ORIGINAL ARTICLE

Targeting Skin Aging: Development of a Phytosomal Polyherbal Gel

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Abstract

Aims: Ageing is a biological process that involves a multitude of external and internal factors, such as genetic and environmental influences, and induces characteristic molecular, cellular, tissular, and clinical changes. Antiaging drugs are mainly responsible for free radical scavenging, supplying vitamin C, suppressing the production of melanin, antioxidant activity, and promoting tissue regeneration. The objective of the present study was to prepare the polyherbal antiaging phytosomal gel. **Materials and Methods:** This formulation hopes for a stable and functionally effective gel and to evaluate for good product performance of the prepared gel. Phytosomes were prepared with active ingredients, such as *Eucalyptus globules* extract, *Clitoria ternatea* extract, *Annona squamosa* extract, *Coriandrum sativum* extract, and *Aloe vera* extract. Physical evaluation, such as pH, viscosity, homogeneity, spreadability, extrudability, characterization of phytosomal gel, stability studies, and *in vitro* antioxidant activity of the antiaging gel was carried out. **Results and Discussion:** These studies suggest that the composition of extracts and base of gel F3 is more stable and efficient, while the remaining formulations were not stable and resulted in the breakdown of the emulsion when stored for a long time. This formulation had a suitable pH, was homogeneous, emollient, non-greasy, and was easily removed after the application. **Conclusion:** In this study, the scavenging activity of the F3 sample was found to be dose-dependent.

Key words: Aloe vera, Annona squamosa, antiaging, Clitoria ternatea, Coriandrum sativum, phytosomal gel

INTRODUCTION

Aging is a biological process that involves a multitude of external and internal factors, such as genetic and environmental influences, and induces characteristic molecular, cellular, tissular, and clinical changes.[1] General atrophy of the extracellular matrix is influenced by the decrease of the number of fibroblasts, while the decrease of the protein synthesis influences types I and III collagen in the dermis and determines an increased breakdown of extracellular matrix proteins.[2] Among all factors, oxidative stress is considered to have a major importance in the aging process. The original free radical theory postulated by Harman in 1956 purported that the reactive oxygen species interfere with the cellular and subcellular systems, inducing molecular degradations. Reactive oxygen species, such as superoxide anion radical, hydrogen peroxide, hydroxyl radical, and oxygen singlet cause oxidative damage to cellular macromolecules including lipids, proteins, and DNA and their oxidized forms.[3] Aging is associated with changes in the molecular structure of these compounds and with other pathways such as spontaneous errors and other protein alterations. At the same time, the production of reactive oxygen species plays an important role in signaling processes and in cellular homeostasis.[4]

The identification of the mechanisms of skin aging and the research of new anti-age cream formulations represent a continuous challenge, which is especially important because skin aging reflects the aging of the entire organism. Herbal extracts present great potential in the development of new anti-age products considering the antioxidant effects of phytochemical compounds. Antiaging agent slows down the degenerative

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processes in the skin which usually occurs with aging. They are believed to play a role in preventing the damage to cell membranes by the Free radicals causing the production of harmful chemicals which results in aging process called glycation. Antiaging drugs are mainly responsible for free radical scavenging, supplying Vitamin C, suppressing the production of melanin, antioxidant activity, and promoting tissue regeneration.[2,5]

The objective of the present study was to prepare the polyherbal antiaging phytosomal gel. This formulation hopes for a stable and functionally effective gel and to evaluate for good product performance of the prepared gel. The formulation of polyherbal gel with leaf extracts of plants would achieve amultipurpose effect, such as anti-wrinkle, antiaging, and sunscreen effect on the skin. This work could be a progress in herbal medicine and contribute a part to the field of science.

MATERIALS AND METHODS

Materials

The leaves of Plant *Annona squamosa*, flowers of *Clitoria ternatea*, and leaves of *Coriandrum sativum* were collected from Tirupati, India in the month of January 2024 and it was identified and authenticated. The following chemicals were obtained such as soy lecithin from Research - laboratories fine chem industries (Mumbai, India), ethyl acetate, carbopol 940, and propylene glycol from chemco.

Preparation of extracts

Preparation of A. squamosa leaf extract

A. squamosa leaves were collected locally from the surrounding area of Tirupathi. Air-dried leaves were grinded to a fine powder in a suitable grinder mixture. Shade-dried powder was extracted using a soxhlet extractor with distilled water, alcohol, and hexane separately to get the semisolid extract. The organic solvents were recovered by steam distillation. The extracts were then concentrated to dryness under reduced pressure and controlled temperature, respectively, and they were preserved in a refrigerator.

Preparation of flower extract

The dried leaves of *C. ternatea* were powdered and sieved through a 40-mesh screen. The fine powder was stored in air-tight containers and left in the refrigerator. Fifty grams of leaf material was soaked in 250 mL (methanol, ethyl acetate, and petroleum ether) for 24 h and filtered using standard filter paper. The filtrate was transferred into vials and allowed to evaporate until completely dry.

Preparation of C. sativum extract

C. sativum leaves are dried overnight in a hot air dryer at 50°C to pulverize into fine powders. Powders of apple mint leaves 20 g is extracted with 2 L of distilled water at 80°C. After filtering supernatants, the residues were freeze-dried to remove the remaining water and obtain final extracts.

Physico chemical parameters

Ash values (total ash value, acid insoluble ash value, and water-soluble ash value) and extractive values were determined as per the World Health Organization guidelines.[6]

Formulation of phytosomal gel

Phytosomes were prepared by reacting the herbal extract and phospholipid such as soy lecithin in a ratio of 1:1 and dissolving them in an aprotic solvent such as ethyl acetate. After solubilization has completed, the complex compounds are removed by solvent evaporation technique. Thus, phytosomes are obtained. Gel was prepared using carbopol 940 as the gelling agent which was dispersed in a small quantity of distilled water and then stored overnight to ensure complete hydration. The active ingredients such as *Eucalyptus globules* extract, *C. ternatea* extract, *A. squamosa* extract, *C. sativum* extract, *Aloe vera* extract in a suitable solvent such as propylene glycol were added to the dispersion (Table 1). Then, preservatives such as methyl paraben and propyl paraben were also added slowly with continuous stirring. Then, the prepared phytosomes were incorporated into the gel, and thus, the phytosomal gel was obtained. This phytosomal gel showed better release of herbal extracts and better penetration to the skin, and as a result, the desired antiaging property was obtained.

Physical evaluation of polyherbal antiaging gel

pH

The pH meter was calibrated using standard buffer solutions, such as pH 4 and 7. About 0.5 g of the cream was weighed and dissolved in 50.0 mL of distilled water and its pH was measured.[7]

Viscosity

The viscosity of the formulation was determined by Brookfield Viscometer at 100 rpm, using spindle no 7.[7]

Homogeneity

The formulations were tested for homogeneity by visual appearance.

Spreadability

Two glass slides of 20 cm × 20 cm were selected. A small amount of sample was sandwiched between the two glass slides. A 100 g weight was placed on the upper slide so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and then fixed to a stand

Table 1: Formulation of antiaging gel

Ingredients	F_1	F_2	F_3	F_4
Annona extract	2 mL	1 mL	2 mL	1 mL
Clitoria extract	1 mL	2 mL	2 mL	1 mL
Coriander extract	3 mL	2 mL	1 mL	2 mL
Aloe vera extract	2 mL	1 mL	1 mL	2 mL
Eucalyptus oil	2 mL	1 mL	2 mL	1 mL
Triethanolamine	1.2 mL	1.2 mL	1.2 mL	1.2 mL
Propylene glycol 400 (5%)	0.1	0.1	0.1	0.1
Vitamin E	0.1 mL	-	0.1 mL	-
Propyl paraben	0.02	0.02	0.02	0.02
Methyl paraben (0.5%)	0.2	0.2	0.2	0.2
Distilled water	Q.s	Q.s	Q.s	Q.s

without the slightest disturbance in such a way that the upper slide slides off freely, to the force of weight tied to it. The time taken for the upper slide to separate away from the lower one was noted using a stop clock. This parallel plate method is the most widely used method for determining and quantifying the spreadability of semisolid preparations.[8] Simplicity and relative lack of expense are the advantages of this method. The following equation was used for this purpose:

$$S = m \times L/T \quad (1)$$

Where, S - Spreadability, m - Weight tied to the upper slide, l - Length of the glass, t - Time taken in seconds.

Extrudability

It is an empirical test to measure the force required for the gel to extrude out from the tube. The prepared gel was filled into a collapsible tube and it was sealed and the weight of the tube was recorded. Placed a 500 g weight on the tube and the amount of gel that extruded out was collected and weighed. Then, the percentage of gel extruded was calculated. The packing of gels has gained a considerable importance in the delivery of a desired quantity of gel; therefore, the measurement of extrudability has become some important criteria for gels.[9]

Characterization of phytosomal gel

The behavior of phytosomes in both physical and biological systems is governed by the factors, such as physical size, shape, stability, and its distribution. Therefore, the phytosomes are characterized for physical attributes, that is, shape, size, and its distribution.

Microscopy

The phytosomes were visualized under Electron microscopy in magnification of ×10 and ×100.[10]

Visualization by scanning electron microscopy (SEM)

Visualization of phytosomes can be achieved using SEM. The morphology of the prepared phospholipid complex was determined by performing SEM at various magnifications ×100, ×250, ×500, ×1,000, ×1,500, and ×2,000.[10]

Entrapment efficiency

The entrapment efficiency of phytosomes was measured using the ultracentrifugation technique in which the proportion of encapsulated extract was subjected to centrifugation at 15,000 rpm for 1 h at room temperature.[11] The Phytosome Complex formed was separated from supernatant and then sonicated with distilled water to measure the encapsulated drug content at 324 nm (λmax). The percentage entrapment efficiency was calculated by the equation.

$$\%E = (ED \setminus AD) \times 100\% \quad (2)$$

Where, %E - percentage entrapment efficiency, AD - amount of added drug, ED - amount of encapsulated drug.

Stability studies

An accelerated stability study was carried out as per ICH guidelines using a Neutronic stability chamber for P5. This formulation was selected as an optimum formulation and the stability study was carried out. Physical parameters, such as color, consistency, and pH were determined at room temperature and 40°C.[12]

Determination of antioxidant activity by *in vitro* antioxidant studies[13-15]

2,2-diphenylpicrylhydrazyl (DPPH) free radical scavenging assay

Different concentrations of standard ascorbic acid and samples, namely, 20, 40, 60, 80, and 100 mcg/mL were prepared in methanol. 0.002% DPPH in methanol was used as a free radical. An equal volume of different concentrations of standards and DPPH was mixed in clean and labeled test tubes separately, and the tubes were incubated at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using ultraviolet-Vis spectrophotometer. The degree of stable DPPH decolorization to DPPH (reduced form of DPPH) yellow indicated the scavenging efficiency of the sample. The scavenging activity of the sample against the stable DPPH was calculated using the following equation:

$$\text{Scavenging activity } (\%) = (A - B/A)\ 100 \quad (3)$$

Where A is the absorbance of the control and B is the absorbance of the sample.

RESULTS AND DISCUSSION

Physicochemical parameters of the plant powders and formulation

The results of the Physicochemical parameters of plant powders obtained from the present research are given in Tables 2 and 3.

Physicochemical parameter evaluation of antiaging gel

pH measurement

It was previously reported that, for gels to be non-irritant and safe for topical application, their pH has to be in the accepted range for topical preparations, that is, pH 6–7 units. pH of various antiaging gel formulations ranged from 6.1 to 6.5, which lies in the normal physiologic range and thus produces no skin irritation.

Viscosity

The antiaging gels were formulated using Carbopol 940. The viscosity of various antiaging gel formulations ranged from 2455.577 to 5134.42.

Spreadability

The spreadability is an important criterion for uniform and ease of application of topical preparations. It also plays a major role from the patient compliance point of view. Application of the formulation to the skin is more comfortable if the base spreads easily, exhibiting maximum "slip" and "drag." The spreadability of gels and gels are measured in terms of the average diameter of the spread circle. The spreadability values for all prepared cream formulations ranged from 23.4–35.7.

Extrudability

It is an empirical test to measure the force required for the cream to extrude out from the tube. For topical preparations, it is an important criterion to check the easiness of the cream to extrude out from the tube. The extrudability values of all prepared gel formulations lie from 90.5 to 93.5.

Microscopy

The phytosomes were visualized under Electron microscopy in magnification of ×10 and ×100 as shown in the Figure 1.

SEM

From Figure 2, it is clear that the particle size of the optimized formulation was confirmed to be 52–115 nm. This was in accordance with the particle size of phytosomes in the literature.

Fourier transform infrared spectroscopy (FT-IR)

The formation of the Extract-phospholipid complex can also be confirmed by infrared spectroscopy by comparing the spectrum of the complex with the spectrum of the individual components and their mechanical mixtures. The FT-IR spectra of the extract and Phytosome are given in Figure 3.

In vitro antioxidant studies

In vitro antioxidant studies were performed for the prepared antiaging gels, gels, and phytosomal gels. The antioxidant activity of the prepared formulations in various concentrations was evaluated using *in vitro* models. It was observed that the test compounds scavenged free radicals in a concentration-dependent manner in all models. The antioxidant activity was expressed as IC_{50} (the amount of antioxidants needed to decrease the radical concentration by 50%), which is negatively related to antioxidant activity. The lower the IC_{50} value, the higher is the antioxidant activity of the tested sample. The *in vitro* antioxidant studies included DPPH assay.

Table 2: Physicochemical parameters of plant powders

S. no.	Plant names	Total ash % W/W	Acid insoluble ash % W/W	Water soluble ash % W/W	Sulfated ash %w/w	Loss on drying % W/W
1	*Clitoria ternatea*	2.7	0.2	1.3	7.9	2
2	*Annona squamosa*	9.2	5.5	7.5	0.9	6.6
3	*Coriandrum sativum*	9.8	0.2	6.5	5.8	7.8

Table 3: Extractive values of plant powders

S. no.	Plant names	Pet. Ether extractive value % W/W	Ethanol soluble extractive value % W/W	Water soluble extractive value % W/W
1	*Clitoria ternatea*	1.1	16.5	18.8
2	*Annona squamosa*	3.7	4.2	6.0
3	*Coriandrum sativum*	1.3	12.1	14.8

Among them, the F3 formulation showed the highest antioxidant activity. From the physicochemical parameter evaluation, it was found that the F3 sample showed optimum values. Hence, the F3 formulation was taken as the optimized formula from all four different formulations. Characterization studies of antiaging phytosomal gel were also performed [Table 4].

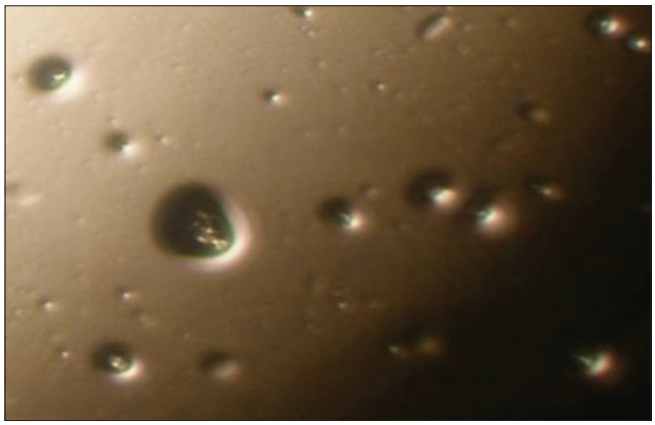

Figure 1: Electron microscopy of phytosomes

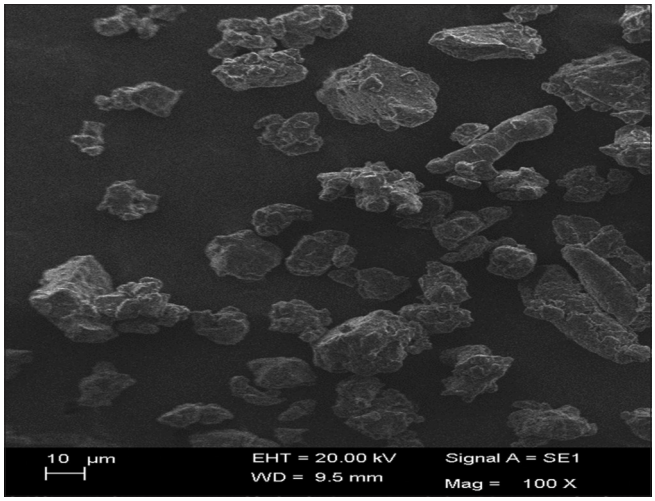

Figure 2: Scanning electron microscopy of optimized formulation

DPPH free radical scavenging activity

The result of the antioxidant activity of different concentrations of samples and standard (ascorbic acid) is shown in Figure 4. The samples exhibited marked antioxidant activity by scavenging DPPH*(free radical) and converting into DPPHH. A dose-dependent radical scavenging activity was observed. The scavenging activity of ascorbic acid was greater than that of all four different samples of gel. Among them, the F3 sample showed the highest antioxidant activity. In the presence of an antioxidant, the DPPH radical obtains one more electron and the absorbance decreases. In this study, the scavenging activity of the F3 sample was found to be dose-dependent, that is, the higher the concentration, the more was the scavenging activity. Although the DPPH radical scavenging abilities of the samples were less than that of ascorbic acid, the study showed that the samples have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Comparison of the phytosomal gel with the marketed formulation

The prepared antiaging phytosomal gel was compared with that of an herbal marketed formulation, antiaging *A. vera* gel. It was found that the antioxidant activity was more for the phytosomal gel. Hence, it was proved that the prepared phytosomal gel containing the antiaging ingredients had significant antiaging properties. The *A. vera* antiaging gel was taken for comparison as it had the antiaging ingredient, *A. vera*. From Table 5, it was evident that the prepared antiaging phytosomal gel had the highest antioxidant property when compared with the conventional dosage forms such as antiaging cream and antiaging gel.

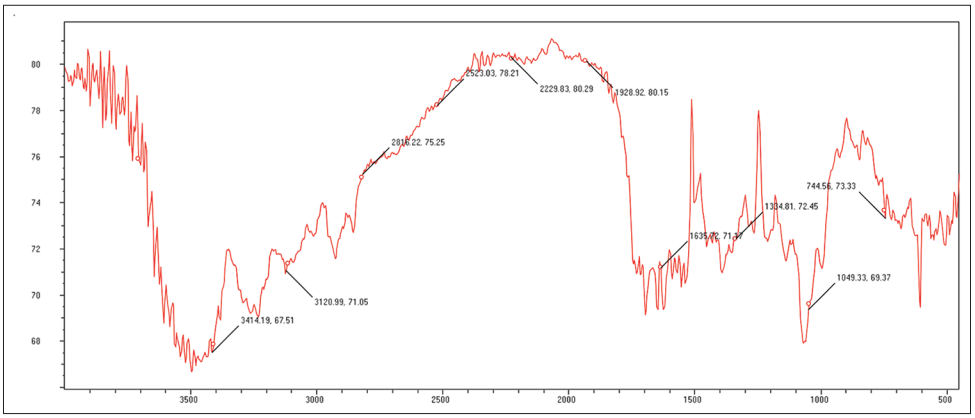

Figure 3: Fourier transform infrared spectroscopy spectra of the extract and Phytosome

Table 4: Stability studies of the optimized formulation

S. no.	Parameter	Observation						
		Initial	1st month		2nd month		3rd month	
			RT	40°C	RT	40°C	RT	40°C
1.	Appearance	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
2.	Color	Blue	Blue	Blue	Blue	Blue	Blue	Blue
3.	PH	6.2	6.2	6.4	6.2	6.4	6.2	6.1
4.	Homogenicity	Good	Good	Satisfactory	Good	Satisfactory	Good	Satisfactory

Table 5: Comparison of phytosomal gel with the marketed formulation

Concentration (µg/mL)	Phytosomal gel (%RSA)	Marketed formulation (%RSA)
0	0	0
20	35.98	32.11
40	46.87	43.09
60	57.99	52.31
80	66.72	60.72
100	71.21	69.06

%RSA: Radical scavenging activity

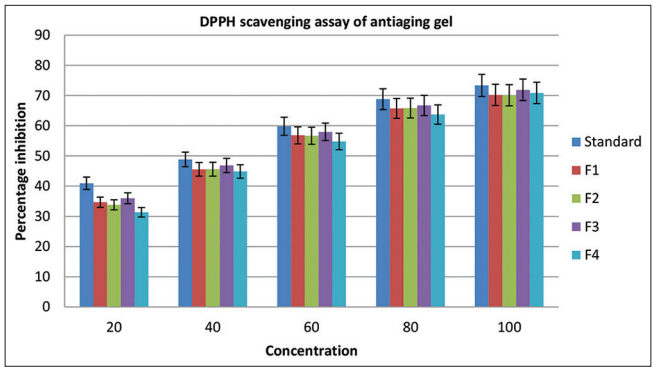

Figure 4: Percentage inhibition of antiaging gel by 2,2-diphenylpicrylhydrazyl assay compared with the standard

CONCLUSION

From the above results, it is concluded that on combining the extracts of *C. ternatea*, *A. squamosa*, *C. sativum*, *A. vera* gel, and Eucalyptus oil have different components in a different ratio to get multipurpose effect such as anti-wrinkle, antiaging and sunscreen effect on the skin. This activity may be mainly due to free radical scavenging, antioxidant activity of the extract is also reported to supplying Vitamin C, which promoting tissue regeneration. The research work suggests that the herbal anti-oxidant formulation and its ingredients were studied to be consistent in quality and purity and can be easily used as a face gel. The validation of the gel was done and was found in limits. From the above discussion, it is concluded that the formulation F3 is safe usable for the skin. This study can be helpful for upcoming researchers to select these herbs for the formulation and evaluation of other cosmetic applications which can be claimed for their efficacy with scientific data.

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