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Formulation and evaluation of herbal antioxidant face cream of *Nardostachys jatamansi* collected from Indian Himalayan region

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ABSTRACT

Objective: To prepare and evaluate a herbal antioxidant face cream which is made by the ethanol extract of *Nardostachys jatamansi* (Valerianceae).

Methods: Antioxidant activity of ethanol extract was assessed by previously reported 2, 2-Diphenyl-1-picrylhydrazyl method. By discovering different types of formulations, such as oil in water, we were able to create several face creams respectively classified from F1 to F6, by incorporating different concentrations of stearic acid and acetyl alcohol. The evaluation of all formulations (F1 to F6) has been done by the analysis of different parameters like pH, viscosity, spread ability and stability.

Results: An ethanol fraction analyzed from a sample of *Nardostachys jatamansi* showed a significant antioxidant activity with an IC_{50} value of 58.39 μg/mL while for ascorbic acid the IC_{50} value was 46.68 μg/mL. Among the six formulations (F1-F6) F5 and F6 showed good spread ability, good consistency, homogeneity, appearance, pH; there is no proof of a separation phase and ease of removal. Also the formulations F5 and F6 showed no redness or edema or erythema and irritation during irritancy studies.

Conclusions: These formulations can be safely used on the skin. Hence, the study suggests that the composition of extract and the base of the cream F5 and F6 are more stable and safe, but it may produce synergistic action.

1. Introduction

Since the ancient times women have started to dress themselves because they wanted to increase their own beauty. Even today, people especially in rural areas, choose natural remedies (plants extracts) for traditional cosmetics. Cosmetics are products which are used to purify and beautify the skin. These products are of active ingredients purporting to have medical and drug-like benefits. A certain number of women are still using herbal cosmetics to beautify their skin. The best reason for using an herbal cosmetic is that it is purely made by herbs and shrubs. The natural content in the herbs does not have any side effects on the human body but these herbal remedies enrich the body with nutrients and other useful minerals. There is now, however, an increased scientific evidence that plants possess a vast and complex arsenal of active ingredients (photochemical) which have the ability to calm or smooth the skin but also to restore actively, heal and protect the skin[1,2].

Nardostachys jatamansi (*N. jatamansi*) (Valerianaceae) is a superb remedy famous in the Indian medicinal system and used for centuries for its healthy benefits, beauty, medicinal and skin care properties. It is composed of two species, *N. jatamansi* and *Nardostachys chinensis* widespread from all the northern part of alpine to sub alpine Himalayan

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region at an altitude of 3 000–5 000 m. The rhizome is the source of spikenard oil. Traditionally, *N. jatamansi* is used as a tonic, stimulant and antiseptic. It has antibacterial, antifungal, antiviral and antioxidant effects and it is also used in treatment of nervous headache, excitement, menopausal symptoms, flatulence, epilepsy, fungal disease, hyperlipidemia and intestinal colic. Nowadays, *N. jatamansi* is a natural product, which is frequently used in the field of cosmetology. It can be applied topically as an emollient for burns, edema, mild abrasion, and for inflammatory skin disorders[3,4]. The phytochemical studies state that the roots of the plant contain essential oil in which you can find sesquiterpenes and coumarins. Jatamansone or valeranone is the principal sesquiterpene. Other sesquiterpenes include nardostachone, jatamansinol, jatamansic acid, jatamansinone, nardostachyin, nardosinone, jatamol A and B *etc*. A new sesquiterpene acid and new pyranocoumarin: 2′, 2′–dimethyl–3′–methoxy–3′, 4′–dihydropyranocoumarin were reported[5]. Actinidine, an alkaloid was also reported[6].

Therefore, the purpose of this study was to develop cosmetic face cream by mixing the ethanol extract of *N. jatamansi*, in order to produce multipurpose effects on skin such as fairness, antiaging and antiseptic effects.

2. Materials and methods

2.1. Plant materials

The rhizomes of *N. jatamansi* were collected from Chamoli District, Uttarakhand, India and authenticated by Dr. J. K. Tiwari, Professor at the Department of Botany, H.N.B. Garhwal (A Central) University, Srinagar Garhwal, Uttarakhand, India. A specimen voucher was deposited at the Department of Pharmaceutical Chemistry, H.N.B. Garhwal (A Central) University, Srinagar Garhwal, Uttarakhand, India.

2.2. Preparation of extracts

The shade dried and coarsely powdered (500 g) *N. jatamansi* was subjected to Soxhlet extractor, first, using petroleum ether and then successively extracted with ethanol. The extracts were then concentrated to dryness under reduced pressure and controlled temperature, respectively and then preserved in a refrigerator.

2.3. Antioxidant activity of N. jatamansi

2, 2–Diphenyl–1–picrylhydrazyl (DPPH) radical scavenging activities of the ethanol extract were investigated following a method previously reported[7]. Briefly, to a methanolic solution of DPPH (100 mmol/L, 2 mL), 2 mL of test sample dissolved in ethanol was added at different concentrations (40–200 μg/mL). Equal amount of ethanol was added to the control. Absorbance was recorded at 517 nm at 5, 15 and 30 min. The scavenging activity was calculated using the formula:

% Scavenging activity=$[(A517_{control}-A517_{sample})/A517_{control}]\times100$.

Ascorbic acid was used as a standard.

2.4. Cream formulation

Oil in water (O/W) emulsion–based cream (semisolid formulation) was formulated. The emulsifier (stearic acid) and other oil soluble components (cetyl alcohol, almond oil) were dissolved in the oil phase (Part A) and heated to 75 °C. The preservatives and other water soluble components (methyl paraban, propyl paraban, triethanolamine, propylene glycol and ethanol extract of *N. jatamansi*) were dissolved in the aqueous phase (Part B) and heated to 75 °C. After heating, the aqueous phase was added in portions to the oil phase with continuous stirring. Perfume was added when the temperature dropped to (45±50) °C[8]. The formula for the cream is given in Table 1.

Table 1

Composition of *N. jatamansi* extract based face cream (g).

Ingredients	F1	F2	F3	F4	F5	F6
Ethanol extract	0.200	0.500	0.500	0.500	0.500	0.500
Stearic acid	1.000	1.000	1.000	1.200	1.000	1.200
Triethanolamine	0.135	0.135	0.135	0.160	0.160	0.165
Rose water	0.300	0.300	0.300	0.400	0.400	0.400
Paraffin oil	0.350	0.350	0.350	0.300	0.300	0.300
Moisturizer conditioner	1.000	1.000	1.000	1.200	1.200	1.200
Cetyl alcohol	–	0.250	0.200	0.150	0.100	0.100
Methyl paraben	0.018	0.018	0.018	0.018	0.018	0.018
Propyl paraben	0.002	0.002	0.002	0.002	0.002	0.002
Ethylene diamine tetraacetic acid	0.010	0.010	0.010	0.010	0.010	0.010
Water	Qs	Qs	Qs	Qs	Qs	Qs

Qs: Quantity sufficient.

2.5. Evaluation of cream

2.5.1. Type of emulsion under dye test

The scarlet red dye is mixed with the cream. A drop of the cream was placed on a microscopic slide, then it was covered with a cover slip and examined under a microscope. If the disperse globules appear red and the ground is colorless, the cream is O/W type. The reverse condition occurs in W/O type cream *i.e.* the disperse globules appear colorless in the red ground[9].

2.5.2. Accelerated stability testing

Accelerated stability can be tested if the prepared formulations were conducted for five stable samples studied for 7 d at room temperature. The formulation numbers were F2–F6 at (40±1) °C for 20 d. The formulations were kept both at room and elevated temperature and observed on 0th, 5th, 10th, 15th and 20th day for the following properties[10–12].

2.5.3. Some properties of the cream

There are some properties of the cream measured in the experiment as followings:

(1) The pH meter was calibrated using a standard buffer

solution. About 0.5 g of the cream was weighed and dissolved in 50 mL of distilled water and its pH was measured[13].

(2) The viscosity of the formulation was determined by Brookfield viscometer at 100 r/min, using the spindle No. 7.

(3) The formulations were tested for the homogeneity by judging their visual appearance and touch affinity.

(4) The appearance of the cream was judged by its color, pearl essence and roughness and graded.

(5) Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked.

(6) Rubout included spread ability and wetness. A fixed amount of cream was applied on the dorsal skin surface of human volunteer and the properties were observed.

(7) After the application of the cream, the type of film or smear formed on the skin was checked.

(8) The ease of removal of the cream applied was examined by washing the applied part with tap water.

(9) The cream was applied on shaved intact skin of albino rabbits and the changes were examined on the skin after 24 h.

2.5.4. Irritancy test

An area (1 cm^2) on the dorsal left hand surface was marked. The cream was applied to the specified area and the time was noted. Irritancy, erythema, edema were checked for regular intervals up to 24 h and reported[14].

2.5.5. Microbial limit test

Microbial analysis was carried out for all the formulations as a procedure of Indian Pharmacopoeia 2010 and WHO Guideline. It included total bacterial count, total fungal count, presence of *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumonia* (*K. pneumonia*) and *Bacillus cereus* (*B. cereus*). Pure culture of *E. coli* (MTCC 0729), *S. aureus* (MTCC 0902), *K. pneumonia* (MTCC 0432), *B. cereus* (MTCC 1272) were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

3. Results

3.1. Antioxidant activity of N. jatamansi

The DPPH radical scavenging activities of the ethanol extract of *N. jatamansi* were assessed. DPPH radicals react with suitable reducing agents which lose color stoichiometrically and the number of electrons consumed was measured spectrophotometrically at 517 nm. An ethanol fraction analyzed from a sample of *N. jatamansi* showed a significant antioxidant activity *i.e.* 58.39 μg/mL (IC_{50}) in comparison to standard ascorbic acid [46.68 μg/mL (IC_{50})]. The scavenging of ethanol extract was found with the highest activity (94.1%) at a concentration of 100 μg/mL observed for 30 min whereas standard ascorbic acid showed 91.27% at 5 μg/mL concentrations.

3.2. Evaluation of the herbal cream

The results of accelerated stability test are shown in Table 2. The results of the Draize test for sensitivity demonstrated that the formulations F5 and F6 were safe and skin irritation and allergic sensitization were scarce or absent. The viscosity of cream was in a range of 28001-27025 centipoises which indicated that the cream is easily spreadable by small amounts of shear. But F5 and F6 shows a better spreadable property than other formulations. The formulations (F5 and F6) show no redness, edema, inflammation and irritation during irritancy studies. These formulations are safe to be used on the skin. The media used for the microbial limit test were of Hi-Media Pvt. Ltd 30-32. The results are as tabulated shown in Table 3.

Table 2

Accelerated stability testing.

Days	Temperature	Formulations	Parameters						
			pH	A1	A2	A3	A4	A5	A6
0	Room temperature	F5	6.50	**	NCC	**	E	NG	ES
		F6	6.55	**	NCC	**	E	NG	ES
	(40±1) °C	F5	6.55	**	NCC	**	E	NG	ES
		F6	6.59	**	NCC	**	E	NG	ES
10	Room temperature	F5	6.51	**	NCC	**	E	NG	ES
		F6	6.60	**	NCC	**	E	NG	ES
	(40±1) °C	F5	6.52	**	NCC	**	E	NG	ES
		F6	6.60	**	NCC	**	E	NG	ES
15	Room temperature	F5	6.51	**	NCC	**	E	NG	ES
		F6	6.60	**	NCC	**	E	NG	ES
	(40±1) °C	F5	6.53	**	NCC	**	E	NG	ES
		F6	6.62	**	NCC	**	E	NG	ES
20	Room temperature	F5	6.53	**	NCC	**	E	NG	ES
		F6	6.62	**	NCC	**	E	NG	ES
	(40±1) °C	F5	6.53	**	NCC	**	E	NG	ES
		F6	6.62	**	NCC	**	E	NG	ES

A1: Homogenity; A2: Appearance; A3: Spread ability; A4: After feel; A5: Type of smear; A6: Removal; **: Good; *: Satisfactory; E: Emollient; NG: Non greasy; ES: Easy; NCC: Not change in colour.

Table 3

Microbial analysis of *N. jatamansi* formulations (F1-F6).

Formulations	TBC (CFU/g)	TFC (CFU/g)	*E. coli*	*S. aureus*	*K. pneumonia*	*B. cereus*
F1	16×10^2	Ab	Ab	Ab	Ab	Ab
F2	4×10^2	Ab	Ab	Ab	Ab	Ab
F3	3×10^2	1×10^2	Ab	Ab	Ab	Ab
F4	10×10^2	3×10^2	Ab	Ab	Ab	Ab
F5	19×10^2	1×10^2	Ab	Ab	Ab	Ab
F6	28×10^2	Ab	Ab	Ab	Ab	Ab

TBC: Total bacterial count; TFC: Total fungal count; Ab: Absent.

The dye test confirms that all formulations were O/W type emulsion cream. But formulation F5 and F6 showed more stability in O/W type emulsion. The pH of the cream was found to be in range of 5.50-6.62 which is good as a skin pH. All the formulations (F1-F6) of cream showed a pH quite similar to that of the skin which was required. All formulations produce a uniform distribution of extracts in cream. This was confirmed by visual appearance and by touch. When formulations were kept for a long time, it was found that there weren't any particular changes in the color of the cream. Emolliency, slipperiness and amount of

residue left after the application of fixed amount of cream was found. After application of cream F5 and F6, the type of smear formed on the skin was not greasy. The cream F5 and F6 applied on skin were easily removable by washing with water.

4. Discussion

N. jatamansi is well known for its medicinal and cosmeceuticals value in the traditional Indian system of medicine. It stimulates fibroblasts to produce collagen and elastin fibers giving more elastic and less wrinkled properties to the skin. It is used in oils and pastes to improve complexion and the general health of the skin. *N. jatamansi* ethanol extract has significant antioxidant activity. So, it can be considered as a natural antioxidant and it is well known that the natural antioxidants have beneficial effects on the process of skin aging, skin sun protection or skin cancer. Many other studies confirmed that an acute exposure of human skin to UV radiation *in vivo* leads to oxidation of cellular biomolecules that could be prevented by a prior antioxidant treatment. Hence, there is an increased demand for herbal cosmetics in the world market and they are invaluable gifts of nature. Therefore, this present study tried to create an antioxidant herbal face cream using the ethanol extract of *N. jatamansi*.

The prepared face cream was O/W type emulsion, hence can be easily washed with plane water which gives better customer compliance. Our study indicated that the formulations (F5 and F6) were more stable, whilst remaining formulations were not stable and resulted in break down of the emulsion when stored for long time. Two out of six formulations were stable with no signs of bleeding and change in color of the product. These formulations (F5 and F6) had almost a constant pH, homogeneity, emollient properties; they were not greasy and easily removable after the application. The stable formulations were safe and skin irritations and allergic sensitizations were scarce. From above results, it is confirmed that the ethanol extract of this plant produce an excellent antioxidant property and it can be used to give whitening, anti-wrinkle and antiseptic effects on the skin. The rose water increases the glow on skin and has emollient properties. All the formulations passed the microbial limit test which included some parameters like total bacterial count and total fungal count; pathogens like *E. coli*, *S. aureus*, *K. pneumonia* and *B. cereus* were also absent.

The extract of *N. jatamansi* is widely used for the formulation of traditional Ayurvedic medicines as well as modern herbal preparation for curing several ailments. The research work suggests that the herbal antioxidant formulation and its ingredients were studied to be consistent in quality and purity and can be easily used as face cream. The validation of cream was done and was found within the limits. From above discussion, it is concluded that the formulation F5 and F6 are safe and usable for the skin.

Conflict of interest statement

We declare that we have no conflict of interest.

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