Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2025, Volume 13, Issue 05
ISSN: 2321–3647(online)

Herbal Sunscreen Lotion Enriched with Custard Apple (Annona squamosa) Seed Oil: Preparation and In Vitro Evaluation.

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ABSTRACT

Herbal sunscreens are widely used by almost everyone on this earth to prevent skin from harmful effects of UV radiation from sun. Herbals are beneficial to the humans and they do not have any side effects and have better safety. The Present work is based on the preparation and evaluation of the herbal sunscreen lotion enriched with the custard apple seed oil which promotes healthy looking skin, provides glow, delays ageing and having healing properties. Lotion were prepared from the extract of the plant material such as, custard apple seed oil, aloe gel, etc which minimizes chemical exposure and this herbal ingredients offer antioxidant, anti-inflammatory and moisturizing properties. Evaluation of the lotion was performed on parameters such as a Organoleptic properties, pH, homogeneity, spreadability and SPF of herbal lotion and it was found to be same as with the sunscreen that were currently available in the market.

Keyword: Herbal Sunscreen, Custard apple seed oil, SPF, Evaluation.

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Received 10 April 2025, Accepted 13 May 2025

Please cite this article as: Ingawale RN *et al*., Herbal Sunscreen Lotion Enriched with Custard Apple (Annona squamosa) Seed Oil: Preparation and In Vitro Evaluation. American Journal of Pharmacy & Health Research 2025.

INTRODUCTION

Sun protection products including sunscreen creams, lotions are designed to absorb or reflect the sun's UV radiation to protect the skin from damage. Ultraviolet light which is responsible for sunburn, suntan and increases the risk of basal cell carcinoma and malignant melanoma [1]. Sunscreens helps to decrease the risk of skin cancer and sunburn-like skin reactions (sun sensitivity). The active ingredients in sunscreens work either by absorbing the sun's ultraviolet (UV) radiation, preventing it from reaching the deeper layers of the skin, or by reflecting the radiation [2]. The UV spectrum lies between 200 nm to 400 nm and is commonly divided into three regions: UV-A: 320-400 nm, UV-B: 280-320 nm, UV-C: 200-280 nm [3]. UVA rays can be divided into two key wavelengths: short-wave UVA (320-340 nm) and long-wave UVA (340-400 nm) [1]. Exposure to solar radiation is recognized to have negative effects on the human skin. Ultraviolet radiations are harmful to skin that result in suppression of skin's immune system. This immunosuppressive effect generates photodermatoses, skin premature aging and skin cancers. Herbal Sunscreen (also known as Herbal sunblock, Herbal suntan lotion) is a lotion, spray or other topical product that helps to protect the skin from the sun's ultraviolet (UV) radiation, and which reduces sunburn and other skin damage, with the goal of lowering the risk of skin cancer with the help of herbes [4]. Herbal extracts provide a healing, softening, regenerating, and sunscreen action. Numerous plant-based substances have been found to be nontoxic, antimutagenic, and anticarcinogenic [5]. The Sun Protection Factor (SPF) measures the length of time a product protects against skin reddening (sunburn) from UV (Ultra Violet) radiation, compared to how long it takes to redden without protection [1]. The higher SPF value indicates maximum protection from sun rays [6].

Annona squamosa, also known as sugar-apple, sweetsop, custard apple or sitaphal. The name sitaphal is originated from the two Sanskrit words "sita" means cold and "phal" means fruit. It belongs to family annonaceae [7]. It represents a tropical, endemic species, which usually grow in West Indies, Central and South America, Brazil, India, Mexico, Taiwan, and Egypt [8]. It is a small semi-sized branched tree usually reach from 3 to 8 m in height [9]. The genus call, 'Annona' is from the Latin word 'anon', which means that 'every year produce' [10]. The seeds are dark brown or black in colour, about 1 cm in size and each fruit contains about 35–45 seeds [7, 11]. The seed of the A. squamosa is mainly composed of a seed kernel (67.7%) and a seed coat (32.4%) [9]. Custard apple seeds contain 23% oil of which 9.8% is a hydroxyl acid, the oil contains 38.6% saturated fatty acids and 61.4% unsaturated fatty acids [12]. Custard apple seed's oil contain a total of 11 fatty acids, among which oleic acid (47.4%), linoleic acid (22.9%),

stearic acid (13.6%) and palmitic acid (12.1%) were present in higher amounts. Heneicosanoic acid (2.3%), eicosanoic acid (0.9%), margaric acid (0.2%), 11-eicosanoic acid (0.2%) and dihydro sterculic acid (0.1%) were all found in lesser amounts in the oil. These 11 fatty acids together constitute nearly 99.8% of the oil. 17-methyloctadecanoic acid (0.1%) and palmitoleic acid (0.01%) were identified in traces or in a minimum amount [9, 12]. Antibacterial constituents present in the seeds of Annona squamosa are Annotemoyin-1, Annotemoyin-2 and squamocin and cholesteryl glucopyranoside [8]. The studies reported that A. squamosa is pale yellow in color, viscid in appearance and with a characteristic smell. Refractive index of oil is 1.474 and specific gravity of oil is 0.922. The oil is freely soluble in petroleum ether.

Various herbal sunscreen ingredients used in present investigation includes Custard apple seed oil, zinc oxide, aloe gel, rose oil, olive oil, vitamin E and rose water. This ingredient provides skin care protection against UV radiations and has soothing properties to heal minor wound. They has potent anti-ageing, moisturizing and emollient effect [13]. Zinc oxide provides physical barrier by reflecting or absorbing or blocking radiations from sun [13]. Aloe vera is a good active ingredient as it prevent burns on skin and blocks UVA and UVB rays. Aloe vera gel is used in herbal lotion for its moisturizing effect and maintains the skin's natural moisture balance. Rose water helps to booster the effectiveness of SPF. It can be used to lighten skin pigmentation and remove oils and dirt from skin. Vitamin E helps to cleanse the skin, remove the impurities and help to improve skin elasticity [2]. Custard apple seed oil has plenty of applications in cosmetic industry. It is good for skin rejuvenation. It naturally heals the skin infections. It delays ageing and keeps skin youthful. It is good for pimple-prone skin [14]. It provides glow to the skin [7]. The seeds of custard apple exhibit antioxidant and anti-inflammatory effect. The seeds account for 3201 ascorbic acid equivalent antioxidant capacity g/100 of dry extract. The seed oil contains significant value of total tocopherols. Tocopherols (Vitamin E), can be beneficial in sunscreen lotions due to its antioxidant properties, which help protect skin from sun damage. The oil can also provides deep hydration and support skin regeneration [8]. Hence the present study aimed that Custard apple seed oil enriched sunscreen lotion promotes youthful, healthy-looking skin, Provides glow, reduces pimple prone skin issues, delays aging and having healing properties. Also offers a plant based alternative to synthetic additives. In this study, herbal sunscreen lotion was prepared using Custard apple seed oil, rose oil, olive oil, rose water, aloe gel, zinc oxide and HPMC as active ingredients. This herbal lotions were evaluated for physicochemical parameters i.e. colour, pH, spreadability and viscosity. Sun protection factor (SPF) of lotion was determined by in-vitro spectrophotometric method [13].

MATERIALS AND METHOD

Plant material and method:

The plant material (CUSTARD APPLE SEED OIL) used in the formulation were purchased from the S. S. Aromas oil store, Budh Vihar, Delhi-110086. COA (Certificate of Analysis) were obtained from manufacturer. The Aloe Vera Gel obtained from the Wellness Forever Medical Store, Satara. Other ingredients used in the present study used were from laboratory of analytical grade.

Instrument:

Instrument used for analysis were pH meter, Brookfield viscometer, Deep Freezer, UV visible Spectrophotometer.

Formulation of the Herbal Sunscreen Lotion:

For preparation of oil phase, accurate quantities of glycerin, stearic acid, cetyl alcohol, zinc oxide and hydroxy propyl methyl cellulose (HPMC) were weighed as per given in Table 1. The oil phase is prepared by heating glycerin, custard apple seed oil, olive oil, cetyl alcohol, stearic acid, zinc oxide, hydroxy propyl methyl cellulose. Accurate quantity of distilled water and rose water was measured and taken in a beaker and 1.0 g of triethanolamine was added in it and stirred well. This water solution was heated upto a temperature of 80°C to 85°C. After the water solution has reached the required temperature, oil phase mixture and propyl paraben was added slowly into the water phase a little at a time, at 80°C with continuous stirring. Stirring was continued until a smooth and uniform paste was obtained. The prepared sunscreen lotion was set aside to cool. Then weighed quantity of Aloe gel and vitamin E were added in lotion and stirred well until all the ingredients mixed uniformly. Finally rose oil was added for a fragrance. A total of three formulations, F1, F2 and F3 were prepared using various formulas (Table 1) [3].

Table 1: Composition of various sunscreen formulations

Sr. No.	Ingredients	Use	F 1 (%)	F 2 (%)	F 3 (%)
1	Zinc oxide	Physical UV blocker	12.0	12.0	12.0
2	Custard apple seed oil	Hydrating and anti-aging agent	6.0	10.0	-
3	Aloe gel	Soothing agent	5.0	5.0	5.0
4	Cetyl alcohol	Emollient, Coemulsifier	2.0	2.0	2.0
5	HPMC	Thickening agent	10.0	10.0	10.0
6	Glycerin	Humectant	2.0	2.0	2.0
7	Stearic acid	Emulsifier	4.0	4.0	4.0
8	Rose water	Toner , moisturiser	3.0	3.0	3.0
9	Rose oil	Fragrance	1.0	1.0	1.0
10	Olive oil	Moisturiser	2.0	2.0	2.0
11	Vitamin E	Antioxidant	1.0	1.0	1.0
12	Triethanolamine	pH adjuster	1.0	1.0	1.0

13	Propyl paraben	Preservative	0.50	0.50	0.50
14	Distilled water	Vehicle	50.50	46.50	56.50

EVALUTION OF THE FORMULATED SUNSCREEN LOTION:

Physicochemical parameter

Physical parameters of prepared lotion formulation such as color, odour, and appearance were visibly observed [13].

Homogeneity

The lotions were tested for homogeneity by visual appearance and by touch [6].

Determination of Viscosity

Viscosity (in cps) profile of each formulated lotion was measured by Brookfield rotational digital viscometer model LVDV-II+P, USA using LV-spindle 64. With the help of the sample holder, the required quantity of lotion (8 ml sample) was taken and the viscosity was determined at 10-100 rotation per minute (RPM) at 25 °C [3, 13].

PH determination

The lotion was checked for its pH as it is responsible for the stability and irritability at the application site. The digital pH meter was used to determine the pH of the prepared lotion. [15]

Spreadability

Spreadability refers to the ease with which product can be spread without losing its firmness and determined therapeutic efficiency of herbal sunscreen lotions. The appropriate amount of herbal sunscreen was applied between two slides, and under specified load directions, and the two sides took the time in seconds to slide off. [13, 17]

$$S = M \times L / T$$

Where M = weight tied to upper slide

L = length of glass slide

T = time taken to separate the slides

Ease of Removal:

The ease of removal of the cream applied was examined by washing the applied part with tap water. [6]

In vitro Occlusive study

The occlusivity of the formulations was determined by occlusion factor. If the occlusion factor is "0", then it indicates that there is no occlusion effect when compared with the reference. The maximum occlusion factor is "100". Occlusive factor is a measure of water flux through the filter paper. The Whatman filter paper with a definite surface area was selected and the sunscreen

cream of about 250 mg was evenly distributed on the filter paper. Approximately 30 ml of distilled water was taken in a beaker and the mouth was covered using a filter paper containing cream (test), and same volume of water was taken in another beaker and covered with the Whatman filter paper, without the sample (control). Both the experimental setup was maintain at a relative humidity of 50–55% and at a temperature of 37°C for a period of 24 h. The occlusion factor F was calculated based on the water evaporated through the filter paper. [15, 16]

$F = 100 \times (A-B)/ A$

Where, A water loss without cream

B = water loss with cream

8. Extruditability study

Extrusion of the cream places an important parameter during its application as well as in patient acceptance. The extrudability of herbal sunscreens was determined in this study by calculating the percentage of formulation extruded from the collapsible tube. In clean aluminum collapsible tubes, a weighed quantity of 10 g was filled. A pressure of 1 kg/cm2 was applied for period of 30 s, and the quantity of the cream extruded was weighed. [15, 17]

Efficacy Analysis

Efficacy of herbal sunscreens was determined by In-vitro method using UV Visible spectrophotometer and is usually expressed as Sun Protection Factor (SPF), which is the ratio of UV energy required to produce a minimal erythemal dose in protected skin to unprotected skin. 0.10 % solution (w/v) each of the three formulated sunscreen lotions in ethanol alcohol was prepared by dissolving 0.050 g of the sunscreen lotion in 50.0 ml of ethanol. 0.10 % solution of the one selected Marketed sunscreen lotions (SPF 15) in Ethanol was also prepared. The Preparation of each formulation prepared were scanned between 290 and 320 nm, with 5 nm interval. SPF can be calculated by applying the following formula known as the Mansur equation. Each sample observed in triplicate. [2, 18]

SPF spectrophotometric = $\mathbf{CF_{290}\sum^{320} EE\ (\lambda)\ X\ I\ (\lambda)\ x\ A\ (\lambda)}$

Where, CF = correction factor (10), EE (λ) = erythemogenic effect of radiation with wavelength λ, I(λ) = intensity of solar light of wavelength λ, A(λ) = spectrophotometric absorbance values at wavelength λ. The value of EE×I is constant.

Stability Testing

All three herbal sunscreen lotions were kept alternatively at 20°C and 40°C in freeze-thaw study for stability determination. Then observe for phase separation and colour change. All the tests were performed three times. [17]

Statistical Treatment

Result was expressed as mean ± S.D. All parameter was analysed by 97% confidences level. [3]

RESULTS AND DISCUSSION:

Physicochemical studies

All formulations F1, F2 and F3 were prepared as per Table 1. F1 contains 6% Custard Apple Seed Oil, F2 contains 10% Custard Apple Seed Oil and while F3 is Blank formulation. The results of physicochemical properties such as color, odour, pH are summarized in Table 2. The PH of formulations range from 6.80 to 6.95 that was found to be within the typical skin pH range and did not cause any irritation upon application. The spreadability of formulations are mentioned in given Table B and were found to be in controlled range justifying its compatibility with skin and confirming good cosmetological property. The homogeneity test confirms the uniform distribution of sunscreen lotion on skin. The formulated lotion exhibited non greasy effect after application of lotion on the skin and was easily removed by washing with tap water.

Table 2: Physicochemical evaluation parameter

Sunscreen	Colour	Odour	pH	Spreadability (gm x cm/sec.)
F1	White	Characteristic	6.87 ± 0.2	57.22 ± 0.40
F2	white	Characteristic	6.86 ± 0.3	61.38 ± 074
F3	white	Characteristic	6.92 ± 0.3	50.27 ± 0.90

Viscosity

The viscosity of the Prepared lotions at various RPM was determined by Brookfield viscometer (LV spindle no 64) is given in Table 3. It showed 4510, 4880 & 4150 cps at the 10 RPM, indicating good flowing rate during handling. The viscosity of all formulations is enlisted in the Table 3. As the speed of rotation increases viscosity of the formulations F1, F2 and F3 decreased. Also, higher viscosity of the lotion reveals more protection from microbial growth.

Table 3: Viscosity profile of formulated sunscreen lotions

Sunscreen	η (cps) 10 RPM	η(cps) 20 RPM	η(cps) 30 RPM	η(cps) 50 RPM	η(cps) 60 RPM	η(cps) 100 RPM
F1	4510	4435	3360	2770	2619	2244
F2	4880	4640	4320	3440	3330	2944
F3	4150	3890	3450	2980	2450	2300

CPS Centipoise; RPM rotation per minute

Occlusive study and Extruditability study

In vitro occlusion studies of the sunscreen lotions (F1 - F3) and reference control (without lotion) were performed. The occlusive factor of all the lotion was found to be 80 and occlusion factor of 80 indicates that the sunscreen lotions provide significant occlusivity, reducing water loss by

80% compared to the control. It suggests the lotion can help to retain moisture in the skin and protect the skin from dryness. The 6 gm - 8 gm of lotion was extruded under 1 kg/cm² pressure, hence the formulation shows good extrudability. A higher extruded quantity of lotion from tube indicates better extrudability.

In vitro SPF

The absorbance values of formulations labeled F1, F2, F3 and one marketed sunscreen (Ayur Herbal Sunscreen SPF 15) were measured using UV spectrophotometer and SPF was calculated. The absorbance readings at various wavelengths (λ max) between 290 and 320 nm for both the formulated and commercial sunscreens are provided in Table E. The results showed that F2 has highest SPF of 15.13 ± 0.45 which may be due to the presence of Custard Apple Seed Oil. The formulations F1 and F3 showed medium SPF (as sunscreens with SPF ranging 11–14 are considered to be medium protection sunscreens) which is sufficient for protection against sun burn. SPF determination of marketed sunscreen by in-vitro method and comparison with its claimed SPF indicated that the method is highly suitable and reliable.

Table4: Absorbance values of formulated and marketed sunscreen lotions

Wavelength(nm)	F1	F2	F3	Marketed Sunscreen SPF(15)
290	2.350	2.590	1.201	2.591
295	2.012	2.280	1.300	2.280
300	1.801	1.805	1.281	1.804
305	1.202	1.488	1.263	1.485
310	1.011	1.105	1.202	1.045
315	0.982	0.788	1.151	0.784
320	0.545	0.505	1.100	0.504

Stability study

The stability study carried out by the Freeze Thaw Method. In this test is observed that, there is no any phase separation in the formulation. The Result of the F1, F2, F3 are mention in the following table 5.

Table 5: Stability evaluation parameter

Sunscreen	Stability
	Freeze Thaw Method
F1	Stable
F2	Stable
F3	Stable

CONCLUSION:

The research focused on formulating a herbal sunscreen lotion using plant extracts from Custard Apple Seed Oil and Aloe Vera and evaluating their effectiveness in protecting against sunburn. Three formulations F1, F2, and F3 were prepared by altering the ingredient ratios and then

assessed for their physical and chemical properties, including Sun Protection Factor (SPF). Among these, formulation F2, which contained 10% Custard Apple Seed Oil, demonstrated greater stability and a higher SPF value, indicating it was the most effective as a sunscreen. Since the SPF values of these herbal formulations ranged between 12 and 15, they are suitable for use on normal skin to help prevent sunburn. The sunscreen effect is likely due to the flavonoids, phenolic compounds, and terpenoids present in the herbal ingredients. Additionally, the formulations were confirmed to be non-mutagenic, setting them apart from synthetic sunscreens. Overall, the study suggests that this approach could lead to improvements in sunburn protection through natural means. It also highlights that UV spectroscopy serves as a fast, reliable, and consistent technique for evaluating the performance of herbal sunscreens. The findings may help guide regulatory bodies, researchers, and manufacturers in establishing consistent standards for herbal sun protection products.

Table 6: SPF of formulated and marketed sunscreens

Sr. No	Sunscreen	SPF ± SD
1	F1	13.91 ± 0.43
2	F2	15.13 ± 0.45
3	F3	12.44 ± 0.40
4	Marketed Formulation	15.00 ± 0.44

All values are represented as Mean ± SD (n=3), $p<0.001$

ACKNOWLEDGEMENT:

We are thankful to S.S. Aroma, Delhi, for generous gift of Custard Apple Seed Oil and we are thankful to the staff of the YSPM, YTC, Satara for their valuable time and support. We are also thankful to my mentor Ms. Aneri Adsul Mam (Assistant Professor), YSPM, YTC, SATARA & My all FRIENDS.

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