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Formulation, Characterization and *In-vitro* Evaluation of Herbal Sunscreen Gel Containing Anthocyanin

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ABSTRACT

Herbal sunscreen gel containing anthocyanins extract of *Beta vulgaries L* and *Garcinia indica* were successfully formulated and characterized. Anthocyanins were extracted from the *Beta vulgaries* L by maceration technique using 0.1% HCl (v/v) in water and *Garcinia indica* in 0.1% HCl (v/v) in water. Anthocyanins gel were formulated using carbopol-934, methyl paraben, propyl paraben, propylene glycol, tri-ethanolamine and distilled water. All the Anthocyanins gel formulations were evaluated for visual appearance and homogeneity, pH, viscosity, spreadability, extrudability, centrifuge and in-vitro evaluation of sun protection factor was determined & formulations were evaluated for pH, which was near to the skin pH. All the formulations were found to be homogeneous and there were no aggregate formation, there were no observable sediment in centrifuge tests. All the gel formulations showed good viscosity and they were capable to remain in the site of application for prolonged time. The values of spreadability indicated that the gel was easily spreadable by small amount of shear. All the gel formulations showed good extrudability. Among these formulations F2 showed excellent extrudability. In-vitro sun protection factor of F3formulation showed enhanced protection against UV radiation as compared toF1 &F2

Keywords: Anthocyanin, *Beta vulgaries* L, *Garcinia indica*, SPF.

I. INTRODUCTION

Anthocyanin's are the most important water-soluble pigments in plants, which stand second, after chlorophyll, amongst the plant pigments that are visible to the human eye. They are responsible for imparting brilliant red, blue and purple hues to fruits, vegetables, flowers and grains. Apart from fruits and vegetables, black soybeans are an excellent source of anthocyanin.[1]

Sun emits a constant flow of energy in the form of electromagnetic radiation spanning an enormous range of wavelength from 10-5 meters down to 10-6 nm.[2]Sunlight is composed of about 40% visible light, 50% infrared light and 10% ultraviolet light.[3] UV Radiation is defined as electromagnetic radiation lies between X-rays and visible light whose wavelength is from 200 to 400nm.[4]Being a component of the electromagnetic spectrum, UV photons fall between the wavelengths of visible light and gamma radiation. UV energy can be sub-divided into UV-A, B and C components based on electro physical properties.[5]

On prolonged exposure to sunlight, skin gets tanned leading to sun burns which is due to depletion of enzymatic and non-enzymatic antioxidants into stratum corneum, epidermis and dermis is caused due to the UV rays mediated photo oxidative damage reaches the dermal capillaries via epidermis and dermis.[6]

The sunscreen protection factor (SPF) is defined as the UV energy required producing a minimal erythema dose (MED) on protecting skin, divided by UV energy required to produce MED on unprotected skin. The MED is defined as the lowest time interval or dosage of UV light irradiation sufficient to produce minimal, perceptible erythema on unprotected skin.[7]

Beta vulgaris L. (Beetroot) belongs to the Chenopodiaceae family. It has bright crimson colour. Beetroot is commonly known as beet, chard, spinach beet, sea beet, garden beet, white beet and Chukander (in Hindi). It has very medicinal properties which give some positive effect on the human body. Beetroot are rich in other valuable compound such as anthocyanins,

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glycine, betaine, Saponins, betacyanin, carotenoids, folates, betanins, polyphenols and flavonoids. Beetroot contributes to consumer's health and wellbeing because it has antioxidant property due to the presence of nitrogen pigment betalain. Beetroot are also known for its antimicrobial and antiviral effects.[8]

Garcinia indica (Kokum) is an under exploited fruit species, which is a rich source of anthocyanins. Kokum contains the following phytonutrients; Anthocyanins such as Cyanidin-3-sambubioside, Cyanidin-3-glucoside.[9]

Anthocyanins are well known for their antioxidant, anti-inflammatory and anti-carcinogenic activity. *Garcinia indica* contains Anthocyanins that prevent ascorbic acid oxidation, scavenge free radicals, show inhibitory effects against oxidative enzymes and reduce the risk of cancer and heart diseases.[10]

The people of early civilization used variety of products made from plants as sun protecting agents. Olive oil was used by Greek people. Rice, Jasmine, Lupine plants were used by the Egyptians. It is obvious that the modern trend of the world to search again for herbal products in all the fields. The consumers are more found of herbal sunscreens which are known to be safe.[11]Hence the objective of the present work was to formulate sunscreen gel from the *Beta vulgaris* L and *Garcinia indica* anthocyanin concentrate and to evaluate the SPF of the formulated gels.

II. MATERIALS AND METHODES

Material

Propylene glycol, Propyl paraben obtained from Genuine chemical Co. Mumbai, Methyl paraben obtained from NR CHEM Mumbai. Himedia Mumbai provided Carbopol934, Lavender from Sisco Research Laboratories Pvt. Ltd. Maharastra, Tri-ethanolamine obtained from SDFCL. Mumbai.

Methods

Anthocyanin Extraction from *Beta vulgaris* L:

Anthocyanins was extracted from fresh taproot portion of beetroot by placing 50gm of chopped beetroot in water which was containing 0.1% v/v of HCl in a beaker. The lid of the beaker was closed with aluminum foil and placed in a dark room for 24 hours at room temperature. After 24 hours filtration was carried out using muslin cloth. The filtrate was then dried by evaporation in a hot air oven at 40°C. The anthocyanin extract was then collected and stored at 4°C.[12]

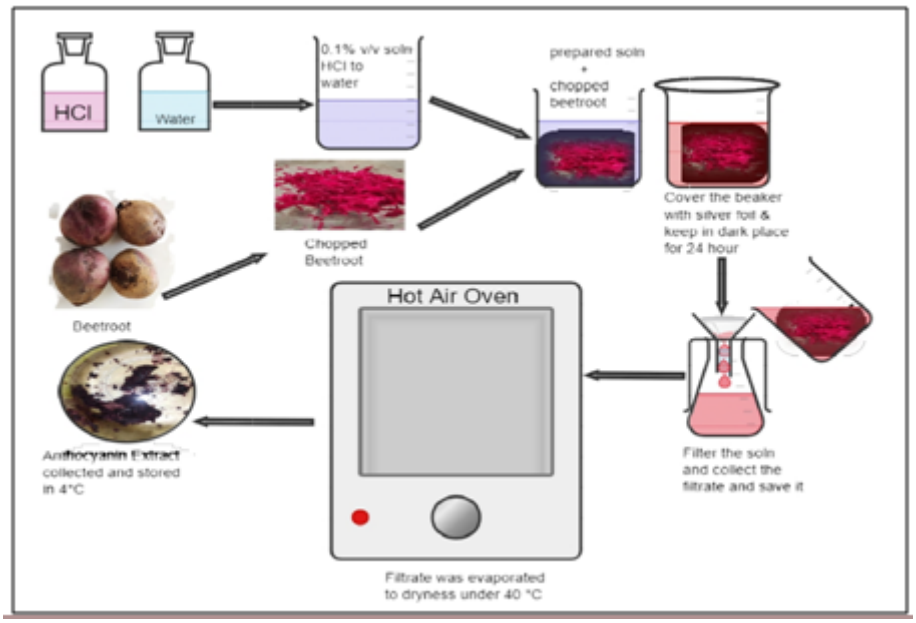

Figure 1: Anthocyanin extract from *Beta valgaris* L.

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Anthocyanin Extraction from *Garcinia indica*.

By soaking 50gm of kokum rind powder in acidifier water that contains 0.1% hydrochloric acid in a beaker. The beaker lid was covered with aluminium foil, and it was left at room temperature for 24 hours in a dark place. Filtration was done using muslin cloth after 24 hours. The filtrate was then dried by evaporation in a hot air oven at 40°C. The anthocyanin extract was then collected and stored at 4°C.[9]

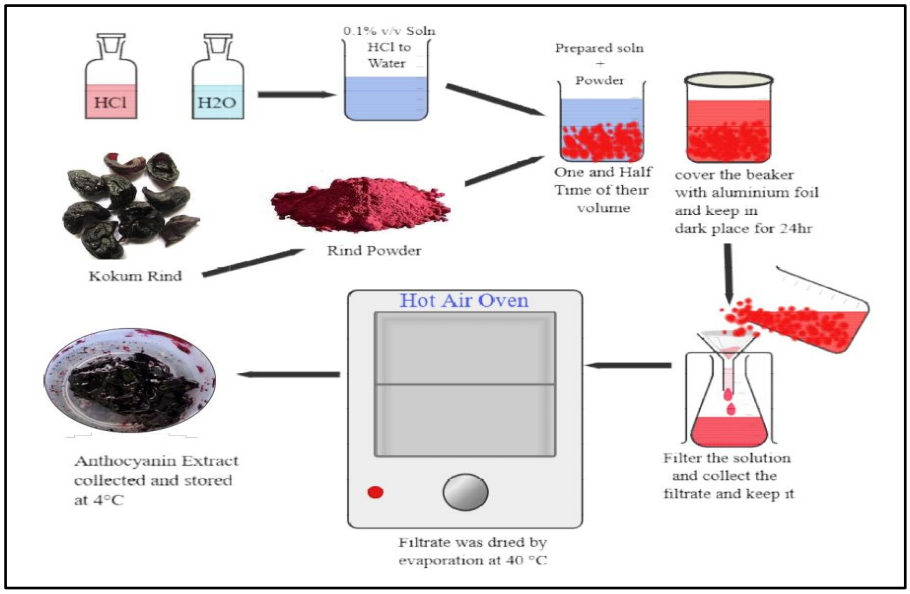

Figure 2: Anthocyanin Extraction from *Garcinia indica*.

Confirmatory Test for Extract[13,14]

1. Presence of Anthocyanin in extract was confirmed by UV-Visible Spectrophotometer using aqueous as blank. A spectrum was taken in UV-Visible range i.e. 200 nm-800 nm.
2. One ml of the *Beta vulgaris* L/*Garcinia indica* extract was mixed with 2ml 2M HCI and heated for 5min at 100°C. When the extract remains the stable purple (magenta) color confirms the presence of Anthocyanin.
3. One ml of the *Beta vulgaris* L/*Garcinia indica* extract mixed with 2ml of 2M NaOH and formation of yellow color indicates the presence of anthocyanin.

Sunscreen preparation method [15]

For gel formulations, the *Beta vulgaris* L and *Garcinia indica* extract (Anthocyanin) was used as the active ingredient, and carbopol-934, was used as gelling agent (polymers). Accurately weighed carbopol-934 was taken in a beaker-a and dispersed in 25gm of distilled water. Kept the beaker aside to swell the carbopol-934 for 24 hours and then stirring was done using lab stirrer at 1200 rpm for 30 min. In another beaker-b 5gm of propylene glycol, anthocyanin concentrate as mentioned in table.1 was added followed by parabens. Contents of beaker-b was transferred to beaker a with continues stirring with lab stirrer and volume was made up to 50gm by adding remaining distilled water and Triethanolamine was added drop wise to the formulations for adjustment of required skin pH (6.8-7) and to obtain the gel of required consistency.

Table1: Composition of Various Sunscreen gel containing Anthocyanin

SI.NO	Ingredients (50 gm)	F1	F2	F3
1	Anthocyanin of *Beta vulgaris* L	3	-	3
2	Anthocyanin of *Garcinia indica*	-	3	3
3	Propylene glycol	2.5	2.5	2.5

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4	Methyl paraben	0.1	0.1	0.1
5	Propyl paraben	0.01	0.01	0.01
6	Carbopol-934	1	1	1
7	Triethanolamine	qs	qs	qs
8	Fragrance	qs	qs	qs
9	Water	qs	qs	qs

***In-vitro* Determination Sun Protection Factor (SPF) by UV-spectrophotometer.**[16]

Gel containing 0.6% of anthocyanin was weighed, transferred to10mlvolumetric flask and diluted to volume 10 ml with methanol. Further, it was kept for sonication for 5 min. Afterwards 1 ml aliquot was transferred to 10 ml volumetric flask and the volume was adjusted with methanol. The absorption data obtained in the range of 290 nm-320 nm every 5 nm interval and 3 determinations were made at each point. The absorbance values of anthocyanin are obtained. SPF was calculated using the formula.

$$\mathbf{SPF(Spectrometry)} = CF \times \sum_{290}^{320} \mathbf{EE}(\lambda) \times \mathbf{I}(\lambda) \times \mathbf{abs}(\lambda)$$

CF= 10 (Correction Factor).
EE (λ)= Erythemogenic effect of radiation at wavelength λ.
I (λ) = Intensity of solar light at wavelength λ.
abs (λ)= Absorbance of wavelength λ.

Evaluation of the formulation

Visual appearance and Homogeneity[17]

The prepared gels were visually analyzed for clarity, color and transparency. The prepared gels were also evaluated for the presence of any aggregation. Gels were prepared and transferred into transparent container then observed under the microscope for the presence of any particle or grittiness.

Centrifuge test[18]

Prepared gel formulations were separately centrifuged in a test tube of 10 cm long and 1 cm width for 5, 15, 30 and 60 minutes and then studied for sedimentation and gel stability.

pH Determination[19]

By using the digital pH meter, pH of the gel was measured. The pH meter was calibrated with standard buffers solution before measurement and every time the measuring was repeated 3 times and the mean was calculated.

Extrudability test[20,21]

About 5 gm of the gel formulation was filled in a clean, lacquered aluminum collapsible tube on crimped end of the tube then clamp was applied to avoid any roll back. And the cap was removed and gel was extruded. The extrudability was then determined by measuring the amount of gel extruded through the tip. The extruded gel was collected and weighed and the percentage of gel extruded was calculated and grades were allotted.

$$\%\ \text{Extrudability} = \frac{\text{Amount of gel extruded from the tube}}{\text{Total amount of gel filled in the tube}} \times 100$$

(>90% Extrudability: Excellent)
(>80% Extrudability: Good)
(>70% Extrudability: Fair)

Viscosity[22]

Viscosity of formulated gel was determined by using Brookfield viscometer. The gels were rotated at 50 rpm using spindle no.64. At each speed, the reading was recorded. The viscosity determination of samples was repeated three times.

Spreadability[23]

Spreadability was determined by the apparatus which consists of a glass plate block, which was provided by a pulley at one end. By this method spreadability was measured on the basis of

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slip and drag characteristics of gels. An excess of gel (about 2 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 20 gm. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. Spreadability was calculated using the following formula:

$$S = M \times L / T$$

Where, S = Spreadability,
M = Weight tied to the upper slide,
L = Length moved by the glass slide (7.5 cm) and
T = Time (in sec.) taken to separate the slide completely each other.

III. RESULTS AND DISCUSSION

The present investigation attempted to formulate sunscreen using Anthocyanin extract of *Beta vulgaris* L and *Garcinia indica* by maceration method. Further gel was formulated by dispersion method and subjected for various evaluation parameters and optimize formulation was subjected for in-vivo determination of Sun Protection Factor by UV-spectrophotometer.

Conformation test for Anthocyanin

1) Anthocyanin extract was analyzed using UV-Visible spectrophotometer and the absorbance between 200nm–800nm indicates the presence of Anthocyanin.

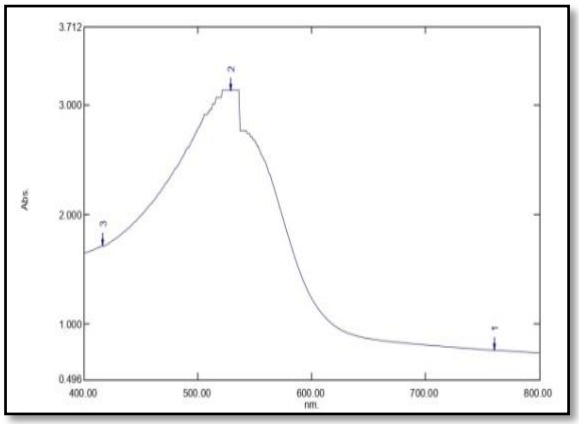

Figure 3a

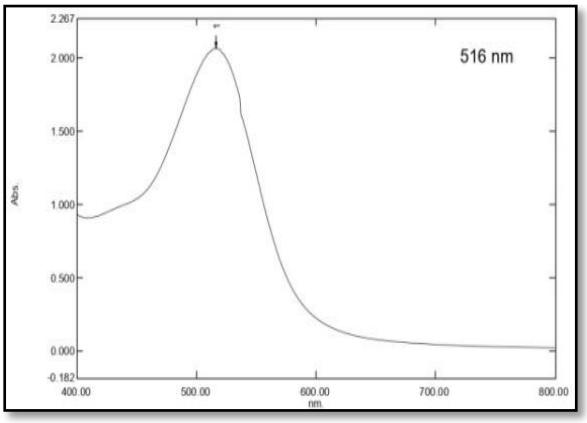

Figure 3b

Figure 3: UV-Spectrum of Anthocyanin of a) *Beta vulgaris* L b) *Garcinia indica*

2). Test for presence of anthocyanin using NaOH and HCl

Figure 4: Test for presence of Anthocyanin using NaOH.

Figure 5: Test for presence of Anthocyanin using HCl.

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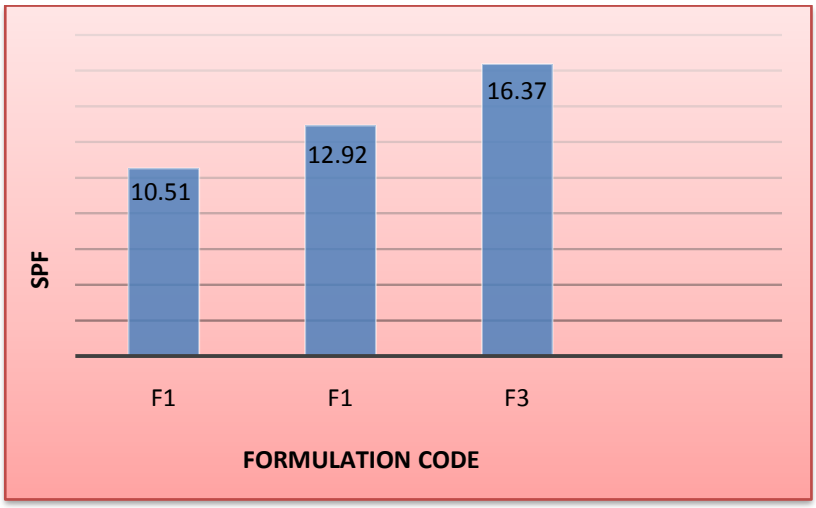
Sun Protection Factor (SPF)[24]

Sun protection factor is a metric for determining how effective a sunscreen product is. In this work, the sunscreen activity of a gel formulation containing *Beta vulgaris* L and *Garcinia indica* anthocyanin extract was assessed using an *in-vitro* SP technique. The SPF number and the presence of UV blocked shown in table 1 and Graph 1 displays the SPF values of broad-spectrum F1 was10.51, F2 was 12.92, F3 was 16.37, by comparing the SPF values its evident that F3 was having the greater UV screening property than other two formulation.

Sl.NO	SPF	Percent of UV blocked
1	2	50
2	4	75
3	8	87.5
4	16	93.75
5	32	96.88
6	64	98.44

Table 2: The SPF Number and the percent of UV blocked



Graph 1: *In-vitro* determination of sun protection factor by UV spectrophotometer of F1: *Beta vulgaris* L, F2: *Garcinia indica* L, F3: *Beta vulgaris* L+ *Garcinia indica*

Visual appearance and Homogeneity:

Formulated gel was investigated for presence of particle, improper mixing aggregation and other residue. So prepared gel was filled in transparent container and visually inspected were all the formulations were found to be homogeneous and there was no aggregate formation of particles.

pH determination:

The pH of Anthocyanin gel was evaluated and it is constant throughout a day. The result of gel pH are determined Table 3.

Extrudability test:

The various anthocyanin gel formulation was subjected to extrudability. The values of extrudability indicate that the gels were showed good extrudability (Table 3). Among these formulations showed excellent extrudability

Spreadability:

To determine the spreading ability of gel the test was performed. The gel having low viscosity showed better spreadability. The various Anthocyanin gel formulations were subjected to spreadability studies. The values of spreadability show that the gel was easily spreadable by small amount of shear.

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Formulation code	pH (Mean ±SD)	Extrudability in percentage (Mean ± SD)	Spreadability in gm.cm/sec (Mean ± SD)	Viscosity in cps
F1	6.4±0.05	97.3±0.1	29.85±0.91	18204 ± 4.509
F2	6.8±0.32	93.96±0.08	20.08 ±0.48	18578± 9.165
F3	6.6±0.05	94.70±0.09	21.26±0.45	18469± 11.532

Table 3: pH, Extrudability, viscosity and Spreadability of Anthocyanin gel formulation.

Centrifuge test:

Centrifugation test for gels is performed to check the phase separation of oil and water in formulation. The prepared formulation was stable and there was not any separation of phases on centrifugation at different speeds which indicates the gels were stable.

IV. CONCLUSION

Herbal sunscreen gel containing anthocyanin extract from different herbs such as *Beta vulgaris* L and *Garcinia indica*.SPF of F3 formulation showed enhanced protection against UV radiation as compared to F1 and F2. The pH of the gel formulations was in the range of 6.6 to 6.8 which lies in the normal pH range of the skin. All the gel formulations were found to be homogeneous and there were now aggregate formation, there were no observable sediment in centrifuge tests. All the gel formulations showed good viscosity and they were capable to remain in the site of application for prolonged time. The values of spreadability indicate that the gel was easily spreadable by small amount of shear. All the gel formulations showed good extrudability. Among these formulations F1 showed excellent extrudability.

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