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Formulation and *In Vitro* Evaluation of Sun Protection Factor of Herbal Sunscreen Cream Containing *Butea monosperma, Neolamarckia cadama* and *Punica granatum Extracts*

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ABSTRACT

Sun radiations are the primary source of light & energy and main causative factor in various skin conditions such as sunburn, photoaging, skin cancer development. These harmful effects may be due to the production of free radicals (Reactive Oxygen Species) . Physical sun protection is not sufficient to get rid of such unwanted changes skin appearance and physiology. Now a day's cosmetics and pharmaceuticals are added with natural sunscreens protecting reagents to provide health benefit apart from beautification. The aim of the present study is to formulate and evaluate natural sun protective topical formulation comprising methanolic extract of *Butea monosperma* (Lam.) flower, *Neolamarckia cadama* (*Roxb.*) leaves and *Punica granatum (Linn) peel.* The formulated cream was evaluated for its Total flavonoid and phenolic content, *In vitro* sun protecting activity and physicochemical parameters. The Total flavonoid and Phenolic content were found to be 42.07±2.03 mg QE/g and 57.03 ± 3.06 mg GAE/g respectively. While the SPF values were found to be 1.65, 2.94, 3.87 for the concentrations 20ug/ml, 30ug/ml and 40ug/ml respectively. From the study we can conclude that cream will enhance the sun protection property and it will significantly contribute to UV absorbing property of conventional sunscreen formulations. It will provide the great advantage of avoiding the adverse and undesirable effects pf synthetic compounds.

Keywords: *Butea monosperma* (Lam.),*Neolamarckia cadama* (*Roxb.*),*Punica granatum (Linn),* sun protection and antioxidant .

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Abbreviations Used:

Methanolic extract of *Butea monosperma (BM),* Methanolic extract of *Neolamarckia cadama (NC) and* Methanolic extract of *Punica granatum (PG).*

INTRODUCTION

Skin is the outermost and the largest part of the body and very sensitive to photo radiations because getting direct expose to solar radiations. Solar radiation causes harmful effects by ultra violet (UV) region of the electromagnetic spectrum. The harmful effects of solar radiation are usually caused by the ultraviolet (UV) region which can be divided into three regions: UVA (320-400 nm), UVB (290-320 nm) and UVC (200-290 nm). UVC radiation is filtered out by the ozone layer before reaching earth. UVA and UVB radiations are not completely filtered out by the ozone layer and causing damage to skin, sunburn and premature aging of the skin.[1].One of the main reasons for the deleterious effects of UV radiations is the formation of reactive oxygen species(ROS), So it is quite recommended that topical application of phytoconstituents with antioxidant effect is an effective strategy for protecting the skin against UV-mediated oxidative damage. In order to minimize these ill effects of ultraviolet radiations on humans, the need for photoprotection has increased. Limiting excessive exposure to sunlight and the use of topical sunscreens which can repair pre-existing skin damage and prevent further damage[2].

During the market survey, it is found that there are many sunscreen formulations available in markets which are used in protection of skin from UV rays. Various formulations having sun protection property on basis of their efficacy of UV ray absorption but maximum formulations are of high cost and incorporated synthetic molecules are with potential toxicity and even carcinogenesis. Hence there is need to develop and evaluate effective and safe sunscreen product which will gives protection from sunburn, wounds, cracks, wrinkles, premature ageing and natural ingredients in protection from long term damaging effects of sunrays mediated free radicals[3]. The efficacy of sunscreens is characterized by the sun protection factor (SPF). The SPF is a numerical rating system calculated by *in vitro* and *in vivo* method to indicate the degree of protection provided by a sun care product like sunscreen, it is defined as the ratio of the minimal erythema dose (MED) of solar radiation measured in the presence and in the absence of a sunscreen agents. Regulatory agencies like the US-FDA and COLIPA (TheComité de Liaison de la Parfumerie in Europe), TGA has made *in vivo* testing on human volunteers using an erythemal endpoint to determine the SPF of topical cream mandatory. Although it is a recommended and recognized method by COLIPA, TGA it beneficial to check the effect of formulation onto the skin[4]. Sometimes this clinical trial is harmful for the humans and also unpredicted scientific accuracy and reproducibility, whereas an *in vitro* measurement has the advantage of not exposing human subjects to harmful UV radiation, is cost effective and provides us with statistically significant data which helps us to develop an effective sunscreen product. Thus, for economical, practical and ethical considerations a suitable method for *in vitro* determination of SPF is used [5,6].

Punica granatum (Punicaceae) commonly known as pomegranate. Pomegranate peel and its chemical components possess various pharmacological and toxicological properties including antioxidant, anti-inflammatory , anti-cancer , anti-angiogenesis activities and ultraviolet radiation-induced skin damage[7]. Scientists from the institute of Hygiene and environmental medicine have reported that the peel offers high yields of phenolics, flavonoids and proanthocyanidins than the pulp[8]. It is stated that Ellagic acid, a polyphenol antioxidant found in large quantities in pomegranates, helps in healing sunburns and is known to reverse sun damage. And it was found that the incredible antioxidant activity in pomegranates may reduce the harmful effects of ultra violet radiations and inhibit the growth of skin tumors [9,10].

Butea monosperma (Fabaceae) widely distributed in India and in all Asian hemispheres. It is a plant that has been electively used in traditional Asian medicines for centuries. Recent *in vivo* and *in vitro* studies have indicated its anti-diabetic, anti-cancer, anti-inflammatory, anti-asthmatic, anti-oxidant, anti-convulsant, anti-microbial, anti-viral and hepatoprotective properties. *Butea monosperma* flowers are known to contain flavonoids and glucosides. Butin, isobutrin and butein are main phytoconstituents of flowers[11]. *Butea monosperma* plant evaluated for sunscreen activity by *in-vitro* method transmission spectroscopy and spectroscopic methods[12].

Neolamarkia cadamba (Rubiaceae) known as kadam. This plant is widely used in the treatment of diabetes mellitus, diarrhoea fever, inflammation, cough, vomiting, wounds, ulcers and antimicrobial activity. Also, as analgesic, antipyretic, anti-inflammatory hypolipidemic and antidiabetic. The leaves extracts were also shown antioxidant properties due to the presence of phenolic and flavonoid compounds [13]. The potential of the different extracts and fractions to scavenge different free radicals in different systems indicated that they may be useful therapeutic agents for treating radical-related pathologic damage [14].

To find new natural sources which could be used natural antioxidants as well as sun protecting agents in pharmaceutical or cosmetic preparations, the main aim of this study is formulation and *In vitro* evaluation of herbal cream for its sun screen protecting activity containing mixture of three plant extracts.

MATERIAL AND METHODS

Collection and authentication of plant material

Fresh flowers of the plant *Butea monosperma* (Lam.) and fresh leaves of the plant *Neolamarckia cadama* (*Roxb*.) were collected from Dist. Ahemdabad . Gujarat in the month of February-March and August -September respectively. The fruit of *Punica granatum* was collected from the medicinal plant garden of Alard College of Pharmacy, Pune in the month of October and pericarp is removed from the fruit for further study. Plant parts were cleaned, sun dried and authenticated at Botanical Survey of India, Western Regional Centre, Pune. The identified plants and their Specimen voucher nos. are BSI/WRC/IDEN.CER./2016/664 for *Butea monosperma* (Lam.) family *Fabaceae*, BSI/WRC/IDEN.CER./ 2016/665 for *Punica granatum (Linn) family* Lythraceae and BSI/WRC/IDEN.CER./2016/666 for *Neolamarckia cadama* (*Roxb*.) family *Rubiaceae.* All plant parts were dried and proceeded for pulverization to coarse powder for extraction.

Extraction of plant material

100 gm of each plant powder was subjected to successive Hot Continuous Soxhlet Extraction with petroleum ether and then with methanol for 36 hrs. The extracts were filtered and evaporated to dryness using rotary evaporator at the temperature of 40°C. All the concentrated extracts were cooled and finally it was placed in the desiccators and were subjected for further studies [15,16,17]. The percentage yield obtained is mentioned in **Table No: 1**

Table 1: Percentage Yield

Sr.No	Name of the plant extract	Name	% Yield (w/w)
1	Methanolic Extract of *Neolamarckia* cadama	PG	11.35
2	Methanolic Extract of *Butea monosperma*	BM	4.54
3	Methanolic Extract of *Punica granatum*	NC	3.79

Instruments

UV spectrophotometer- JASCO, (V 630), Brookfield Viscometer (DV-II + Pro) , pH meter - ELICO (LI 20), Microcentrifuge- Bio Lab(BL 135 D), Sonicator- Equitron , Electroni Balance -Wensar (PGB 200).

Formulation of sunscreen cream

Step I: Aqueous Phase Preparation

Required quantity of water was weighed and from that 80% quantity was heated at 70°C. Chlorocresol and Sodium Dihydrogen Phosphate Dihydrate were added and stirred on electric water bath till the solution became clear.

Step II: Oil Phase Preparation

Light Liquid Paraffin, white Soft Paraffin, Cetostearyl alcohol , Cetomacrogol 1000 were added to another beaker and temperature was maintained to 70°C under constant high speed stirring on electric water bath till all the waxes melts completely.

Step III- formation of emulsion

Oil Phase was transferred to aqueous phase with continuous the mixing of emulsion at high speed for 10 min with homogenizer till both phases get mixed thoroughly and temperature was recorded.

Step IV- Preparation of Active Solution

Propylene Glycol heated to 70ºC and mixture of extracts was added to it under continuous stirring till clear solution formed. Active solution was then added to mixing phase and quantity was adjusted with remaining water (20%). Composition of the cream is mentioned in **Table No: 2** and formulated cream is shown in **Figure No 1.**

Table No: 2 Composition of Herbal Sunscreen Cream

Sr.No	Ingredients	Uses	Components (% w/w)
1	Herbal Extract Mixture	Active	2
2	Cetomacrogol 1000	Emulsifier	3
3	Cetostearyl Alcohol	Emulsifier	8
4	Methylparaben	Preservative	0.15
5	Propylparaben	Preservative	0.5
6	Light Liquid Paraffin	Emollient	5
7	White Soft Paraffin	Emollient	15
8	Propylene Glycol	Humectant	5
9	Chlorocresol	Preservative	0.038
10	Sodium Dihydrogen Phosphate Dihydrate	Buffer	0.0252
11	Purified Water	Vehicle	q.s

Figure No: 1 Herbal Sunscreen Cream

Evaluation of physicochemical parameters of cream

Cream was evaluated for its physicochemical parameters and the results are mentioned in **Table No .3**

Determination of physical appearance

The cream was observed for its color, odour and appearance.

Type of emulsion

When sample readily mixes with mineral oil, it is w/o and if it mixes with water then it is o/w emulsion on.

Determination of pH

5 g of the cream was weighed accurately in a 100ml beaker. 45ml of water was added & the cream was dispersed in it. The pH of the suspension was determined at 27° C using the pH meter. pH was also determined for accelerated stability testing done for 6months.

Homogeneity

The formulation was tested for the Homogeneity by visual appearance and by touch.

After feel

Emollient, slipperiness and amount of residue left after the application of fixed amount of cream was checked.

Irritancy Test

About 1-3gm of material to be tested was placed on a piece of fabric or funnel and applied to the sensitive part of the skin e.g. skin behind ears. The site of patch is inspected after 24 hrs.

Viscosity

Viscosity of the formulation was determined using rotational-type viscometer (Brookfield DVII+ Pro, spindle No 7 at 25±1°C). Measurements were taken in 3 replications in 100 rpm [18,19]. Viscosity values were recorded in centipoise (cp).

Spreadability

Spreadability is expressed in terms of time in seconds taken by two slides to slip off from the formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of the two, better the spreadability. The other slide was placed on top of the formulation was sandwiched between the two slides across the length of 5 cm along the slide. 100 g weight was placed up on the upper slide so that the formulation between the

two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of formulation adhering to the slides was scrapped off. One of the slides was fixed on which the formulation was placed. The second movable slide was placed over it, with one end tied to a string to which load could be applied by the help of a simple pulley and a pan. A 30g weight was put on the pan and the time taken for the upper slide to travel the distance of 5.0cm and separate away from the lower slide under the direction of the weight was noted. The spread ability was then calculated from the following formula [20].

Spread ability= m ×l /t

m = weight tied to the upper slide (30g)

l =length of glass slide (5cm)

t =time taken in seconds to separate them.

Test for microbial limit

The formulated cream was inoculated on the plates of agar media by streak plate method and a control was prepared by omitting the cream. The plates were placed in to the incubator and are incubated at 37 ^{0C} for 24 hours. After the incubation period, plates were taken out and check the microbial growth by comparing it with the control. *Pseudomonas aeruginosa, Staphylococcus aureus, Canidida albicans* [21].

Thermal Stability

Thermal stability of the formulation was determined by the humidity chamber controlled at 60- 70% RH and 37 ± 1^{o}C .

Accelerated Stability Study

Formulated cream was subjected for accelerated stability studies for 6 months, at 25^{o}C & 60 %RH for 3 months and further at40^{o}C & 75 %RH for next 3 months and evaluated for its stability for the parameters like viscosity, $_{P}$H, phase separation, liquefication and Stability on Centrifugation. Evaluated parameters on accelerated stability studies are mentioned in **Table No: 4.**

Stability on Centrifugation

During the centrifugation studies, sunscreen was centrifuged at 3500- 13,500 rpm at interval of 500 rpm for 10 minutes[22].

Estimation of Total flavonoid content

$AlCl_3$ colorimetric method

Preparation of stock solution

10 mg of the cream formulation was accurately weighed and transferred to 50 ml volumetric flask and the volume was adjusted with methanol (80%) and then diluted to 100,50 and 25 µg/ml.

Determination of Total flavonoid content

A volume of 0.5 ml of cream formulation of various concentration 100, 50, 25 µg/ml were separately mixed with 1.5 ml methanol (80%), 0.1 ml of $AlCl_3$ (10%), 0.1 ml potassium acetate solution (1 M) and 2.8 ml distilled water and incubate at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm. Blank was prepared in similar way by replacing $AlCl_3$ with distilled water[23]. The total flavonoid content of the cream was expressed in terms of standard quercetin equivalent (QE) µg/g.

Determination of Total phenolic

Total polyphenol content was measured using the Folin-Ciocalteu colorimetric method 100 µL cream was mixed with Folin-Ciocalteu reagent (0.2 mL) and H_2O (2 mL) and incubated at room temperature for 3 min. Following the addition of 20% sodium carbonate (1 mL) to the mixture, total polyphenols were determined after 1 h of incubation at room temperature. The absorbance of the resulting blue colour was measured at 765 nm with a UV-VIS spectrophotometer. Quantification was done with respect to the standard curve of gallic acid[24]. The results were expressed as Gallic Acid Equivalents (GAE), milligrams per g of dry weight. All determinations were performed in triplicate (n = 3)[24]. The results for total flavonoid content and phenolic content are mentioned in **Table No: 5**.

Determination of Sun protection factor

Sample preparation

1.0 g of cream was weighed, transferred to 100 ml volumetric flask, diluted to volume with methanol followed by ultrasonication for 5 min and then filtered through cotton, rejecting the first 10 ml. A 5.0 ml aliquot was transferred to 50 ml volumetric flask and diluted to volume with methanol. Then 5.0 ml of aliquot was transferred to 25 ml volumetric flask and the volume completed with methanol. Thereafter, absorbance values of each aliquot prepared were determined from 290-320 nm at 5 nm interval, taking methanol as a blank. The measurements were taken in triplicates and the determinations were made at each point, followed by application of Mansur equation.

Mansur et al. (1986) developed a very simple mathematical equation which substitutes the in vitro method proposed by Sayre et al. (1979), utilizing UV Spectrophotometry and the following equation.

$$SPF_{in\ vitro} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times abs(\lambda)$$

Where CF=Correction factor (10), EE (λ) = Erythmogenic effect of radiation with wavelength λ, Abs (λ) = Spectrophotometric absorbance values at wavelength λ.

The aliquots prepared were scanned between 290-320 nm and the obtained absorbance values were multiplied with the respective EE (λ) X I values[25]. Then, their summation were taken and multiplied with the correction factor (10). The values of EE×I are constant, they were determined by Sayre et al. The absorbance for the different concentrations and respective SPF values were calculated[25].

Statistical analysis

The analyses were performed in triplicate, and the results expressed as mean ± SEM and mean ± SD.

RESULT AND DISCUSSION

Evaluations of physical parameters

The cream was observed for its color, odour and appearance.

Colour

Color of the cream was greenish and the odor was not found to be obnoxious and appeared smooth.

Type of emulsion

The prepared cream is of O/W type of emulsion and it was easy to wash off.

Determination of pH

The pH of the cream was found to be in range of 6-7 which is excellent for skin pH.

Homogeneity

The cream found to be homogenous and uniform. It appeared same on accelerated stability testing.

After feel

It has good emollience and slipperiness.

Irritancy test

The cream shown no redness, oedema, inflammation and irritation during irritancy studies. Thus, the cream found to be safe to use for skin

Viscocity

Measurements were taken in 3 replications in 100 rpm (n: 3). Viscosity values were recorded in centipoise (cp) and it is found to be 914(cp).

Spreadability

The formulated cream was determined for Spreadability which indicates good spreading property when cream was applied.

Test for microbial limit

The formulated cream has shown no presence of microorganisms when inoculated on the plates of agar media by streak plate method.

Thermal Stability

Thermal stability of the formulation was determined by the humidity chamber controlled at 60- 70% RH and 37 ± 1°C and it was found stable during the study.

Accelerated stability study

The parameter observed after the accelerated stability studies were found stable and no more parameters got changed during the period of 6 months in context with color, pH, viscosity , stability on centrifugation etc.

Table 3: Physicochemical Evaluation of the Cream

Sr.No	Parameters	Observations
1	Colour	Slight greenish
2	Type of emulsion	o/w
3	pH	6.3
4	Homogeneity	Uniform and homogenous
5	After feel effect	Smooth and slippery
6	Irritancy test	No irritation
7	Viscosity	914cp
8	Spreadability	20.6
9	Consistency	Good
10	Grittiness	No
11	Washability	Easy to wash
12	Microbial Limit	No microorganism growth

Table No: 4 Accelerated Stability Studies of Sunscreen Cream

Sr. No	Day	Colour	pH	Viscosity	Homogeneity	Centrifugation	liquefication
1	0	Greenish	6.37	914cp	Uniform and homogenous	Stable, No Phase separation	No liquefication
2	3 Months (25°C & 60 %RH)	Greenish	6.33	913cp	Uniform and homogenous	Stable, No Phase separation	No liquefication
3	3 Months (40°C & 75 %RH)	Greenish	6.35	911cp	Uniform and homogenous	Stable, No phase separation	No liquefication

The Total flavonoid content and Total Phenolic content

The cream was found to be 42.07±2.03 mg QE/g and the Total Phenolic content of the cream was found to be 57.03 ± 3.06 mg GAE/g. The flavonoid and phenolic compounds are main source of antioxidants and free radical scavengers hence, there should be a close correlation between the phenolic content and antioxidant activity. It will help naturally for sun protection.

As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Flavonoids, including flavones, flavanols and condensed tannins are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups.

Table 5 Total Flavonoid and Phenolic content

Sr.No	Parameter	Result
1	Total Flavonoid Content	42.07±2.03 mg QE/g
2	Total Phenolic Content	57.03 ± 3.06 mg GAE/g

Determination of Sun protection factor

Sun protection factor determined by spectrophotometric method with increasing concentrations given increasing SPF values. The concentration 20ug/ml ,30 ug/ml and 40ug/ml shown significant increase in SPF values and which indicates that the combination of three extracts having property to block UV radiations. The results obtained are mentioned in **Table No 6**. The proposed UV spectrophotometric method for determination on in vitro Sun Protection Factor is rapid and cost effective.

Table 6: *In vitro* SPF values of cream at different wavelength.

		Conc. 20ug/ml		Conc. 30ug/ml		Conc. 40ug/ml	
Wave length (nm)	**EE (λ) × I (λ)**	**Absorbance (λ)**	**EE (λ) × I (λ) × Abs (λ)**	**Absorbance (λ)**	**EE (λ) × I (λ) × Abs (λ)**	**Absorbance (λ)**	**EE (λ) × I (λ) × Abs (λ)**
290	0.015	0.2305± 0.0002	0.003458	0.1417± 0.0240	0.002125	0.52213± 0.0004	0.007832
295	0.0187	0.1939± 0.0001	0.015841	0.3529± 0.0646	0.028831	0.44850± 0.0002	0.036642
300	0.2874	0.1749 0.0002	0.0502662	0.3166± 0.0021	0.090799	0.40790± 0.0001	0.117230
305	0.3278	0.1625 0.0003	0.053278	0.2922± 0.0006	0.0958050	0.38040± 0.0001	0.124695
310	0.1864	0.15133 0.0002	0.028223	0.2734± 0.0012	0.0509953	0.35673± 0.0004	0.066530
315	0.0837	0.1421 0.0001	0.011922	0.25713± 0.0045	0.0215734	0.33827± 0.0001	0.0283805
320	0.018	0.1326 0.0004	0.002386	0.2418± 0.0346	0.0043524	0.32030± 0.0002	0.005765
	Total 1		0.165377± 0.35843		0.294483± 0.014602		0.387077± 0.0002
SPF			**1.65377**		**2.944829**		**3.870768**

CONCLUSION

The present research work gives us a better platform to use the natural source of material for their sun protection property. As the mixture of all the three plant extracts in cream formulation exhibited good amount of flavonoid and phenolic content. Now a day there is great market potential for sunscreen chemicals either synthetic or natural or in combination due to awareness of protection from hazardous UVA as well as UVB rays. Stable and uniform UVA/UVB protective sunscreen product with potential SPF can definitely provide effects especially against free radical generated skin damages along with UV-rays blocking. All results suggested that formulated sunscreen cream is physically stable and may be served as active sun screening agent in order to protect the skin from harmful UVR.

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