Research Article

FORMULATION AND EVALUATION OF CAMELLIA SINENSIS LEAF EXTRACT INCORPORATED VANISHING CREAM FOR PHOTO-CHEMO PROTECTIVE ACTIVITY

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ABSTRACT

Objective: Natural substances extracted from plants have recently been considered as potential sunscreen resources because of their ultraviolet ray absorption in the UVA region and their antioxidant activity. In the present study, the UV protective effects of Camellia sinensis leaves extract were evaluated by calculating Sun Protection Factors. **Methods:** Camellia sinensis leaves extract were prepared using soxhlet extraction apparatus. Camellia sinensis leaf extract incorporated vanishing cream was formulated and evaluated. **Results:** From sun protection factors, it was found that Camellia sinensis leaves extract can block ultra violet radiations. The evaluations indicates that the formulated cream were within the acceptable limit. **Conclusion:** Camellia sinensis leaf extract incorporated vanishing cream most promising formulation for photo-chemo protective activity.

Keywords: Camellia sinensis leaves extract, Sun Protection Factors (SPF), Photo-chemo protective activity, Vanishing cream.

INTRODUCTION

In the past, sun exposure was thought to be a healthy benefit of outdoor activity. However, studies have shown many unhealthy effects of sun exposure, such as early ageing of skin and skin cancer .Sunburn is a visible type of damage, which appears just a few hours after sun exposure. In many people, this type of damage also causes tanning, or increased pigmentation of the skin, which is noticeable a few days after sun exposure [1]. Part of the suns energy that reaches earth is composed of rays of invisible UV light. When UV light rays enter the skin, they damage skin cell, causing visible and invisible injuries resulting in increased numbers of moles, freckles, wrinkles, and skin cancers. Sun burns are caused by exposure to too much UV light [2]. Melanin is a dark pigment in the outer layer of skin that gives your skin its normal color. When you are exposed to UV light, your body protects by accelerating the production of melanin. The extra melanin creates the darker color of a tan. Many people simply don't produce enough melanin to protect the skin well. Eventually, UV light causes the skin to burn, bringing pain, redness, and swelling [3].

Sun protection factor (SPF) is a measure of sunscreen protection from UV rays, the kind that cause sunburn and contribute to skin cancer [4]. UV radiation reaches the earth in the form of UVB and UVA rays. UVB radiation plays a key role in skin cancer, and SPF refers mainly to the amount of UVB protection a sunscreen offers. An SPF 15 sunscreen blocks 93 percent of UVB radiation, while an SPF 30 sunscreen blocks nearly 97 percent [5]. The tea plant is an evergreen of the Camellia family. There are two main varieties of the tea plant. The small leaf variety, known as Camellia sinensis, thrives in the cool, high mountain regions, where it was used as a medicinal drink. The leaves are then fully oxidized before they are dried, which is how they get their dark color and rich flavor [6]. A cream is a preparation usually for application to the skin. The use of the fingertip unit concept may be helpful in guiding how much topical cream is required to cover different areas [7].

METERIALS AND METHODS

MATERIALS

The following materials were used (Grade-LR): Stearic acid, Cetyl alcohol, Propylene glycol, Borax, Glycerine, Triethanolamine, Benzoic acid (Nice Chemicals Pvt Ltd Cochin-2)

METHODS

Collection

The leaves of Camellia sinensis leaves were collected from Kattapana, Idukki district, Kerala, India in the month of November 2017. The plant material was identified and authenticated by Dr. K K Anilkumar, Assistant Professor, Department of Botany, N.S.S. Hindu College, Changanacherry, Kottayam.

Preparation of extract

The Camellia sinensis leaves were dried in the shade at room temperature. The ethanolic extract was obtained using 30 gm of dried powdered plant material with ethanol (400 ml) in soxhlet extraction apparatus under reflex for 10 hrs. The ethanolic extracts were concentrated [8, 14].

Chemical tests for Epicatechins

Flame Test

A small amount of Camellia sinensis leaves extract were taken at the tip of a match stick. The match stick were dipped in hydrochloric acid and leave it for two seconds. Then the stick were warmed near the flame and colour was observed [9].

Vannilin - Hydrochloric acid Test

A small amount of Camellia sinensis leaves extract were added to the mixture of vannilin and hydrochloric acid. Then the colour of sample were observed [9].

Determination of Sun protection factor

One gm. of Camellia sinensis leaves extract were taken and 100 ml of ethanol were added to it and diluted. From the above solution about 1ml were taken out and add 100ml of ethanol were added. The absorbance for UVB were measured at the range 290-320 nm [10].

SPF were calculated using the formula [10]

$$SPF = C \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Formulation of Cream

Oil in water (O/W) emulsion-based cream (semisolid formulation) was formulated. The oil phase stearic acid and cetyl alcohol were mixed (part A) and melted at 75° C in a water bath. The preservatives and other water soluble components like tri ethanolamine, glycerin, Propylene glycol, benzoic acid and borax were dissolved in the aqueous phase (Part B) and heated to the same temperature as of oil phase. The heated aqueous phase was added to the hot oil phase with continuous stirring at low speed using the mechanical stirrer to get oil in water emulsion cream. Allow the cream to cool while stirring continued. During the cooling phase Camellia sinensis leaves extract were added to the cream and was cool down to room temperature [11].

Evaluation of the Cream

The formulated cream was subjected to the following evaluation such as pH determination, *invitro* drug diffusion study, homogeneity, appearance, viscosity, spreadability etc.

pH

The pH meter was calibrated using standard buffer solution. About 0.5gm of the cream was weighed and dissolved in 50ml of distilled water and the pH of the solution was measured [12].

Dilution

A small quantity of cream was taken in a test tube and diluted with water, and checked for its miscibility with water [12].

Homogeneity

The formulations were tested for homogeneity by visual appearance and feel on the skin [12].

Appearance

Appearance of the cream was visually judged by its color, roughness, and pearlscence [12].

Viscosity

Viscosities of the formulations were determined using Brookefield synchroelectric viscometer (RTV model) at 100rpm with spindle no 7. [12]

Spreadability

1 gm of the cream was sandwiched between two glass slides having fixed dimension and provided with the hook for the upper slide. 1kg weight was placed on the top of the slides for 5min to expel out air and to provide a uniform film of the cream between the slides. Excess of cream was scrapped off from the edges. The top plate was then subjected to pull by 180gms weight with the help of string passed over. The time (in seconds) required by the top slides to cover a distance of 7.5cm to be noted. A short interval indicates better spreadability.

$$\text{Spreadability, } S = M * L/T$$

"M" is the weight applied to pulley, "L" is the length moved by the upper glass slide

(7.5cm), "T" is the time (in seconds) taken to cover 7.5cm by the upper slide [12].

Particle Size Determination

1 gm of the prepared cream is dissolved in 10 ml of glycerin. It is mounted on a glass slide and observe under microscope. The diameter of the particles were determined by using an eye piece micrometer. The eye piece micrometer was caliberated with the help of stage micrometer. Then diameter of 20 particles were measured and average diameter was calculated [12].

In vitro drug diffusion study of cream

The *in vitro* drug release studies were carried out in a Franz diffusion cell with capacity of 25 ml using cellophane membrane as diffusion media. A magnetic bead with 12.5mm was placed in the receptor compartment filled with 25ml of phosphate buffer pH 7.4. A piece of cellophane membrane was placed in between receptor and donor compartment. The whole assembly was kept on a magnetic stirrer at a speed of 100 rpm and temperature condition of the cell was controlled at 32±50c by circulating water jacket. Then the formulated cream (5gm) was placed on the diffusion media. The samples were withdrawn at definite time interval and replaced with equal amount of fresh phosphate buffer solution pH 7.4. Then the samples filtered through a Whatman filter paper, diluted appropriately with buffer solution and analyse spectrophotometrically at 254 nm. Then the amount of drug diffused at each time interval was calculated. [12]

Kinetics of in vitro drug release

To study the release kinetics of in-vitro drug release, data obtained from in-vitro release study were plotted in various kinetic models: Zero order as % drug released Vs time, First order as log % drug retained Vs time, Higuchi as % drug released Vs √time, Korsmeyer-Peppas as log %drug released Vs log time and Hixson-Crowell as (% drug retained)$^{1/3}$ Vs time. By comparing the r-values obtained, the best-fit model was selected [13].

Stability studies

Stability study for the Camellia sinensis leaf extract incorporated vanishing cream was carried. The samples were stored in polythene cover in screw capped glass bottles at 40⁰C and 75% RH, in Lab top stability testing equipment for a period of 30 days. Samples were withdrawn periodically and were visually examined for any physical change. The samples were analyzed for pH, homogeneity, appearance [13, 15].

RESULTS AND DISCUSSION

Chemical tests for Epicatechins

Flame Test

Flame test was done to determine the presence of epicatechin. Flame test produced pinkish red colour. And the presence of red colour conform the presence of epicatechin.

Vannilin - Hydrochloric acid Test

Vannilin and hydrochloric acid test was done to determine the presence of epicatechin. Then the colour of sample were observed. The sample shows pink or red colour, which indicates presence of epicatechin.

Determination of Sun protection factor

Sun protection factor (SPF) were calculated using the formula and sun protection factor of Camellia sinensis leaves extract was found to be 30.2. SPF 30 sunscreen can block 97% of ultra violet radiations.

Formulation of Cream

Camellia sinensis leaf extract incorporated vanishing cream was formulated. Formulation code of Camellia sinensis leaf extract incorporated vanishing cream was given in table no. 1

Table1: Formulation code of Camellia sinensis leaf extract incorporated vanishing cream

Sl. No	Ingredients	Percentage quantity in cream
1	Camellia sinensis leaf extract	1 gm.
2	Stearic acid	1.2 gm
3	Cetyl alcohol	0.05 gm.
4	Borax	0.1 gm.
5	Glycerine	0.2 ml
6	Triethanolamine	0.2 ml
7	Benzoic acid	0.01gm
8	Propylene glycol	0.2 ml
9	Distilled water	7.04 ml

Evaluation of the Cream

pH

The pH of the cream at room temperature (25°C) was 6.4.

Dilution

In the dilution test the water was easily miscible and resulted in a homogenous solution. This confirmed that the creams were O/W type.

Homogeneity

The formulation showed good homogeneity with absence of lumps and greasiness homogeneity test reveals that the creams were homogenous with good feel.

Appearance

The prepared cream formulated was viscous creamy preparation with a smooth and homogeneous appearance. It was easily spreadable with acceptable bioadhesion.

### Viscosity

The viscosity was found to be 7200cps.

Spreadability

The spreadability was found to be 31.28 gm/sec.

In-vitro drug diffusion study of cream

The *in-vitro* drug diffusion of the cream through the cellophane membrane was determined by using Franz diffusion cell. The percentage of drug diffused as a function of time was determined.

Graph 1: Cumulative Drug Diffusion of Camellia sinensis leaf extract incorporated vanishing cream
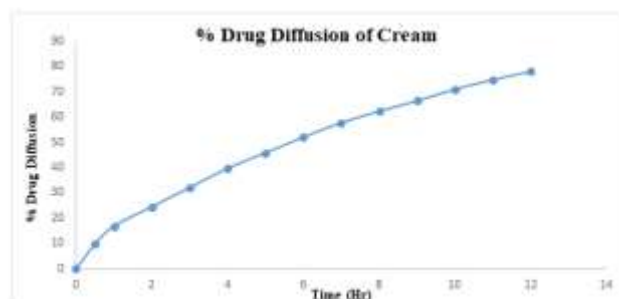
Kinetics of in vitro drug release

The data were processed for regression analysis using MS-EXCEL statistical functions. Evaluation of release kinetics and application of best fit by correlation coefficient shows that the drug release follows Higuchi's equation. And their high Regression coefficient indicating the mechanism of release was diffusion controlled. From Korsemeyer-Peppas equation it was found that system follows non fickian diffusion.

Stability studies

pH, Appearance and *in vitro* diffusion study of Formulation before and after stability study are given in Table 2. It can be seen that there is no considerable change in these parameters.

Table 2: Comparison parameters before and after stability studies

Parameters	Before stability study	After stability study
Invitro diffusion study at 12th Hour	78.12 %	77.96 %

	smooth and homogeneous appearance	smooth and homogeneous appearance
Appearance		
pH	6.4	6.4

CONCLUSION

The leaves of Camellia sinensis leaves were collected from Kattapana, Idukki district, Kerala. Camellia sinensis leaves extract were prepared using soxhlet extraction apparatus. Alcoholic extract was used to analyze the prescence of catechin which was confirmed by the chemical test such as flame test, vanillin hydrochloride test. Camelia sinensis extract incorporated vanishing cream was prepared and determined for its sun protection activity using UV spectrophotometric method. Sun protection factor was found to be 30.2, it indicates that the prepared extract was having a good Photo-chemo protective activity. The prepared vanishing cream was subjected to evaluation studies, from which it was concluded that it passes the test for pH (6.4), miscibility, homogenicity, particle size (60μm). From Korsemeyer-Peppas equation it was found that system follows non fickian diffusion. The formulation was found to be stable even after accelerated stability study as per ICH guidelines.

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