

DEVELOPMENT AND EVALUATION OF HERBAL ANTIAGING CREAM FORMULATION CONTAINING *ANNONA SQUAMOSA* LEAF EXTRACT

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ABSTRACT

Objective: The objective of this research article is to develop and evaluate herbal anti-aging cream using *Annona squamosa* leaf extract due to its antioxidant potential.

Methods: Free radical scavenging activity of *A. squamosa* aqueous was determined by 2,2-diphenylpicrylhydrazyl (DPPH) method. 0.004% ethanolic DPPH solution was prepared and from this solution 3 ml was poured to each solution of the concentrated sample, after 30 min the absorbance of the anti-aging cream sample preparations was taken at 517 nm by a ultraviolet spectrophotometer and comparison is made between the absorbance of a sample and ascorbic acid (standard). The qualitative phytochemical analysis of *A. squamosa* (custard apple) leaf extract shows the presence of flavonoids, tannins, alkaloids, and phenols and absence of terpenoids and steroids

Results: Formulation A₃ shows good DPPH scavenging activity as compared to formulation A₁ and A₂ and ascorbic acid. Formulation A₃ is stable for 3 months.

Conclusion: Due to high antioxidant values of *A. squamosa*, it is concluded that it is possible to develop anti-aging cream using an aqueous extract of *A. squamosa* leaf.

Keywords: Skin aging, Free radicals, Antioxidants, *Annona squamosa*, 2,2-Diphenylpicrylhydrazyl assay.

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INTRODUCTION

Aged skin is a usual progression, and it is a consequence of the continual decaying process because to the harm of cellular DNA and protein [1]. Air, harsh sun rays, other environmental pollutants or other mechanical and chemical insults, induce the generation of free radicals (FR) as well as reactive oxygen species (ROS) of our own metabolism when human being exposed to it. Thus, skin aging is divided into these two categories: Natural or sequential and extraneous or photo-aging into common terms [2,3].

A FR is any chemical species (atom, ion, or molecule) that contains an unpaired electron and in biological systems the common source of FR is oxygen [4]. FR are created as a consequence of ATP production by the mitochondria when cells use oxygen to generate energy, and they are generally called as ROS that result from the cellular redox process and play a dual role as both toxic and advantageous compounds [5].

FR are major causative factors in the induction of various skin diseases, including skin tumors, skin wrinkling, and skin aging. Ultraviolet (UV) mainly produces FR/ROS through interaction with endogenous photo sensitizers which leads together to cumulative structural and physiological alterations and progressive changes in each skin layer as well as changes in skin appearance [6,7].

Antioxidative defense mechanisms are the most effective path to eliminate and diminish the action of FR which cause oxidative stress [8]. They are natural scavengers of FR, known to cause cellular damage and consequent age-related medical disorders (including wrinkling of skin) [9]. Topical application of antioxidants may be beneficial for protecting the skin against environmental factors. Antioxidant compounds play an important key ingredient in skin caring products such as creams and lotions which have received importance in the present scenario [10,11]. Antioxidants

such as Vitamin E and C, carotenoids, flavonoids, and tannins are present in various plants insubstantial amount and can be utilized to scavenge the overindulgence FR from the human body. Custard apple plant is one of them which contains potent antioxidants [12].

Annona squamosa, one of the important medicinal plants, commonly called "custard apple," belonging to the family Annonaceae. It possesses a wide variety of pharmacological activities and is used in traditional applications [13]. Flavonoids are natural products, which have been shown to possess various biological properties related to the antioxidant mechanism and leaves of *A. squamosa* are rich in flavonoids such as rutin and hyperoside [14]. Presence of acetogenins, polyphenols in leaves of *A. squamosa* probably play a role as an effective FR scavenger [15]. *A. squamosa* extract increases cellular proliferation and increases collagen [16]. Since custard apple has tremendous antioxidants, it is good if custard apple is being developed as pharmaceutical products, especially as cosmetics. Thus, the current research study focused to protect the skin against oxidative damage and eliminate the FR.

METHODS

Leaves of custard apple (*A. squamosa* Linn.) were collected from local regions near Ratlam city (M.P.). The leaves were authenticated by Dr. S.N. Mishra (head AINP on M and AP) KNK college of Horticulture, Mandsaur (M.P.). Chemicals were collected from the storehouse of college.

Extraction of *A. squamosa* (custard apple) leaves by means of distilled water

Leaves of *A. squamosa* were shade dried at room temperature for 4-5 days. The dried leaves were crushed to coarse powder. *A. squamosa* leaves extract was prepared by boiling 30 g leaves of *A. squamosa* in 800 ml of water for 2 h and then filtered by muslin cloth. Then, the

liquid obtained was poured into a China dish and heated gently over the water bath. When the liquid gets evaporated to cool the content and scratches it, the dark brown extract was obtained. The extract stored in a refrigerator at 2-8°C for subsequent experiments (Fig. 1) [17,18].

Phytochemical screening of leaf extract of *A. squamosa* (custard apple)

Phytochemical screening of the freshly prepared crude extract was qualitatively tested for the presence of chemical constituents, and it was performed using the following reagents and chemicals [19].

Test for flavonoids

A few drops of dilute sodium hydroxide were added to 1 ml of the extract. An intense yellow color was produced in plant extract, which becomes colorless in an addition of a few drops of dilute acid indicates the presence of flavonoids [20].

Test for terpenoids

Chloroform (2 ml) and concentrated H_2SO_4 were added with plant extract to form a layer. The presence of terpenoids is determined when a reddish-brown coloration at the interface was formed [21].

Test for tannins and phenols

The test extract was taken in water, warmed and filtered. 5 mL of the filtrate were allowed to react with 1 mL of 5% ferric chloride solution. Dark green or deep blue color shows the presence of tannins and phenols [22].

Test for the steroid

From 0.5 ml of acetic anhydride to 0.5 ml of chloroform, the extract was treated and then concentrated solution of sulfuric acid was added slowly, and the green-bluish color was produced which shows the presence of steroids [23].

Test for alkaloids

About 1 ml of extract adds to 2 ml of Wagner's reagent (iodine in potassium iodide) a reddish brown precipitate confirms the presence of alkaloids [24].

A procedure of anti-aging cream

An anti-aging cream contains an aqueous phase and oil phase. Oil phase like (stearic acid an emulsifier and other oil miscible components olive oil, and cetyl alcohol) was mixed (Part A) and heated up to 75°C. Components of aqueous phase (Part B) mixed together and warmed to about the same temperature of an oil phase. After heating, the aqueous phase and oil phase were mixed by continuous stirring in a magnetic stir. The therapeutically active ingredient and preservative sodium benzoate were added after cooling to 40°C. Perfume was added (Table 1 and Fig. 2) [25,26].

Determination of antioxidant activity

The FR scavenging activity of the H-donor ability was assessed using an ethanol solution of 2,2-diphenylpicrylhydrazyl (DPPH), a stable nitrogen-centered FR. FR scavenging activity of an anti-aging cream formulation was determined by DPPH which acts as a stable FR.

Sample preparation

About 10 mg cream formulations were weight and dissolve in ethanol. Prepared sample was filtered by the Whatman filter paper, and volume makes up to 10 ml in a volumetric flask.

Preparation of standard

Accurately weighed 10 mg of ascorbic acid and dissolved in 10 ml of ethanol. From this solution, take 2.5 ml and volume make up to 25 ml with ethanol thus the stock solution is prepared. This stock solution was serially diluted separately to obtain different concentrations.

Procedure

Up to 3 ml of 0.004%, ethanolic DPPH solution was added with the 0.5 ml sample solution. At 517 nm DPPH shows its absorbency and at the same wavelength sample absorbance was taken by a UV spectrophotometer after 30 min and then comparison was made between the absorbance of a sample and the absorbance of ascorbic acid (standard) Then, the percentage inhibition was calculated by the following equation [27,28].

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of the sample})}{(\text{Absorbance of control})} \times 100$$

Evaluation parameters of anti-aging herbal cream

Organoleptic properties

The organoleptic test of a cream preparation was performed visually including color, odor, and clarity [29].

Stability of a formulation

Stability of a formulation was evaluated by placing formulation at different storage conditions, that is, 8, 25, and 40°C for 3 months [30].

Homogeneity

The prepared cream formulation was evaluated by visual appearance for its homogeneity.

After feel

The quantity of cream formulation left after the application and slipperiness and emolliency was also reported [31].

Washability

To determine the washability of a formulation a small amount of cream applied to the skin and wash under tap water with minimal force to remove the cream [32].



Fig. 1: Herbal extract of custard apple



Fig. 2: Formulated anti-aging cream containing custard apple leaf extract

Table 1: Composition of herbal anti-aging cream formulation containing *Annona squamosa* (custard apple) extract

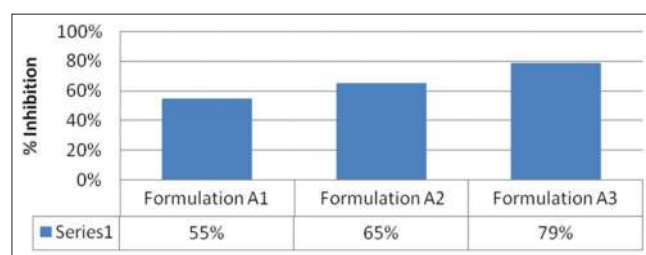
Ingredient	A ₁	A ₂	A ₃	A ₄	A ₅
<i>Annona squamosa</i> (g)	1	2	3	4	5
Stearic acid (g)	6.2	6.2	6.2	6.2	6.2
Cetyl alcohol (g)	0.75	0.75	0.75	0.75	0.75
Olive oil (ml)	1	1	1	1	1
Polyethylene glycols (ml)	1.5	1.5	1.5	1.5	1.5
Glycerine (ml)	2	2	2	2	2
Triethanolamine (ml)	0.5	0.5	0.5	0.5	0.5
Tween 80 (ml)	2.5	2.5	2.5	2.5	2.5
Sodium benzoate (g)	0.25	0.25	0.25	0.25	0.25
Ethylene diamine tetra acetic acid (g)	0.020	0.020	0.020	0.020	0.020
Rose water (ml)	3	3	3	3	3
Water	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.

Table 2: Qualitative phytochemical analysis of *Annona squamosa* (custard apple)

S. No.	Phytochemicals	Custard apple (aqueous extract)
1	Flavonoids	+
2	Terpenoids	-
3	Tannin	+
4	Phenols	+
5	Steroids	-
6	Alkaloids	+

Table 3: Physical Properties of herbal anti-aging cream formulations

S. No.	Properties	A ₃ (Custard apple extract)
1.	Color	Brown in color
2.	Odor	Characteristic
3.	State	Semi-solid
4.	Texture	Smooth
5.	Grittiness	No grittiness

**Fig. 3: 2,2-Diphenylpicrylhydrazyl scavenging activity of anti-aging cream formulations**

Type of smear

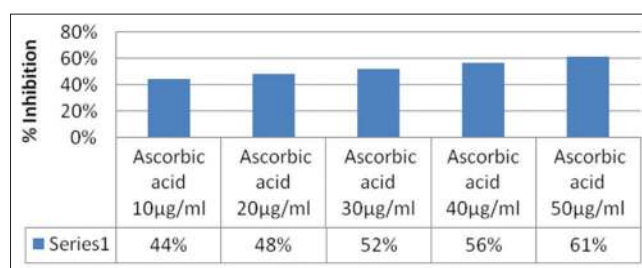
After applying the cream formulation to the skin a film or smear formed which was determined [33].

pH of the cream

To determine pH of anti-aging cream formulation a pH meter is used. Put 50.0 ml water in a beaker and dissolved the weighed amount of cream (5 g) in it and its pH was measured [34]. The pH study of a cream formulation was determined in triplicate, and the average of three reading is recorded [35].

Irritancy test

The cream was applied on the skin, and time was noted. Irritancy, erythema, was checked after application of cream formulation on a specified area of skin and reported at regular intervals up to 24 h [36].

**Fig. 4: 2,2-Diphenylpicrylhydrazyl scavenging activity of ascorbic acid (standard)**

RESULTS AND DISCUSSION

Phytochemical screening of leaf extract of *A. squamosa* (custard apple)

The qualitative phytochemical analysis of *A. squamosa* (custard apple) leaf extract shows the presence of flavonoids, tannins, alkaloids, and phenols and absence of terpenoids and steroids as shown in Table 2.

DPPH radical scavenging activity

The antioxidant activity of *A. squamosa* (custard apple) cream formulation was assessed using DPPH radical scavenging activity and by taking ascorbic acid as standard. Formulation A₃ (custard apple) shows maximum percentage inhibition of DPPH of about 78%, as compared to ascorbic acid which shows maximum 61% inhibition (50 µg/ml) of DPPH. Fig. 3 shows DPPH scavenging activities of formulation A₁, A₂, and A₃ containing custard apple extract in different concentrations and Fig. 4 shows DPPH scavenging activities of ascorbic acid at different concentrations.

Evaluation parameter of herbal anti-aging cream formulations

Physical properties

Physical properties of formulation A₃ are shown in Table 3.

Stability studies

Table 4 depicts that all the physicochemical parameters were well maintained during the period of accelerated stability studies at temperatures 8°C in the refrigerator and at 25°C, 40°C in an incubator for 3 months. Formulation A₃ containing custard apple extract shows good stability in color, odor, and consistency until the end of the accelerated study. Moreover, Table 5 represents the pH study of a cream formulation at different temperature for 3 months.

CONCLUSION

Due to constant exposure of human skin to the UV radiations present in sunlight, several pathobiological alterations in cells occur such as irregular pigmentation, increased wrinkling, loss of elasticity, dryness, and roughness. For the protection of this signs of aging herbal cosmetic are used as a therapy. Various active constituents such as flavonoids and phenolic acids appear efficient against UV radiation-induced damage.

Table 4: Accelerated Stability Studies of formulation A₃

Formulation A ₃									
Time duration	Temp erature (°C)	Appear ance	Homogeneity	Spreadability	After feel	Type of smear	Removal	Irritation	
Initial month	4-8	No change in color	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil	
	25	No change in color	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil	
	40	No change in color	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil	
After 1 month	4-8	No change in color	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil	
	25	No change in color	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil	
	40	No change in color	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil	
After 2 months	4-8	No change in color	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil	
	25	No change in color	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil	
	40	No change in color	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil	
After 3 months	4-8	No change in color	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil	
	25	No change in color	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil	
	40	No change in color	Good	Good	Emollient and slipperiness	Non-greasy	Easy	Nil	

Table 5: pH study of formulation A₃

Formulation	Time duration	Temperature (°C)	pH
A ₃	Initial month	4-8	5.4±0.25
		25	5.6±0.40
		40	5.3±0.23
	After 1 month	4-8	5.7±0.36
		25	5.1±0.20
		40	5.6±0.44
	After 2 months	4-8	5.1±0.30
		25	5.8±0.30
		40	5.0±0.20
	After 3 months	4-8	4.8±0.24
		25	5.6±0.36
		40	5.4±0.26

The use of natural compounds in skin protection especially topical application of antioxidants indicates their popularity in decreasing the effect of aging on the skin. Thus, the aim of this research article is to develop and evaluate herbal anti-aging cream containing *A. squamosa* extract. *A. squamosa* leaves are rich in antioxidants, and they thicken and stimulate the collagen to reduce wrinkles and fine lines, improving the elasticity of the skin and providing a healthy skin. Antioxidants fight the harsh rays of the sun, protecting from sun damage.

The present research study revealed that the custard apple leaves were having higher radical scavenging activity compared to that of ascorbic acid which was taken as standard. The evaluation test reveals that the formulated anti-aging cream from custard apple leaves extract showed that it is safe to be used in the skin to protect from intrinsic and extrinsic aging.

Moreover, our research study presented that formulation of A₃ is stable for 3 months. The formulations A₃ were homogeneous, emollient, non-greasy, and easily removed after the application and showed good spreadability, no evidence of phase separation and good consistency during the study period. Formulation A₃ shows the proper pH range which confirms the compatibility of the formulation with skin secretions. From this study, it is concluded that it is possible to develop anti-aging cream containing custard apple leaf extract and it will help in reducing oxidative damage and give the antioxidant effect to our skin due to its high antioxidant values.

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AUTHORS' CONTRIBUTION

All the authors have equally contributed.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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