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Formulation and Evaluation of Moisturizing Cream using *Amaranthus Cruentus Seed* oil

Miss. Riya A. Patil[1], Dr. Suchita G. Mahalle[1] & Mr. Aditya S. Pawar[2]
Department of Cosmetic Technology,
R. C. Patel Institute of Pharmaceutical Education and Research. Shirpur, Dhule. (425405)
Email id: 11adityam@gmail.com

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Abstract

Our world is geographically divided into 7 Continents and all continents' climatic conditions are different and in that our Asia also has many regions and their climatic conditions also vary from one another. Indian climate is generally divided into 3 seasons, summer, rainy, and winter. The winter seasons are mainly responsible for making our skin dry and dehydrated. Skin is the most exposed and largest organ of our body. Skin plays a very important role in protecting our body from physical, chemical &biological assailants and it helps to reduce excess water loss of our body. Climatic conditions and surrounding environment make our skin dry and un-flexi, it needs to be moisturized and hydrated as always for its better health and it's also dependents our daily activities and our daily diet. Amaranthus Cruenthus is an herbaceous plant that reproduces by seeds and its growing period is 4-6weeks. It has a large central root, thick stem, and straight & branched herb, its matured flower generally in red color, each flower containing 50000 seeds. These seeds are rich in zinc, copper, selenium, flavonoids & Squalene. Squalene is an acyclic polyunsaturated hydrocarbon of triterpene nature (C30H50) containing 12 double bonds. Squalene is a great emollient, it's quickly and efficiently absorbed deep into the skin and helps the skin to stay healthy, provides suppleness, flexibility, and hydration without leaving any oily residue. This Amaranthus Cruentus oil purity test performs a phytochemical screening, which finds the presence of a flavonoid, phenolic acid, Gallic acid, fatty acids, omega-3, omega-6 & Squalene, etc.

Keywords: Amaranthus Cruenthus oil, moisturizing agent, Squalene.

Introduction

The skin is the biggest organ of our body, it's made by many layers of tissues & muscles. The skin contains hair follicles, sweat glands, sebaceous glands, Blood vessels, connective tissue, veins, arteries, arrector pili muscle, and sensory nerve. Skin performs various functions like sensation, protection, Heat regulation, absorption, excretion and also helps to reduce excessive water loss of ourbody. The skin helps to protect our body from any physical, chemical, and biological assailants. A skin forms a waterproof layer on this surface, it helps to protect against UV light & dehydration; it acts like a water-resistant barrier, water is the most important for skin gloss. Water makes the horny layer of healthy skin, skin contains 10-20% of water in it. Without the use of natural moisturizer factors, these water molecules easily evaporate on the surface and make it dehydrate, un-Flexi or dry; the innermost skin layer supplies water and normal sweating maintaining the skin moisture level. But mostly our hand, neck, face, legs, etc. parts are exposed to the environment and they suffer from dryness, anhydrate. When the winter season comes this is a big problem for skin to avoid dryness because in winter environmental humidity is very low. [1, 2, 3, 4.]

For this big skin problem, the solution is the natural moisturizer; that is essential to avoid dry, un-Flexi, anhydrate skin. Natural moisturizer gives an emollient, hydrated, smooth, glossy, Flexi effect to the skin. Amaranthus cruentus seed oil for natural moisturizer of skin. This oil is extracted from Amaranthus cruentus matured seeds this oil contains many phytochemicals that are very good for our body. Amaranthus cruentus seed oil contains antioxidants, anticancer, anti-allergic, antihypertensive, moisturizing properties, its contain zinc, copper, selenium, flavonoids, Gallic acid, phenolic acid, omega-3, omega-6, and it contains

Squalene. Squalene acts as a very good moisturizing agent as well as it can easily penetrate to the deep skin and gives anemollient effect without leaving an oily residue.[5,6.]

Fig no.1 Dry & hydrated skin.

Fig no.2. Dry skin.

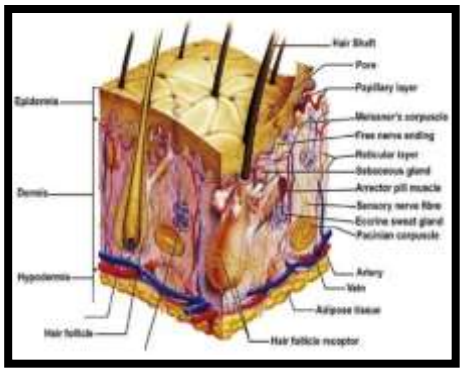


Fig no.3. Skin diagram.

1.2 About active

Active name: Amaranthus cruentus LCommon name: Rajgira

Family: *Amaranthaceae*

Genus: Amaranthus L.

Fig no 4. Amaranthus cruentus seed & oil

It is the fully mature flowered seeds extracted by the solvent extraction techniques i.e. n-hexane Soxhlet extraction technique byusing Amaranthus cruentus seeds belonging to the **family** *Amaranthaceae.*

Amaranthus cruentus is a yearly herb that reproduces seeds by taken a short period of 4 to 6 weeks, it produces one large central root and thick straight small branches it's 0.1 to 2.0 M in height greenish to red-dyed leaves and their shape varies from ovate to rhombi-ovate. Small fine hairs cover leaf and steam surfaces. Its immature flowers are greenish-red and when they matured they turn into a reddish color. Each flower contains around 50000 seeds. Amaranthus are cultivated a very long time ago in central & Latin America, South America, Europe, Russia, India, Nepal, North America, China, etc. Amaranthus seeds are rich in nickel, chromium, zinc, copper (1.25mg/100g), and selenium. They contain iron (29.35 mg/100g) manganese (425.2 mg/100g) it also contains flavonoids,phenolic acid, Gallic acid, fatty acids 24.58-24.84, palmitic acid (20.34-20.68%) linoleic (43.0-43.21%) and oleic acids (22.57- 22.39%) the oils from Amaranthus cruentus grain have been reported to contain a larger amount of Squalene (2.4-8.0%) than other common vegetable oils.[7,8,9,10.]

1.3 Chemical composition of *Amaranthus cruentus* oil. [7]

Table no.1 Chemical composition of Amaranthus cruentus seed oil

Composition	Quantity	Composition	Quantity
Unsaponifiable residue	8.5 %	Stearic acid	3.9%
Squalene	6.8 %	Palmitic acid	20.50%
Saturated total fatty acid	26.1 %	Linoleic acid	38.15%
Monounsaturated Fatty acids	34.7 %	Omega 6	315.9 mg/g
Polyunsaturated total fatty acid	38.9%	Omega 3	6.96 mg/g
Oleic acid	32.1%	Phytosterols	24 mg
Alpha tocopherol	18%	Delta tocopherol	32%

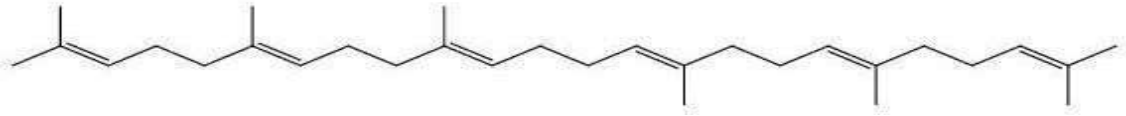


Fig No 5. Chemical Structure of Squalene

Benefits of the product:

- Squalene is a great skin moisturizer. And it is also a natural emollient and similar to skin oil so they lock moisture into your skin and control excessive oil secretion.
- This moisturizing cream helps to prevent fine lines, and ease dry patches.
- Squalene provides the perfect combination of hydration, SPF, and coverage to give your face a natural-looking, clear complexation.
- Boosting hydration can help your skin appear plumper and healthier.
- Antioxidants like vitamin E are present in this cream to fight skin damage and free radicals, which can stop fastening theaging process.[11]

Material methods

2.1. Materials

Amaranth seed oil was procured from Shri Hari Aromatics, Pvt. Ltd., Delhi (India). Stearic acid, Cetyl alcohol, Sodium Benzoate, Triethanolamine, and other excipients were purchased from SD Fine Chemicals Pvt Ltd, Mumbai.

2.2. Methods

2.2.1 Analysis of Amaranth seed oil.

Physical analysis.[13]

Colour- clear yellow-goldish colorOdour- pleasant seed like
Taste- agreeable.
Melting point- -27^{o} C.
Solubility- Soluble in alcohol and fixed oil

2.3 Preliminary Phytochemical Screening

All the extracted oils were subjected to preliminary phytochemical screening for evaluation of phytochemical constituents such as fats and oil, flavonoids, vitamins, fatty acid using the standard procedure of analysis [13,18,19,20]. phytochemical screening of the Amaranthus seed oil

Qualitative phytochemical tests were carried out to identify some bioactive components of the oil. The main bioactive groups (flavonoids, fats & fatty acids, vitamins) were identified in each oil sample using different standard methods.

Test for flavonoid [14-15]

Shinoda test:

5ml of 95% ethanol, 2 drops of conc. HCl and 0.5g of magnesium were added to 2mg of oil. The appearance of pink color indicates thepresence of flavonoids.

Fats and oil test [16]

Spot test:

A drop of each extract was placed between folds of filter paper and pressed. Permanent stains of oils on filter paper indicatethe presence of fats and oil.

Test for vitamin E determination [17]

Weigh accurately about 0.5 gm of a sample, dissolve it in 100ml of 0.25 M ethanolic sulphuric acid, add 20 ml of water and 0.1 mlof 0.25% w/v solution of diphenylamine in sulphuric acid and titrate with 0.01M ceric ammonium nitrate until the blue color is produced that persist for at least 5 seconds. Repeat the operation without the substance being examined. The difference in titration values represents the amount of ceric ammonium nitrate required.

Each ml of 0.01m ceric ammonium nitrate is equivalent to 0.002154 g of tocopherol.

Determination of Squalene [22] Isolation of the Unsaponifiables

The extracted *Amaranthus* oil was used for Squalene isolation and purification by two steps. The first step was to remove the saponifiable. *Amaranthus* oil (3 g) was dissolved in a mixture of 30 mL of 95% ethanol and 5 mL of 50% KOH and refluxed for 1 h. The reaction mixture was transferred to an extraction cylinder and washed to 40 mL with 95% ethanol and then diluted to 80 ml with distilled water. The unsaponifiable was extracted five times, using 50 mL of petroleum ether each time. The combined extracts were washed using 25 mL portions of 10% ethanol in water until the wash solution no longer gave a pink color after the addition of phenolphthalein solution (1% in ethanol, wt/wt). The petroleum ether layer was dried by anhydrous sodium sulfate. Thesolution was filtered, and the solvent was removed in a rotary evaporator. The unsaponifiable fraction in the oil was then expressedin weight percent

Column Chromatography.

The unsaponifiable were further purified for squalene by column chromatography on a silica gel (24 g, 70-230 mesh, Sigma Co.) column. A solution of 0.18 g of unsaponifiable in 5 mL of petroleum ether was loaded and eluted by washing the column with 1% diethyl ether in petroleum ether, at a flow rate of approximately 1.0 mL/min. Test tubes (10 mL with screw cap) were used for fraction collecting. Thin-layer chromatography (TLC) was used for detection. Squalene appeared completely in the 1% diethyl etherin petroleum ether eluate. The eluate was evaporated by vacuum evaporation to give colorless squalene liquid. The residues in the column were washed out by chloroform. The results are interpreted in graph no-1.

2.5 Selection of base

Cosmetic moisturizing cream is a high-level performance product based on water and oil. The use of the application to achieve the effect of cosmetics that contain physical satisfaction Results will be seen instantly Squalene is a great skin moisturizer, and it is also natural emollient and similar to skin oil so they lock moisture into your skin and control excessive oil secretion Due to Squalene, it provides the perfect combination of hydration, SPF and coverage to give your face a natural-looking, clear complexation. Boosting hydration can help your skin appear plumper and healthier.11]

2.6 Preparation of base

1. All the apparatus should be washed and cleaned properly.
2. All ingredients were weighed properly in a separate phase.
3. Oil in water type emulsion cream was prepared by initially melting the oil phase at 70-80°C, ingredients such as Stearic acid, Beeswax, Mineral oil, and Cetyl alcohol.
4. Along with it, in another beaker, aqueous phase propylene glycol, isopropyl myristate, Triethanolamine, glycerin, and water and preservatives were heated at the same temperature.
5. Both the phases are mixed to form homogenous dispersion.
6. Perfumes and Amaranthus oil were added when the temperature goes down to 35°C.
7. Then the preparation was transferred into the container and it was examined for physical evaluation for 24 hours at 25°C [11,12]

Table No.2. Formulation of moisturizing Cream.

Sr. No.	**Ingredients**	**Quantity 100%**		
		A	**B**	**C**
1	Stearic acid	7gm	6 gm	5 gm
2	Cetyl alcohol	2 gm	1 gm	2 gm
3	Beeswax	2 gm	2 gm	1 gm
4	Mineral oil	4 gm	3 gm	2 gm
5	Isopropyl myristate	3 ml	3 ml	2 ml
6	Glycerine	6 ml	4 ml	6 ml
7	Triethanolamine	1ml	1 ml	1 ml
8	Sodium benzoate	0.25gm	0.50gm	0.50 gm
9	Distilled water	75.2ml	78.50ml	77.50ml
10	Perfume	Q.S	Q.S	Q.S
11	*Amaranthus cruentus* Seed oil	3 ml	2 ml	3 ml

Table No.3. Formulation of Moisturizing Cream.

Sr. No.	Ingredients	Quantity 100%
1	Stearic acid	5%
2	Cetyl alcohol	2%
3	Beeswax	1%
4	Mineral oil	2%
5	Isopropyl myristate	2%
6	Glycerine	6%
7	Triethanolamine	1%
8	Sodium Benzoate	0.50%
9	Distilled water	77.50%
10	Perfume (Odour)	Quantity sufficient
11	*Amaranthus cruentus* Seed oil	3%

Fig No 6. Formulated moisturizing cream.

3. Analysis of Cream Base -The analysis of the product was carried out by the following methods. [11]

A) Physical Appearance

1. Colour
2. Odor
3. Consistency

B) Determination of pH
C) Determination of Thermal Stability
D) Determination of Total Fatty Matter
E) Skin irritation test
F) Spreadability
G) Determination of Viscosity:
H) In- vitro occlusivity test
I) Determination of moisturizing property of cream

A) Physical Appearance

1) Colour and Appearance**:** The color and appearance of the formulation were observed visually.

2) Odor: The Odour of the formulation is pleasant/characteristic.

3) Consistency: It is found to be semi-solid with visual observation.

B) Determination of pH

The pH of the developed cream base was measured on a standardized digital pH meter at room temperature by taking an adequate amount in a 50 ml beaker.

C) Determination of Thermal Stability

Thermal stability (20^0C, 30^0C, and 40^0C) of the preparation and formulation was determined according to (BSI) Indian standard guideline.

D) Determination of Total Fatty Matter

Weigh accurately about 2 g of the material into a conical flask, add 25 ml of dilute hydrochloric acid, fit a reflux condenser into the flask, and boil the contents until the solution is clear. Pour the contents of the flask into a 300-ml separating funnel and allowit to cool to 28^0C. Rinse the conical flask with 50 ml of petroleum ether in portions of 10 ml. Pour the Petroleum ether rinsing into the separating funnel, shake the separation funnel well and leave until the layers separate. Separate the aqueous phase and shake it out with 50 ml portions of Petroleum ether twice. Combine all the Petroleum ether extracts and wash them with water until free of acid (when tested with methyl 1 orange indicator solution). Filter the Petroleum ether extracts through a filter paper containing sodium sulphate into a conical flask which has been previously dried at a temperature of 90 + 2^oC and then weighed. Wash the sodium sulphateon the filter with Petroleum ether and combine the washing with the filtrate. Distill off the Petroleum ether and dry the material remaining in the flask at a temperature of 90 + 2^oC to constant mass.

CALCULATION

Total fatty substance, percent by mass = 100(M1/M2)Where,

M1 = mass in g of the residue,

M2 = mass in g of the material taken for the test.

E) Skin irritation test

An irritation test was performed on human volunteers with their consent. Five volunteers were selected and 1.0 g of formulated cream was applied on an area of 2 square inches to the back of the hand. The volunteers were observed for lesions or irritation.

F) Spreadability

The spreadability of test samples was determined using the following technique: 0.5 g test formulation was placed within a circle of 1cm diameter pre-marked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. Spreadability refers to the area covered by a fixed amount of cream sample after the uniform spread of the sample on the glass slide. The increase in the diameter due to the spreading of the test formulation.

G) Determination of Viscosity. [21]

Apparatus: Brook field viscometer DV2T model.

Procedure: The cream sample (50g) was placed in the sample holder of the viscometer and allowed to settle for 5 min, and the viscosity measured at a rotating speed of 10,20,30,40,50,60,100 rpm at room temperature (25–27°C). An average of three triplicates was computed.

H) in-vitro occlusivity test [20]

Beakers having a height of 4.6 cm and a diameter of 3.2 cm were taken. For performing the test 10 ml distilled water is poured in each beaker and the open end of the beaker s is closed by the Whatman filter paper 0.45 pore size on the upper surface of one of this paper 200mg of sample was evenly distributed. After this, these two beakers were placed in conditions like at 37-+2 C/607_+5% RH for 48 hrs. And another beaker was kept uncovered in the same conditions.
The occlusion factor F was calculated as,
F = (A – B)/A .100
Where,
A = water flux through an uncovered filter (percent water loss)
B = Water flux through the filter when covered with test preparation (% water loss).

I) Determination of moisturizing property of cream [23]

The skin moisture content was measured on the based Corneometer also called as Capacitance method. The moisture content of the stratum corneum is measured with a skin capacitance meter (Corneometer CM 825). The instrument determines the moisture contentof the superficial epidermal layers, down content to a depth of about 0.1 mm, and the values are obtained in arbitrary units.[23]
In experimentation, the Corneometer is a technique used to determine the Corneum hydration before and after application of the cosmetic product. It is the most reliable, easy, and effective method, used in industries for evaluation of moisture content determination and gives reading and prepared graphs.

Procedure –
1) The evaluation of moisture content is done on the inner forearm of the selected volunteer.
2) The inner forearm of the volunteer was cleaned.
3) 5 Blocks of 2X2 cm were drawn on the inner forearm of the volunteer, for blank (Skin Moisture), Control (Base), 1%, 2%, and 3% withactive respectively.
4) The probe head was placed vertically on the skin surface on marked sites with little pressure for one second and reading as displayed by Corneometer was noted as Blank.
5) As such readings were taken for Blank, Control, 1%, 2%, and 3% samples at 0 minutes, 30 minutes, and 60 minutes.
6) After every interval, the average was taken and a graph was plotted.

Fig.No.7 Corneometer CM 825

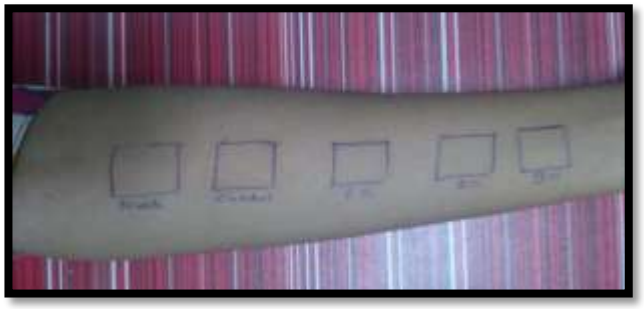
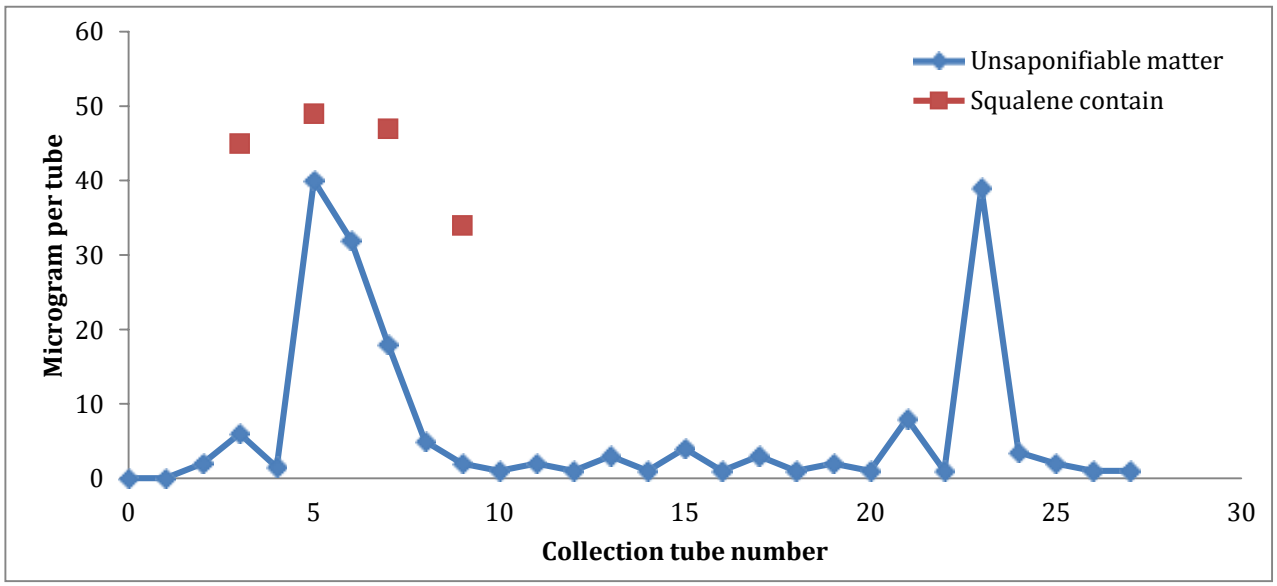


Fig.No.8 Moisture content determination using Corneum meter on inner forearm of a volunteer

Result

Table No 4. Phytochemical screening of the Amaranthus cruentus seed oil

Sr.no.	Phytochemical	Test	Result
1.	Flavonoid	Shinoda test	+ pass
2.	Vitamin E	Vitamin E test	+ pass
3.	Fat	Spot test	+ pass
4	Determination of Squalene	CC,TLC	+ pass



***Graph 1*- a ration of the unsaponifiable matter of *Amaranthus cruenthus* seed oil by silica gel coloum. (0-10 diethyl ether and 11-28 chloroform assolvent**

Table.No.5. Evaluation parameters

Sr .No.	Parameters	Results		
		1 Week	2 Week	3 Week
1.	Colour	White	White	White
2.	Odor	Pleasant	Pleasant	Pleasant
3.	Consistency	Semi-solid	Semi-solid	Semi-Solid
4.	pH	5.5	5.6	5.6

Evaluation of cream according to BIS

Table.No.6. Evaluation of cream according to BIS

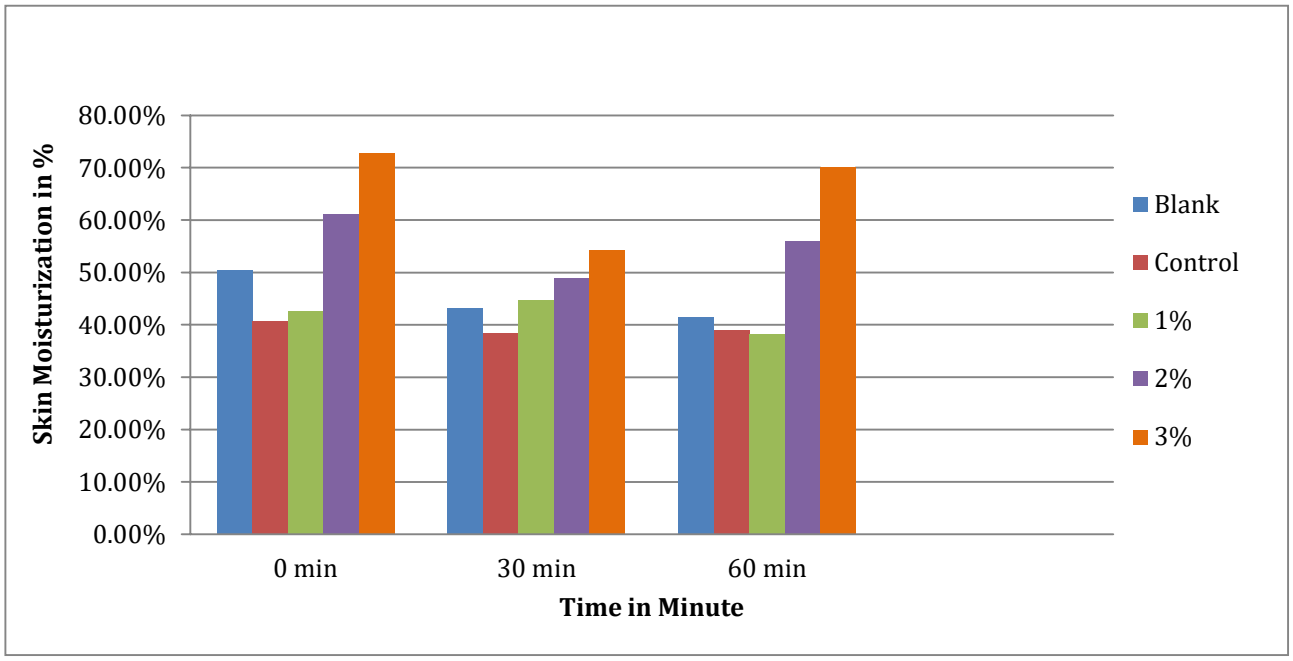
Sr.No.	Parameters	Results
1.	Colour	White
2.	Odor	Pleasant
3.	Consistency	Semi-Solid
4.	pH	5.7
5.	Spreadability	6.74 g.cm/sec
6.	Skin irritation test	Non-irritant
7.	Thermal stability	To pass the test(20,30,40^{0}C)
8.	Total fatty matter	4.8
9.	In- vitro occlusivity %	32.06_+2.86
10.	Determination of Viscosity	1.350 poise.

Determination of moisturizing property of cream

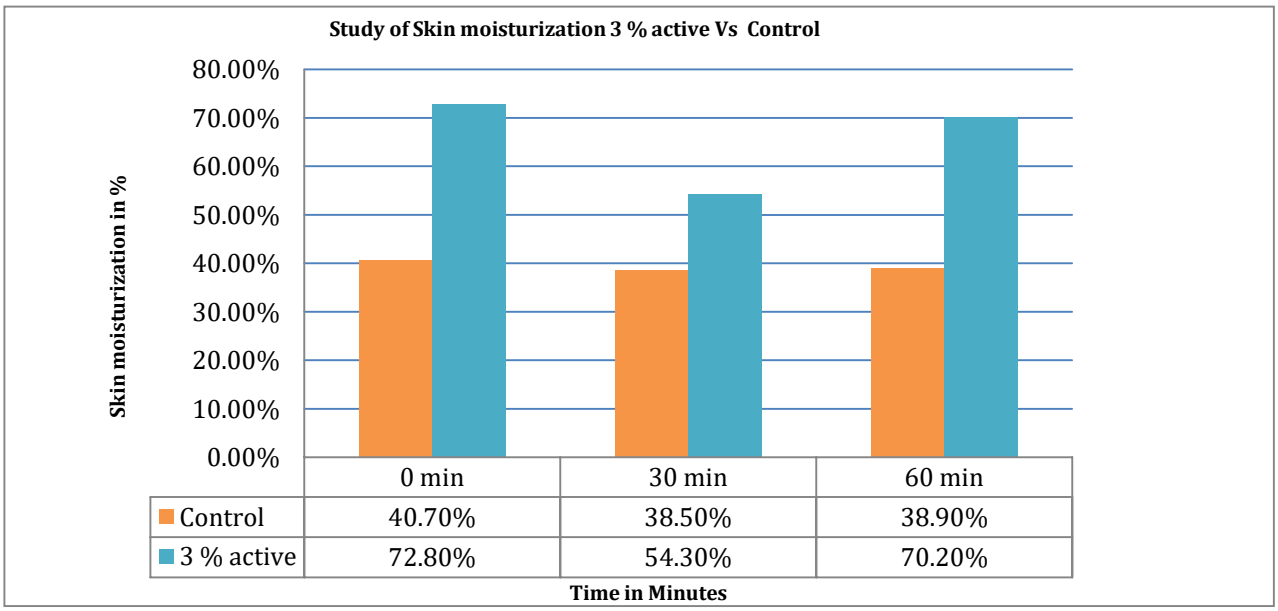
Observation and Result-

Table no.7. Study of moisturizing property of moisturizing cream with Amaranthus cruentus seed oil.

Volunteer	ntration ofactive	Moisture content in % against time		
		0 min	30 min	60 min
Volunteer-1	Blank	50.5%	43.2%	41.5%
	Control	40.7%	38.5%	38.9%
	1%	42.6%	44.7%	38.2%
	2%	61.2%	48.9%	56%
	3%	72.8%	54.3%	70.2%
Blank: Skin, Control: Base without active				



Graph 2- Water content of stratum corneum at 0 min, 30 min and 60 min after the application of the formulation with active.



Graph 3- The skin miniaturization difference between 3% Active and Control product.

Result

Preliminary phytochemical screening showed the presence of Squalene, fatty acids, fat, flavonoids, and vitamin-E Amaranthus cruentus seed in the extract which is recorded in table no.4. The cream formulated was evaluated as per BIS standards for thermal stability, pH, viscosity, spreadability, etc. Moisturizing cream showed good stability and no change in color, pH, and viscosity at different temperatures. The skin hydration was determined by the Corneometer. It was observed that the cream containing 3% active(Amaranthus Cruentus seed oil) has more hydration property as compared to the control and other listed concentration. After performing this experiment and interpreting the result it was found that 3% moisturizing cream gives a better moisturizing effect. Thus, it was proved that the prepared product showed good cosmetics desired properties and moisturizing property to human skin.

Discussion

The standardization of exact was done by using standardized methods such as phytochemical screening and organoleptic properties. The phytochemical study of Amaranthus cruentus seed oil showed the presence of Squalene, fat, fatty acids, flavonoids, vitamin E. The present study demonstrated that Amaranthus cruentus seed oil has moisturizing properties. The moisturizing property was determined with help of a Corneometer by measuring the skin moisture content. The active is incorporated at different concentrations 1%, 2%, and 3% then skin moisture content study compared with Blank and Control. After conducting this test the result was found that 3% moisturizing cream gives the better result of moisturization.

Conclusion

The present study showed Amaranthus seed oil contains Squalene, fat, flavonoids, fatty acid, and vitamins E which are responsiblefor the moisturizing property. Moisturizing cream with 3% Amaranthus Cruentus seed oil gives good moisturization as comparedwith control (simple cream base). Thus, it was concluded that the prepared product showed good cosmetics desired properties and moisturizing property to human skin. Further, the result of the present study demonstrated that Amaranthus cruentus is one of the Amaranthus species that merit more investigation, research and can be used in various cosmetics for other properties.

Acknowledgment

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