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FORMULATION AND EVALUATION OF KARANJA-BASED HERBAL SUNSCREEN

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ABSTRACT

Sunscreens work as a shield for skin against the harmful effects of the sun. In today′s situation sunscreens have become a vital part of each person's skin care routine. Most marketed sunscreen products are expensive and contain synthetic molecules that can be toxic or carcinogenic. For this reason, there was a necessity to develop and evaluate effective and safe sunscreen products containing antioxidants that will help prevent long-term damage by free radicals caused by sun rays and prevent sunburn, scars, cracks, wrinkles, and premature aging. Karanja oil increases the protection power of sunscreens, mainly against UVB. It has anti-aging, soothing, and antibacterial properties. The basis moto of this study was to invent Karanja oil-based photoprotective herbal sunscreen with antioxidant property, high SPF andprovide UVA/UVB shield to protect from harmful sun rays. The prepared formulation was evaluated for its physical, rheological, antifungal, and antibacterial properties. To know the efficacy of formulation; the in vitro SPF value of prepared sunscreen was calculated and its stability study was also carried out.

KEYWORDS: Karanja oil, Sunscreen, SPF, UV rays, Antimicrobial.

1. INTRODUCTION

The people of early civilization used a diversity of products made from plants as sun agents. UV protection is extremely popular because of the photo protective property of sunscreens.[1]

since its main components are flavonoids, phenolic compounds or vegetable oils, it provides antioxidant, wound healing, antibacterial, premature aging, moisturizing, anti-inflammatory and anti-UV-A protection and is UV protected.[2]

Historically, Karanja oil has been used as a folk medicinal plant, mostly in Ayurveda and Siddha systems of Indian medicine.[3] As a molecule of interest, Karanja is anantioxidant and contains bioflavonoid that possesses many differing properties, including anti-inflammatory and antisolar effects.[3] Karanja (or Pongamia pinnata) is described in Ayurvedic literature for its antibacterial properties. The antibacterial and antifungal properties of karanja are attributed to pongaroten (degenin) and karanjin (a flavanol). It also denotes its action on skin diseases Staphylococcus aureus, E. coli, Aspergillosis, and Candidiasis are the main pathogens that cause skin infections.[4]

The intend of present study was formulation of novel Karanja seed oil-based photoprotective herbal sun protective novel cream having antioxidant properties, elevated SPF value and obligatory homogenous UVA/UVB protection. The prepared sun protective cream was evaluated for its physical, rheological, antifungal, antibacterial properties. To recognize the effectiveness of prepared formulation, SPF value calculated and its stability study was also carried out.

2. MATERIALS AND METHODS

2.1. Chemicals and solvent

Karanja oil (Pongamia pinnata), paraffin wax, beeswax,zinc oxide, titanium dioxide, methylparaben, distilled water.

2.2. Test microbial strains

Strains used for study: E. coli and S. aureus (Bacterial)

Aspergillus sp and C. albicans (Fungal)

Table: 1 Finalized Sunscreen formulation.

Sr. no .	Phases	Ingredients	Quantity(50g)
1.	A	Karanja oil	24
2.	A	Beeswax	1
3.	A	Paraffin wax	1
4.	B	Distilled water	6.5
5.	C	Zinc oxide	10
6.	C	Titanium dioxide	3.3

7.	C	Methyl paraben	0.2

Table: 2 Development of Sunscreen formulation (50 gm).

Sr. no	Ingredients	Formulation A	Formulation B	Formulation C	Formulation D	Formulation E
1.	Karanja oil	12	12	15	20	24
2.	Beeswax	5	4	4	1	1
3.	Paraffin wax	5	4	2	1	1
4.	Distilled water	7.8	9.8	11	7.8	6.5
5.	Zinc oxide	5	5.2	7	8	10
6.	Titanium dioxide	5	4.8	3.8	5	3.3
7.	Methyl paraben	0.2	0.2	0.2	0.2	0.2

2.3. Procedure

1. Add zinc oxide and titanium dioxide in a mortar and mix them evenly.
2. Add phase A ingredients into a labelled beaker 1.
3. Similarly add phase B ingredients into a labelled beaker 2.
4. Simultaneously, boil both the beaker A and B on water bath.
5. Heat both the beakers until the temperature of both the beakers reaches 75˚C. Mix beaker 2 contents with beaker 1 content and mix them continuously for 3 minutes.
6. Add phase C in this solution.
7. Milky, white appearance is seen.
8. Place the beaker in cold water (Ice bath) for 3-4 minutes. Then until the solution reaches the body temperature keep it still.

2.4. Characterization of Karanja oil based herbal sunscreen

2.4.1. Evaluation of physical parameters

Parameters like colour, appearance and odour of formulated sunscreens were evaluated.

2.4.2. pH determination

Formulated sunscreen's pH was calculated using pH meter.2gm of each sunscreen was accurately weigh and dispersed in 20ml distilled water & stored it for 2 hrs. The pH estimation was carried out and results were noted.

2.4.3. Type of emulsion under dye analysis

The Scarlet dye mixed in sunscreen thoroughly. Single drop of sunscreen-dye added on a glass slide and covered it using cover slip. Further observed under the microscope lens; to evaluate the type of emulsion had been formed.

2.4.4. Irritancy study

Mark an area (1cm²) on the left dorsal face. Apply cream on marked area. Irritation, erythem, edema were then monitored periodically for up to 24 hours, and the duration was recorded an d reported.

2.4.5. Spreadability

Place the preparation on two glass slides and place a 50 g weight on the top slide for 5 minute s to compress the cream into a thick layer. Add 50 grams of weight to pot. The time (in secon ds) required for the separation of these slides is used to measure spread.

2.4.6. Viscosity measurement

It is an vital parameter in evaluation. The viscosity of
an adhesive, its flexibility, and ability of glue to flow out of container etc ;
It describes many properties, such as the viscosity of a formulation measured using a Brookfi eld viscometer, which is in the range of 28,000 to 32,000 cm^3.

2.4.7 Rancidity

Bitterness was due to entire or partial oxidation of oils and fats which causes an nasty taste and smell when showing to light, air and moisture or bacteria. Rancidization typically did using fluoroglucinol. The rancidity occurred because of the hydrolysis of oils and fats; free form of fatty acids are on the loose during oxidation. These free fatty acids respond to fluoroglucinol and turn red, representing that the product is rancid. Take around 10 ml cream, add fluoroglucinol solution of 10 ml with addition of 10 ml conc.HCL and shudder for 1minute. If red color is not visible, the adhesive must pass the test.

2.4.8 Microbial test

Nutrient agar and nutrient broth media were used for microbial escalation study. Here one blank and sample Petri plates were used and in which Sunscreen sample was germfree pass on to sample plates in cross pattern, then the microbial growth on plates was observed and noted. Antimicrobial (Bacterial and fungal) activity was assessed against staphylococcus aureus, E. coli, Candida, and Aspergillus sp. strains after 24hrs, 48hrs and 72hrs, found to exhibit significant antimicrobial activity.

2.4.9 Stability study

For in vitro assessment herbal sunscreen was positioned at different temperatures to cream check stability; at 8˚C, 25˚C and around 40˚C in stability chamber having 75% relative humidity, were placed for about 28 days. Any changes so far in pH, colour, dissolution, phase dividing & conductivity were observed and were recorded.

3. RESULT AND DISCUSSION

3.1. Physical evaluation

Colour, smell, and appearance of sunscreens were checked and is mentioned below in Table 3.

3.2. PH value determination

PH was determined via pH meter which was digital. Creams were weighed of 2.5 gm and diffuse in 50 ml double distilled / filtered water. PH meter was used to take values and were shown in Table 3.

3.3. Type of emulsion

The scarlet dye was mixed in prepared creams completely. Then taken on microscopic slide with covering of cover slip and seen under microscope lens. The scattered globules appeared red and ground as pale. On the basis of this experiment cream was proved as o/w i.e oil in water emulsion type. Furthermore results were recorded and reported in Table 3 given below.

3.4. Irritancy

A skin area of ($1cm^2$) was selected on the left dorsal face. The cream was applied to marked spot. Irritation, erythema and edema were then monitored periodically every 24 hours, and the results were noted and reported in below Table 3.

3.5. Spreadability

Cream spreadability test was performed. The time (in seconds) required to split two petri plated was recorded (fig 1) & reported in (Table 3).

3.6. Viscocity

The viscosity was determined via Brookfield viscometer and was found to be in scope of 25000-32000 cubic meters and results were noted and reported in (Table 3).

3.7. Rancidity

concentrated HCL of 10 ml and fluoroglucinol solution of 10 ml was added in prepared formulation of 10 ml and mixed upto 1minute duration, result was noted (Fig 2) and reported in (Table 3).

3.8. SPF Determination

Formulated herbal cream of 10% was developed using 95 % of ethanol. Absorbance result taken for every formulation at wavelength starting from 290 upto 320 nm with 5 nanometer of gap by operating UV-Visible type of spectrophotometer.[5-8] Furthermore at 5 nm of gap the absorbance result which were noticed was calculated and mentioned in (Table:3). Characterization of herbal Karanja oil-based sunscreen

3.9. Microbial test

Nutrient agar (NA) medium was used in bacterial growth study, while Potato dextrose agar (PDA) was used in fungal growth related study. In this study the blank along with the sample petri plates were used and sunscreen samples were aseptically transferred on sample plates, the Bacterial and Fungal growth was observed (Table 4,5and Fig.3,4)

Sr.No	Test	Result
1	**Physical evaluation**	
	Colour	Yellowish white
	Odour	Characteristics
	Appearance	Semisolid with smooth texture
	Consistency	Good
	Texture	Smooth
2	pH determination	6.5(acidic)
3	Type of emulsion under dye test	Oil – Water type (O/W)
4	Irritancy test	No irritation on skin
5	Spreadability	17.02 gm/cm/sec.
6	Viscosity	30202
7	Rancidity	No pink colour
8	SPF by UV	38
9	Extrudability	Good

Fig1: Spreadability test.

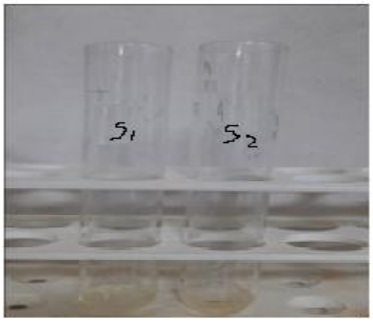


Fig 2: Rancidity Test.

Table: 4. Microbial test (Bacterial).

Sr.No.	Test Bacterial Strain	24 hrs	48hrs	72hrs
1.	Nutrient Agar medium	Growth not found	Growth not found	Growth not found
2.	Nutrient Agar medium + *Staphylococcus aureas* + sunscreen	Growth not found	Growth not found	Growth not found
3.	Nutrient Agar medium + *E. coli* + sunscreen	Growth not found	Growth not found	Growth not found

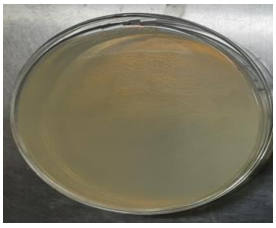
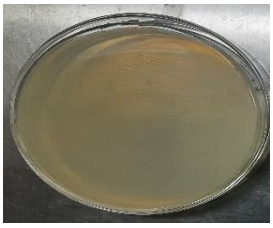
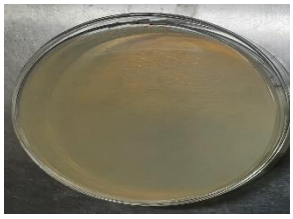
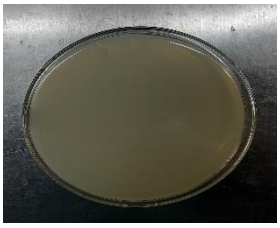
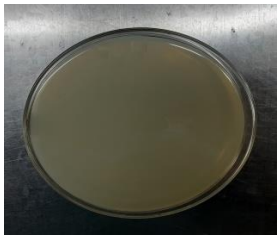
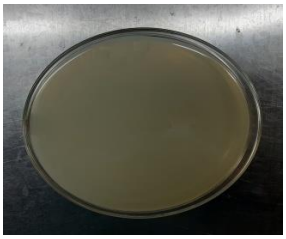
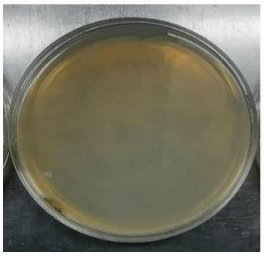
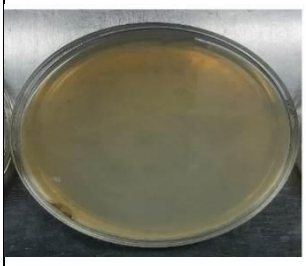
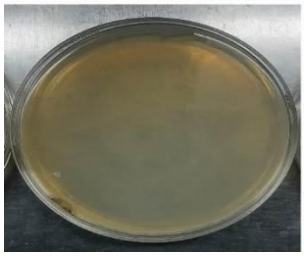
	24 hrs	48 hrs	72 hrs
Nutrient agar media+ sunscreen			
Nutrient agar media + S. aureas + sunscreen			
Nutrient agar media + E. coli + sunscreen			

Figure: 3.Microbial Test (Bacterial).

Table: 5. Microbial test (Fungal).

Sr.No.	Test Bacterial Strain	24 hrs	48hrs	72hrs
1.	Nutrient Agar medium	Growth not found	Growth not found	Growth not found
2.	Nutrient Agar medium + *Aspergillus* + sunscreen	Growth not found	Growth not found	Growth not found
3.	Nutrient Agar medium + *Candida albicans* + Sunscreen	Growth not found	Growth not found	Growth not found

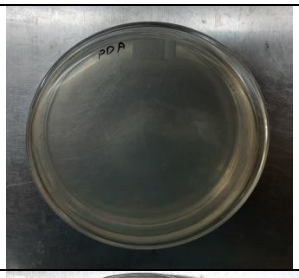
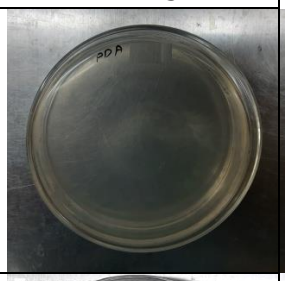
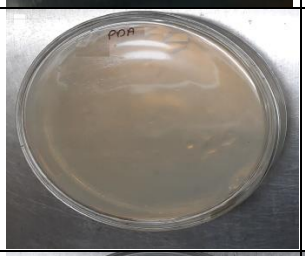
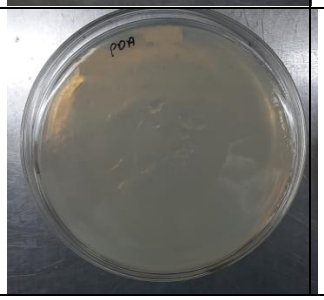
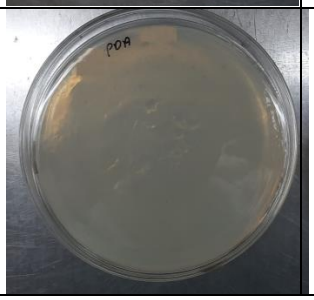
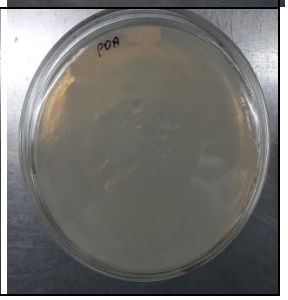
	24 hrs	48 hrs	72 hrs
Potato dextrose agar media + Sunscreen			
Potato dextrose agar media+Staphylococcus aureas + cream			
Potato dextrose agar media + E. coli + cream			

Figure: 4.Microbial Test (Fungal).

3.10. Stability study

For in vitro evaluation Karanja oil based herbal sunscreen, it was positioned at various temperatures to check stability; like at 40˚C, 25˚C and 8˚C in stability chamber having 75% relative humidity, were placed for about 28 days (Table 6). No changes found in type of liquefaction, color and phase parting; the result of electrical conductivity experiment was also negative and pH of herbal sunscreen at various temperature and humidity condition was in range of our skin pH. PH of freshly prepared sunscreen formulation was found to be 6.5, respectively. The formulations showed a continuing decrease in pH from after 3rd day to 28thday in study time. At the ending of the study (on 28th day) pH of cream sample decreased up to 6.40 only.

Table 6: Stability characteristics of Herbal Karanja oil-based sunscreen.

		Fresh	After 24 hr.	After 3 days	After 7 days	After 14 days	After 21 days	After 28 days
Colour	A	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowis h white	Yellowish white
	B	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white
	C	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white
	D	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white
	E	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white
Liquefaction	A	No	No	No	No	No	No	No
	B	No	No	No	No	No	No	No
	C	No	No	No	No	No	No	No
	D	No	No	No	No	No	No	No
	E	No	No	No	No	No	No	No
Phase separation	A	No	No	No	No	No	No	No
	B	No	No	No	No	No	No	No
	C	No	No	No	No	No	No	No
	D	No	No	No	No	No	No	No
	E	No	No	No	No	No	No	No
Conductivity	A	No	No	No	No	No	No	No
	B	No	No	No	No	No	No	No
	C	No	No	No	No	No	No	No
	D	No	No	No	No	No	No	No
	E	No	No	No	No	No	No	No
pH	A	5.6	5.6	5.61	5.53	5.51	5.50	5.48
	B	6.1	6.0	5.6	5.38	5.31	5.30	5.23
	C	6.3	6.3	6.57	6.43	6.37	6.34	6.32
	D	6.5	6.5	6.51	6.46	6.43	6.41	6.38
	E	6.5	6.5	6.48	6.47	6.47	6.41	6.40

Fig.5: Final Product of Karanja-oil based herbal Sunscreen.

4. CONCLUSION

This study was conducted with aim to develop and estimate Karanja oil- based photoprotective herbal sunscreen. The formulated Karanja oil-based cream exhibited no

reddishness, irritation inflammation and edema throughout irritancy studies in animals. The test of red dye affirms that the prepared sunscreen was o/w emulsion type. Also the homogeneity test confirms the oil evenly distributed in cream. When sunscreen was kept for longer period, it was found that there was no alter in colour of cream. Also the formulated cream exhibited non greasy effect, later application of cream on the skin. The slipperiness, emolliency and quantity of rest left after applied on skin was prominent. The cream was with no trouble removed by washing under tap water. Studies have proven that sunscreens made with karanja oil consistently block UV rays and increase UV protection. Additional clinical studies are needed for further confirmation.

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