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Formulation and Evaluation of a Moisturizing Cream Using *Aegle Marmelos* Leaves Extract

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Abstract: ***Aegle marmelos (Linn) Correa commonly known as Bael belonging to family Rutaceae, is a moderate sized, slender and aromatic tree, widely available in India with great known medicinal uses. The known potential pharmacological activity of the leaves of Aegle maermelos are hypoglycemic, anti-inflammatory, antimicrobial, anticancer, chemopreventive and anti-oxidant activity due to its rich content. Phytochemical screening of Aegle marmelos leaves extract confirmed the presence of alkaloid, saponin, flavonoid, phytosterol, tannins, phenol, fatty acid, phenolic compound. It was thought worthwhile to explore its application in skin care as a Moisturizer. The objective of the present study was to analyse the phytochemical composition and moisturizing property of Aegle marmelos. The focus of the study was also to formulate a moisturizing cream using Aegle marmelos extract and evaluate its moisturizing property. A simple cream base was formulated by taking different trials and evaluated. The cream base showing the best results with respect to pH and thermal stability was used for the incorporation of the active ingredient in different concentrations and was then evaluated for its moisturizing property using Corneometer.***

Keywords: *Aegle marmelos*, Moisturizing property, Phytochemical analysis, Skin hydration analysis, Stability study

1. Introduction

Aegle marmelos (L) Correa commonly known as Bael or Bilva belonging to family Rutaceae has been widely used in indigenous systems of Indian medicine due to its various medicinal properties. *Aegle marmelos* tree is held sacred by Hindus and offered in prayers to deities Lord Shiva and Parvati and thus the tree is also known by the name Shiva duma (the tree of Shiva). The Bael tree has its origin from Eastern Ghats and Central India. It is also Indigenous to Indian subcontinent and mainly found in tropical and subtropical regions.[1]

Bael has been known to be one of the most important medicinal plants of India. More than 100 phytochemical compounds have been isolated from various parts of plant, namely phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, and tannins. These compounds are well known to possess biological and pharmaceutical activity against various chronic diseases such as cancer and cardio vascular and gastrointestinal disorder. Antioxidant, Antiulcer, Antidiabetic, Anticancer, Anti-inflammatory, Antimicrobial effects have been reported on various animal models by crude extract of this plant. Every part of *Aegle marmelos* plant such as fruit, stem, bark and leaves possesses medicinal property and is used for treating various eye and skin infections. Leaf is considered to be one of the highest accumulatory parts of plant containing bioactive compounds which are synthesized as secondary metabolites. However there is little information about the uses of Bael in external care for properties like moisturization. The present study was, therefore, aimed at evaluating the phytochemical potential and moisturizing property of *Aegle marmelos* aqueous and chloroform leaf extract.[2]

2. Material and Method

2.1 Collection of *Aegle marmelos*:

The fresh leaves of *Aegle marmelos* were collected from the local market, Nagpur.

2.2 Authentication of Herb:

The leaves were authenticated botanically from the Department of Botany of Rashtrasant Tukdoji Maharaj Nagpur University' *Aegle marmelos: 10126*

Slide 1: Authentication Certification of Leaves of *Aegle marmelos*

2.3 Preparation of Extract:[2]

The fresh leaves of the plant were taken washed with water and grounded into a paste for further extraction

2.3.1 Extraction with water (Active A):

- 300g of fresh *A. marmelos* leaves paste prepared above was taken.
- The leaves were extracted with water for 12 hours at room temperature.
- Extraction was carried out by Maceration method.
- The supernatant after 12 h was filtered out.
- The extract was labelled as Active A (Liquid form).

2.3.2 Extraction with chloroform (Active B):

- The supernatant was filtered out and leaves were air dried for further extraction process with chloroform.
- The extraction method was carried out by Maceration method.
- This process was repeated successively with chloroform for 72 hour at room temperature until the color of the extract appeared pale.
- The extract obtained was filtered using Whatmann No.1 filter paper.
- The extract was dried on water bath until the constant weight with dry mass was obtained for solvent extract.
- This residual extract was stored in refrigerator at 4°C in small sterile glass bottle and labelled as Active B (Dried form)
- The percent extractive values were calculated by the following formula.

Percent extract = weight of dried extract/weight of leaf materia×100

2.4 Phytochemical Screening of *Aegle marmelos* Extract:[5,9]

Qualitative phytochemical analysis of both the extracts was performed by following the protocol.

2.4.1. Tannins

200 mg of plant material was boiled in 10 ml of distilled water and few drops of $FeCl_3$ were added to the filtrate; a blue black precipitate indicated the presence of tannins.

2.4.2. Alkaloids:

- Mayer's test:

To a few ml of filtrate, a drop or two of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate indicated the test as positive.

- Wagner's test:

To a few ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish- brown precipitate confirmed the test as positive.

- Hager's test:

To a few ml of extract, 1 or 2 ml of Hager's reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive.

- Dragendorff's test:

To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent was added. A prominent yellow precipitate indicated the test a positive.

2.4.3. Saponins (frothing test):

5ml distilled water was added to 200mg of plant material. 0.5ml filtrate was diluted to 5ml with distilled water and shaken vigorously for 2 minutes. Formation of stable foam indicates the presence of saponins.

2.4.4. Cardiac Glycosides(Keller-Killani test):

2ml of filtrate was treated with 1 ml of glacial acetic acid containing few drops $FeCl_3$. Conc. H_2SO_4 was added to the above mixture giving green color depicting the positive result for presence of cardiac glycosides.

2.4.5. Phytosterols:

The extract (50mg) was dissolved in of 2ml acetic anhydride. To this, 1 or 2 drops of concentrated sulphuric acid was added slowly along with the slides of the test tube. An array of color change shows the presence of phytosterols.

2.4.6. Terpenoids:

To 200mg plant material 2ml of chloroform and 3 ml of concentrated sulphuric acid were carefully added. A reddish brown coloration signifies the presence of terpenoids.

2.4.7. Flavonoids:

To the aqueous filtrate 5ml of dilute ammonia solution was added, followed by concentrated H_2SO_4. A yellow coloration indicated the presence of flavonoids.

2.4.8. Phenolic compound (Ferric chloride test):

The extract was dissolved in 2ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green color indicated the presence of phenolic compounds.

2.4.9. Fatty acids

- 1 g of Sudan III is mixed with 5 ml of distilled water and mixed with 1 ml of extract. The appearance of dark red oil droplet in the upper layer indicates the presence of fatty acids.
- A few drops of 0.5N alcoholic potassium hydroxide solution were added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Table 1: Phytochemical Screening of *Aegle marmelos* Leaves Extract:

Sr. No.	Test	Observation	Result	
			Active A	Active B
1.	Alkaloid	White precipitate	+	+
2.	Cardiac glycosides	Green colour	+	+
3.	Saponin	Foam appear	+	-
4.	Flavonoid	Yellow colour	+	+
5.	Phenolic compound	Dark green colour	+	+
6.	Phytosterols	Colour change	+	+
7.	Fatty acid	Soap formed	-	+

- : absence, + : presence

Slide 2: Phytochemical result of Active A

Slide 3: Phytochemical result of Active B

2.5. Formulation of Moisturizing Cream Base:

1) Simple oil in water base was selected, so that it doesn't interfere with the evaluation of moisturizing property of Active.
2) The cream base was formulated by the given procedure-
   - All the ingredients of phase A (oil phase) and phase B (water phase) were taken in separate beakers.
   - They were allowed to melt completely by heating up to a temperature of 70-80◦C.
   - Then the oil phase was added to the water phase with constant stirring until a cream is formed.
   - The cream was triturated to the desired consistancy and appearance.

Table 2: Trial for selection of Base

Ingredients	Quantity (%w/w)	
	Trial 1	Trial2
Phase 1		
Stearic acid	7	7
Cetyl alcohol	1.5	1.5
Mineral oil	10	10
Propyl paraben	0.5	0.5
Phase 2		
Triethanolamine	1.5	1.5
Glycerine	5	10
Water	65	70
Methyl paraben	0.5	0.5

Trial 1: The product appeared dry.

Trial 2: The quantity of humectant was increased by 5%. The trial 2 was selected for the further procedure and named as 'Control'.

2.5.1. Incorporation of Active in cream base:
The cream with Bael leaves extract was prepared by adding varying concentration of Active A as 1%, 2% and 3% in formulation respectively.

Table 3: Incorporation of Active A in cream base

Ingredients	Quantity(%W/W)		
Phase1	1%	2%	3%
Stearic acid	7	7	7
Cetyl alcohol	1.5	1.5	1.5
Mineral oil	10	10	10
Propyl paraben	0.5	0.5	0.5
Phase 2			
Triethanolamine	1.5	1.5	1.5
Glycerine	10	10	10
Water	67	66	65
Active A (Liquid form)	1	2	3
Methyl paraben	0.5	0.5	0.5

The cream with Bael leaves extract was prepared by adding varying concentration of Active B as 0.1%,0. 2% and0.3% in formulation respectively.

Table 4: Incorporation of Active B in cream base

Ingredients	**Quantity (%w/w)**		
Phase 1	**0.1%**	**0.2%**	**0.3%**
Stearic acid	7	7	7
Cetyl alcohol	1.5	1.5	1.5
Mineral oil	10	10	10
Active B (Dried form)	0.1	0.2	0.3
Propyl paraben	0.5	0.5	0.5
Phase 2			
Triethanolamine	1.5	1.5	1.5
Glycerine	10	10	10
Water	69	68	67
Methyl paraben	0.5	0.5	0.5

2.6 Stability Study: [10]

The accelerated stability study was carried out for 8 days by keeping the samples under room temperature, refrigerator (4◦C) and oven (45±2◦C). The parameters for stability study were: color, odor, pH, and viscosity. Stability test observations for moisturizing cream with Active A and Active B were as follows:

2.6.1. Color change: Appearance of color of the product was observed visually with naked eye. The samples were kept at different temp changes i.e., room temperature, oven and fridge. No change in color was noted.

2.6.2. Odor change: Change in its odor was observed by smelling the products kept at different temperature conditions. No change was noted.

2.6.3. Viscosity change: Change in the viscosity at different temperature of the sample was observed by viewing the productfor its consistency through naked eye. No change was noted.

2.6.4. pH: pH changes were determined using pH meter of all the samples kept at different temperature, conditions and changes were noted.

Table 5: pH change of moisturizing cream (Base) at different temperature conditions

Time in days	Moisturizing cream (Base)		
	RT	OVEN	FRIDGE
0	6.31	6.37	6.48
2	6.33	6.39	6.47
4	6.37	6.45	6.49
6	6.35	6.49	6.51
8	6.35	6.42	6.53

Table 6: pH change of 1%, 2% and 3% of cream with Active A at different temperature conditions

	1% moisturizing cream			2% moisturizing cream			3% moisturizing cream		
Time in days	RT	OVEN	FRIDGE	RT	OVEN	FRIDGE	RT	OVEN	FRIDGE
0	6.71	6.27	6.68	6.75	6.65	6.79	6.85	6.81	6.86
2	6.73	6.29	6.67	6.77	6.66	6.81	6.85	6.82	6.85
4	6.73	6.35	6.69	6.79	6.68	6.81	6.86	6.83	6.87
6	6.75	6.39	6.71	6.75	6.70	6.82	6.86	6.83	6.88
8	6.75	6.42	6.73	6.79	6.74	6.83	6.88	6.85	6.89

Table 7: pH change of 0.1%, 0.2% and 0.3% of cream with Active B at different temperature conditions

	0.1% moisturizing cream			0.2% moisturizing cream			0.3% moisturizing cream		
Time in days	RT	OVEN	FRIDGE	RT	OVEN	FRIDGE	RT	OVEN	FRIDGE
0	6.20	6.39	6.71	6.75	6.70	6.82	6.88	6.90	6.89
2	6.20	6.42	6.73	6.79	6.70	6.83	6.88	6.92	6.90
4	6.83	6.45	6.71	6.79	6.74	6.84	6.89	6.92	6.91
6	6.85	6.47	6.71	6.80	6.75	6.86	6.90	6.94	6.92
8	6.84	6.47	6.69	6.81	6.76	6.88	6.92	6.93	6.92

2.7 Determination of Moisturizing Effect of Cream with Bael Extract: [11, 12]

The measurement of skin moisture content was based on Corneometer also known as Capacitance method. The water content of the stratum corneum can be measured with a skin capacitance meter (Corneometer CM 825). The device determines the water content of the superficial epidermal layers down content to a depth of about 0.1mm and expresses the values in arbitrary units.

In practice, the use of Corneometer is the technique, which measures Corneum hydration before and after application of a cosmetic. It is used in industries as it is one of the easy and most effective as well as reliable method which gives reading and prepared graph. The moisturizing effect of cream containing Bael leaves extract on skin was done using Corneometer.

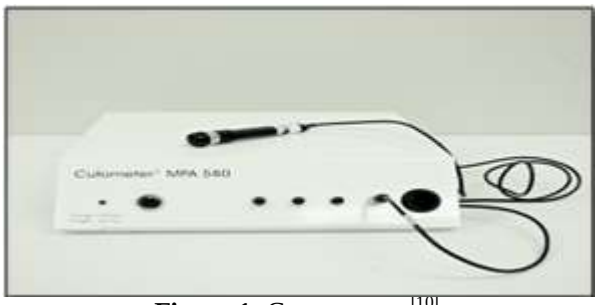
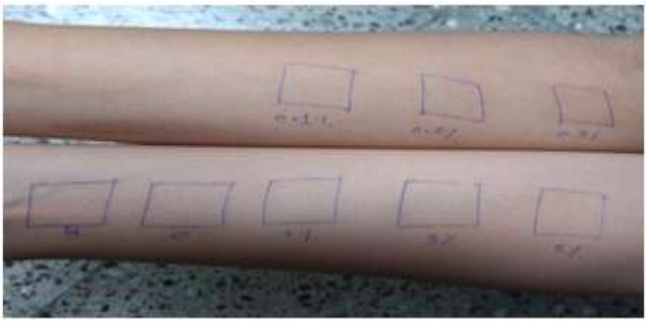


Figure 1: Corneometer [10]

Procedure

1) The evaluation for moisture was done on inner forearm of selected subject.
2) The volar forearm of subject was cleaned.
3) 8 blocks of 2×2cm were drawn on the inner hands of subjects for Blank (Skin Moisture), Control (Base), 1%, 2% and 3% with Active A and 0.1%, 0.2% and 0.3% with Active B moisturizing cream.
4) The probe head was placed vertically on the skin surface on the marked sites with little pressure for one second and reading as displayed by Corneometer was noted as a Blank
5) Similarly the readings were taken for the Blank, Control, 1%, 2% , 3% , 0.1%, 0.2%, and 0.3% sample at 0 minutes, 30 minutes and 60 minutes
6) The average was taken after every interval and graph was plotted.



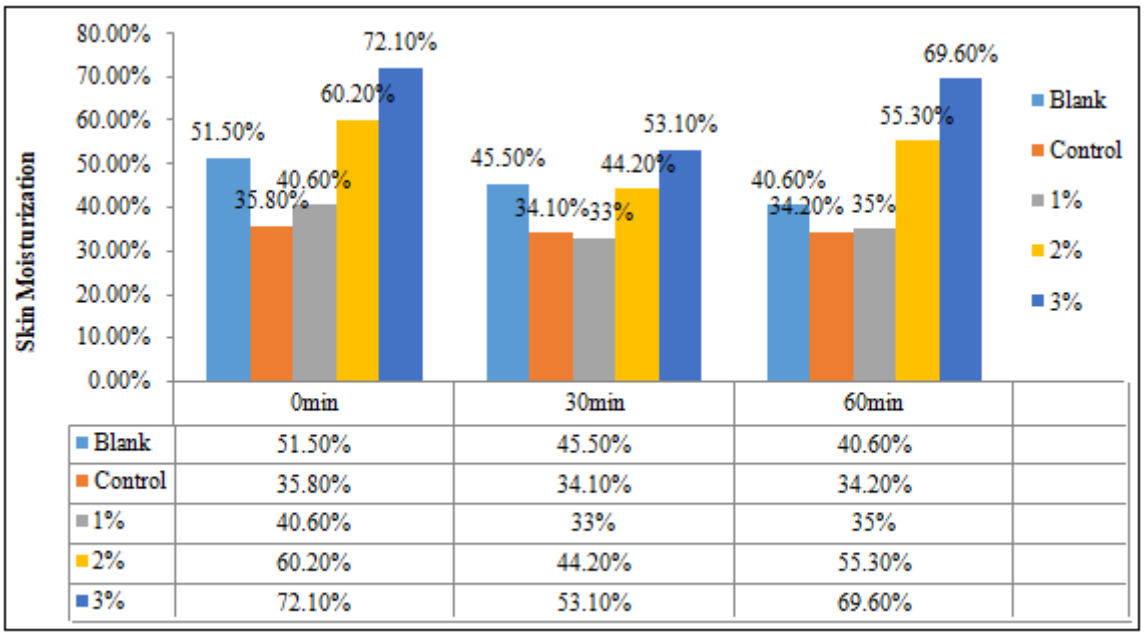
Slide 4: Moisture Measurement using Corneometer on Volar forearm of subject

2.8. Observation and Results

Table 8: Study of skin moisturizing property of moisturizing cream with Active 'A'

Subject	Concentration of active	Water content in % against time		
		0min	30min	60min
Subject 1	Blank	51.5%	45.5%	40.6%
	Control	35.8%	34.1%	34.2%
	1%	40.6%	33%	35%
	2%	60.2%	44.2%	55.3%
	3%	72.1%	53.1%	69.6%

Blank: Skin, Control: Base without active,



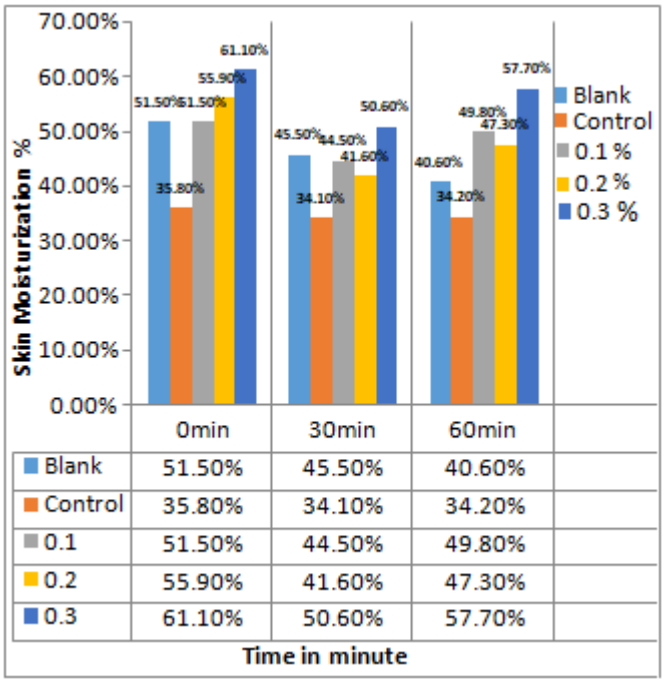
	0min	30min	60min
Blank	51.50%	45.50%	40.60%
Control	35.80%	34.10%	34.20%
1%	40.60%	33%	35%
2%	60.20%	44.20%	55.30%
3%	72.10%	53.10%	69.60%

Graph No. 1: Water content of the stratum corneum at 0min, 30min and 60min after the application of the formulation with Active A.

Table 9: Study of skin moisturizing property of moisturizing cream with Active 'B'

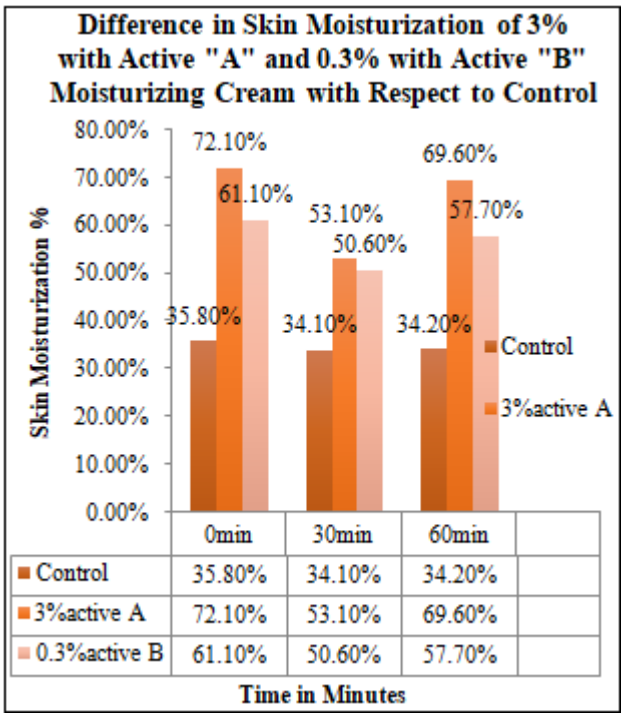
Subject	Concentration	Water content in % against time		
		0min	30min	60min
Subject 1	Blank	51.5%	45.5%	40.6%
	Control	35.8%	34.1%	32.2%
	0.1%	51.5%	44.5%	49.8%
	0.2%	55.9%	41.6%	47.3%
	0.3%	61.1%	50.6%	57.7%

Blank: skin moisture, Control: base without active



	0min	30min	60min
Blank	51.50%	45.50%	40.60%
Control	35.80%	34.10%	34.20%
0.1	51.50%	44.50%	49.80%
0.2	55.90%	41.60%	47.30%
0.3	61.10%	50.60%	57.70%

Graph 2: Water content of the stratum corneum at 0min, 30min and 60min after the application of the formulation with Active B



Graph 3: Difference in skin moisturization in 3% with Active A and 0.3% with Active B moisturizing cream with respect to control.

2.8.1 Result Interpretation

1) Graph 1 show that 3% Active A (Liquid extract) shows maximum moisturization when compared with 1%, 2%, Blank and Control. Also it was observed 3% Active A shows maximum moisturization at 0min than at 30 and 60 min after application on skin.
2) Graph 2 shows that 0.3% Active B (Dried extract) shows maximum moisturization as compared to Blank, Control, 0.1% and 0.2%. Also it was observed 0.3% Active B shows maximum moisturization at 0min than at 30 and 60 min after application on skin.
3) Referring to Graph 3 it was seen that skin moisturization content for 3% Active A MC was 72.1% which is greater than that for 0.3% Active B which is 61.1% only when compared to Control at 0 minute.
4) After 30 minute interval, the skin moisturization in 3% MC was 53.1% which is greater than 50.6% for 0.3% MC when compared with Control.
5) After 1 hour of application of the product, the skin moisturization for 3% MC was 69.6% which is greater than for 0. 3% MC with a value of 57.7% when compared to Control.

MC: Moisturizing Cream

Conclusion

1) Thus, from the results, in the study *Aegle marmelos* was explored for its moisturization property on skin.
2) It can be concluded *Aegle marmelos* extract increases the skin hydration. It can be effectively used as skin moisturizing agent in formulated cream.
3) Moisturizing cream with 3% *Aegle marmelos* extract gives good moisturization as compared to simple cream base (Control)
4) The water extract of *Aegle marmelos* gives better skin hydration as compared to chloroform extract.

3. Discussion and Conclusion

In daily life, human skin is exposed to variety of factors that have detrimental effects on dermal integrity resulting in dry skin and wrinkles. The most common protective and preventive step taken against dry skin is the use of emollients and moisturizing creams and lotions. Knowing the gravity of the problem the study was planned to exploit the properties of *Aegle marmelos* leaves extract. During the literature survey it was observed that Bael leaves extract can act as antioxidant, antiseptic, insecticidal, carminative, antimicrobial agent, and moisturizing agent. Considering this fact it was thought to study the properties of the extract in cream based formulation.

The initiation of work was done by the extraction of Bael leaves with water (active A) and chloroform (active B) solvent by maceration process. Standardization of extract was done by employing standardized method namely phytochemical screening and organoleptic properties. After standardization, it was concluded that the sample of extract complied with standards indicating that the extract can be used in the formulation.

The next step was to formulate a suitable cream base for the incorporation of the active. Cream formulation was selected because it provides better application Property and stability. The cream base was formulated using trial and error method. Since the properties of the ingredients of the base can also affect the activity of extract it was thought to keep the base ingredients as minimal as possible.

The cream base formulated was evaluated for its stability for 8 days by subjecting it to accelerated stability study conditions i.e. Oven (45±2°C), Refrigerator (4 ± 4^0C) and Room temperature (27◦C ±2◦C)). No change in color, odor, pH, and viscosity was observed within 8 days. The active was then incorporated in the cream base in selected concentrations of 1%, 2%, and 3% for Active A (Liquid extract) and Active B (Dried extract) in concentrations of 0.1%, 0.2% and 0.3% respectively.

Skin hydration determination was performed on both moisturizing creams by using Corneometer. It was observed that the cream containing 3% Active A gave more skin hydration property as compared to 0.3% Active B and Control. After conducting this test and interpreting the results it was found that 3% moisturizing cream was giving better moisturization. Thus it was concluded that the product showed satisfactory moisturuization ability on human skin.

4. Future Scope

1) Bael leaves extract can be studied and evaluated for other such pharmacological action.
2) Further other extraction methods can be explored for preparation of Bael leaves extract and some study can be conducted.
3) Concentration can be further varied of the extract and its effect studied.
4) It can be also studied for antimicrobial activity.
5) It can be incorporated in other products like: hand sanitizers, deodorants and antiperspirants, foot products,

and in skin care products, cosmeceutical and can also enter in formulation for babies and kids products.

6) Herbs stepping forward in today's market are the future diamonds for better personal, oral, skin, laundry etc. care areas.

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