseline to 64.30±14.41%, 62.23±16.83%, 60.39±16.46% at 15,30, and 60 days respectively. The average wrinkle value decreased 57.62±11.42% at baseline to 53.32±10.72% on the 60th day, with a significant difference (P<0.05). The skin age significantly reduced (P<0.05) from 50.75±8.71 years at baseline to 50.08±8.76 years at day 60. Analysis of these four parameters indicated that a significant difference was mainly observed at day 60. These data showed that jellose serum not only had great effectiveness in reducing spots, wrinkles and skin roughness, but also was safe to use without any adverse effects or irritations in volunteers.

3.6 Volunteer Satisfaction Questionnaire of Tamarind Jellose Serum

Responses to the satisfaction questionnaire are summarized in Fig. 6. The appearances of jellose formulation such as color, texture and spread

Table 2. Effect of jellose serums on keratinocyte cell viability

Formulation	% viability of keratinocyte cell at various concentrations (µg/ml)							
	0	**0.039**	**0.078**	**0.156**	**0.312**	**0.625**	**1.25**	**2.5**
O/W	100±0.0	125.9±5.3	129.5±3.6	118.5±2.7	114.8±8.3	112.6±5.3	117.6±1.3	92.4±1.5
Oil free	100±0.0	124.0±13.9	115.3±0.8	118.4±5.3	107.6±11.3	102.1±6.0	97.1±3.6	75.9±3.7

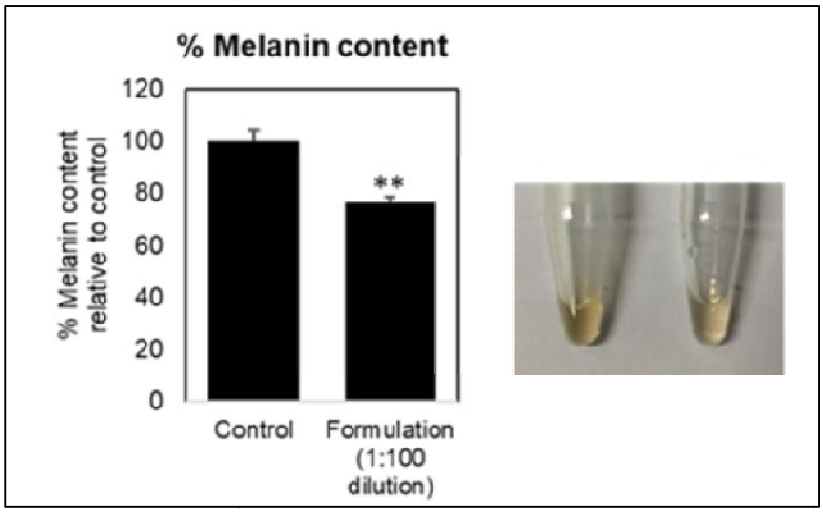

Fig. 3. Tamarind jellose formulation reduced melanin production in B16 melanoma cells. Relative melanin contents were measured at 48 h after treatment. Each bar represents Mean±SD (N=3), **P<0.01 denote significant differences when compared to control (Student's t-test)

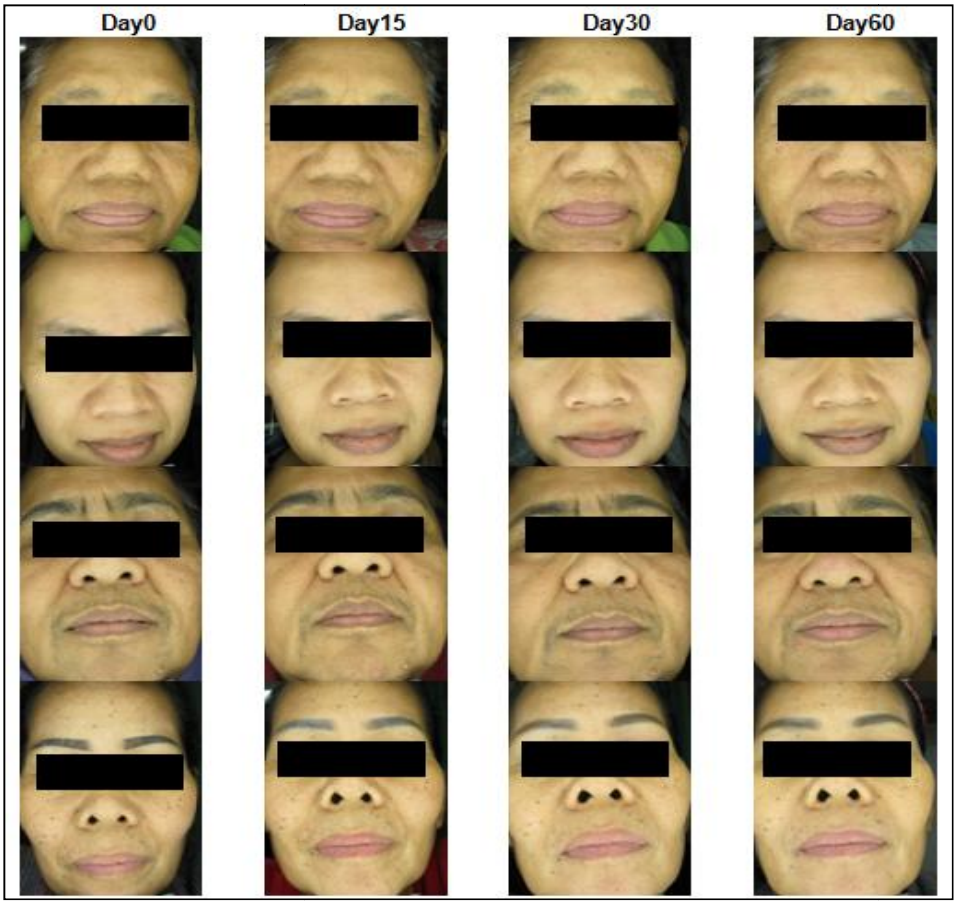

Fig. 4. Volunteers' facial images at baseline and after 15, 30 and 60 days of jellose serum use
***The images are revealed with consent from the volunteers**

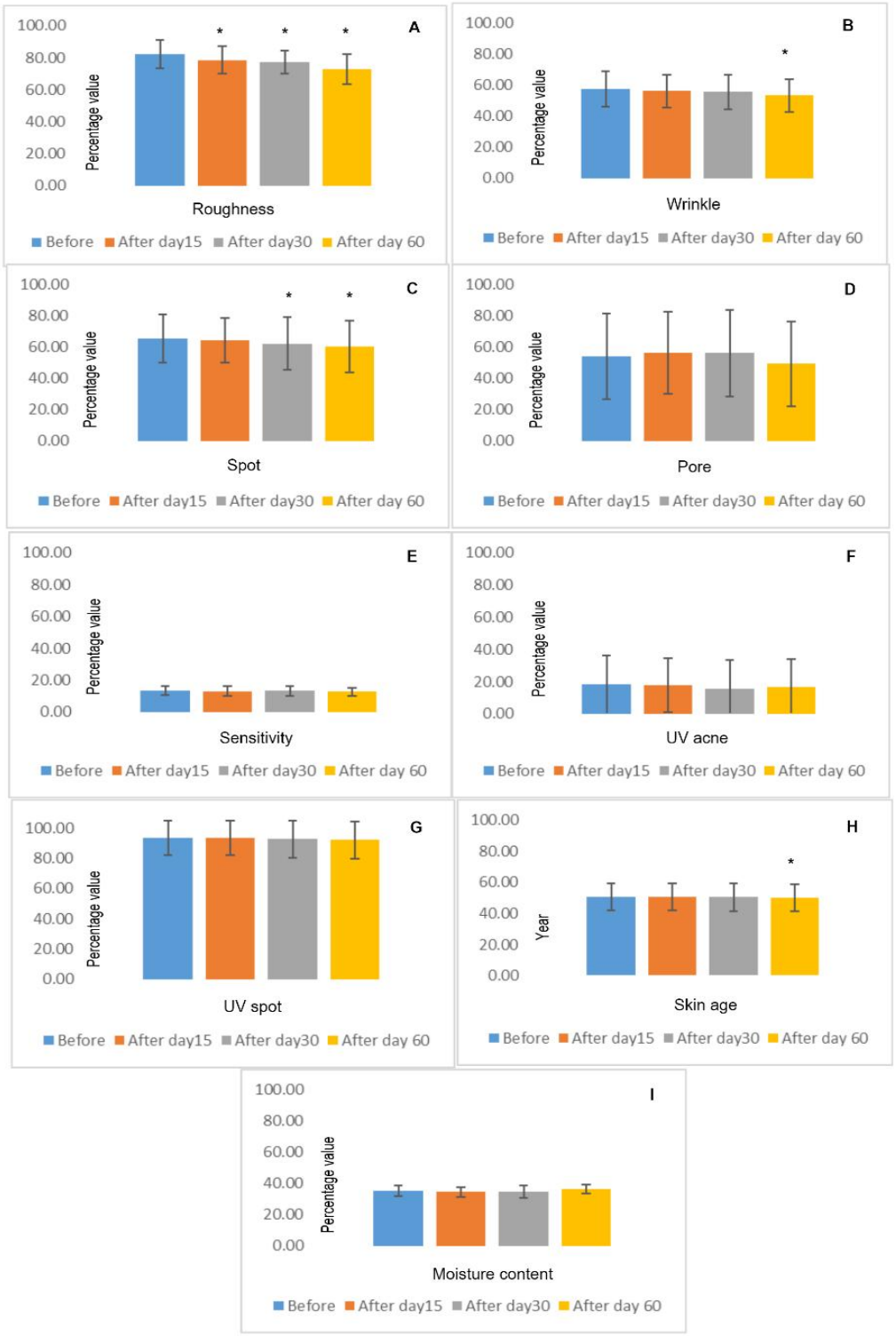

Fig. 5. The mean values of all parameters from Visia skin analysis: skin roughness (A), wrinkle (B), spot (C), pore (D), sensitivity (E), uv acne (F), uv spot (G), skin age (H), and moisture content (I) at day 0, and after application of product for 15, 30 and 60 days(* *P*<0.05 when compared to day 0)

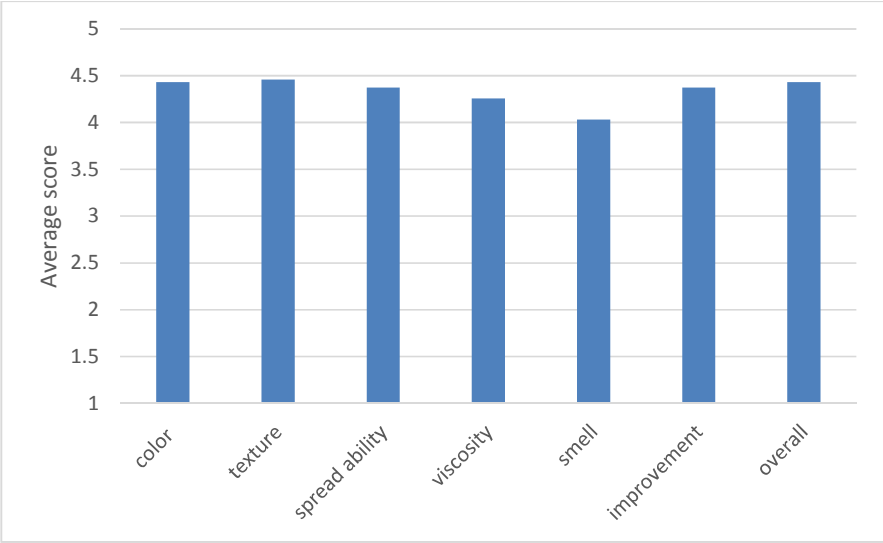

Fig. 6. Satisfaction average scores of using jellose serum after 60 days treatment

ability were good enough, but most of the volunteers wanted the formulation with lower viscosity for easier absorption, and the volunteers also preferred to have a different smell for the formulation. The volunteers' impressions of skin improvement after using product was good, including the overall quality of tamarind jellose formulation. Furthermore, the volunteers were interested for the jellose serum to be launched in the market, because of its anti-wrinkle and whitening effect.

The most prominent clinical result of the jellose serum was its ability in decreasing skin roughness, wrinkles and spots. The good properties of tamarind jellose serum that was developed in this study makes it attractive as a cosmeceutical product, that can be used for anti-aging and skin lightening [17-18]. Furthermore, our findings were similar to the findings of Waqas, et al. [19], where they used tamarind seed extract in the formulation for improving photo-aged skin. Their formulation reduced the fine wrinkles and improved the appearance of the skin. However, in our study jellose was mainly composed of xyloglucan, glucose, xylose and galactose which was different from tamarind seed extract. The ability of jellose to inhibit melanin production in melanoma cells, resulted in good skin whitening property. Furthermore, jellose also had a good antioxidant activity, which resulted in anti-aging property, as per the above-mentioned study, and also another study proved that it plays an important role in body health because of its antioxidant activity [20].

4. CONCLUSION

Jellose can be used as an effective ingredient and has a great potential in cosmeceutical applications**.** In vitro and facial skin evaluation studies results gave similar results that confirmed the effectiveness of cosmeceutical formulation containing jellose on human facial skin**.** Long term use of serum formulation containing jellose is safe, and no irritation or allergy was observed in volunteers after application of the serum as seen in this study**.** Thus we concluded that jellose is valuable ingredient in cosmeceuticals and we can develop products that are stable, pleasant in appearance and provide great benefits to the skin such as lightening and antiaging.

CONSENT AND ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the ethics committee of faculty of pharmaceutical science (prince of songkla university, thailand) (moe.0521.1.07/2367) and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of helsinki. A formal informed consent was obtained from each subject before the study for publication of this clinical study result and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

ACKNOWLEDGEMENT

This research was supported by Thailand Research Fund (TRF)- Researchers and Research Funds for Industries (RRi) (Grant No. RDG6050082). Jellose powder in this study was supported by Pinphet Corporation Co, Ltd. (Phetchabun, Thailand).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/52459