IJPSR (2020), Volume 11, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 05 April 2019; received in revised form, 05 July 2019; accepted, 19 July 2019; published 01 January 2020

RUBBER SEED CLEANSING OIL FORMULATION AND ITS EFFICACY OF MAKEUP REMOVER

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Keywords:

Rubber seed oil, Cleansing oil, Cosmetic application, Make-up remover

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ABSTRACT: The objective of this study was to determine the safety of rubber seed oil in terms of toxicity, residual solvent and to develop rubber seed cleansing oil as a makeup remover. The cytotoxicity of rubber seed was determined on human fibroblast cell. It was not cytotoxic to human fibroblast cell at >1000 µg/ml. Rubber seed oil was further examined for the presence of linamarin toxin and determining the solvent residual. FTIR spectrum revealed that there was no presence of cyanide peak and, n-hexane residual was 60.35 ± 1.12 ppm. Based on these results, it was suggested that rubber seed oil was safe to be developed as a product for cosmetic applications. The rubber seed cleansing oil was formulated in various compositions of rubber seed oil (5, 10 and 15%) combined with sorbeth-30 tetraoleate (HLB 11.5) and sorbitan sesquioleate (HLB 3.7) and the ratio of surfactant was 10, 15 and 20% respectively. The most suitable formulation with good physical properties was obtained from 5% of rubber seed oil, and 15% of surfactant. The makeup removal efficacy of rubber seed cleansing oil was 89.89 ± 2.75% against the liquid foundation.

INTRODUCTION: Cleansing oil is a cosmetic formulation that can be used as a makeup remover for daily routine. The main composition of cleansing oil contains oil from two different sources; synthetic and natural. Cleansing oil also plays an important role as an emollient and can easily dissolve cosmetic preparations [1, 2]. Synthetic oil is often the choice for commercial use in cleansing oil because of its lower cost of production.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(1).146-55

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(1).146-55

An example of synthetic oil that is often found in cleansing oil is mineral oil. However, mineral oil which is obtained from distillation of petroleum may lack biological compounds which are essential for cosmetic application. Natural oil is a more preferable choice to formulate as cleansing oil because of its antioxidant property, non-toxicity, and it contains biological compounds such as essential fatty acids and active compounds which are compatible with the skin.

Another component of cleansing oil is a surfactant, which is an ingredient that interacts with oil, facial makeup and surface debris on the skin in a manner that allows them to be rinsed off when they come in contact with water. The availability of different or various sources of natural oil from which a cleansing oil is formulated will always be

beneficial to the cosmetic market, as it provides the consumers more options to choose from, depending on their preference. The oil derived from plants, which are non-toxic and contain essential fatty acids is more preferable for use in cosmetic applications [3]. It was reported that the rubber seed oil extracted from *Hevea brasiliensis* contains a significantly high value of essential fatty acid.

Many researchers have been conducted regarding the cosmetic applications of rubber seed oil. Kittigowittana *et al*., [4] studied the potential source of antioxidant oil for cosmetic application, non-toxic, and antimicrobial property. They reported that the major components of fatty acids found in rubber seed oil were linoleic and oleic acids, which could inhibit the scavenging radical. Lourith *et al*., [5] showed that rubber seed oil is unconventional oil for cosmetic products due to the high level of fatty acid compositions. The fatty acids in rubber seed oil comprise linoleic acid, oleic acid, linolenic acid, palmitic acid, and stearic acid. These fatty acids are widely used in cosmetic in many products such as hair products, face preparations.

Moreover, the utilization of rubber seed oil is facing various challenges; for example, the toxin and solvent residual in the oil can be harmful to the users. The toxin in the rubber seed is linamarin and cyanogenic glucoside ($C_{10}H_{17}O_6$) which can be catalytically hydrolyzed and release hydrogen cyanide (CHN). However, different methods such as soaking, boiling, and drying could reduce cyanide content in rubber seed. Organic solvents extraction plays a vital role in oil production because it gives good physicochemical properties and high percentage yield of oil, but the solvents cannot be eliminated by manufacturing techniques. As there is no benefit from residual solvents, all residual solvents should be removed to the maximum extent possible.

Therefore, residual solvents testing become one of the important parts of quality control in the product. Particularly, in this study, the solvent which was extracted from rubber seed oil was n-hexane. According to USP30-NF25 Chapter 467[6], *n*-hexane is classified as a class 2 solvent, which should be limited at 290 ppm. The rubber seed oil was analyzed to determine the toxicity and n-hexane residual, before use in cosmetic applications. Subsequently, the rubber seed cleansing oil formulation was developed, and makeup removal efficacy of the developed product was compared with commercial product available in the market.

MATERIALS AND METHODS: The rubber seed oil was provided on special request by Thai-China Flavors and Fragrances Co., Ltd. Almond oil, jojoba oil, macadamia oil, sunflower seed oil, caprylic/capric triglyceride, ascorbyl palmitate, phenoxyethanol and sorbitan sesquioleate (HLB 3.7) was purchased from PC drug, Thailand. Sorbeth-30 tetraoleate (HLB 11.5), cetyl ethyl hexanoate, vitamin E acetate, and light mineral oil were purchased from Namsiang, Thailand.

Physicochemical Properties Study of Rubber Seed Oil: Physicochemical properties of rubber seed oil were determined by standard methods. The viscosity was measured in triplicate using a cup and bob viscometer (Brookfield® model DV-III ultra-programmable rheometer, USA) at room temperature (25 ± 2 °C) at a speed of 200 rpm. The refractive index and specific gravity were determined by AOAC method number 921.08 [7] and USP30-NF25 Chapter 401, [8,] respectively. The chemical properties of rubber seed oil like acid, iodine, peroxide and saponification value were determined by USP30-NF25. [8]

The antioxidant activity was determined by ABTS assay, which is more related to lipid peroxidation and widely applied in the antioxidant evaluation of the vegetable oils and expressed as trolox equivalent antioxidant capacity (TEAC). The fatty acid composition of rubber seed oil was determined by converting all fatty acids to the corresponding fatty acid methyl esters (FAME) followed by GC analysis [5, 9]. Color parameters (CIELAB), which are L*, a* and b*, were measured by CM-700d Spectrophotometer (KONICA MINOLTA, INC., Japan). The L* corresponds to the light ranging from white (100) to black (0) color, the a* is for the green (-) and red (+) color, whereas b* is the hue of blue (-) and yellow (+).

Cytotoxicity Test of Rubber Seed Oil: The cytotoxicity assay of rubber seed oil against skin fibroblast cells was measured using a sulforhodamine B (SRB) assay. This assay relies on

the property of SRB, a bright-pink aminoxanthene dye, which binds to basic amino acid residues in protein components of cells under mildly acidic conditions, and then can be extracted under basic conditions. The amount of dye extracted from stained cells is directly proportional to the cells mass. Briefly, the cells were seeded onto a 96-well plate at the density of 3×10^3 cells/well. The cells were incubated at 37 °C, 5% CO_2 for 24 h. The cells were then treated with or without various concentrations (0-1000 µM) of rubber seed oil for 48 h. After incubation, 40% (w/v) trichloroacetic acid (TCA) was gently added to each well and was then incubated at 4°C for 1 h.

Then the medium was removed and the cells were washed with tap water. After the last wash, the plate was allowed to air dry at room temperature (25 ± 2 °C). 0.4% (w/v) SRB solution (100 µl) was dropped into each well, incubated at room temperature for 1 h and then the plates were quickly rinsed 3 times with 1% (v/v) acetic acid to remove unbound dye. The plates were then allowed to dry at room temperature, and after drying, 10 mM tris base solution was added to each well to dissolve the protein-bound dye and absorbance was calculated at 492 nm using a microplate reader [10, 11].

Determination of Linamarin: Fourier transform infrared spectroscopy (FTIR) was carried out according to Salimon *et al.* [12] Rubber seed oil was applied as films on a NaCl disc (25 mm i.d. × 4 mm thickness) for this analysis. FTIR spectra of rubber seed oil were determined and recorded on an FTIR spectrophotometer (Perkin Elmer Spectrum GX, USA). The recording was done between 400-4000 cm^{-1}. FTIR was used to determine functional groups of rubber seed oil [13], *i.e.*, nitrile (C≡N) groups, at wavelength number 2260-2220 cm^{-1}.

Residual Solvent in Rubber Seed Oil: This method was used for analyzing n-hexane levels that were involved in the production of rubber seed oil according to the USP30-NF25 Chapter 467. [6] Instrument used in this study was Gas Chromatography-Flame Ionization Detector GC/ FID (GC6850 Hewlett Packard, USA). The determining column used was CP-SIL 5CB (50 m x 0.32 mm i.d., 5 µm, Chrompack, Middleburg EA, The Netherlands). The gas flow rate was constant at 1.0 ml/min; the split mode was set at 1: 10 and the temperature were set at 250 °C, for the detection. The determination was carried out at an ion source temperature of 200 °C and ionization energy of 70 eV.

Oxidative Stability Study of Rubber Seed Oil: Accelerated oxidative stability study of samples was determined using a Metrohm Rancimat model 743 (Herisau, Switzerland) according to the AOCS Official method Cd 12b-92. [14] Approximately, 3 gm samples of rubber seed oil and cleansing oil were placed separately in reaction glass test tubes. These samples were analyzed under a constant airflow of rate 20 l/h within the thermostat controlled-block heater at a temperature of 110 °C. The volatile oxidation products were carried through the detector chamber and transported to measuring vessel containing 60 ml of Millipore water, where the conductivity was measured as a function of time. The volatile compounds can be detected by an increase in conductivity. The time that was taken to detect the volatile compounds was calculated as the oxidative stability index (OSI) [15].

Preparation and Characterization of Rubber Seed Cleansing Oil: Rubber seed cleansing oil was prepared by varying the composition of rubber seed oil at the ratio of 5, 10 and 15 wt. %. Different concentrations of non-ionic surfactants including sorbeth-30 tetraoleate (HLB 11.5) and sorbitan sesquioleate (HLB 3.7) were used at 10, 15, and 20 wt. % as emulsifying agent based on the HLB value of each formulation **Table 1**. All ingredients were mixed at room temperature (25±2 °C) until a completely homogenous and clear liquid was produced. The most suitable rubber seed cleansing oil was chosen according to physicochemical properties, cleansing property, and satisfying evaluation of product efficacy (emulsification, greasiness, removal property).

Study of Physicochemical Properties and Stability Test of Rubber Seed Cleansing Oil: The physical properties of rubber seed cleansing oil like pH and viscosity were measured by pH meter (Seven Compact, Mettler Toledo, Switzerland) and Brookfield rheometer (DV-III Brookfield Engineering Laboratories Inc., Middleboro, MA, United States) at 25 °C, respectively. The color measurement was examined by CM-700d Spectro-

photometer (KONICA MINOLTA, INC., Japan) at 25 °C. Further, the selected formulations were subjected to stability test by freeze-thaw cycle test (4 °C for 24 h and 45 °C for 24 h) for 6 cycles. All parameter measurements in terms of pH, viscosity, and color were done at the initial time and also after the stability test period for product screening. The selected formulations of rubber seed cleansing oil were analyzed at accelerated conditions of temperature and humidity to assess their stability again, according to the ICH guidelines Q1A R2[16].

TABLE 1: COMPOSITION OF RUBBER SEED CLEANSING OIL

Ingredients (w/w)	Formulation no.								
	1	2	3	4	5	6	7	8	9
Rubber seed oil	5	10	15	5	10	15	5	10	15
Mixed oil	30	30	30	30	30	30	30	30	30
Sorbeth-30 tetraoleate	6.24	5.3	4.36	8.75	7.19	5.65	10.74	8.44	6.17
Sorbitan sesquioleate	3.76	4.7	5.64	6.25	7.81	9.35	9.26	11.56	13.82
Caprylic/capric triglyceride	√	√	√	√	√	√	√	√	√
Cetyl ethylhexanoate	√	√	√	√	√	√	√	√	√
Vitamin E acetate	√	√	√	√	√	√	√	√	√
Ascorbyl palmitate	√	√	√	√	√	√	√	√	√
Phenoxyethanol	√	√	√	√	√	√	√	√	√
Light mineral oil q.s. to	100	100	100	100	100	100	100	100	100

Physical and chemical stability of rubber seed cleansing oil was examined for 6 months at 3 different conditions of room temperature which were 25 ± 2 °C, 30 ± 2 °C/ 75 ± 5% RH, and 40 ± 2 °C/ 75 ± 5% RH respectively. After the storage period, the rubber seed cleansing oil was subjected to a test by the Rancimat method.

Evaluation of Cleansing Oil Product Efficacy: The cleansing efficacy of all the 9 formulations of rubber seed cleansing oil prepared were determined. To evaluate cleansing efficacy, a slightly modified method of NIKKOL group [17] was followed; 2 × 2 cm^2 areas with lipstick and 4 × 3 cm^2 areas with liquid foundation were made on the Petri dishes, left on for 5 min and then, pictures were captured. Next, 0.5 ml and 1 ml of rubber seed cleansing oil were dropped on the lipstick and liquid foundation respectively, and massaged by a gloved hand; 10 times on lipstick, and 25 times on liquid foundation. Then, 5 ml of water was poured in both the areas and was gently massaged again for 25 times. Next, the Petri-dishes were dipped in water and immediately taken out. Pictures were taken before and after, for comparison. The best formulations were selected after comparison, for testing the cleansing ability by UV-spectrophotometer method.

UV-Spectrophotometer Validation of the Rubber Seed Cleansing Oil Efficacy: The cleansing efficacy of the selected rubber seed cleansing oils were determined by measuring the content of liquid foundation (True Match, L'Oréal, China) in 95% ethanol using a UV-visible spectrophotometer (UV-1800, Shimadzu, Japan). Validation method to evaluate the specificity, linearity, accuracy, and precision of rubber seed cleansing oil on liquid foundation removal were performed according to the method followed by Chaloennit and Lourith[18] with slight modifications.

Specificity: Each of the selected rubber seed cleansing oil or commercial product was weighted 0.080 g in a conical flask, 95% of ethanol was added and then sonicated for 2 min. The mixture was filtered through a Whatman no. 1 paper and adjusted to the final volume of 50 ml. Also, the liquid foundation was weighted 0.035 gm and extracted by 95% ethanol in the same method as described above. Absorbance patterns were recorded for comparing each of these cleansing oil products in ethanol.

Linearity: Different amounts of a liquid foundation in the range of 0.04-1.4 g were weighted into a series of 50 ml volumetric flasks, and adjusted to the final volume with ethanol to obtain the concentration 0.8-2.8 mg/ml, respectively. The extraction process was performed as the previous conditions mentioned, and then the absorbance was reported for the specified wavelength. The correlation coefficient (r^2) was calculated, which had to be equal to or greater than 0.995 to demonstrate acceptable linearity of the analytical method.

Accuracy: Three different concentrations of liquid foundation (1, 1.4 and 2.2 mg/ml) were applied onto cotton sheets, then extracted using 95% ethanol (10 ml × 4), and filtered through Whatman no. 1 filter paper into 50 ml volumetric flasks. The recovery (%) of the extracted liquid foundation was assessed.

Precision: This experiment was done to evaluate and compare the precision of various formulations of rubber seed cleansing oil and a commercial product. In this experiment, liquid foundation (3 × 3 cm) was applied on to a Petri dish, then 2 drops of cleansing oil were dropped, and a cotton sheet folded into 4 layers was placed over it. A standard weight (100 g, Class F2) was placed on top of the cotton sheet. Next, the cotton sheet was moved horizontally in all directions without taking off the standard weight atop. The residues in the cotton sheet were extracted as previously mentioned. The experiment was done in triplicate for each cleansing oil product.

Statistical Analysis: All experiment data were presented as mean ± SD. Analysis of variance (ANOVA) was used to test the statistical significance of difference among the group. The significance of the difference between the mean was tested using the Student's t-test. The differences were considered statistically significant when $p<0.05$.

RESULTS AND DISCUSSION:

Physicochemical Properties and Fatty Acids Content of Rubber Seed Oil: The physicochemical properties of rubber seed oil are shown in **Table 2**. The viscosity, refractive index, and the specific gravity was in the same range as previously reported by Lourith *et al.*, [5] and Raknam *et al.* [19] The acid value (6.91 ± 0.00 mg/KOH/g) and iodine value (128.49 ± 0.59 mg I_2/g) was similar to the general seed oils used in cosmetics such as sunflower (<6 mg KOH/g) and wheat germ (128 mg I_2/g) [5, 20]. Peroxide and saponification value of rubber seed oil were in an acceptable range for natural oil in cosmetic products [21]. All parameters of chemical properties of rubber seed oil were in the normal range for a natural oil, and comparable to macadamia (<10 mg eq of peroxide/kg, 150-200 mg KOH/g), rice bran (<15 mg eq of peroxide/kg, 190-200 mg KOH/g) and avocado oil (<10 mg eq of peroxide/kg, 177-198 mg KOH/g) [22, 23]. The fatty acid of rubber seed oil consisted of saturated fatty acid (17.07%) and unsaturated fatty acid (78.73%). The saturated fatty acids were palmitic acid (9.33 ± 0.01%) and stearic acid (7.74 ± 0.01%), and unsaturated fatty acids were mainly oleic acid (23.60 ± 0.01%), linoleic acid (40.83 ± 0.02%) and linolenic acid (14.30 ± 0.00%). The color of rubber seed oil presents as a pale yellow based on b* value (9.30 ± 0.02).

TABLE 2: THE PHYSICOCHEMICAL PROPERTIES AND FATTY ACID CONTENT OF RUBBER SEED OIL

Parameter	Rubber seed oil
Viscosity 25 °C, 200 rpm	75.31±0.54
Refractive index	1.4632-1.4812
Specific gravity	$0.9244±8.11×10^{-5}$
Acid value (mg KOH/g)	6.91±0.00
Iodine value (mg I_2/g)	128.49±0.59
Peroxide value (mg eq of peroxide/kg)	2.45±0.01
Saponification value (mg KOH/g)	183.46±2.25
TEAC (mg/100g oil)	42.23±3.53
Palmitic acid (%)	3.47±0.01
Stearic acid (%)	8.33±0.01
Oleic acid (%)	32.05±0.01
Linoleic acid (%)	37.09±0.02
Linolenic acid (%)	16.40±0.00
Color L*	30.65±0.00
Color a*	1.99±0.01
Color b*	9.30±0.02

Cytotoxicity Effect of Rubber Seed Oil on Skin Fibroblast Cells: Cytotoxicity test of rubber seed oil on skin fibroblast cells was tested by SRB assay. Skin fibroblast cells were treated with or without various concentrations of rubber seed oil (0-1000 µg/ml).

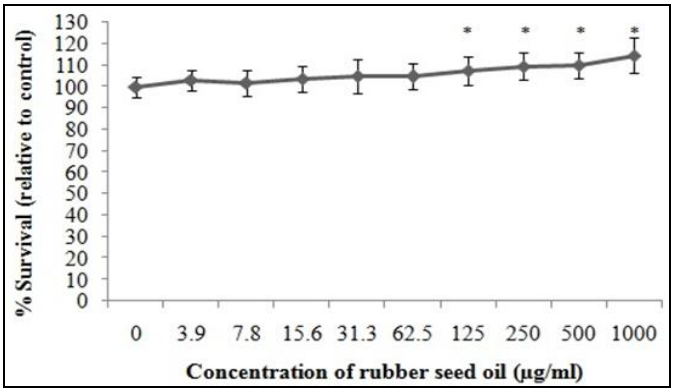

FIG. 1: CYTOTOXICITY OF RUBBER SEED OIL ON SKIN FIBROBLAST CELL

After 48 h of treatment, the result showed that IC_{50}>1000 µM in these concentrations, which meant that rubber seed oil was not toxic to the skin fibroblast cell. Furthermore, the results also showed

that the high dose of rubber seed oil (125-1000 µg/ml) **Fig. 1** significantly enhanced fibroblast cell growth. These results indicated that rubber seed oil was safe to apply to human skin.

Analysis of Linamarin: Linamarin in rubber seed oil was determined by FTIR technique. The result of the characteristic absorbance band and their assignment to functional groups are shown in **Fig. 2**. The FTIR spectrum indicates the various contents of rubber seed oil. Wavenumber (1743 cm^{-1}) indicates the presence of the ester carbonyl functional group (C=O). Spectrum peaks at 3009, 2922 and 2853 cm^{-1} indicates the presence of CH_2 and CH_3 in rubber seed oil. The spectra ranging between 1461-1419 cm^{-1} belongs to double bond (C=C), and 1376-1236 cm^{-1} indicates the presence of carboxylic acid. The obtained FTIR spectra of rubber seed oil did not show regions which indicated the presence of cyanide. It meant that linamarin or hydrogen cyanide was eliminated during the process of extraction with an adequately high temperature.

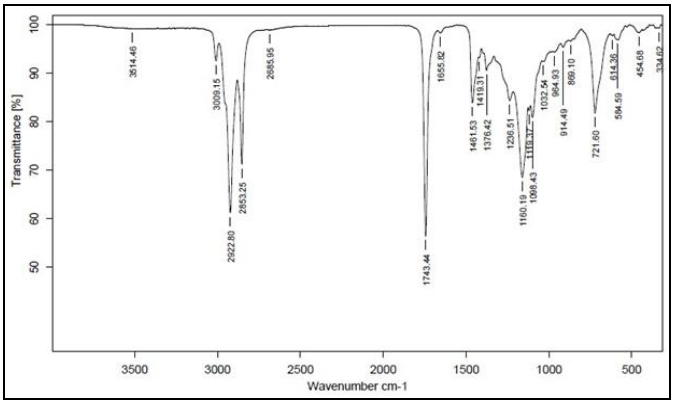

FIG. 2: THE FTIR SPECTRUM OF RUBBER SEED OIL

Residue Solvent in Rubber Seed Oil: Hexane is one of the most commonly used solvents, not only in the edible oil industry but also in the process of production of herbal medicine and bioactive components. So, it is possible that hexane may be present in trace amounts in these kinds of products. A study showed that 2, 5-hexanedione, which is derived from the metabolism of hexane, is neurotoxic. According to USP30-NF25 concentration limit, n-hexane concentration should be lower than 290 ppm. In this study, n-hexane residual in rubber seed oil was determined. It was found that n-hexane in rubber seed oil was 60.35 ± 1.12 ppm, which is acceptable and not harmful.

Oxidative Stability of Rubber Seed Oil: Accelerated oxidative stability test of rubber seed oil was carried out by measuring the oxidative stability index (OSI). Higher OSI indicates that it takes a longer time to create the volatile oxidation products, which mean the stability would also be higher. The OSI of rubber seed oil in this study was 5.21 ± 0.1 h at 110 °C. This showed that the OSI of rubber seed oil was even better than other oils which are used commercially, such as sunflower oil (2.40 h) and grape seed oil (3.20 h) [24]. Moreover, the presence of adequate content of saturated fatty acids and moderately low monounsaturated fatty acids in rubber seed oil might support a high thermal stability [25].

Physicochemical Properties and Stability of Rubber Seed Cleansing Oil: Nine formulations of rubber seed cleansing oils were prepared and tested for their stability by freeze-thaw cycle, as shown in **Table 3**. The physical appearance, color, viscosity, pH, and cleansing power were used as the criteria to select the product of rubber seed cleansing oil with the highest quality. Rubber seed cleansing oil was stable in terms of appearance, pH, and color, with ΔE less than 1.5 [26, 27], which means the color change was not visually identifiable. All the formulations showed good results in terms of physical appearance and stability after the freeze-thaw cycle. However, the best product was selected based not only on good physical appearance and stability but also on good cleansing efficacy and overall property.

Evaluation of Rubber Seed Cleansing Oil Product Efficacy: The various properties of rubber seed cleansing oil related to its cleaning efficacy is reported in **Table 4**. The results show the efficacy in terms of the ability to emulsify when in contact with water, non-greasiness after use, and efficacy as a makeup remover. Formulation no. 2, 4, and 7 showed a high score in the ability to emulsify, removal property and efficacy power with low greasiness after evaluation **Fig. 3**. This result shows that formulation no. 2, 4, and 7 were superior over the other formulations. Therefore, the formulation no. 2, 4, and 7 were selected to evaluate their cleansing efficacy by UV method. A blind test was conducted between rubber seed cleansing oil formulation no. 2, 4, 7, and the commercial

product. Their efficacy was compared, as shown in **Fig. 4** and **5**.

Oxidative Stability of Rubber Seed Cleansing Oil Formulation: Oxidative stability of cleansing oil is one of the most important parameters for its quality assessment. Cleansing oil formulation no. 4 was selected for oxidative stability study by Rancimat method at 3 conditions for 6 months to determine the shelf life of cleansing oil, as shown in **Table 5**.

TABLE 3: PHYSICAL APPEARANCES OF RUBBER SEED CLEANSING OIL BEFORE AND AFTER FREEZE AND THAW CYCLE (6 CYCLE)

Formulation no.	Before			After	
	pH	Viscosity	Color ΔE	pH	Viscosity
1	7.90±0.23	34.27±0.000	0.15±0.00	7.40±0.12	34.43±0.000
2	8.00±0.45	35.55±0.069	1.21±0.01	7.11±0.32	35.87±0.000
3	7.58±0.64	36.71±0.000	1.11±0.01	7.00±0.53	36.19±0.183
4	8.43±0.67	37.87±0.069	0.95±0.10	7.70±0.45	38.07±0.069
5	7.38±0.33	39.75±0.183	0.86±0.02	7.15±0.64	38.79±0.069
6	8.32±0.21	38.79±0.069	0.27±0.01	6.72±0.11	39.55±0.346
7	8.73±0.15	47.23±0.069	0.08±0.00	7.58±0.46	42.75±0.183
8	8.29±0.53	46.91±0.120	1.24±0.01	6.93±0.33	45.91±0.183
9	7.82±0.32	51.47±0.120	0.31±0.01	6.66±0.23	51.51±0.069

TABLE 4: EVALUATION OF RUBBER SEED CLEANSING OIL PRODUCT

Formulation no.	Evaluation protocol		
	Ability to emulsify	Greasiness	Removal property
1	+	++	++++
2	++	+	++++
3	++	+++	+++
4	+++	+	+++++
5	+	+	++++
6	+	++++	++
7	+++	++	++++
8	++	+	++
9	+	+++++	+

+++++ the highest value, ++++ very high, +++ high value, ++ fair value and + less value

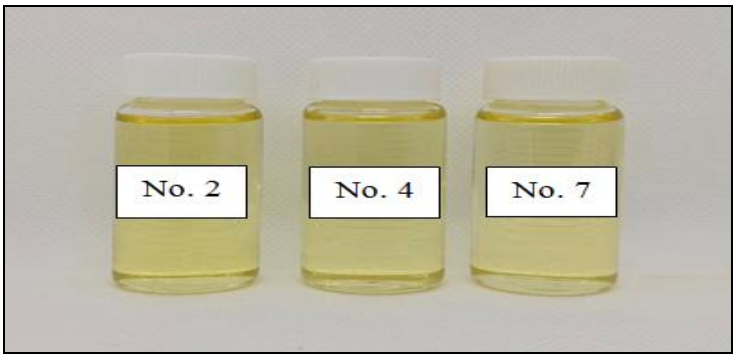

FIG. 3: THE APPEARANCE OF RUBBER SEED CLEANSING FORMULATION NO. 2, 4 AND 7

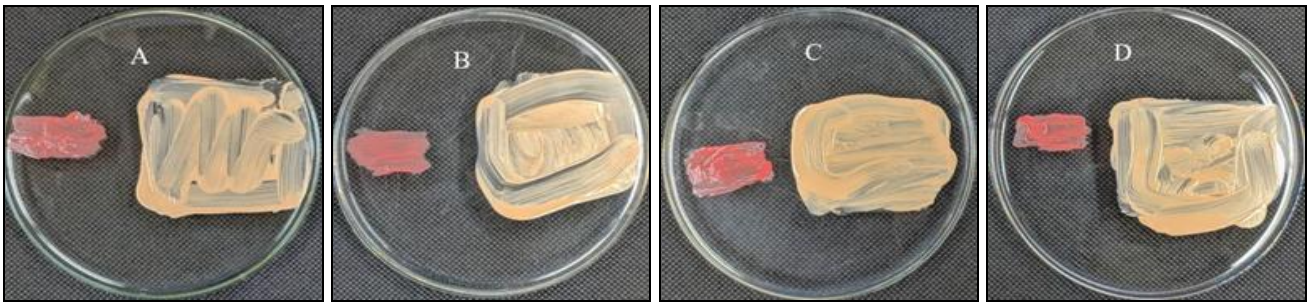

FIG. 4: THE PETRI DISHES BEFORE EVALUATION (FORMULATION NO. 2, 4, 7 AND COMMERCIAL PRODUCT WHEN APPLIED ON A, B, C, AND D RESPECTIVELY)

FIG. 5: THE PETRI DISHES AFTER EVALUATION (FORMULATION NO. 2, 4, 7 AND COMMERCIAL PRODUCT WHEN APPLIED ON A, B, C AND D RESPECTIVELY)

The result revealed that when the cleansing oil was stored at room temperature (25 ± 2 °C) for 6 months, OSI was the highest (66.64 ± 1.45 h). This may be due to the antioxidant in the formulation such as vitamin E and ascorbyl palmitate [21,] which delayed the oxidation in the product. Also, the process of drying rubber seed before oil extraction helped the oil have good stability for long time storage [28].

After storing for 6 months at 30 ± 2 °C / 75 ± 5% RH, OSI was 55.75 ± 0.11 h, and after storing at 45 ± 2 °C / 75 ± 5% RH, OSI was 48.35 ± 0.62 h, which indicated that rubber seed cleansing oil was stable in terms of oxidative stability.

Moreover, we can minimize the variations between shelf life predictions and long-term storage study by selecting the correct levels in the Rancimat method according to the product type. Thus, using the Rancimat technique, we can rapidly evaluate the quality of cleansing oil, and determine if it can be stored at room temperature for 6 months.

TABLE 5: OXIDATIVE STABILITY OF RUBBER SEED CLEANSING OIL FORMULATION NO. 4 FOR 6 MONTHS

Storage condition	Oxidative stability index (h)
Room temperature (25±2˚C)	66.64±1.45
30±2˚C/75±5% RH	55.75±0.11
45±2˚C/75±5% RH	48.35±0.62

UV-Spectrophotometer Validation of the Rubber Seed Cleansing Oil Efficacy:

Specificity: The wavelength 250 nm was found to be the most suitable for measuring the content of liquid foundation. This wavelength only indicated the content of liquid foundation and was not interfered by any of cleansing oil product in extracted solution.

Linearity: The solutions were scanned on a spectrophotometer in the UV range of 200-400 nm. The spectrum was recorded at 250 nm as maximum (λ_{max}). The calibration plot was plotted as concentration *vs.* absorbance. The coefficient of determination (r^2) was found to be 0.9995 (y= 0.2634x + 0.0050). This method indicated good linearity and also represented the precision of the extracted amount of liquid foundation.

Accuracy: A known amount of three different solutions that represent low, medium and high (1, 1.4 and 2.2 mg/ml) concentrations of a liquid foundation in cotton sheets were extracted and tested to validate the accuracy of this method. Recovery (%) was found in the range of 88-95%, as shown in **Table 6**. The validity and reliability of this method were obtained by calculating the % RSD, which was < 2% (0.25-0.76%) [29], indicating that this analytical method was accurate.

TABLE 6: RECOVERY (%) OF THIS UV-SPECTROPHOTOMETRIC DETERMINATION (N=3)

Added amount (mg/mL)	Mean of measured conc. (mg/mL)	Average recovery (%)	% RSD
1	0.89±0.02	88.88±1.78	0.31
1.4	1.32±0.03	94.40±2.18	0.76
2.2	2.11±0.06	95.53±2.51	0.25

Precision: As shown in **Table 7,** the % RSD of the three formulations of rubber seed cleansing oil and the commercial product was less than 2, which indicates that the method used was precise. The rubber seed cleansing oil was compared to commercial product, and the result found that formulation no.4 exhibited the highest efficacy (89.89 ± 2.75%) in makeup removal against the liquid foundation.

After blind test evaluation, the result indicated that the efficacy of formulation no 4 was almost identical to the commercial product, and was better than formulation 2 and 7. Therefore, formulation no 4 was the best cleansing oil product.

TABLE 7: PRECISION OF EFFICACY OF RUBBER SEED CLEANSING OIL AND COMMERCIAL PRODUCT AND SATISFIED EVALUATION

Formulation no.	Recovery (%)	% RSD
2	87.28±3.23	1.00
4	89.89±2.75	1.01
7	85.17±3.33	0.56
Commercial product	88.08±6.09	0.55

CONCLUSION: This study proved that the rubber seed oil obtained from solvent reflux extraction was safe for use in topical applications. Hence, we were able to develop rubber seed oil into a cleansing oil product. It showed good physicochemical properties, good stability, high ability in emulsification, removal property, and cleansing efficacy with low greasiness. The best rubber seed cleansing oil product composed of 5% of rubber seed oil and 15% of surfactants, which had a high quality similar to a commercial product.

ACKNOWLEDGEMENT: This research was financially supported by the Natural Rubber Innovation Research Institute (Grant No. PHA581138S) and Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

CONFLICTS OF INTEREST: The authors declared that they have no conflict of interest.

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How to cite this article:
Raknam P, Pinsuwan S and Amnuaikit T: Rubber seed cleansing oil formulation and its efficacy of makeup remover. Int J Pharm Sci & Res 2020; 11(1): 146-55. doi: 10.13040/IJPSR.0975-8232.11(1).146-55.

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