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DESIGN AND DEVELOPMENT OF NOVEL COSMECEUTICAL CONTAINING ROYAL JELLY

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ABSTRACT

The purpose of this research work is to formulate and evaluate cosmeceutical containing Royal Jelly. According to World Health Organization (WHO) about 80% population of most developing countries still rely on traditional herbal medicines for their primary health care needs. Keeping the vision of more preference to natural and bee products, in this work the Royal jelly: a bee product has been chosen to formulate cream as it is a good source of flavonoids, proteins and has potential for antioxidant and sunscreen activity which can prove to be a better cosmeceutical i.e. cosmetic with medicinal properties. The cream was developed by incorporating Royal jelly powder into o/w emulsion based vanishing cream base. Preparation of cream of Royal jelly was challenging job because desired consistency and stability were the major factors of concern. Different types of formulations namely F1 to F4 were formulated by incorporating different concentrations of honey and preservatives. The evaluations of all formulations (F1 to F4) were done on different parameters like pH, spreadability, viscosity, total microbial count, stability, antioxidant activity, sunscreen activity etc. The study shows that both honey and preservatives were needed for its stability and after that all four prepared formulations were found to be stable while F4 have better antioxidant activity and sun protection factor (SPF) than other three (F1, F2, F3) formulations. Our study recommends cream of royal jelly for UV-rays protection (sunscreen activity) and in the management of skin aging as it shows better antioxidant activity.

KEYWORDS: Royal Jelly cream, Antioxidant activity, Sun Protection Factor(SPF), Flavonoids.

INTRODUCTION

Cosmeceuticals

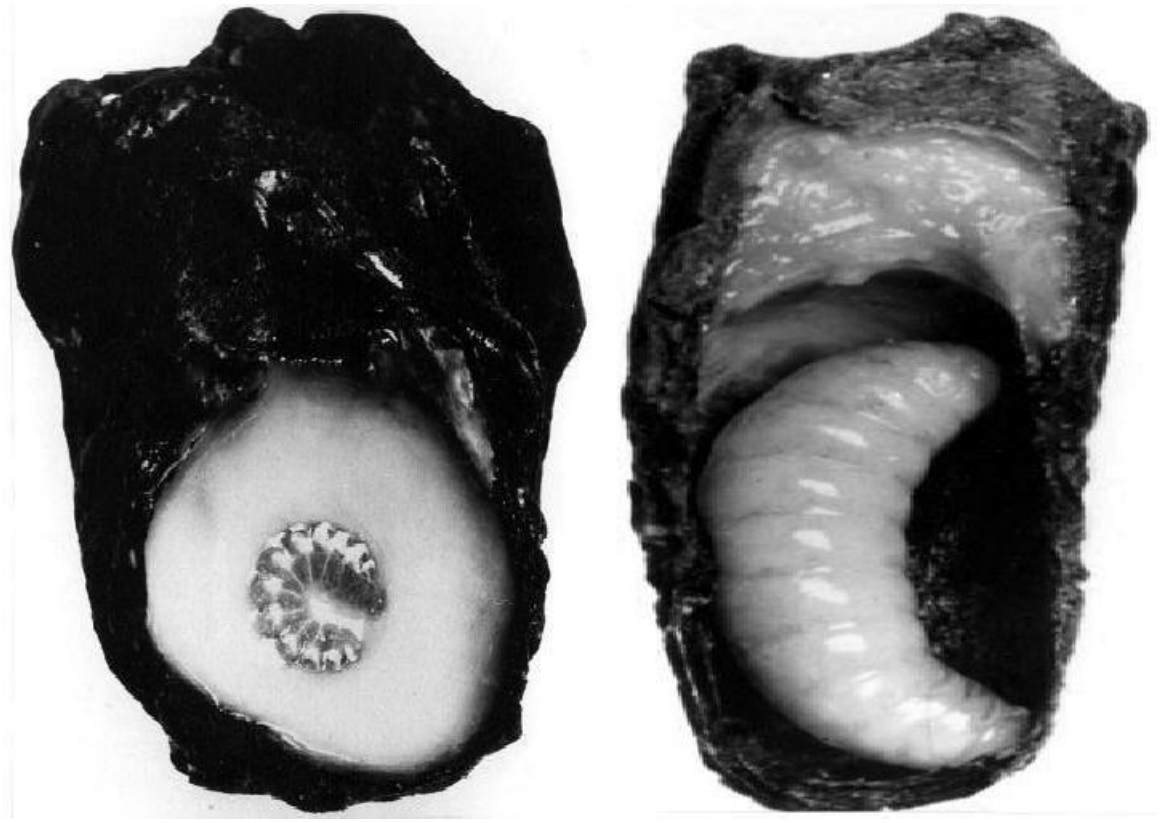
Cosmeceuticals represent the marriage of cosmetics and pharmaceuticals. Examples of products typically labeled as cosmeceuticals include anti-aging creams and moisturizers. It encompasses cosmetic, therapeutic, disease fighting and healing properties, serving as a bridge between personal care products and pharmaceuticals. Like cosmetics, cosmeceuticals are topically applied but they contain ingredients that influence the biological function of the skin.[1]

Profile of Royal Jelly

Royal jelly is one of the nature's best kept secret which is produced by young nurse worker bees mixing honey and bee pollen with enzymes in the hypo pharyngeal and mandibular endocrine glands. The power of Royal jelly is well-documented yet little is known about this mysterious elixir, Called "youth tonic" by the ancient Mayans, it is created by worker bees to feed the queen bee and queen bees live exclusively on royal jelly and it accounts for their incredible size and longevity.[2] All bee larva's are fed royal jelly for the first three days after they are laid. Thereafter, only that larvae destined to become queens get royal jelly. With royal jelly a larva turns into a queen bee which can live for up to 6 years and can lay up to 2500 eggs a day. She is fed only royal jelly for the rest of her life. Deprived of royal jelly a larva turns into a worker bee with a life expectancy of 6-8 weeks. It cannot be reproduced in a laboratory and there is no man-made element that comes close to its powerful regeneration properties. It is often believed that royal jelly may be a useful supplement because of the queen bee's superior size, strength, stamina, and longevity compared to other bees.[3]

Source

Royal jelly is secreted by the hypo pharyngeal gland of young worker (nurse) bees, to feed young larvae and the adult queen bee. The only situation in which harvesting becomes feasible is during queen rearing, when the larvae destined to become queen bees are supplied with an over-abundance of royal jelly. The queen larvae cannot consume the food as fast as it is provided, and royal jelly accumulates in the queen cells (see Figure 1). The exact definition of commercially available royal jelly is therefore related to the method of production: it is the food intended for queen bee larvae that are four to five days old.[4]



a **b**

"Fig. 1": a) A 3-day old queen larva floating in royal jelly. The cell is almost ready for harvesting. b) A 5-day old queen larva in a newly sealed cell just before pupation. Not much royal jelly is left.

Table 1: Composition of Royal jelly.

	Minimum	Maximum
Water	57%	70%
Proteins	17% of dry weight	45% of dry weight
Sugars	18% of dry weight	52% of dry weight
Lipids	3.5% of dry weight	19% of dry weight
Minerals	2% of dry weight	3% of dry weight

Table 2: Vitamin content of Royal jelly in µg per gram of fetch weight.[4]

	Thiamin e	Riboflavi n	Pantothenic acid	Pyridoxine	Niacin	Folic acid	Inosit ol	Biotin
Min.	1.44	5	159	1.0	48	0.130	80	1.1
Max.	6.70	25	265	48	88	0.530	350	19.8

The above properties are reason for the selection of Royal jelly in the preparation of cosmetic cream to produce multipurpose effect on skin such as fairness, sunscreen, antiaging, antioxi dant and antiwrinkle properties.

MATERIALS

The chemicals used in research work are:

Royal Jelly, honey (Hi- Tech Naturals Ltd, New Delhi), Stearic Acid, Glycerine (S. D. fine-Chem Ltd, Mumbai), Cetyl Alcohol, Glyceryl Monostearate (Alpha Chemicals, Mumbai),Triethanolamine, Methyl Paraben, Propyl Paraben, Olive Oil, Trichloroacetic Acid, Hydrochloric Acid, Ethyl Ether, Sodium Sulphate (Thomas Baker Chemicals Ltd, Mumbai),Lanoline (Rolex Lanoline Products), Thiobarbituric Acid (Research lab Fine Chem Industries, Mumbai), Ethanol (Chinachangshu Yangyuan Chemical), Soyabean Casein Digest Agar (Hi-Media Laboratories Pvt. Ltd, Mumbai). All chemicals were of analytical grade.

METHODOLOGY

Analysis of raw materials[5]

Each ingredient used in formulation was tested in accordance with IP monograph for various parameters such as color, odor, solubility, clarity, acidity, alkalinity, heavy metals etc.

Preparation of Cream[6]

The cream was developed by incorporating Royal jelly powder into o/w emulsion based vanishing cream base. The formula of the cream with good consistency, appearance and spreadability was optimized and selected for further study.

Table 3: The optimized formula of cream.

Ingredients	F1	F2	F3	F4
Royal jelly	0.9%	0.9%	0.9%	0.9%
Honey	4%	8%	4%	8%
Olive oil	4%	4%	4%	4%
Stearic acid	10%	10%	10%	10%
Cetyl Alcohol	2%	2%	2%	2%
Glyceryl mono stearate	2%	2%	2%	2%
Triethanolamine	0.5%	0.5%	0.5%	0.5%
Glycerine	10%	10%	10%	10%
Methyl Paraben	0.25%	0.5%	0.25%	0.5%
Propyl Paraben	0.25%	0.5%	0.25%	0.5%
Lanoline	0.25%	0.25%	0.25%	0.25%
Distilled water to make 100 gm	q.s.	q.s.	q.s.	q.s.

Procedure for preparation of cream

Oil in water (O/W) emulsion-based cream (semisolid formulation) was formulated. The emulsifier (stearic acid) and other oil soluble components (Cetyl alcohol, glycerol mono stearate, lanoline, olive oil) were dissolved in the oil phase and heated to 75 ± 5 ^{0}C. The preservatives and other water-soluble components (Methyl paraben, Propyl paraben, Triethanolamine, Glycerin) were dissolved in aqueous phase and heated to 75^{0}C. and then royal jelly mixed with honey and added into aqueous phase once it gets cooled upto 40^{0}C.After this, the aqueous phase was added in portions to the oil phase with continuous stirring until the cooling of emulsifier took place. Perfume was added when the temperature dropped to 45^{0}C.

Evaluation of cream: Physical observation

Physical parameters such as color, appearance and feeling on application were recorded.[8]

1. Homogeneity

The homogeneity of all developed creams was checked visually for the presence of any aggregates or clumps and for appearance.[8]

2. Determination of pH

The pH was determined by using digital pH meter. Accurately weighed 5 gm of cream was dispersed in 45ml of water to determine the pH of the suspension. The determinations were carried out in triplicate and the averages of three readings were noted.[9]

3. Viscosity

The viscosity of cream was determined by LVT Brookfield viscometer. The sample was placed in a clean and dried container and viscosity was checked as per standard operating procedure of viscometer by using spindle no. 4. Following formula is used for the calculation of the viscosity:

Viscosity in centipoises (cps) = Dial reading × Factor.

For calculation of viscosity put the factor value corresponding to the speed and the spindle number.[10]

4. Rheological Studies[11]

Rheograms

For studying rheology of cream, both ascending and descending readings were noted down i.e. firstly, by increasing shear stress and then by decreasing.

Rheograms obtained were plotted by taking RPM on Y- axis and Dial reading on X- axis.

5. Determination of spreadability

The spreadability was determined by parallel plate method. spreadability was calculated as follows:[12]

S = W × L/T

Where, S = Spreadability

L = Length of the glass plate (14.5 cm) W = Weight tied to upper plate (50g)

T = Time taken to separate the slide completely from each other.

6. Test for Thermal Stability[13]

20 mm broad and 5 mm thick strip was spread from material to be tested on the internal wall of a beaker of 100 ml capacity along its total height. Beaker was kept for 8 hrs. in the humidity chamber at 60 to 70 percent relative humidity and temperature of 37± 1^0C to observe any oil separation on removal from thermostat.

7. Determination of Total Fatty Matter[14]

2.0 g of the material was accurately weighed into a conical flask, to this 25 ml of dilute hydrochloric acid was added. The contents were refluxed until solution was perfectly clear. The contents of the flask were poured into 300ml separating flask and allowed to cool to 20^0C. The conical flask was rinsed with 50ml of ethyl ether in portions of 10ml. Ether rinsing were poured in separating flask and the separating flask was shaken. All the ether extracts were combined and washed with water. The ether extracts were filtered through a filter paper containing sodium sulphate into a conical flask which is previously dried at a temperature of 60 ± 2^0 C and then weighed. The sodium sulphate on the filter was washed with ether and washing were combined with filtrate. The ether was distilled off and the material was dried at temperature 60± 2^0 C to constant mass and total fatty matter was calculated.

Calculation

Total Fatty Matter% = 100 × M1/M2

Where, M1 = mass in gram of residue & M2= mass in gram of material taken for test.

8. Determination of total microbial count in formulated creams: Preparation of culture media (Soyabean casein Digest Agar)

The medium was prepared as per direction stated on bottle. 4gm of soyabean digest agar with 2 gm of agar-agar powder was dissolved in 100ml distilled water. The solution was sterilized by heating in an autoclave at 121^0C for 15 mins. Test tubes, pipettes and Petri dishes were also sterilized by heating in an autoclave at 121^0C for 15-20 mins.

9. Stability study

The present stability studies are carried out according to guidelines given by International council of Homonisation.[15]

10. *In vitro* SPF (Sun Protection Factor) Determination[16]

1 gm of all sample was weighed, transferred to a 100 ml volumetric flask, diluted to volume with ethanol, and then filtered through cotton, to give 10000 ppm solution. Rejecting the first 10 ml, a 5.0 ml aliquote was transferred to 50 ml volumetric flask and diluted to volume with ethanol to produce 1000 ppm solution. Then a 2 ml aliquote was transferred to a 10 ml volumetric flask and the volume completed with ethanol to give 200 ppm solution. The absorption spectra of each aliquote prepared were determined.

11. Study of Antioxidant activity of Cosmetic formulations[17]

Thiobarbituric Acid Method

The test was conducted according to the method of Kikuzaki and Nakatani (1993).

12. Evaluation for safety assessment Patch Tests on healthy volunteers

The single and repeated patch tests was performed on two age groups; in first age group 2 healthy female volunteers between 21-25 years while in second age group 2 female volunteers ages between 50-65 years were selected for testing skin reaction/irritation. The dorsal skin was cleaned with 70% alcohol before application of cream. Two patches on the right forearm was saturated with F1 & F3 respectively while the patch for left forearm was saturated with other two formulations i.e. F2 and F4. The formulation was applied to the 5× 4 cm marked region on forearm. The regions were covered with the surgical dressing after application. The patches were removed after 48 hrs. (single patch test) and the forearms were washed with physiological saline and observed. The formulations were reapplied 3 times for various time periods (repeated patch test). The cutaneous reactions were evaluated by monitoring the reactions of erythema, edema, pruritis and urticaria, skin allergy and irritation at 15 min, 1 hr.

and 24 hrs. after removal of the patch of test sample.[18]

RESULT

From all developed creams F1 & F4 are antique white in color while F2 & F3 have almond like color and the creams were non- greasy and had a smooth feeling on application (Table 4).

1. Homogeneity

From the results all cream formulations showed the good homogeneity without any lumps (Table 4).

Table 4: Evaluation of Physical parameters of developed creams.

Sr. No.	Color	Homogeneity
F1	Antique white	Good and non- greasy
F2	Almond	Good and non- greasy
F3	Almond	Good and non- greasy
F4	Antique white	Good and non- greasy

2. Determination of pH

The pH of the cream formulations was maintained constant throughout the study in the range 6.2 to 6.7 which lies in the normal pH range of the skin. The pH of creams was found to be 6.36±0.02, 6.21±0.02, 6.20±0.01, 6.32±0.01 respectively (Table 6).

3. Viscosity

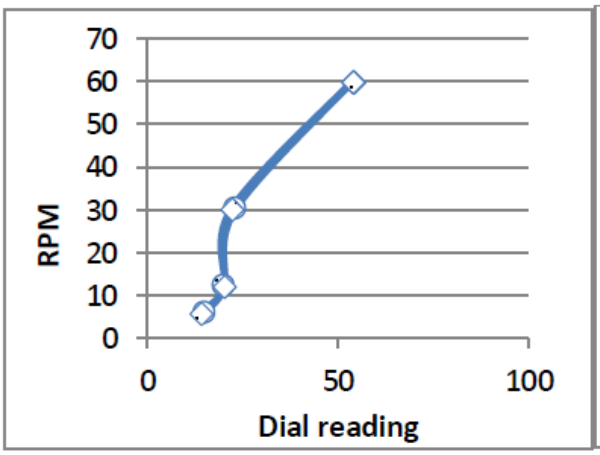
Viscosity for respective creams was found to be 6490± 40.92, 11236± 14.01, 9250± 20.45 and 7625± 25.16.

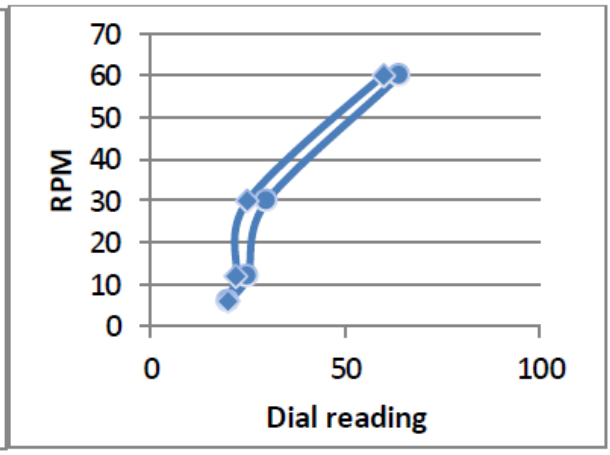
4. Rheological Studies

The rheograms depict that all formulations F1, F2, F3, and F4 show the thixotropic behaviors, since the down curve shifted to the left of the upcurve when r.p.m. plotted against dial reading. Thus, formulations F1, F2, F3, and F4 are shear thinning systems. The results are shown in table no.5 and rheograms of formulations F1, F2, F3 and F4 are shown in fig.2,3,4 and 5 respectively shows ascending and descending curves.

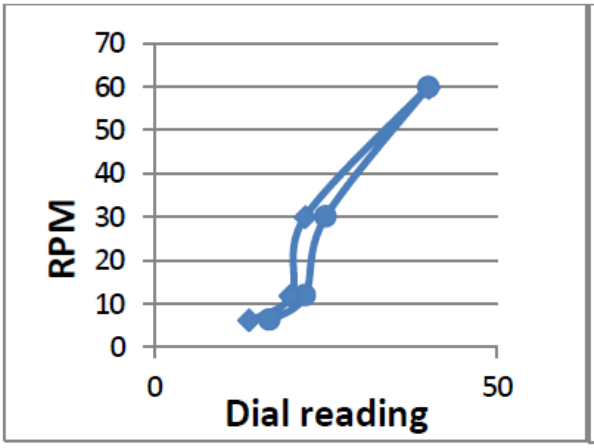
Table 5: Rheological observation of various formulations.

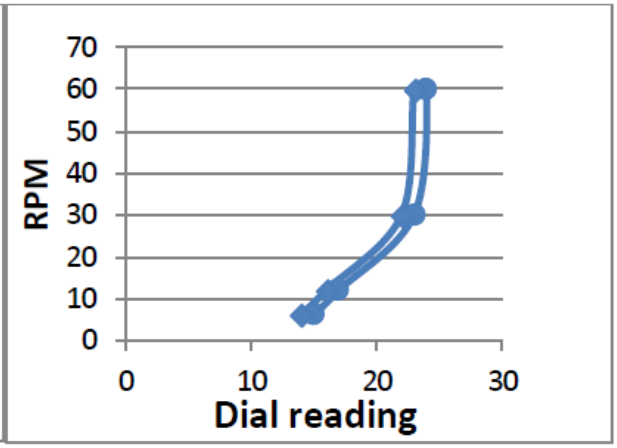
Formulations		RPM			
		6	12	30	60
F1	Ascending	15	20	23	54
	Descending	14	20	22	54
F2	Ascending	20	25	30	64
	Descending	20	22	25	60
F3	Ascending	17	22	25	40
	Descending	14	20	22	40
F4	Ascending	15	17	23	24
	Descending	14	16	22	23





"Fig.2": Rheological behaviour of F1. "Fig.3": Rheological behavior of F2.





"Fig.4": Rheological behaviour of F3. "Fig.5": Rheological behaviour of F4.

5. Determination of Spreadability

The result of spreadability was found to be 12.10± 0.09, 12.24± 0.02, 11.34±0.01, 11.5 ± 0.05.

Table 6: Evaluation parameters of developed creams.

Sr. No.	pH	Spreadability	Viscosity	TFC	Thermal stability
F1	6.36±0.02	12.10±0.09	6490± 40.92	2.6%	No oil separation
F2	6.21±0.02	12.24± 0.02	11236± 14.01	3.0%	No oil separation
F3	6.20±0.01	11.34±0.01	9250± 20.45	2.9%	No oil separation
F4	6.32±0.01	11.5± 0.05	7625± 25.16	2.3%	No oil separation

6. Determination of Total Microbial Count (TMC)

The result of experiment of total microbial count showed that 213, 221, 205 and 211 cfu/gm for F1, F2, F3, F4 formulations respectively when checked on the same day of preparation while results of TMC after stability studies showed 225, 278, 224 and 243 cfu/gm for respective formulations.

Table 7: Total microbial count of developed creams.

	Total microbial count (cfu/ gm)							
	F1		F2		F3		F4	
	0 day	3 months	0 day	3 months	0 day	3 months	0 day	3 months
Total count Bacterial	213	225	221	278	205	224	211	243

7. Stability study

The stability test was carried out for three months and results revealed that all the creams showed better stability at 40^0C and 30^0C (Table 8 & 9).

Table 8: Accelerated stability testing of developed creams at 40^0 C.

Batch	Day	pH	Viscosity	Spreadability	Thermal stability
F1	1	6.36 ± 0.02	6490 ± 40.92	12.10±0.09	No oil separation
	15	6.42 ± 0.06	6536 ± 43.68	12.12± 0.06	No oil separation
	30	6.48 ± 0.07	6555 ± 32.52	12.22± 0.09	No oil separation
	60	6.50 ± 0.1	6540 ± 36.05	12.09 ± 0.01	No oil separation
	90	6.50 ± 0.15	6542 ± 21.37	12.3 ± 0.02	No oil separation
F2	1	6.21± 0.02	11236±14.01	12.24± 0.02	No oil separation
	15	6.25 ± 0.02	11238±15.27	12.26±0.04	No oil separation
	30	6.30 ± 0.03	11248±25.16	12.20±0.01	No oil separation
	60	6.32 ± 0.01	11245±10	12.22±0.15	No oil separation
	90	6.30 ± 0.05	11244±10.01	12.25± 0.05	No oil separation
F3	**1**	6.20 ±0.01	9250± 20.45	11.34±0.01	No oil separation
	15	6.22 ± 0.03	9255± 21.44	11.30± 0.04	No oil separation
	30	6.23 ± 0.04	9252± 20.33	11.32± 0.02	No oil separation

	60	6.20 ± 0.02	9222± 30.22	11.33± 0.12	No oil separation
	90	6.22 ± 0.12	9212 ± 25.32	11.34± 0.02	No oil separation
F4	**1**	6.32±0.01	7625 ± 25.16	11.50± 0.05	No oil separation
	15	6.34± 0.13	7620 ± 25.11	11.40± 0.03	No oil separation
	30	6.30± 0.02	7615 ± 20.78	11.33± 0.08	No oil separation
	60	6.36± 0.04	7610± 25.22	11.40 ± 0.02	No oil separation
	90	6.34± 0.08	7620 ± 30.05	11.42± 0.01	No oil separation
All the values are in mean of three readings					

Table 9: Stability results of developed creams at 30^{0}C.

Batch	Day	pH	Viscosity	Spreadability	Thermal stability
F1	1	6.36 ± 0.02	6490 ± 40.92	12.10 ±0.09	No oil separation
	15	6.40 ± 0.06	6534 ± 43.60	12.14 ± 0.07	No oil separation
	30	6.46 ± 0.07	6550 ± 32.51	12.15 ± 0.09	No oil separation
	60	6.49 ± 0.1	6545 ± 35.01	12.08 ± 0.02	No oil separation
	90	6.50 ± 0.15	6542 ± 21.40	12.32 ± 0.03	No oil separation
F2	1	6.21± 0.02	11235±14.02	12.25 ± 0.03	No oil separation
	15	6.23 ± 0.03	11238±15.11	12.27 ± 0.05	No oil separation
	30	6.31 ± 0.04	11246±23.16	12.22 ± 0.02	No oil separation
F3	60	6.34 ± 0.02	11245±10.13	12.23 ± 0.15	No oil separation
	90	6.30 ± 0.05	11244±10.01	12.24 ± 0.06	No oil separation
	1	6.20 ±0.01	9250± 20.45	11.34 ± 0.01	No oil separation
	15	6.24 ± 0.05	9254± 21.50	11.32 ± 0.05	No oil separation
	30	6.23 ± 0.04	9252± 20.33	11.34 ± 0.06	No oil separation
	60	6.22 ± 0.03	9226± 30.40	11.33 ± 0.13	No oil separation
	90	6.25 ± 0.12	9218± 24.32	11.34 ± 0.05	No oil separation
F4	1	6.32±0.01	7625± 25.16	11.50 ± 0.05	No oil separation
	15	6.35± 0.14	7622 ± 26.11	11.45 ± 0.04	No oil separation
	30	6.32± 0.04	7618 ± 20.58	11.35 ± 0.09	No oil separation
	60	6.34± 0.05	7612 ± 25.55	11.40 ± 0.04	No oil separation
	90	6.36± 0.09	7622 ± 30.05	11.45 ± 0.02	No oil separation
All the values are in mean of three readings					

8. Absorption spectroscopy by Mansur equation (Determination of SPF): Cream containing Royal jelly

SPF of all developed creams was determined and depicted in table10. For determination of SPF the spectrum from 290 nm- 320nm for all formulations were taken. SPF of F1 cream was found to be 1.36, 1.73, 1.03 for 10000ppm, 1000ppm, 200ppm respectively. SPF of F2 cream at 10000ppm, 1000ppm, 200ppm was found to be 2.79, 1.20, and 1.03 respectively. SPF of F3 cream was found to be 3.96, 3.19 and 1.40 for 10000ppm, 1000ppm, 200ppm respectively and SPF of F4 cream at 10000ppm, 1000ppm, 200ppm was found to be 4.08, 2.95 and 1.53 respectively.

Table 10: SPF (sun protection factor) values of all developed formulations.

Sample	Conc.	Wavelength(λ)	290	295	300	305	310	315	320	SPF
	ppm	EE(λ)×I(λ)	0.015	0.0817	0.2874	0.3278	0.1864	0.0839	0.018	1
F1	10000	(A) EE(λ)×I(λ)×(A)	0.3098±0.001 0.04647	0.2232±0.0004 0.1823	0.1621±0.0003 0.4658	0.1219±0.0004 0.3995	0.0986±0.0003 0.1837	0.0775±0.0004 0.0650	0.0601±0.0002 0.0108	1.36
	1000	(A) EE(λ)×I(λ)×(A)	0.1485±0.0003 0.0222	0.1254±0.0004 0.01024	0.1168±0.0004 0.3356	0.1085±0.0003 0.3556	0.1023±0.0003 0.1906	0.0954±0.0004 0.8004	0.0921±0.0003 0.0165	1.73
	200	(A) EE(λ)×I(λ)×(A)	0.1375±0.0003 0.0206	0.1202±0.0003 0.0982	0.1107±0.0002 0.3181	0.1017±0.0002 0.3333	0.0944±0.0002 0.1759	0.0895±0.0003 0.0750	0.0843±0.0002 0.0151	1.03
F2	10000	(A) EE(λ)×I(λ)×(A)	0.5330±0.0002 0.0799	0.3321±0.0001 0.2713	0.2976±0.0002 0.8553	0.2808±0.0002 0.9204	0.2408±0.0001 0.4488	0.2205±0.0003 0.1849	0.1937±0.0003 0.0348	2.79
	1000	(A) EE(λ)×I(λ)×(A)	0.1573±0.0002 0.0235	0.1368±0.0003 0.1117	0.1258±0.0002 0.3615	0.1197± 0.0002 0.3923	0.1121±0.0002 0.2089	0.1047±0.0002 0.0878	0.1027±0.0003 0.0184	1.20
	200	(A) EE(λ)×I(λ)×(A)	0.1374±0.0003 0.0206	0.1200±0.0001 0.0980	0.1106±0.0002 0.3178	0.1017±0.0002 0.3333	0.0946±0.0004 0.1763	0.0894±0.0002 0.0750	0.0843±0.0002 0.0151	1.03
F3	10000	(A) EE(λ)×I(λ)×(A)	0.7420±0.0002 0.1113	0.4979±0.0002 0.4067	0.4175±0.0002 1.1998	0.3783±0.0002 1.2400	0.3592±0.0002 0.6695	0.3316±0.0002 0.2782	0.3151±0.0002 0.0567	3.96
	1000	(A) EE(λ)×I(λ)×(A)	0.0295±0.0002 0.0044	0.1281±0.0002 0.1046	0.4031±0.0002 1.1585	0.4248±0.0002 1.3924	0.2276±0.0002 0.4242	0.0953±0.0002 0.0799	0.1917±0.0002 0.0345	3.19
	200	(A) EE(λ)×I(λ)×(A)	0.1757±0.0001 0.0263	0.1539±0.0002 0.1257	0.1502±0.0007 0.4316	0.1307±0.0002 0.4284	0.1228±0.0002 0.2288	0.1768±0.0002 0.1483	0.1053±0.0001 0.0189	1.40
F4	10000	(A) EE(λ)×I(λ)×(A)	0.7436±0.0002 0.1115	0.4964±0.0002 0.4055	0.4294±0.0001 1.2340	0.3972±0.0002 1.3020	0.3663±0.0001 0.6827	0.3438±0.0002 0.2884	0.3221±0.0002 0.0579	4.08
	1000	(A) EE(λ)×I(λ)×(A)	0.0263±0.0002 0.0039	0.1154±0.0002 0.0942	0.3689±0.0002 1.0602	0.4008±0.0003 1.3138	0.2164±0.0003 0.4033	0.0929±0.0002 0.0779	0.0194±0.0003 0.0034	2.95
	200	(A) EE(λ)×I(λ)×(A)	0.2036±0.0002 0.0305	0.1746±0.0002 0.1426	0.1639±0.0002 0.4710	0.1506±0.0003 0.4936	0.1424±0.0003 0.2654	0.1346±0.0002 0.1129	0.1268±0.0002 0.0228	1.53

9. Study of Antioxidant activity of Cosmetic formulations:

The TBA Inhibition values of all four creams are less as compared to Standard Vit C (80.5%).

Table 11: Inhibition of TBA activity.

	Control	Absorbance	Percent Inhibition
F1	0.1250±0.007	0.0837±0.002	33
F2	0.1250±0.007	0.0980±0.005	21.6
F3	0.1250±0.007	0.0672±0.002	46.4
F4	0.1250±0.007	0.0385±0.003	69.2
Vit C	0.1250±0.007	0.0243±0.004	80.5

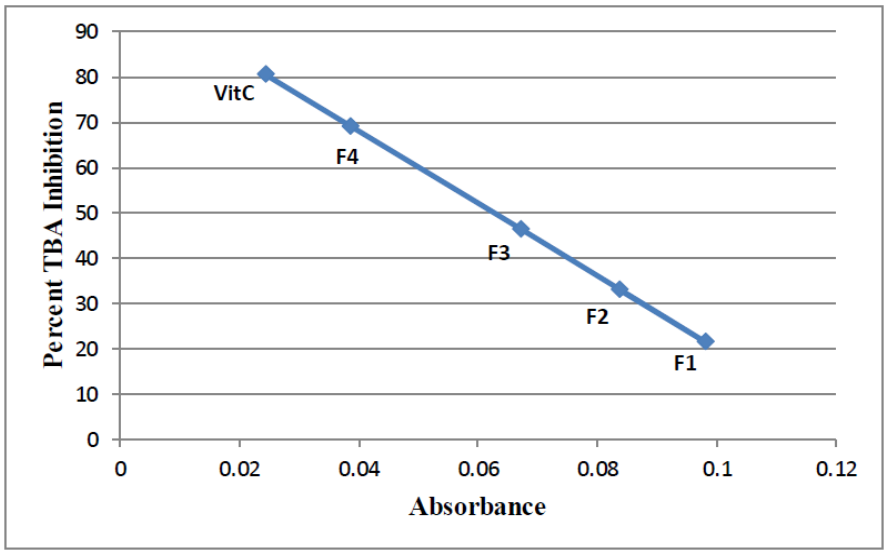


Fig. 6: Inhibition of TBA activity of creams of Royal Jelly (The values are mean of three readings ± SD).

10. Patch Tests on healthy volunteers

The single and repeated patch tests showed no irritation and sensitization potential of the all formulations on healthy volunteers.

Table 12: Cutaneous reaction after application of all 4 creams (F1, F2, F3 & F4) in patch test on human volunteers.

Parameters	Single patch test (48 hrs.)	Repeated Patch test		
		15 min.	60 min.	24 hrs.
Erythema	Nil	Nil	Nil	Nil
Edema	Nil	Nil	Nil	Nil
Pruritis and Urticaria	Nil	Nil	Nil	Nil
Skin allergy	Nil	Nil	Nil	Nil
Irritation	Nil	Nil	Nil	Nil

DISCUSSION

The Royal jelly was found to possess physical and chemical properties which were suitable for being used in cosmetic formulations.

Preparation of cream of Royal jelly was challenging job because desired consistency and stability were the major factors of concern. Formulation of cream containing Royal jelly powder did not pose a serious problem and the formulations were acceptable regarding consistency as well as stability. Firstly, the effect of honey and preservatives on the stability of Royal jelly powder was studied and it showed that both honey and preservatives were needed

for its stability and after that all four prepared formulations were found to be stable but F4 have better antioxidant activity and sun protection factor (SPF) than other three (F1, F2, F3) formulations. In addition to the antioxidant activity the cream has showed better sunscreen activity. On storage the creams were seen to retain their all properties. The pH of the prepared cream with Royal jelly was found to be around 6 which is suitable for topical application because the pH of the skin is between 4.5– 6. The spreadability studies showed that formulation have better spreadability. The formulation showed no redness, edema, inflammation and irritation during Patch Test studies. These formulations are safe to use for skin. The formulated creams were tested for the presence of pathogenic microorganisms by culturing it with agar medium. Hence, from all the results it was found that further work in such area would be of a great relevance.

CONCLUSION

From all these findings, the present article concluded that formulated cream of royal jelly has potential to use for functional cosmetics. Due to availability of antioxidant activity of royal jelly cream has potential to use in various skin disorders related free radicals. Our study also recommends the cream of royal jelly for UV- rays protection (sunscreen activity) and applicable for different types of skin when using different concentration as per SPF obtained the study also recommended the royal jelly cream in the management of skin aging as it showed better antioxidant activity. While the anti-infective (anti- bacterial) activity of royal jelly cream may be useful for the acne treatment. The results of safety assessment of formulation on human skin concluded that none of human subject demonstrated the skin irritation. The skin irritancy results concluded that the cream will be safe for use.

Finally, the study concluded that royal jelly has potential to use in various skin cosmetics. The royal jelly has greater applicability for functional cosmetics as well as colored cosmetics. However, to enhance the skin cosmetics utility of royal jelly there is need further study in future to isolate active compounds from it for various functional application.

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