INTRODUCTION

Aging is a very common phenomenon to all living beings. It is signified by progressive deterioration and degeneration of cells and organs. As far as human beings are concerned, aging is a complex phenomenon of physical, psychological, and social changes. According to biologists, aging is a sum of all changes that occur in a living being with the passage of time and lead to a decreasing ability to survive stress, to functional impairment, and finally to death [1,2].

The effect of aging on the world population does have an indirect effect on the worldwide medical care and health scenario [3]. The United Nations have stated that the number of people worldwide of the age group 60 or above will be rising one in 10 to the current one in five ratios by 2050 and the ratio in developing nations and developed nations are going to double and triple, respectively. These demographic statistics suggest healthcare scenario to be more careful with the changes associated with aging and to concentrate on more antiaging therapies [4,5].

The theories involved in the aging process are mutation accumulation and antagonistic pleiotropy theory. The symptoms include atrophy, laxity, wrinkling, sagging, dryness, blemishes, and sparse gray hair. Being the largest organ of the body, in case of body weight and surface area, skin has the most profound and observable changes of aging [6].

Cosmeceuticals are the most mushrooming arena of natural personal care industry. However, the sad part of story is that a large percentage of these products do have a lot of carcinogenic agents such as diethanolamine and triethanolamine (TEA). Due to these side effects, researchers have now turned to the natural side of the field. Herbal extracts to phosphatidylcholine (acts as a carrier and nourishes the skin). They had a phospholipid molecular structure which included a water-soluble head and two fat-soluble tails, and due to this dual solubility, it was better absorbed and so it was used for the treatment of skin disorders, antiaging, and skin carcinomas.

CONCLUSION

From the present study, it can be concluded that the prepared antiaging phytosomal gel was safe, convenient, and efficient carrier to deliver the herbal extracts. It also showed better penetration into the skin. Hence, the desired antiaging property was obtained. Hence, the desired antiaging property was obtained and is used in skin care cosmetics.

Keywords: Antiaging, Phytosome, gel, Flavonoids, Herbal extracts.
specifically protect the body from the damages caused by these reactive oxygen species [10,11].

Cytokines are a group of plant growth hormone, involved in cell growth and differentiation. These are also known as antiaging hormones as aging of plants is affected by these hormones. Coconut water was found to have antiaging properties. It was found that antioxidants and cytokines in coconut water have profound action against the aging phenomenon [12].

Antiaging cream reduces the wrinkles and blemishes on the skin. The advantage is that these have lesser penetrations and can be removed by sweating or a mild washing of the face. Gel is a semisolid preparation with two interpenetrating phases: A gelling phase and a liquid. The continuous liquid phase allows free diffusion of molecules through the skin. Gels are preferred to creams as they have less emollient effects and no pore clogging. There is also enhanced penetration of drugs [26,28].

Phytosomes are very recent introduction into herbal formulation as they are better absorbed and have higher bioavailability. The term phyto means "plant" while 'some' means cell like. This are advanced forms of herbal formulation that contains bioactive phytoconstituents of herbal extract surrounded by a lipid. Phytosomes have a better pharmacokinetic and pharmacodynamic profile. In short, phytosomes are a bridge of connection between the conventional drug delivery system and the novel drug delivery system [30].

Advantages of phytosomes are increased bioavailability, efficient nutrient safety, and optimal entrapment efficiency. Phosphatidylcholine in phytosomes has a role both as a skin nourisher and also nourishes the skin. The phytosomes have better clinical benefits. The structural properties are also superior and are unique. Phytosomes also can cross cell membranes and enter cells. Being a complex between a natural products and natural phospholipid, there is the formation of hydrogen bond between the two compounds. With water, phytosomes assume a micelle shape. These are better absorbed, utilized, and have better results. It has higher bioavailability than conventional drug. There are increased pharmacokinetic and pharmacodynamic properties [32,33].

Phytosomes are used in liver diseases and heart diseases. It is also used as anti-inflammatory, lipolytic, vasokinetic, trophodermic, immunomodulatory, and antioxidant agents. Phytosomes are a patented technology developed by a leading manufacturer of drugs and nutraceuticals to incorporate standardized plant extracts or water-soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes called as phytosomes and so vastly improve their absorption and bioavailability [35].

MATERIALS AND METHODS

Materials

Tender coconut water was obtained from CDB lab, South Ernakulum. Aloe vera extract was obtained from Ekelir Extracts, Nellad Kochi.

Methods

Formulation of O/W cream

Required quantities of glycyril monostearate (GMS), light liquid paraffin, isopropyl palmitate, emulsifying wax, stearic acid, and cetyl alcohol were weighed and transferred into a 200 ml beaker. To this, a required amount of jojoba oil and Vitamin E were added and heated in a water bath. Another 200 ml beaker was weighed, and required quantities of glycerin, TEA, methylparaben, and propylparaben were added followed by A. vera extract, grape seed extract, and tender coconut water. The beaker was heated to a temperature up to 60–70°C. After both the solutions had attained their respective temperature, beaker was removed from heat and poured the oily phase to water phase with continuous stirring. The stirring was continued until the mixture forms a creamy emulsion. A few drops of perfume with a small quantity of propylene glycol were mixed and were added to the creamy emulsion after cooling (Table 1).

Formulation of gels

Antiaging gel was prepared using carbopol 940 as the gelling agent. Gelling agent was dispersed in a small quantity of distilled water and then stored overnight to ensure complete hydration. The active ingredients such as tender coconut water, A. vera extract, grape seed extract, Vitamin E, and jojoba oil in a suitable solvent such as propylene glycol were added to the dispersion. Other excipients such as methylparaben and propylparaben were also added slowly with continuous stirring. In carbopol gels, pH of the vehicle was brought to neutral by adding TEA. The final weight of the gel was adjusted to 50 g with distilled water. Entrapped air bubbles were removed by keeping the gels in vacuum desiccator (Table 2).

Formulation of phytosomes

Phytosomes are prepared by reacting the herbal extract and phospholipid such as soy lecithin in a ratio 1:1 and dissolving them in an aprotic solvent such as ethyl acetate. After solubilization has completed, the complex compounds are isolated by removing the solvent under vacuum, by freeze drying or by precipitation with non-solvents such as n-hexane. Thus, the obtained complexes are lipophilic in character and soluble in a polar and aprotic solvent, in which the individual components of the complex are normally insoluble [62].

Formulation of phytosomal gel

Phytosomes are prepared by reacting the herbal extract and phospholipid such as soy lecithin in a ratio 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 tender coconut water, A. vera extract, grape seed extract, Vitamin E, and jojoba oil in a suitable solvent such as propylene glycol were added to the dispersion. Then, preservatives such as methylparaben and propylparaben 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, desired antiaging property was obtained.

Evaluation studies

In vitro antioxidant studies [40]

2,2-diphenylpicrylhydrazyl (DPPH) free radical scavenging assay [41] Different concentrations of standard ascorbic acid and sample, namely, 20, 40, 60, 80, and 100 mg/ml were prepared in methanol. 0.002% DPPH in methanol was used as a free radical. Equal volume of different concentrations of standards and DPPH was mixed in a 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 UV-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:

\[ \text{Scavenging activity (\%)} = \frac{(A - B)}{A} \times 100 \]

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

Reducing power assay

Different concentrations of standard ascorbic acid and sample, namely, 20, 40, 60, 80, and 100 mg/ml in 1ml of methanol were mixed with 2.5ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1% potassium ferricyanide separately. The mixtures were placed in a water bath for 20 min at 50°C, cooled rapidly, mixed with 2.5 ml of 10% tri
A solution of hydrogen peroxide (20 mM) was prepared in PBS (pH 7.4). Various concentrations of 1 ml of the sample was added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min against blank solution that contained extracts in PBS without hydrogen peroxide.

\[
{\text{IC}}_{50} = \frac{A_0 - A}{{	ext{A}}_0} 
\]

Where \( A_0 \) was the absorbance of the standard (ascorbic acid) and \( A \) was the absorbance of samples.

### Physical evaluation of antiaging cream and gel

#### pH

The pH meter was calibrated using standard buffer solution 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.

#### Viscosity

Viscosity of the formulation was determined by Brookfield Viscometer at 100 rpm, using spindle no 7.

#### Homogeneity

The formulations were tested for the homogeneity by visual appearance and by determining the homogeneity of the preparation by visual inspection.

#### 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 top of the sample, and the slides were allowed to stand for 10 min. The spreadability was determined by the distance covered by the sample under the weight of 100 g.
was placed on the upper slide so that the cream between the two slides was pressed uniformly to form a thin layer. The weight was removed and then fixed to a stand without 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. Simplicity and relative lack of expense are the advantages of this method. The following equation was used for this purpose:

\[ S = m \times L/T \]

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 cream to extrude out from the tube. The prepared cream 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 cream that extruded out was collected and weighed. Then, the percentage of cream extruded was calculated. The packing of creams has gained considerable importance in the delivery of desired quantity of cream; therefore, measurement of extrudability has become some important criteria for creams.

**Characterization of phytosomal gel**

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

**Visualization**

Visualization of phytosome can be achieved using scanning electron microscopy (SEM).

Vesicle size and zeta potential

The particle size and zeta potential can be determined by dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy.

Vesicle stability

The stability of vesicles can be determined by assessing the size of the vesicles overtime. Mean size is measured by DLS.

**Comparison studies of antiaging cream, gel, and phytosomal gel**

The prepared formulations of cream, gel, and phytosomal gel were compared to find out which formulation had the highest antioxidant activity. The comparison study was done on the basis of in vitro antioxidant models.

**Comparison studies based on the IC50 values of each formulation**

A comparative study was also done on the basis of IC50 value of each formulation. The IC50 value is the concentration of the sample which scavenges 50% of the free radicals. The lower the IC50 value, the highest the antioxidant activity.

**Stability studies**

Stability studies were performed according to the ICH guidelines. The optimized formulation was kept at two different temperatures 30°C ± 2°C and 4°C ± 2°C for 45 days.

**Comparison of the optimized phytosomal gel with marketed antiaging gel**

The prepared antiaging phytosomal gel was compared with a marketed formulation, antiaging A. vera gel. This marketed formulation contained A. vera as astringing ingredient because of the presence of phenol compounds and Vitamin E and C. The phytosomal gel containing tender coconut water and A. vera extract was compared with the antiaging A. vera gel.

**RESULTS AND DISCUSSIONS**

**Physicochemical parametric evaluation studies**

Physicochemical parameters such as pH, viscosity, homogeneity, spreadability, and extrudability of antiaging creams and gels were found out, and it was found that F2 formulation showed optimum value.

**Physicochemical parameter evaluation of antiaging cream pH measurement**

It was previously reported that, for creams and gels to be non-irritant and safe for topical application, their pH has to be fall in the physiologic accepted range for topical preparations, i.e., pH 6–7 units. Table 3 shows that pH of various antiaging cream formulations ranged from 6.1 to 6.5 which lies in the normal physiologic range and thus produces no skin irritation.

**Viscosity**

The prepared antiaging creams and gels were formulated using Carbopol 940. Table 4 shows that the viscosity of various antiaging cream 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 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.” Spreadability of creams and gels are measured in terms of average diameter of the spread circle. Table 5 shows that

| Table 3: pH of various antiaging cream formulations |
|-----------------------------------------------|
| **Formulation code** | **Trial 1** | **Trial 2** | **Trial 3** | **Average pH** |
|----------------------|-------------|-------------|-------------|----------------|
| F1                   | 6.4         | 6.5         | 6.6         | 6.5 ± 0.1      |
| F2                   | 6.2         | 6.4         | 6.6         | 6.4 ± 0.2      |
| F3                   | 6           | 6.2         | 6.4         | 6.2 ± 0.15     |
| F4                   | 6.1         | 6.3         | 6.4         | 6.2 ± 0.15     |
| F5                   | 6           | 6.1         | 6.2         | 6.1 ± 0.1      |
| F6                   | 6.4         | 6.5         | 6.6         | 6.5 ± 0.1      |
| F7                   | 6.5         | 6.7         | 6.8         | 6.6 ± 0.15     |
| F8                   | 6.2         | 6.3         | 6.4         | 6.3 ± 0.1      |
| F9                   | 6.1         | 6.2         | 6.4         | 6.2 ± 0.5      |

The values are expressed as mean ± SD. SD: Standard deviation.

| Table 4: Viscosity of various antiaging cream formulations |
|----------------------------------------------------------|
| **Formulation code** | **Viscosity (cps)** | **Average viscosity (cps)** |
|----------------------|---------------------|----------------------------|
| **Trial 1** | **Trial 2** | **Trial 3** |                       |
| F1                   | 2453.33 | 2462.88 | 2450.52 | 2455.577 ± 6.4 |
| F2                   | 2454.21 | 2471.89 | 2454.64 | 2465.288 ± 6.7 |
| F3                   | 2461.19 | 2482.91 | 2468.75 | 2491.387 ± 6.8 |
| F4                   | 2494.74 | 2487.43 | 2499.13 | 2490.476 ± 5.9 |
| F5                   | 2498.82 | 2489.51 | 2499.82 | 2491.534 ± 4.3 |
| F6                   | 3289.76 | 3277.56 | 3284.10 | 3283.807 ± 6.1 |
| F7                   | 3294.88 | 3279.67 | 3292.48 | 3287.745 ± 5.2 |
| F8                   | 3414.72 | 3420.45 | 3417.04 | 3417.403 ± 2.8 |
| F9                   | 5132.78 | 5129.78 | 5140.71 | 5134.423 ± 5.6 |

The values are expressed as mean ± SD. SD: Standard deviation.
the spreadability values for all prepared cream formulations ranged 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. Table 6 shows that the extrudability values of cream are ranged from 90.5 to 93.5.

**Physicochemical parameter evaluation of antiaging gels**

**pH measurement**

Table 7 shows that pH of various antiaging gel formulations ranged from 6.13 to 6.5 which lies in the normal physiologic range and thus produces no skin irritation.

**Viscosity**

Table 8 shows that the viscosity of various antiaging gel formulations ranged from 2446.577 to 5334.423.

| Formulation code | Spreadability (g/s) | Average spreadability (g/s) |
|------------------|---------------------|-----------------------------|
|                  | Trial 1 | Trial 2 | Trial 3 |                      |
| F1               | 32.2    | 32.4    | 32.6    | 32.4±0.2             |
| F2               | 35.5    | 35.7    | 35.9    | 35.7±0.2             |
| F3               | 31.1    | 31.2    | 31.3    | 31.2±0.1             |
| F4               | 34.9    | 35.0    | 36.5    | 35.4±0.89            |
| F5               | 26.2    | 27.3    | 28.7    | 27.4±1.25            |
| F6               | 24.3    | 25.4    | 26.5    | 25.4±1.1             |
| F7               | 28.4    | 29.1    | 30.8    | 29.4±1.2             |
| F8               | 22.4    | 23.3    | 24.6    | 23.4±1.1             |
| F9               | 29.9    | 30.2    | 31.8    | 30.6±1.02            |

The values are expressed as mean±SD. SD: Standard deviation

| Formulation code | Extrudability | Average extrudability |
|------------------|---------------|-----------------------|
|                  | Trial 1 | Trial 2 | Trial 3 |                      |
| F1               | 89.9    | 90.7    | 91.2    | 90.6±0.6             |
| F2               | 89.5    | 90.9    | 91.3    | 90.5±0.9             |
| F3               | 91.2    | 92.8    | 93.4    | 92.4±1.13            |
| F4               | 91.2    | 92.9    | 93.8    | 92.6±1.32            |
| F5               | 92.8    | 93.2    | 94.5    | 93.5±0.88            |
| F6               | 92.4    | 93.1    | 94.5    | 93.3±1.06            |
| F7               | 90.2    | 91.6    | 92.3    | 91.3±1.06            |
| F8               | 92.3    | 93.9    | 94.5    | 93.5±1.13            |
| F9               | 90.3    | 91.8    | 92.3    | 91.3±1.06            |

The values are expressed as mean±SD. SD: Standard deviation

| Formulation code | pH | Average pH |
|------------------|----|------------|
|                  | Trial 1 | Trial 2 | Trial 3 |                      |
| F1               | 6.4    | 6.5      | 6.6     | 6.5±0.1              |
| F2               | 6.3    | 6.4      | 6.5     | 6.4±0.1              |
| F3               | 6.1    | 6.3      | 6.5     | 6.3±0.2              |
| F4               | 6.0    | 6.2      | 6.4     | 6.2±0.2              |
| F5               | 6.0    | 6.1      | 6.3     | 6.13±0.15            |
| F6               | 6.5    | 6.6      | 6.7     | 6.6±0.1              |
| F7               | 6.5    | 6.7      | 6.9     | 6.7±0.2              |
| F8               | 6.4    | 6.6      | 6.8     | 6.6±0.2              |
| F9               | 6.1    | 6.2      | 6.3     | 6.2±0.1              |

The values are expressed as mean±SD. SD: Standard deviation

**Spreadability**

Table 9 shows the spreadability values for all prepared gel formulations ranged from 22.4 to 38.2.

**Extrudability**

Table 10 shows that the extrudability values of all prepared gel formulations lie from 90.5 to 93.5.

**In vitro antioxidant studies**

In vitro antioxidant studies were performed for the prepared antiaging creams, 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 concentration-dependent manner in all models. The antioxidant activity was expressed as IC₅₀ (the amount of antioxidant needed to decrease the radical concentration by 50%), which is negatively related to antioxidant activity. The lower the IC₅₀ value, the higher is the antioxidant activity of the tested sample. The in vitro antioxidant studies included DPPH assay, reducing power assay, nitric oxide scavenging assay, and hydrogen peroxide assay. Among them, DPPH assay method was found to be the easiest, sensitive, and...
rapid method to assess the antioxidant activity of compounds. Nine different formulations of antiaging creams, gels, and phytosomal gels were prepared. Although the radical scavenging activity of the standard was found to be higher than that of the samples, the study showed that the samples have the proton-donating ability and could serve as free radical inhibitors or scavengers. Among them, F2 formulation showed the highest antioxidant activity. Physicochemical parameters such as pH, viscosity, homogeneity, spreadability, and extrudability were evaluated for both antiaging creams and gels. From the physicochemical parameter evaluation, it was found that F2 sample showed optimum values. Hence, the F2 formulation was taken as the optimized formula from all the nine different formulations. Characterization studies of antiaging phytosomal gel were also performed.

**Antiaging cream**

**DPPH free radical scavenging activity**

The result of antioxidant activity of different concentrations of samples and standard (ascorbic acid) is shown in Table 11. 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 the nine different samples of cream. Among them, F2 sample showed the highest antioxidant activity. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases. In this study, the scavenging activity of F2 sample was found to be dose dependent, i.e., higher the concentration, 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.

**Reducing power assay**

The result of antioxidant activity of different concentrations of samples and standard (ascorbic acid) is shown in Table 12. In this study, the absorbance was found to increase with the dose of samples and standard which is suggestive of reducing power. In the Fe3+ reducing assay, the reducing power of antiaging cream and gel samples was found to increase with the dose. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe3+/ferricyanide complex to the ferrous form. Therefore, Fe2+ can be monitored by measuring the formation of Perl’s Prussian blue at 700 nm. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the antioxidant activity of putative antioxidants has been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical.

Fig. 2 shows the reducing power assay of antiaging cream when compared with that of the standard ascorbic acid.

**Nitric oxide scavenging assay**

The result of antioxidant activity of different concentrations of samples and standard (ascorbic acid) is shown in Table 13. Nitric oxide free radical catalyzes the decomposition of peroxides, prevention of continued hydrogen abstraction, and radical.

**Table 11: DPPH scavenging assay of antiaging cream and the standard ascorbic acid**

| Concentration | Standard | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---------------|----------|----|----|----|----|----|----|----|----|----|
| 0             | 0.0      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 20            | 26.54±1.38 | 20.1±1.30 | 24.08±1.41 | 22.04±1.90 | 21.98±1.32 | 20.77±1.37 | 20.62±1.48 | 20.33±1.48 | 20.21±1.49 | 20.19±1.46 |
| 40            | 38.87±1.38 | 30.06±1.42 | 36.68±1.41 | 33.99±1.32 | 32.06±1.51 | 31.05±1.37 | 34.87±1.34 | 33.87±1.40 | 32.23±1.39 | 31.17±1.49 |
| 60            | 46.76±1.40 | 35.13±1.40 | 44.45±1.42 | 41.6±1.49 | 40.56±1.32 | 40.77±1.35 | 42.02±1.32 | 42.28±1.36 | 42.19±1.38 | 40.01±1.49 |
| 80            | 56.66±1.39 | 43.9±1.36 | 55.01±1.47 | 53.67±1.30 | 52.34±1.29 | 51.09±1.31 | 53.66±1.36 | 53.82±1.22 | 53.34±1.29 | 50.87±1.39 |
| 100           | 58.87±1.41 | 51.03±1.45 | 56.98±1.41 | 52.99±1.37 | 51.09±1.39 | 50.6±1.39  | 52.09±1.37 | 52.11±1.44 | 52.77±1.39 | 52.46±1.34 |

**Table 12: Reducing power assay of antiaging cream and the standard ascorbic acid**

| Concentration | Standard | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---------------|----------|----|----|----|----|----|----|----|----|----|
| 0             | 0.0      | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 20            | 25.88±1.49 | 22.98±1.48 | 24.64±1.43 | 23.53±1.49 | 22.74±1.49 | 22.63±1.43 | 22.31±1.40 | 21.74±1.49 | 20.79±1.44 | 20.32±1.33 |
| 40            | 38.98±1.50 | 33.27±1.42 | 36.73±1.43 | 35.55±1.47 | 34.72±1.47 | 33.33±1.48 | 32.87±1.47 | 31.08±1.49 | 30.73±1.42 | 30.28±1.32 |
| 60            | 45.53±1.41 | 42.01±1.47 | 43.99±1.44 | 42.82±1.47 | 41.66±1.41 | 40.83±1.42 | 40.65±1.40 | 40.03±1.47 | 40.27±1.41 | 40.19±1.31 |
| 80            | 52.22±1.46 | 50.09±1.48 | 51.07±1.41 | 50.71±1.41 | 50.75±1.41 | 50.65±1.40 | 50.36±1.39 | 50.02±1.46 | 50.16±1.40 | 50.12±1.30 |
| 100           | 57.75±1.45 | 52.22±1.44 | 55.98±1.40 | 54.75±1.39 | 53.87±1.39 | 52.55±1.44 | 51.64±1.39 | 51.08±1.46 | 50.91±1.39 | 50.26±1.34 |
radicals are released from the endothelial cells which are necessary for the inflammatory actions of skin. The results signify that the level of nitric oxide was reduced to a considerable amount by the samples. Thus, the prepared formulation can be used effectively for topical purposes.

Table 13 suggests the potency of nitric oxide scavenging activity of the formulations with that of the standard ascorbic acid.

Fig. 3 shows the nitric oxide scavenging activity of the antiaging cream when compared with the standard.

**Nitric oxide scavenging assay**

The results given in Table 13 show the potency of nitric oxide scavenging activity of the formulations with that of the standard ascorbic acid.
Table 15: DPPH assay of antiaging gel and standard ascorbic acid

| Concentration | Standard | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---------------|----------|----|----|----|----|----|----|----|----|----|
| 0             | 0        | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 20            | 31.11±1.39 | 33.82±1.45 | 38.87±1.45 | 31.33±1.48 | 30.29±1.11 | 34.66±1.47 | 32.97±1.47 | 31.64±1.13 | 31.33±1.49 | 30.29±1.41 |
| 40            | 48.85±1.48 | 44.98±1.39 | 47.67±1.37 | 40.43±1.34 | 40.28±1.05 | 45.57±1.48 | 43.74±1.40 | 41.85±1.17 | 40.43±1.41 | 40.28±1.47 |
| 60            | 59.83±1.32 | 55.83±1.32 | 58.64±1.34 | 51.82±1.28 | 50.47±1.20 | 56.83±1.28 | 54.82±1.41 | 52.64±1.13 | 51.82±1.44 | 50.47±1.38 |
| 80            | 68.83±1.40 | 64.85±1.41 | 67.75±1.36 | 60.38±1.34 | 60.11±1.14 | 65.74±1.32 | 63.71±1.28 | 60.72±1.17 | 60.11±1.27 | 60.11±1.32 |
| 100           | 73.39±1.42 | 70.22±1.47 | 72.29±1.28 | 70.05±1.43 | 70.18±1.01 | 70.11±1.47 | 70.21±1.34 | 70.09±1.13 | 70.05±1.39 | 70.18±1.41 |

DPPH: 2,2-diphenylpicrylhydrazyl

Table 16: Reducing power assay of antiaging gel and standard ascorbic acid

| Concentration | Standard | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---------------|----------|----|----|----|----|----|----|----|----|----|
| 0             | 0        | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 20            | 40.98±1.34 | 33.82±1.45 | 38.87±1.45 | 31.33±1.48 | 30.29±1.11 | 34.66±1.47 | 32.97±1.47 | 31.64±1.13 | 31.33±1.49 | 30.29±1.41 |
| 40            | 48.85±1.48 | 44.98±1.39 | 47.67±1.37 | 40.43±1.34 | 40.28±1.05 | 45.57±1.48 | 43.74±1.40 | 41.85±1.17 | 40.43±1.41 | 40.28±1.47 |
| 60            | 59.83±1.32 | 55.83±1.32 | 58.64±1.34 | 51.82±1.28 | 50.47±1.20 | 56.83±1.28 | 54.82±1.41 | 52.64±1.13 | 51.82±1.44 | 50.47±1.38 |
| 80            | 68.83±1.40 | 64.85±1.41 | 67.75±1.36 | 60.38±1.34 | 60.11±1.14 | 65.74±1.32 | 63.71±1.28 | 60.72±1.17 | 60.11±1.27 | 60.11±1.32 |
| 100           | 73.39±1.42 | 70.22±1.47 | 72.29±1.28 | 70.05±1.43 | 70.18±1.01 | 70.11±1.47 | 70.21±1.34 | 70.09±1.13 | 70.05±1.39 | 70.18±1.41 |

Table 17: Nitric oxide scavenging assay of antiaging gel and standard ascorbic acid

| Concentration | Standard | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---------------|----------|----|----|----|----|----|----|----|----|----|
| 0             | 0        | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 20            | 40.98±1.34 | 33.82±1.45 | 38.87±1.45 | 31.33±1.48 | 30.29±1.11 | 34.66±1.47 | 32.97±1.47 | 31.64±1.13 | 31.33±1.49 | 30.29±1.41 |
| 40            | 48.85±1.48 | 44.98±1.39 | 47.67±1.37 | 40.43±1.34 | 40.28±1.05 | 45.57±1.48 | 43.74±1.40 | 41.85±1.17 | 40.43±1.41 | 40.28±1.47 |
| 60            | 59.83±1.32 | 55.83±1.32 | 58.64±1.34 | 51.82±1.28 | 50.47±1.20 | 56.83±1.28 | 54.82±1.41 | 52.64±1.13 | 51.82±1.44 | 50.47±1.38 |
| 80            | 68.83±1.40 | 64.85±1.41 | 67.75±1.36 | 60.38±1.34 | 60.11±1.14 | 65.74±1.32 | 63.71±1.28 | 60.72±1.17 | 60.11±1.27 | 60.11±1.32 |
| 100           | 73.39±1.42 | 70.22±1.47 | 72.29±1.28 | 70.05±1.43 | 70.18±1.01 | 70.11±1.47 | 70.21±1.34 | 70.09±1.13 | 70.05±1.39 | 70.18±1.41 |

Nitric oxide assay of phytosomal gels

Table 21 shows the percentage of free radicals scavenged by the sample and standard at increasing concentrations.

Hydrogen peroxide assay of phytosomal gel

Table 22 shows the percentage of free radicals scavenged by the sample and standard at increasing concentrations (Fig. 11).

All the formulations showed potent antioxidant activity in all the assay methods. From the figures, the result represents that all the nine formulations had the capacity to scavenge the free radicals, but among them, the F2 formulation containing both tender coconut water and Aloe vera extract showed the highest antioxidant activity and also the radical scavenging activity by DPPH assay method showed highest antioxidant activity than the other methods. Hence, it was concluded that the prepared phytosomal gel could be used for the purpose of anti-wrinkle treatment as it could scavenge the harmful free radicals.

IC50 values of optimized formulation, F2 of antiaging cream, gel, and phytosomal gel

IC50 value, i.e. the concentration of the sample which is required to scavenge 50% of the free radicals of the optimized formulation of the prepared antiaging cream, F2 is found to be 70.5 µg/ml (Fig. 12).
Comparison studies of antiaging cream, gel, and phytosomal gel on the basis of in vitro antioxidant studies

The antioxidant property of the prepared formulations of antiaging cream, gel, and phytosomal gel was compared on the basis of in vitro antioxidant studies. From the comparison study, it was clear that antiaging phytosomal gel had the highest antioxidant activity, as it could be concluded that the prepared phytosomal gel possessed the antiaging property. Comparison studies are shown in Table 23.

Fig. 15 indicates the graph in which it is clear cut that the antiaging phytosomal gel had the highest antioxidant activity.

Comparison studies of IC_{50} values of antiaging cream, gel, and phytosomal gel [41]

IC_{50} values of the optimized formulations (F2) of antiaging cream, gel, and phytosomal gel were compared. The IC_{50} value of antiaging cream was found to be 70.5 µg/ml, the IC_{50} value of antiaging gel was found to be 47.0 µg/ml, and the IC_{50} value of antiaging phytosomal gel was found to be 47.0 µg/ml. Therefore, it can be concluded that the antiaging phytosomal gel proved to show the highest antioxidant activity as it had an IC_{50} value of 47.0 µg/ml. The lower the IC_{50} value, the higher the antioxidant activity (Fig. 16).

Table 18: Hydrogen peroxide assay of antiaging gel and standard ascorbic acid

| Concentration | Standard | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---------------|----------|----|----|----|----|----|----|----|----|----|
| 0             | 0        | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 20            | 40.98±1.51 | 34.66±1.34 | 35.98±1.50 | 34.72±1.45 | 33.82±1.49 | 32.97±1.42 | 31.33±1.44 | 30.99±1.45 | 31.64±1.44 | 31.33±1.44 | 30.29±1.13 |
| 40            | 48.85±1.45 | 45.57±1.39 | 47.73±1.33 | 44.98±1.36 | 43.74±1.41 | 40.43±1.41 | 40.32±1.39 | 41.85±1.34 | 40.43±1.42 | 40.28±1.13 |
| 60            | 59.83±1.45 | 56.83±1.41 | 55.83±1.38 | 55.83±1.43 | 54.82±1.39 | 51.82±1.30 | 50.81±1.43 | 52.64±1.36 | 51.82±1.45 | 50.47±1.12 |
| 80            | 68.83±1.39 | 65.74±1.38 | 64.22±1.38 | 64.85±1.39 | 63.71±1.46 | 60.38±1.39 | 60.19±1.40 | 61.72±1.45 | 60.38±1.34 | 60.11±1.19 |
| 100           | 73.39±1.38 | 70.11±1.38 | 70.01±1.39 | 70.22±1.37 | 70.21±1.33 | 70.05±1.37 | 70.01±1.41 | 70.09±1.37 | 70.05±1.38 | 70.18±1.12 |

Table 19: DPPH assay of phytosomal gel and standard ascorbic acid

| Concentration | Standard | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---------------|----------|----|----|----|----|----|----|----|----|----|
| 0             | 0        | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 20            | 40.98±1.51 | 34.66±1.44 | 35.98±1.50 | 34.72±1.45 | 33.82±1.49 | 32.97±1.42 | 31.33±1.44 | 30.99±1.45 | 31.64±1.44 | 31.33±1.44 | 30.29±1.13 |
| 40            | 48.85±1.45 | 45.57±1.39 | 47.73±1.33 | 44.98±1.36 | 43.74±1.41 | 40.43±1.41 | 40.32±1.39 | 41.85±1.34 | 40.43±1.42 | 40.28±1.13 |
| 60            | 59.83±1.45 | 56.83±1.41 | 55.83±1.38 | 55.83±1.43 | 54.82±1.39 | 51.82±1.30 | 50.81±1.43 | 52.64±1.36 | 51.82±1.45 | 50.47±1.12 |
| 80            | 68.83±1.39 | 65.74±1.38 | 64.22±1.38 | 64.85±1.39 | 63.71±1.46 | 60.38±1.39 | 60.19±1.40 | 61.72±1.45 | 60.38±1.34 | 60.11±1.19 |
| 100           | 73.39±1.38 | 70.11±1.38 | 70.01±1.39 | 70.22±1.37 | 70.21±1.33 | 70.05±1.37 | 70.01±1.41 | 70.09±1.37 | 70.05±1.38 | 70.18±1.12 |

DPPH: 2,2-diphenylpicrylhydrazyl
Characterization of antiaging phytosomal gel

Visual inspection
The prepared phytosomal gel was visually inspected and it showed that the formulation was homogeneous without any gritty particles and was of optimum consistency.

Globule size determination
Microscopic evaluation
The prepared antiaging phytosomal gel was observed under optical microscope at 100× and observed that the formed vesicles were of uniform size.

Size and size distribution:
The vesicular size and size distribution was evaluated using DLS, and the results showed that increase in extract phospholipid concentration increases the mean vesicular size. The vesicular size was between 52 nm and 115 nm. The polydispersity index was found to be low, and the obtained vesicles were of uniform size [Figs. 17-21 and Table 24].

Zeta potential determination
The magnitude of zeta potential gives a potential stability of the colloidal dispersion. If the particles have large positive or negative charge reveals that they repel each other and there is dispersion stability. The zeta potential of the optimized formulation showed that the particle size was highly stable. It was found as -22.21, and hence, this indicates that the prepared formulation is stable.

SEM
From Fig. 22, 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.
Stability studies

The best formulation of the phytosomal gel was kept at varying conditions of temperature. The system was stable at 25°C. There were no significant changes in the formulation when kept at room temperature (30°C±2°C) and also at refrigerated temperature (4°C±2°C). No much change of pH, viscosity, homogeneity, spreadability, extrudability, and degradation of the samples were observed during 45 days period. Table 25 shows the data for stability studies for antiaging phytosomal gel F2. There was no much change in the zeta potential of the sample, and this proves that the phytosomal gel system remains stable.

Comparison of the phytosomal gel with 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 such as tender coconut water and A. vera had significant antiaging properties. The A. vera antiaging gel was taken for comparison as it had the antiaging ingredient, A. vera.

From Fig. 23, 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...
to the ICH guidelines, and it was found that the optimized formulation was stable under both room temperature and refrigerated temperature. The optimized formulation was compared with a marketed antiaging gel and it was clear cut that the prepared antiaging phytosomal gel had highest antioxidant activity than the marketed formulation, and hence, gel and it was identified that the prepared antiaging phytosomal gel had all the parameters as optimum. Stability studies were done according
Table 25: Stability studies of phytosomal gel, F2

| Days | Temperature | Formulation | Parameters | pH | Viscosity | Homogeneity | Spreadability | Extrudability |
|------|-------------|-------------|------------|----|-----------|-------------|--------------|--------------|
| 0    | RT          | 1           |            | 6.5±0.1 | 23108±e | Homogeneous | 32.4±0.2 | 90.7±0.6 |
| 30°C±2°C | 5           | 6.4±0.2    | 27634±e    | 35.7±0.2 | 90.9±0.9 |
| 8    | 6.3±0.1    | 30192±e    | Homogeneous | 31.2±0.1 | 92.8±1.1 |
| 1    | 6.1±0.1    | 26345±e    | Homogeneous | 35±0.8  | 92.9±1.3 |
| 4°C±2°C | 2           | 6.5±0.1    | 26889±    | 25.4±1.1 | 91.3±1.0 |
| 5    | 6.7±0.1    | 27512±     | Homogeneous | 29.1±1.2 | 91.6±1.0 |
| 8    | 6.8±0.1    | 28129±     | Homogeneous | 23.3±1.1 | 93.9±1.1 |
| 10   | RT          | 1           |            | 6.7±0.1 | 30198±e | Homogeneous | 30.2±1.0 | 91.8±1.1 |
| 30°C±2°C | 5           | 6.5±0.1    | 31279±     | 36.1±1.1 | 90.3±1.2 |
| 8    | 6.3±0.2    | 35583±     | Homogeneous | 33.3±1.3 | 93.1±1.0 |
| 1    | 6.8±0.1    | 39153±     | Homogeneous | 38.9±1.1 | 92.9±1.0 |
| 4°C±2°C | 2           | 6.3±0.1    | 27736±     | 24.3±1.0 | 92.5±1.0 |
| 5    | 6.1±0.1    | 28102±     | Homogeneous | 29.1±1.1 | 93.8±0.9 |
| 8    | 6.8±0.1    | 29710±     | Homogeneous | 20.1±1.1 | 93.1±1.4 |
| 14   | RT 30°C±2°C | 1           |            | 6.4±0.2 | 23509±e | Homogeneous | 21±1.1    | 93.2±1.1 |
| 2    | 6.3±0.2    | 39127±     | Homogeneous | 37.1±1.1 | 93.5±0.9 |
| 5    | 6±0.1      | 52198±     | Homogeneous | 39.4±0.9 | 92.6±1.1 |
| 8    | 6.2±0.2    | 50123±     | Homogeneous | 38.7±1.1 | 92.1±1.1 |
| 4°C±2°C | 1           | 6.1±0.1    | 33671±     | 24.3±0.8 | 97.2±1.2 |
| 2    | 6.2±0.1    | 48193±     | Homogeneous | 26.7±1.1 | 92.5±0.9 |
| 5    | 6.5±0.1    | 49100±     | Homogeneous | 28±1.1  | 93.1±0.8 |
|     | 6±0.2      | 52475±     | Homogeneous | 27.1±1.1 | 93.4±1.1 |
| 30   | RT 30°C±2°C | 1           |            | 6.4±0.2 | 20918±e | Homogeneous | 31.9±1.2 | 92.8±1.2 |
| 2    | 6.1±0.1    | 37162±     | Homogeneous | 34.2±1.3 | 91.5±1.2 |
| 5    | 6±0.2      | 39188±     | Homogeneous | 38.4±1.2 | 90.2±1.1 |
| 8    | 6.5±0.2    | 51092±     | Homogeneous | 39.2±1.3 | 90.4±0.9 |
| 4°C±2°C | 1           | 6.4±0.2    | 38190±     | 39.4±0.09 | 91.4±0.9 |
| 2    | 6.8±0.1    | 51092±     | Homogeneous | 31.5±0.8 | 93.2±0.8 |
| 5    | 6.6±0.2    | 50162±     | Homogeneous | 37.5±1.3 | 93.9±1.1 |
| 8    | 6.3±0.1    | 53851±     | Homogeneous | 35.5±1.4 | 93.2±1.2 |
| 45   | RT 30°C±2°C | 1           |            | 6.2±0.1 | 29133±e | Homogeneous | 20.2±1.2 | 90.2±1.1 |
| 2    | 6.1±0.1    | 39012±     | Homogeneous | 27.1±1.1 | 90.5±1.1 |
| 5    | 6.2±0.2    | 42386±     | Homogeneous | 24.3±1.1 | 90.7±1.2 |
| 8    | 6.8±0.2    | 51893±     | Homogeneous | 29.2±1.1 | 90.9±1.1 |
| 4°C±2°C | 1           | 6±0.2      | 21677±     | 30.2±1.1 | 93.3±0.9 |
| 2    | 6.1±0.2    | 25643±     | Homogeneous | 34.3±1.1 | 91.4±1.1 |
| 5    | 6.8±0.1    | 36710±     | Homogeneous | 35.6±1.1 | 91.2±0.9 |
| 8    | 6.8±0.1    | 48176±     | Homogeneous | 33.2±1.1 | 91.2±0.8 |

Parameters are derived using mean±SD. SD: Standard deviation

Table 26: Comparison of phytosomal gel with 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

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Hence, the prepared herbal antiaging phytosomal gel which is non-toxic, safe, and effective is found to be highly acceptable.

ACKNOWLEDGMENT

We would like to show our gratitude to the Verjina CU and Dr. Deepa T Vasudevan for sharing their pearls of wisdom with us during the course of this research, and we thank the "anonymous" reviewers for their so-called insights and their comments on an earlier version of the manuscript.

AUTHOR’S CONTRIBUTIONS

Verjina CU, Deepa T Vasudevan developed and optimized the formulations. Julie Mariam Joshua and Athira Anilkumar wrote the manuscript with the support and supervision of Saritha A Surendran.

CONFLICT OF INTEREST

All authors have none to declare.

REFERENCES

1. Harman D. Aging: Overview. Ann N Y Acad Sci 2001;928:1-21.
2. Winker MA. Aging in the 21st century: A call for papers. Arch Intern Med 2002;162:745-52.
3. Sahli ME, Yates FE. Kinetics of Human Aging: I. Rates of senescence between ages 30 and 70 in healthy people. J Gerontol 2001;56:198-208.
4. Hughes KA, Alipaz JA, Drnevich JM, Reynolds RM. A Test of Evolutionary Theories of Aging. Proceedings of the National Academy of Sciences of the United States of America; 2002. p. 14286-91.
5. Gilchrest BA. Skin aging 2003: Recent advances and current concepts. Cuts 2003;72:5-10.
6. Lapiere CM. The ageing dermis: The main cause for the appearance of old skin. Br J Dermatol 1990;122:5-11.
7. Bossert S, Barre P, Chalon A, Kurlast R, Bonté F, André P, et al. Skin aging: Clinical and histopathologic study of permanent and reversible wrinkles. Eur J Dermatol 2002;12:247-52.
8. Rojas J, Londoño C, Ciró Y. The health benefits of natural skin uva photoprotective compounds found in botanical sources. Int J Pharm Pharm Sci 2016;8:13-23.

9. Ali SS, Kasoju N, Luthra A, Singh A, Shananasabava H, Sahu A, et al. Indian medicinal herbs as sources of antioxidants. Food Res Int 2008;41:1-15.

10. Kumpulainen JT, Salonen JT. Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease. Great Britain: The Royal Society of Chemistry; 1999 p. 178-87.

11. Ge L, Yong JW, Tan SN, Yang XH, Ong ES. Analysis of cytokingin nucleotides in coconut (Cocos nucifera L.) water using capillary zone electrophoresis-tandem mass spectrometry after solid-phase extraction. J Chromatogr A 2006;1133:322-31.

12. Squadrato GL, Pryor WA. Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite, and carbon dioxide. Free Radic Biol Med 1998;25:392-403.

13. Clark AM, Hufford CD. Discco and development of novel prototype antibiotics for opportunistic infections related to the acquired immunodeficiency syndrome. In: Human Medical Agents from Plants. Washington, DC: American Chemical Society; 1993. p. 228-41.

14. Gutteridge JM. Free radicals in disease processes: A compilation of cause and consequence. Free Rad Res Comm 1995:19:141-2.

15. Halliwell B. How to characterize an antioxidant: An update. Biochem Soc Symp 1995:67:131-18.

16. Leong LP, Shui G. An Investigation of Antioxidant capacity of fruits in Singapore markets. Food Chem 2002;76:69-75.

17. Prior RL. Fruit and vegetables in the prevention of cellular oxidative damage. Am J Clin Nutr 2003;78:570-90.

18. Devasagayam TP, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD, et al. Free radicals and antioxidants in human health: Current status and future prospects. J Assoc Physicians India 2004;52:794-804.

19. Zérola M, De Lima TC, Sonaglio D, González-Ortega G, Limberger RP, Petzovich PR, et al. CNS activities of liquid and spray-dried extracts from Lippia alba verbenaceae (Brazilian false melissa.). J Ethnopharmacol 2002;82:207-25.

20. Büyükokuroğlu ME, Gülcin I, Oktay M, Kürşüoğlu Ö. In vitro antioxidant properties of dantrolene sodium. Pharmac Res 2001;14:44-91.

21. Shahidi F, Wanasundara PK. Phenolic antioxidants. J Food Sci Technol 1992;32:67-103.

22. Prathapan A, Rajamohan T. Antioxidant and antithrombotic effect of tender coconut water in myocardial infarction. J Nutr Biochem 2011;22:1-6.

23. Branen AL. Toxicology and biochemistry of butylated hydroxyanisole. J Toxicol Environ Health 1987;127:127-476.

24. Joyce DA. Oxygen radicals in disease. Adv Drug React Bull 1988;4:392-403.

25. Tiwari A. Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidant therapy. Curr Sci 2011;81:1179-85.

26. Choubey A. Phytosome-a novel approach for herbal drug delivery. Int J Curr Pharm Res 2011;3(3):7066-79.

27. Balasubramanian SS, Venugopal S. A novel approach of herbal phytocapsules. Int J Curr Pharm Sci 2013;2(3):267-74.

28. Nair AJ, Soman P, George A, Surendran SA. Formulation of phytosome gel using 3% herbal extract of Aloe vera leaf. Int J Pharm Tech Res 2016;8:11065-76.

29. Kataoka S, Ishibashi S, Furusawa J, Ito N, Fukushima S, Hagiwara A, Shibata M, Ogiso T. Carcinogenicity of phosphorus compounds in coconut water. Food Chem Toxicol 1988;26:579-90.

30. Prathapan A, Rajamohan T. Antioxidant and antithrombotic effect of tender coconut water in myocardial infarction. J Nutr Biochem 2011;22:1-6.

31. Gupta NK, Dixit VK. Development and evaluation of vesicular system loaded proniosomal gel using 3% herbal extract of Aloe vera leaf. Int J Med Res Publ 2012;80:731-48.

32. Nair SS, Majeed S, Sankar S, Jeejamol, Mathew M. Formulation of some antioxidant herbal gels. Hygyna 2009;1:44-6.

33. Prior RL. Fruit and vegetables in the prevention of cellular oxidative damage. Am J Clin Nutr 2003;78:570-90.

34. Zérola M, De Lima TC, Sonaglio D, González-Ortega G, Limberger RP, Petzovich PR, et al. CNS activities of liquid and spray-dried extracts from Lippia alba verbenaceae (Brazilian false melissa.). J Ethnopharmacol 2002;82:207-25.

35. Büyükokuroğlu ME, Gülcin I, Oktay M, Kürşüoğlu Ö. In vitro antioxidant properties of dantrolene sodium. Pharmac Res 2001;14:44-91.

36. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. Botany. London: Academic Press; 1986. p. 450-90.

37. Kaur C, Kapoor HC. Power of antioxidants. J Food Sci Technol 2001;36:703-4.

38. Kinsella JE, Frankel E, German B, Kanner J. Herbal drug delivery system. J Food J Food Sci Technol 1993;34(8):843-5.

39. Bernattieni J, Masteikova R, Daavoliene J, Cecura R, Gauyliene R, Bernattieni R, et al. Topical application of Calendula officinalis (L.). Formulation and evaluation of hydrophilic cream with antioxidant activity. J Med Plants Res 2011;5:868-77.

40. Madhavi DL, Salunke DK. In: Madhavi DL, Deshpande SS, Salunke DK, editors. Antioxidant Rich Foods. New York: Dekker; 1996. p. 239-45.

41. Wadher KJ, Lakhotia CL, Umekar MJ. Formulation and evaluation of cream of Azadirachta indica leaves extract on skin renewal rate. Int J Chemtech Res 2009;1:88-95.

42. Gazzani G, Papetti A, Massolini G, Daglia M. Review on antioxidant containing foods. Food Chem 1998;64:4118-9.

43. Das S, Haldar PK, Pramanik G. Formulation and evaluation of herbal gel containing Clerodendrum infortunatum leaves extract. Int J PharmTech Res 2011;1:140-3.

44. Ceve G, Vierl U. Nanotechnology and the transdermal route: A state of the art review and critical appraisal. J Control Release 2010;141:277-99.

45. Wickett RR, Visscher MO. Structure and function of the epidermal barrier. Dermatol Surg 2006;34:98-110.

46. Das K, Dang R, Machale MU. Formulation and evaluation of a novel herbal gel of stevia extract. Int J Pharm 1988;1:1-10.

47. Bhattachar SC, Herbal chemistry. Int J Pharm Herbal Drugs 1998;23:122-33.

48. Tirtha SS, The Ayurveda Encyclopedia. Bayville, NY: Herbal Medicines 1998. p. 33-62.

49. Gupta NK, Dixit VK. Development and evaluation of vesicular system for curcumin delivery. Arch Dermatol Res 2011;303:89-101.

50. Saurabh KY, Kesari A. Herbosome-a novel carrier for herbal drug delivery. Int J Curr Pharm Res 2011;3(3):7066-79.

51. Chouhey A. Phytosome-a novel approach for herbal drug delivery. Int J Pharm Sci Res 2011;2:807-15.

52. Kumari P, Singh N, Cheriyan BP, Neelam J. Phytosome: A novel approach for phytomedicinal gels. J Inst Pharm Sci 2011;1:84-93.

53. Nair AJ, Soman P, George A, Surendran SA. Formulation of Myristica fragrans (Nutmeg) topical gel and its in vitro evaluation for anti-inflammatory activity. Int J Pharm Techol 2016;8:11065-76.

54. Pillai AB, Nair JV, Gupta NK, Gupta S. Microemulsion-loaded hydrogel formulation of but enaline hydrochloride for improved topical delivery. Arch Dermatol Res 2015;307:625-33.

55. Thomas L, Viswanad V. Formulation and optimization of clotrimazole-loaded prionsomal gel using 3 2 factorial design. Sci Pharm 2012;80:731-48.

56. Ganesh N, Hanna C, Nair SV, Nair LS. Enzymatically cross-linked alginic-hyaluronic acid composite hydrogels as cell delivery vehicles. Int J Biol Macromol 2013;55:289-94.

57. Finkel T, Holbrook NJ. In vitro antioxidant studies. Nature 2000;408:239-40.

58. Nair NC, Henry AN. Analysis of Cytokinins. Flora of Tamil Nadu. Karnatakata: Botanical Survey of India; 1983. p. 13-20.