Research Article

Sunn Hemp *(Crotalaria juncea)* Seed Oil: A Potential Antioxidant for Emulgel Applications

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ABSTRACT

Sunn hemp (*Crotalaria juncea*), a valuable legume, is used to improve soil quality, boost crop yields, and reduce pests. Its abundant seeds hold promise for cosmetic applications due to their content of beneficial compounds. This study investigated the antioxidant activity and fatty acid profile of sunn hemp seed oil. The oil was extracted using five different solvents: hexane, dichloromethane, ethyl acetate, acetone, and ethanol. The ethanol extract exhibited the strongest antioxidant activity, measured by its ability to scavenge free radicals (DPPH assay), with a 50% scavenging concentration (SC_{50}) of 53.69 ± 0.02%. This ethanol extract was chosen to formulate an emulgel using a cold process technique. Different seed oil concentrations were tested. Evaluations of the prepared emulgels revealed that the one containing 1% seed oil extract displayed the most desirable properties, demonstrating good physical and chemical stability. The results suggested that sunn hemp seed oil might possess an ability to be used as an active ingredient in the emulgel formula. Further s.tudies about other formulation and clinical efficacy of formulated products should be considered.

Keywords: *Crotalaria juncea*, antioxidant, 2,2-diphenyl-1-picrylhydrazyl (DPPH), emulgel, cosmetics

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Introduction

Crotalaria juncea or sunn hemp is an important legume crop in Fabaceae family. *C. juncea* is a short-day, erect shrubby annual plants with a height about 1 to 4 cm, which has strongly tapped root with the lateral roots and plentifully branched. The stems are cylindrical. The leaves are arranged in the form of spiral along the stem, simple, hairy pod, short petiole, and has a shape in oblong-lanceolate or elliptical form. The flowers are deep yellow. The cylindrical fruits have a high amount of seed, hairy pod, and can turn to a light brown color when grow up. The cordiform seed has a dark brown to black color. It is commonly planted as a rotational crop in many tropical and subtropical countries including India, Bangladesh, Brazil, and Thailand. It has many agronomical benefits, such as nitrogen fixing property, which can enhance the soil quality. *C. juncea* has been used for traditional medicines such as in a treatment of anemia, impetigo, menorrhagia, and psoriasis [1]. Many biological activities of *C. juncea* were reported. The seeds possess antioxidants and anti-inflammatory activities. Flowers and seeds were reported with antibacterial activity. The leaves were reported with anti-diarrheal, antiarthritic, and anti-ulcerogenic activities [2–4].

The seeds of *C. juncea* contain carbohydrates, protein and oil. In previous studies, gas chromatographic analysis of seed oils revealed that they contain fatty acid components such as stearic acid, linolenic acid, oleic acid, linoleic acid, and palmitic acid [5]. The protein components of the seeds consisted of several essential amino acids and non-essential amino acids. The major components of these amino acids are lysine, leucine, isoleucine and aspartic acid, glutamic acid, arginine, alanine [3]. The extracts of *C. juncea* seeds consist of fatty acids which affect to the moisturizing quality of the skin [6]. Linoleic acid was reported as the main component in the seed oil. Since linoleic acid is an essential fatty acid, and responsible in a formation of ceramide 1, which is the most important on skin barrier function [7,8].

Emulgel is the dosage form which is a combination of gel and emulsion. It is an interesting topical delivery system. It contains the benefits from both emulsion and gel. The emulgel is the emerging drug delivery system nowadays and popular to use for the delivery of cosmetically active ingredients and drugs, both hydrophilic and hydrophobic [9–12].

Based on these reasons, this research was aimed to evaluate the antioxidant activity, fatty acids constituents of *C. juncea* seed oil and preparation of an emulgel containing *C. juncea* seed oil by cold process emulsification technique. Physical and chemical properties of formulated emulgel were also evaluated.

Materials and Methods

Plant materials

C. juncea seeds were purchased from agriculturist in Ubon Ratchathani, Thailand in June, 2020. The collected seeds were identified by a botanist. After authentication process, the seeds were dried in tray dryer at temperature 60 $^{\circ}$C until dried and stored in the cool temperature and dry place until further used.

Chemicals and reagents

Mineral oil (Carnation #70, UPI chemicals, North Carolina, USA), butylene glycol (Chemecosmetics, Thailand), Viscolam AT 100P (INCI name: sodium polyacryloyldimethyl taurate, hydrogenated polydecene, trideceth-10; Rita Corporation, Illinois, USA) and Kem E (INCI name: benzyl alcohol, water, potassium sorbate, sodium benzoate; Akema Fine Chemicals, Italy) were purchased from their local distributors in Thailand in the highest quality available of cosmetic grades. Deionized (DI) water was prepared with Elix® Essential Water Purification System (Merck KGaA, Darmstadt, Germany).

Extraction of C. juncea seeds

Maceration technique was used in the extraction process. The *C. juncea* seeds (2 kg) were ground into fine powders. They were then macerated in 1:5 (seed:solvent) weight ratio with various solvents including hexane, dichloromethane, ethyl acetate, acetone and ethanol, in a cold temperature and dark chamber for 7 days. After the duration was reached, the extracts were filtered with Whatman® No.1 filter paper. The extracts were concentrated under controlled pressure and temperature using a rotary evaporator (Eyela, Tokyo, Japan) in order to obtain crude extracts. These extracts were stored in 4 °C refrigerator until further used.

Evaluation of antioxidant activity

For evaluation of antioxidant activity of *C. juncea* seed extract, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method was selected. The extracts from hexane, dichloromethane, ethyl acetate, acetone, and ethanol solvents were used to test. Firstly, the 60 μM DPPH reagent was prepared by dissolving DPPH (0.00236 g) in methanol (100 mL). In this study, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a positive control. Methanol was used as a negative control. The *C. juncea* seed extracts samples were diluted into appropriate concentration with methanol. To determine DPPH free radical scavenging activity, 170 μL of DPPH was mixed with 30 μL sample solution in a 96-well microplate. After that, the mixture was incubated at room temperature in a dark room for 30 min, before measured the absorbance at 517 nm with FLUOstar Omega Microplate Reader. The DPPH radical scavenging activity was calculated using the following formula;

$$\%\text{Inhibition} = \frac{(\text{Abs of control-Abs of sample})}{\text{Abs of control}} \text{ X } 100$$

50% scavenging concentration (SC_{50}) value was calculated from this equation and used in comparable of antioxidant activity between extracts.

Preparation of emulgel

Emulgel was prepared with the formula composition described in Table 1. The oil content was fixed at 5% w/w and the amount of Viscolam AT100P was controlled at 4% w/w for similar viscosity and texture.

Table 1 Composition of emulgel formulations (F1-F4) containing *C. juncea* seed oil extract.

Phase	Ingredients	Concentration (%w/w)				
		Base	F1	F2	F3	F4
A	Mineral oil	5.00	4.75	4.50	4.25	4.00
	C. juncea seed ethanolic oil extract	-	0.25	0.50	0.75	1.00
	Viscolam AT100P	4.00	4.00	4.00	4.00	4.00
B	Butylene glycol	4.00				
	DI water	86.00				
C	Kem E	1.00				

For the preparation technique, oils were mixed together until homogenous liquid was obtained. After that, Viscolam AT100P was added into the oil phase (phase A). In another vessel, butylene glycol and water (phase B) were mixed. After that, the ingredient mixture in phase B was slowly incorporated into phase A with moderate agitation until the homogenous emulgel was obtained. Finally, phase C. was added.

Evaluation of formulated emulgels

Physical characteristics and pH

Physical appearance of the formulated emulgels was evaluated by an observation. Color of the emulgel was analyzed by ColorQuest XE, HunterLab, in Commission on Illumination (CIE) Lab system [13]. Viscosity of prepared emulgels was analyzed at room temperature using Brookfield viscometer (model RVDV-II+P [14]. The dynamic viscosity measurements were performed with spindle R0.5 at rotational speed of 10 rpm. pH of formulated emulgels was evaluated with pH meter (pH-3c pH Meters for Hydroponic Grow pH Meter Aquarium) under room temperature.

Sensory evaluation

Sensory characteristics of formulated emulgels were evaluated by researchers. Parameters of color, odor, viscosity, spread ability, greasiness, absorb ability, and feeling after used were rated with five-point hedonic scale of sensory evaluation. The score from 1, the least favorite, to 5, the most favorite, were given [15].

Stability of prepared emulgels

The accelerated stability test was conducted by heating/cooling cycles. The samples were kept in a refrigerator (4 °C) for 24 h and kept in hot air oven (45 °C) for another 24 h. This was counted as 1 cycle. Six consecutive cycles were used. The changes in physical appearances and sensory characteristics were evaluated i.e. color intensity, feeling after used, odor strength, absorb ability, viscosity, greasiness, and spread ability.

Statistics

All of the data were reported in the term of mean ± SD obtained from triplicate samples. The statistical analysis was undertaken using one-way ANOVA test followed by Tukey's test ($p < 0.05$).

Results and Discussion

Extraction of C. juncea seed

The *C. juncea* seeds were extracted with various solvents. All of the obtained extracts showed yellow color with nutty odor characteristic. The ethyl acetate extract had the highest yield of extraction (7.98%w/w of dry seeds) followed by dichloromethane (5.01 %w/w), ethanol (4.65 %w/w), acetone (3.58 %w/w), and hexane (2.26 %w/w), respectively (Table 2).

Table 2 Yield of extraction of *C. juncea* seed oil extracts in various solvents (n=3).

Solvents used	Oil yield (%w/w)
Hexane	2.25 ± 0.06
Dichloromethane	5.01 ± 0.03
Ethylacetate	7.98 ± 0.02
Acetone	3.58 ± 0.05
Ethanol	4.65 ± 0.07

Antioxidant activities

Antioxidant activity is the inhibition property of free radicals that are produced by cells and environment. This study identified antioxidant activity by using the method of DPPH radical scavenging activity. DPPH is a stable hydrophobic free radical, which have purple color. Once it reacts with an antioxidant, the neutralized DPPH changes its color into yellow. By measuring the reduction of the visible light absorbance in purple color (517 nm), free radical scavenging activity can be identified. In this experiment, the *C. juncea* seed oil extracts from various solvents were studied their antioxidant capacity in the concentration at 50 mg/mL. The results showed that ethanolic extract had the highest antioxidant activity (53.69 ± 0.02% inhibition) (Table 3). The SC_{50} of *C. juncea* seed oil ethanolic extract is 32.20 mg/mL and the SC_{50} of standard trolox used in this study was 333.25 µM.

Table 3 DPPH free radical scavenging activities of *C. juncea* seed oil extracts (50 mg/mL) obtained from several solvents (n=3).

Solvents	% Inhibition
Hexane	5.70 ± 0.60
Dichloromethane	16.10 ± 0.29
Ethylacetate	11.07 ± 0.59
Acetone	23.15 ± 0.37
Ethanol	53.69 ± 0.02

*Positive control is standard trolox (SC_{50} = 333.25 μM)

Preparation of emulgel

Emulgel was selected as a vehicle in this study, based on its properties. Emulgel contains oil or lipophilic material and water or hydrophilic material. This makes emulgel suitable for utilizing as an appropriate carrier for both lipophilc and hydrophilic materials. Compared with emulsion, emulgel has lighter sensory feel, which makes it more consumer susceptible. In this study, Viscolam AT100P was selected as a thickener and emulsifier. This is a blended cosmetic raw material, made from 3 chemicals, which are sodium polyacryloyldimethyl taurate, hydrogenated polydecene, trideceth-10. Hydrogenated polydecene is a long chain saturated hydrocarbon that used as occlusive agent in cosmetic formula. In this combination this compound serves as a major vehicle for other raw materials. Sodium polyacryloyldimethyl tuarate is a polymer which is a gelling agent that produces thickening effect to the formula. Trideceth-10 is an emulsifier which can help emulsifies oil into water, and create emulsion. Combination of trideceth-10 and hydrogenated polydecene create appropriate emulgel. In this study, emulgels were prepared with four different concentrations of *C. juncea* seed oil ranging from 0.25% - 1.00% w/w. Mineral oil was used as a control. Total weight of oil phase was fixed at 5% w/w. All prepared formula had the cream-gel liked appearance as shown in Figure 1.

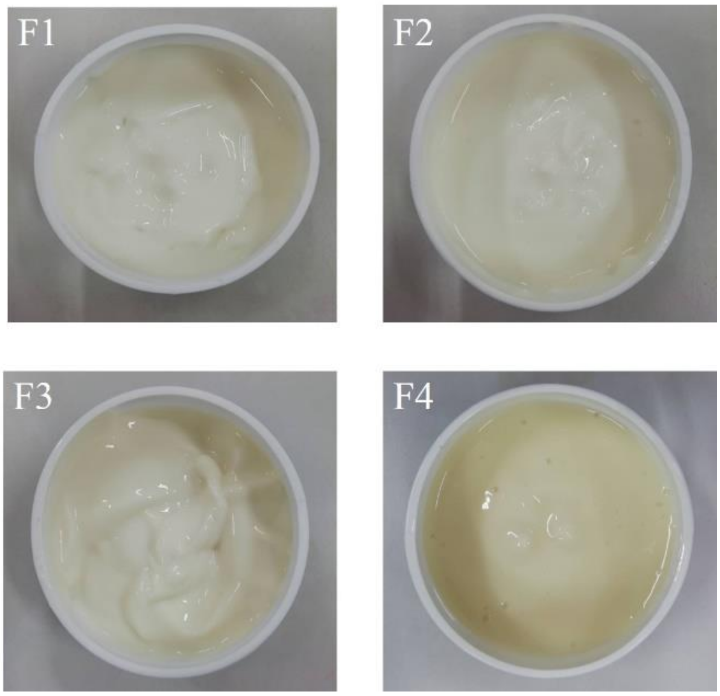

Figure 1 Appearance of the emulgel formulation, Formulation 1-4 (F1-F4).

Color parameter measurement

Color parameters of formulated emulgels in CIELab scale are shown in Table 4. The intensity of the yellow color was increased according to the increased in the concentration of *C. juncea* seed oil used. The results were in agreement of the increased in b* value. According to CIELab [16], b* indicates the intensity of color scale from blue to yellow. Negative b* value shows blue color, while positive b* value shows yellow color. Decrease in a* was observed when the concentration of *C. juncea* seed oil was increased. According to CIE, a* indicates the color scale from red to green. Negative a* value shows the green color, while positive a* value shows the red color. Since increasing in the negative value of a* was observed, this suggested that the emulgel appeared greener according to the increased concentration of *C. juncea* seed oil used. L* represents the brightness value ranging from 0 (black) to 100 (white). The brightness of the cream was decreased when the concentration of *C. juncea* seed oil increased. Nutty odor characteristic of the seed was observed in the formulation containing the seed oil, with increased odor intensity according to the concentration used. The pH of each formula was measured with pH meter. The pH of prepared formulations was in the range between 6.38 – 6.93, which are in the normal range of the commercially available moisturizers (pH 5 – 7). Addition of *C. juncea* seed oil into the extract did not affect the pH of the formulation. Viscosity of prepared emulgels was evaluated with the spindle no. 0.5 at room temperature with rotational speed at 10 rpm. The results was shown in Table 5. There was no significant effect of concentration of *C. juncea* seed oil used in the formulation on the measured viscosity. However, preference test suggested that F4 had the highest viscosity.

Table 4 Color parameters of formulated emulgels (n=3).

Emulgel formula	CIELab color scale		
	L*	a*	b*
Base	82.30 ± 0.13	-0.56 ± 0.01	-1.58 ± 0.03
Formula 1	86.74 ± 0.07	-1.77 ± 0.03	4.06 ± 0.03
Formula 2	86.12 ± 0.08	-2.34 ± 0.03	7.03 ± 0.02
Formula 3	82.65 ± 0.06	-2.64 ± 0.02	10.74 ± 0.06
Formula 4	77.57 ± 0.05	-2.97 ± 0.03	12.25 ± 0.13

Note: Color measurement was report in term of Commission International de l'Eclairage (CIE), L*a*b* scale. L* represents the brightness value ranging from 0 (black) to 100 (white). a* represents the color from green (-a) to red (+a). b* L* represents the color from blue (-b*) to yellow (+b*).

Table 5 Viscosity of emulgel (n=3)

Property	Base	Formula 1	Formula 2	Formula 3	Formula 4
Viscosity (cP)	2945 ± 35	37947 ± 260	38680 ± 92	18280 ± 20	16600 ± 20
%Torque	58.2 ± 1.3	94.9 ± 0.7	96.6 ± 0.2	44.4 ± 0.9	45.5 ± 0.9

Sensory characteristics, which were color, odor, viscosity, spread ability, greasiness, absorb ability and after feel, were rated with five-point hedonic scale of sensory evaluation. The score from 1, the least favorite, to 5, the most favorite, were given. The results are shown in Figure 2.

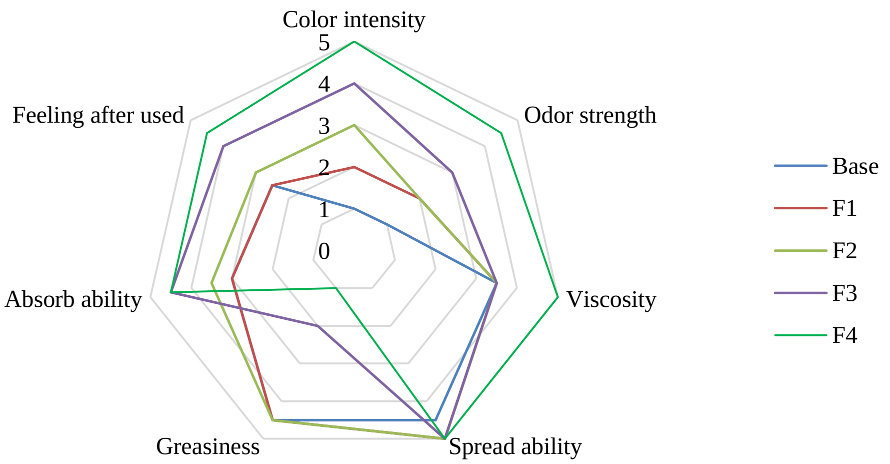

Figure 2 Sensory profile of formulated emulgels.

From Figure 2, the color intensity of the formulated emulgels increased according to the concentration of *C. juncea* seed oil used in the formula. The nutty odor of *C. juncea* seed oil also increased according to the concentration of *C. juncea* seed oil used in the formula. Since *C. juncea* seed oil had intense yellow color with unique nutty odor, increased in its concentration lead to the more intense yellow color and stronger nutty odor. For viscosity, the perceived scores for the base, F1, F2 and F3 were not different. However, the perceived viscosity of the formulated product with 1.0% *C. juncea* seed oil had the highest score. Perceived greasiness was given based on the Likert scale from 1 which is less greasy feel to 5 which was more greasy feel. The perceived greasiness was increased according to the concentration of *C. juncea* seed oil used, as F4 showed the highest greasiness on application, followed by F3 which contain 0.75% of *C. juncea* seed oil. However, the skin absorption for F3 and F4 had the highest score. This may be a result from *C. juncea* seed oil which is an emollient and can be absorbed into the skin. Finally, for the feeling after used, F4 had the highest score, which may come from the emollient effect of *C. juncea* seed oil.

Conclusions

C. juncea or sunn hemp is mainly used for mothering out weeds and soil renovation due to high nitrogen content. It is a fast-growing plant with low economic value. The *C. juncea* seed oil ethanolic extract contains high composition of the beneficial fatty acids such as linoleic acid, oleic acid, and palmitic acid which can improve skin penetration, moisturization, wound healing and antioxidants activities. The emulgel containing *C. juncea* seed oil ethanolic extract in this research can preserve the benefits of the *C. juncea* seed oil. The physical studies of all emulgel formulas showed that the formula 4, which contained 1.0 %w/w of *C. juncea* seed oil ethanolic extract, showed the better preferences and stabilities than the others. These results showed that the prepared emulgel with *C. juncea* seed oil ethanolic extract is a good candidate for further development of skin care products with active ingredients from natural sources. Moreover, these developed products may also be an approach to increase the value of *C. juncea* in the future.

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