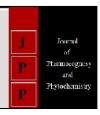


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Development of skincare formulations using flaxseed oil and mucilage

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Abstract

The majority of the peoples prefer natural products for their personal care rather than chemical products because natural products are free from harmful chemicals, improves the health of the skin, and provides the nutrients, satisfaction for users. Thus, the research has been initiated on utilizing flaxseed oil and mucilage in the formulation of cosmetic products. As a result, the Flaxseed-based Moisturizer and Multipurpose skin cream have been developed and evaluated. The formulation contains mucilage and flaxseed oil due to the highly skin beneficial fatty acid composition which is effective in many inflammatory skin disorders and the potential of water and oil holding capacity. Also, it was observed that Moisturizer and Multipurpose skin cream were reduced fine wrinkles and provided a soft and smooth surface while keeping the skin hydrated for up to 8-10 hours after application and the physiochemical parameters are appropriate for skin creams that are safe, nourishing, and naturally moisturizing.

Keywords: Skin cream, flaxseed oil, mucilage, fatty acids, water holding capacity

1. Introduction

Cosmetics are substances that are used to clean, beautify, and provide a sense of well-being. Use of cosmetics is quite old [2]. Since ancient times, women have used numerous products to improve their complexion and enhance the beauty of their eyes [3]. Today even men are not behind women in the use of cosmetic product & many specialty items for example after shave lotion, skin care & hair care products have been developed for the use of men [4]. Skin care is essential for both Men and women because, as a result of our widespread exposure to pollution, may cause oxidative stress, early ageing, inflammation, and illnesses. Excessive heat causes the skin to dry out, which can lead to the development of wrinkles, freckles, pimples, pigmentation, and sunburns. The severe winter frequently results in a variety of unpleasant side effects, including cracks, sores, maceration, and infections. Skin diseases can affect people of any age and have a variety of potential causes, including contact with microorganisms, chemical agents; biological contaminants present in the environment [1]. Thus, to prevent the skin from environmental effect and to beautify the skin, use of cosmetic product is considered as an essential component of life. Numerous issues have emerged as a result of the widespread use of cosmetics, including poor cosmetic quality, high levels of toxic substances, and the presence of various metals [7]. To improve the quality and effectiveness of these products, the formulators are utilizing various additives, including preservatives, stabilizers, mineral pigments, dye, and shine, etc. However, some of these compounds may cause allergic reactions, irritability, and harm to human health [8]. This is because natural products are highly acceptable by many customers, as they are free from synthetic chemicals and have relatively fewer side effects compared to synthetic cosmetics. [9]. Natural products the name itself suggests that these cosmetics are natural and free from all the harmful synthetic chemicals which otherwise may prove to be toxic to the skin. The plant parts used in cosmetic preparation should have properties like antioxidant, antiseptic, antibacterial, etc. The best cosmetic product must contain following properties antioxidant property, anti-inflammatory, antibiotic, whitening agent, moisturizing properties etc. [10]. Therefore, the flax seed oil and mucilage have been chosen for research due to its outstanding potential for use in skin cream, based on the aforementioned viewpoints. The plant included in the composition was chosen based on a reported scientific property.

2. Cosmetic Benefits of Flaxseed oil and Mucilage

Flax (*Linum usitatissimum*) is an annual plant in the linaceae family. Flaxseed, often known as linseed, is a seed found in the fruit.

Flaxseed has been consumed by people since the beginning of civilization [1]. Flaxseed is one of the richest plant sources of omega-3 fatty acids (ALA) and lignans (phytoestrogens). Canada, China, the United States, India, and Ethiopia are major flaxseed producers. With an output of 0.42 million tonnes in 2010 (FAO 2012), Canada is the world's top producer, accounting for approximately 80% of worldwide flaxseed commerce. With 0.15 million tonnes of total flaxseed production, India ranks fourth (FAO2012). Flaxseeds are available in two basic varieties: (1) brown (2) yellow or golden. Brown flax is better known as an ingredient in paints, varnish, fiber and cattle feed. Various edible forms of flax are available in the food market whole flax seeds, milled flax, roasted flax and flax oil. According to its physico-chemical composition, flaxseed is a multicomponent system with bioactive plant substances such as oil, protein, dietary fiber, soluble polysaccharides (mucilage), lignans, Phenolic compounds, vitamins (A, C, F and E) and mineral (P, Mg, K, Na, Fe, Cu, Mn and Zn) [12]. Flax seed contain soluble and insoluble dietary fibers in a proportion that varies between 20: 80 and 40:60. The major insoluble fiber fraction consists of cellulose and lignin and the soluble fiber fractions are the mucilage gums [35].

2.1 Flax seed oil

Flaxseed oil has low saturated fatty acid content (9%), moderate monounsaturated fatty acid content (18%), and high polyunsaturated fatty acid content (73%). The primary fatty acid in flaxseed oil is - linolenic acid, which ranges from 39.00 to 60.42%, followed by oleic, linoleic, palmitic, and stearic acids, which offer an excellent Omega-6: Omega-3 fatty acid ratio of roughly 3:1. Flaxseed oil is naturally high in anti-oxidant like tocopherols and beta-carotene. [12]. Flax oil provides hair and skin care benefits to renew and revitalize. Flax oil is known to reduce dull-looking skin and soften the appearance of fine lines and wrinkles. The natural advantages of the anti-inflammatory properties and omega-3 fatty acids oil can help with dry skin, eczema symptoms and even sunburned skin pain and reduce red skin irritation. Essential fatty acids increase moisture to keep skin soft and hydrated. Flax seed oil has been used medicinally to treat skin conditions such as acne, psoriasis, rosacea and eczema. Flax seed is high in fiber and antioxidant properties to protect and rejuvenate the skin [14].

2.2 Flax seed mucilage

Mucilage is a typical plant component that can be isolated from seeds and soft stems. Together, the flaxseed coat and endosperm comprise six layers. Mucilage comes from the secondary wall material in the outermost layer [16]. It is easily extracted from the seed coat by soaking in water. When hydrated, the mucilage cells swell, and their content exude on the surface of the seeds. Mucilage makes up approximately 8% of the total seed weight. Flax seed mucilage contains between 50-80% carbohydrates and 20% and 4% of proteins and ash. The major constituent of flax mucilage consists of two polysaccharide components, neutral and acidic. The neutral fraction contains L-arabinose, D-xylose and Dgalactose in a mole ratio of 3:5:6.2:1 and the acidic fraction contains L-rhamnose, L-fucose, L-galactose, and D galacturonic acid in a mole ratio of 2.6:1:1.4:1.7 [17]. Flax seed mucilage has unique functional properties, such as viscosity, emulsifying and foaming properties, gelation and water binding as well as on their bio-active role in the prevention and/or treatment of certain diseases. Flax mucilage also

exhibits surface activity and the ability to stabilize oil/water emulsions and foams ^[18]. It has been used in emulsion preparation in order to enhance stability. It stabilizes the emulsions by increasing the viscosity and decreasing the interfacial tension ^[15]. The properties of this plant extracts will support it, as a part of ingredient in cosmetic formulation.

3. Methodology

3.1 Material and method

Two types of cosmetic cream formulation have been carried out from flax seed;

- Moisturizing lotion/cream(S₁)
- Multipurpose cream (S₂)

3.2 Material required

Flax seed and flax seed oil were collected from the farmers in the wardha district, Maharashtra India. The other raw materials and accessories are procured form the local supplier in Wardha districts, Maharashtra India.

3.3 Sample preparation

Flax seeds were cleaned with water, dried in shade for 48 hours, crushed and milled to the desired size and then stored in air tight storage container for further work at room temperature.

3.4 Extraction of flax seed Mucilage By Heating Method

10 grams of the brown flaxseed grains were boiled in 300 ml of distilled water for 60 min at 80 -90°C while being stirred at a speed of 500 rpm on a magnetic stirrer. The seeds were then separated from the colloidal solution by filtration through Nylon cloth. In order to preserve the filter sample for future research, 1% sodium benzoate was added to an airtight container [19].

3.5 Preparation of Moisturizing cream (S1)

The five formulations of the Moisturizing cream were prepared by oil in water (O/W) emulsification process. The water phase (water soluble ingredients) and oil phase (oil soluble ingredients) were heated separately up to 80°C. The water-soluble mucilage is a component of the water phase. After heating, the aqueous phase was added to the oil phase while being continuously stirred to complete the emulsification process. The homogenized cream texture, is then cooled down and preservatives, perfumes were added. Finally, the Moisturizer is stored in air tight container for the study of stability test and other physiochemical parameters.

Table 1: Formulation of Moisturizing cream

Ingredients		Formula % w/w								
ingredients	$\mathbf{F_1}$	\mathbf{F}_2	\mathbf{F}_3	$\mathbf{F_4}$	\mathbf{F}_{5}					
Stearic acid	5	4	3	3	3					
Paraffin oil	3	9	3	0	0					
Olive oil	0	0	0	3	3					
GMS	2.5	2.5	1	1	2.5					
Flax seed oil	10	8	6	4	2					
TEA	1	1	1	1	1					
Glycerin	3	2	2	2	2					
Water	65	58	64	60	56					
Flax seed mucilage	10	15	20	25	30					
Sodium Benzoate	0.5	0.5	0.5	0.5	0.5					
Essence/perfume	Qs	Qs	Qs	Qs	Qs					
Total	100.0	100.0	100.0	100.0	100.0					

3.6 Preparation of Multipurpose cream based (S2)

The five formulations of the Multipurpose cream were also prepared by above same procedure with different composition of ingredients. In order to study stability tests and other physiochemical parameters, the Multipurpose cream is then kept in an airtight container.

Table 1: Formulation of Multipurpose Cream

Ingradiants	Formula % w/w									
Ingredients	F ₁	\mathbf{F}_2	F ₃	F4	F 5					
Stearic acid	11.5	10.5	9.5	7.5	6.5					
Olive oil	5	5	5	5	5					
Flax seed oil	20	17	15	12	10					
TEA	1.0	1.0	1.0	1.0	1.0					
Glycerin	17	17	17	12	07					
Water	35	34	32	37	40					
Flax seed mucilage	10	15	20	25	30					
Sodium Benzoate	0.5	0.5	0.5	0.5	0.5					
Essence/perfume	Qs	Qs	Qs	Qs	Qs					
Total	100.0	100.0	100.0	100.0	100.0					

4. Evaluation of Physiochemical Parameters of creams

The quality of the cream is dependent on a variety of different factors, including the types of oils and emulsions that were utilized in its production (for example, coconut oil, palm oil, olive oil, and so on). In accordance with the criteria of the Indian standards, many parameters including moisture, thermal stability, pH, total fatty substances, total residue, heavy metals (Pb, As), microbial count and skin Irritation test were examined and evaluated [22].

- **4.1 Skin Irritation Test:** The irritancy test was used to observe the allergic reactions that were caused by creams. The purpose of this test was to evaluate the irritative potential of the product when applied to the skin. The irritancy test was performed by applying cream to a small section of the left hand, and then examining that section after a period of twenty-four hours to look for any evidence of allergy or irritancy. The test was repeated for each formulation of S_1 and S_2 sample $^{[21]}$.
- **4.2 Skin sensitization test by patch-test methods:** Skin sensitization test was conducted through occlusive patch-test methods, in which an individual develops probability of reacting in an allergic (abnormal) manner to a particular substance. It is helpful to point out moderate or strong irritants. The study was done on 40 healthy people between the ages of 18 and 65, both men and women. Firstly, a 70% alcohol solution is used to clean the skin, and then the samples are placed on the skin and covered with adhesive strips. The Skin reactions are observed 15 min and 24 hr after the strips have been removed [23].
- **4.3 Skin Moisturizing Test:** Skin Moisturizing test was performed on 40 healthy volunteers, male and female, between the ages of 18 and 65. The Significant use of the creams during the one-week period and the users report was recorded.

5. Results

Analysis of chemical composition of Flax seed

5.1 Proximate composition flax seed: According to approved AOAC techniques, the moisture, ash, crude fiber,

fat, protein, and total carbohydrate (measured by difference) contents of flax seeds were determined [34].

Table 3 and 4 shows proximate chemical composition of raw material (Flax seed and its mucilage) for the formulation of cream. From this analysis it was observed that flax seed contain 10.49% moisture, 25.04% crude fiber, 24.28% fat content, 20.12% protein, 26.29% carbohydrate and 4.27% ash content were as its, mucilage contain 99.07% moisture, 4.95% ash, 0.015% protein and its pH was found to be 6.47 which is good for skin.

Table 3: Profile of Proximate Composition of flax seeds

Sr. No.	Parameters	Composition of flax seeds (%)
1.	Moisture	10.49
2.	Crude Fiber	25.04
3.	Ash content	4.27
4.	Fat content	24.28
5.	Protein content	20.12
6.	Carbohydrate	26.29

5.2 Proximate composition of flax seed mucilage

The moisture, ash and protein contents of the flax seed mucilage were determined according to official methods described by AOAC [34].

Table 4: Profile of Proximate Composition of flax seeds mucilage

Sr. No.	Parameters	Composition of flax seeds
1.	Moisture (%)	99.07
	pН	6.47
3.	Ash content (%)	0.027
5.	Protein content (%)	0.015

A study was conducted on the physicochemical properties of a multipurpose and moisturizing cream made up of flax seed oil and mucilage, and the outcomes are summarized in tables 5 and 6. The table illustrates a comparison of the five formulated samples $(F_1, F_2, F_3, F_4, \text{ and } F_5)$.

5.3 Cream removal and appearance (organoleptic evaluation)

On washing the hand with tap water, the prepared moisturizer and multifunctional creams were easily removed from the applied area. The organoleptic test revealed that the colours of all cream formulations were identical, i.e., pale yellow due to flax seed oil, and that each cream had a characteristic odour. The organoleptic evaluations of the prepared creams are listed in tables 5 and 6, respectively.

5.4 Homogeneity and smear test

The homogeneity and smear tests revealed that the moisturizing and multipurpose creams that had been prepared would have a uniform composition overall. At zero time and one-week period for three months, the homogeneity was evaluated under a various temperature condition. After three months, the prepared creams were found to be stable, and there was no noticeable change in the homogeneity of the emulsion, regardless of the temperature. In a similar manner, the smear tests that were performed on the formulations revealed that the formulation F_3 , F_4 , and F_4 , F_5 for the S_1 and S_2 samples could be absorbed quickly and have a non-greasy consistency respectively.

Table 5: Profile of Physio- Chemical parameters of Moisturizer cream

Sr.		D	Formulation									
No.		Parameters	F ₁	\mathbf{F}_2	F ₃	$\mathbf{F_4}$	F ₅					
1.		Color	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow					
2.		Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic					
3.	I	Iomogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous					
4.		Smear type	greasy	greasy	Non – greasy	Non – greasy	Non – greasy					
5.		State	Solid	Solid	Semi-solid	Semi-solid	Semi-solid					
6.	N	Moisture (%)		72.2	73.0	73.8	73.1					
7.	pН		7.8	7.4	6.8	6.4	6.4					
8.	Acid Value		6.4	6.2	5.8	5.7	5.4					
9.	Thermal stability		Passed	Passed	Passed	Passed	Passed					
10.	Total fa	atty substances (%)	20	18	16	15	14					
11.	To	tal residue (%)	26.4	27.8	27	26.2	26.9					
	Heavy metals	Lead (PPM)	Nil	Nil	Nil	Nil	Nil					
12.	neavy metais	Arsenic (PPM)	Nil	Nil	Nil	Nil	Nil					
13.	Microbial Content	Total viable count (cfu/g)	7.8×10^{2}	7.5×10^{2}	7.6×10^{2}	7.5×10^{2}	7.6×10^2					
	<u> </u>	Gram Negative pathogen(cfu/g)	BDL*	BDL	BDL	BDL	BDL					

BDL* - Below detection limit

5.5 Thermal Stability test

The thermal stability test was determined in humidity chamber/incubator at 60 to 70 percent relative humidity and 45 ± 1 °C. The sample was filled up to two third capacity of bottle and inserted the plug and tightened the cap. Kept the filled bottle in incubator at 45 ± 1 °C for 48 hrs. The results showed that there is no oil separation or any other phase separation in the formulation.

5.6 pH test

The pH of cream is an important parameter for its efficiency. The pH values must be in a range which is suitable for skin. The pH of moisturizer creams was in the range from 7.8-6.4, pH of F_3 , F_4 , F_5 sample was near to neutral hence suitable for skin (Table 5). The pH of all prepared multipurpose creams was in the range of 7.0-6.7 which is also near to neutral hence suitable for skin (Table 6)

Table 6: Profile of Physico- Chemical parameters of Multipurpose Cream

Sr.		Parameters	Formulation									
No.		rarameters	$\mathbf{F_1}$	\mathbf{F}_2	\mathbf{F}_3	$\mathbf{F_4}$	F ₅					
1.		Color	Pale Yellow									
2.		Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic					
3.		Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous					
4.		Smear type	Slightly greasy	Slightly greasy	Slightly greasy	Non – greasy	Non – greasy					
5.		State	Solid	Solid	Solid	Semi-solid	Semi-solid					
6.		Moisture (%)	36.8	36.8 36.4 35.9		37.5	38.0					
7.	pН		7.0	6.9	6.7	6.5	6.5					
8.	Acid Value		7.8	7.8 7.7		7.4	7.3					
9.	Thermal stability		Passed	Passed	Passed	Passed	Passed					
10.	Total fatty substances (%)		35	30	25	20	19					
11.	7	Total residue (%)	63.2	63.6	64.1	62.5	62					
	Heavy metals	Lead (PPM)	Nil	Nil	Nil	Nil	Nil					
12.	Arsenic (PPM) Nil	Nil	Nil	Nil	Nil							
13.	Microbial content	Total viable count (cfu/g)	6.8×10^2	6.5×10^2	6.7×10^2	6.1×10^2	6.2×10^2					
13.	Wherobial content	Gram Negative pathogen(cfu/g)	BDL*	BDL	BDL	BDL	BDL					

BDL* - Below detection limit

5.7 Total fatty substances (TFS)

Total fatty substance maintains and nourishes the dry skin and, hence, it was one of the most important ingredients that

determine the quality of creams $[^{24}]$. The TFS percentage of all prepared formulation of S_1 and S_2 sample were noted in table 5 and 6 respectively and its comparison was shown in figure 2

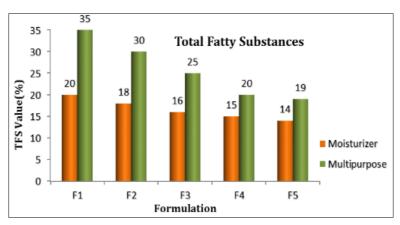


Fig 2: Comparison of TFS Value of Moisturizer and Multipurpose creams

5.8 Total residue

The total residue was determined by dried the sample at constant mass at 105 $^{\circ}$ C in hot air oven and weighed the dried sample after cooling. Table 5 and 6 indicate the total residue percentage in all formulation of S_1 and S_2 sample respectively.

5.9 Heavy metal

The heavy metal analysis was performed in Atomic Absorbtion Spectrometer to determine the presence of hazardous elements such as lead and arsenic. According to IS specifications, the maximum levels of lead and arsenic are 20 parts per million (ppm) and 2 ppm, respectively. Each formulation of samples S_1 and S_2 passed the test.

5.10 Microbial content/limit

The microbial test for Total Viable Count and the test for presence of gram-negative pathogens was illustrate in table 5 and 6 for S_1 and S_2 sample respectively. As per the IS specification the Total viable count (TVC) should not be more than 1,000 cfu/g in skin cream which was found to be in the range of 750-780 and 610-680 cfu/g in S_1 and S_2 sample respectively. While gram-negative pathogens should be less than 10 cfu/g, both samples were found to be below the detection limit.

5.11 Skin Irritation Test

The prepared moisturizer & multipurpose creams were non-irritant, caused no side effects on the applied area after 24 h of application in formulation F_3 , F_4 and F_4 , F_5 respectively, as shown in table 7 and 8.

Table 7: Scale for assessing the skin reaction to Moisturizer and Multipurpose cream

Sr. No	Skin reaction	Moisturizer creamMultipurpose cream										
		\mathbf{F}_1	\mathbf{F}_2	F ₃	F ₄	F 5	\mathbf{F}_1	\mathbf{F}_2	F ₃	F ₄	F 5	
1.	No Reaction	0	0	0	0	0	0	0	0	0	0	
2.	Very slight reaction	1	1	0	0	1	1	1	1	0	0	
3.	Slight reaction	ı	ı	ı	ı	ı	ı	1	ı	ı	_	
4.	Moderate reaction	ı	ı	ı	ı	ı	ı	1	ı	ı	_	
5.	Severe reaction	_	_	_	_	_	_	_	_	_	_	

5.12 Skin sensitization test by patch-test methods

To determine whether a given product is causing allergic skin irritation, a patch test is typically performed (contact dermatitis). Patch tests can identify delayed allergic reactions, which may not manifest for a few days $^{[26]}$. The profile of patch test of all developed formulation of S1 and S2 sample was depicted in table 8. The $F_3,\,F_4$ and $F_4,\,F_5$ formulation of S1and S2 sample shows no redness, edema, Inflammation and irritation during irritancy studies. These formulations are safe to use for skin.

 Table 8: Profile of patch test for Moisturizer and Multipurpose

 cream

Sr. No	Skin reaction	Moisturizer creamMultipurpose cream										
		\mathbf{F}_1	\mathbf{F}_2	F 3	\mathbf{F}_4	F 5	\mathbf{F}_1	\mathbf{F}_2	F ₃	\mathbf{F}_4	F 5	
1.	No Reaction	ı	ı	0	0	0	ı	ı	ı	0	0	
2.	Discrete Erythema	1	1	0	0	0	1	1	1	0	0	
3.	Moderate erythema	-	-	-	-	-	_	_	_	_	_	
4.	Intense erythema	_	_	_	_	_	_	_	_	_	_	

^{* 0-} No reaction, 1 – Mild reaction

6. Discussion: According to the results of the proximate analysis of Flaxseed oil and mucilage, which compared the

results to the standard values and the sources of 25.04% crude fibre, 24.28% fat content, and 20.12% protein could be beneficial for the skin. The homogeneity test of S₁ and S₂ sample showed that the prepared creams had uniform texture. The thermal stability test showed that there is no oil separation or any other phase separation in the formulation. The organoleptic evaluation showed that all formulations of S_1 and S_2 sample have same color i.e. pale yellow in color due to presence of flax seed oil. All creams had a characteristic acceptable odor and semi-solid state for F₃, F₄, F₅ and F₄, F₅ in S_1 and S_2 sample respectively. The smear test confirmed that the formulations F₃, F₄ in S₁ sample and F₄, F₅ in S₂ sample was non-greasy in nature and had good moisturizing effects. The pH of F₃, F₄ and F₄, F₅ formulation of S₁and S₂ sample was observed to be 6.8 and 6.7 which suitable for skin. Comparatively, it was found that both creams entire formulations stayed homogenous and did not vary in pH over the course of a week at various temperatures. As an emollient, fatty acids improve skin-hydration by sealing in the skin's moisture and reducing evaporation [31]. In sample S1 (F3), the total fatty substances were determined to be 16%, while in sample S2 (F4), they were found to be 20%. The heavy metal test findings show that neither the S1 sample formulation nor the S2 sample formulation contain any lead or arsenic. The microbiological test showed total viable count less than 1000 cfu/g and gram-negative pathogen is below detection limit. The irritancy and Skin sensitization test by patch-test methods confirmed that the prepared formulations F₃, F₄ in S₁ sample and F4, F5 in S2 sample did not showed the evidence of allergic reaction and skin irritation, which indicated that the formulated both skin creams are very safe to apply on the all type of skin.

7. Conclusion

The present work concluded that, economically based skincare cream containing flax seed oil and flax seed mucilage can be prepared by simple method and ingredients. The creams were made with safer chemicals and natural ingredients, so they are found excellent for the skin. The formulation of flax seed based Moisturizing cream and Multipurpose cream provides a good base for treating the skin and skin related disorder. Five formulations were prepared with different ratio of flax seed oil, flax seed mucilage, to get cost effective product. Out of five formulations; F3 and F4 in S_1 sample (Moisturizer) and F_4 and F_5 in S_2 sample (Multipurpose) shows a creamy, soft appearances with satisfactory result of all test. From the experiment it could be concluded that flaxseed oil and mucilage were economically useful sources for cosmetic skin care formulations. On the basis of this work, it is possible to develop flax seed mucilage as a cosmetic emulsifier and gelling agent in further study.

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