WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 7, 1202-1213. Research Article ISSN 2277– 7105

SUNSCREEN AND ANTI-OXIDANT ACTIVITY OF HERBAL GEL OF CUCUMBER EXTRACT

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Article Received on
15 May 2016,

Revised on 05 June 2016,
Accepted on 26 June 2016

DOI: 10.20959/wjpr20167-6542

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ABSTRACT

As in daily life humans are exposed to harmful UV radiations which cause tanning, sunburns, edema and various other allergic reactions it becomes a need to use such agents which can protect the human body from the above mentioned side effects. Since it is more beneficial to use natural agents in comparison to synthetic ones we have taken cucumber as an ingredient to prepare a sunscreen gel and evaluated it for its effectiveness as a sunscreen preparation. The formulated gel was evaluated for its SPF (Sun Protection Factor) i.e. protection against UVB rays and UVA: UVB ratio i.e. protection against UVA rays and it was found that the cucumber gel has potential to provide protection against both UVA rays and UVB rays. Additionally it was also found to have antioxidant properties which may contribute to sunscreen activity. The gel was found to have good spreadability, homogeneity and pH within the range of skin pH and thus suitable to use on skin.

KEYWORDS: Sun Protection Factor (SPF), Boot star rating, DPPH, UVA/UVB ratio

INTRODUCTION

Cucumber, scientifically known as *Cucumis sativus* (family- cucurbitaceae) are obtained throughout India. The flesh of cucumber is primarily composed of water but also contains ascorbic acid (vitamin C) and caffeic acid, both of which help soothe skin irritations and reduce swelling. The silica in cucumber is an essential component of healthy connective tissue, which includes muscles, tendons, ligaments, cartilage and bone.[1] Cucumber juice is a source of silica which can improve the skin complexion and health and also give natural

glow to skin due to its hydrating property. Cucumber had been used traditionally for swelling under the eyes, dark circles and sunburn etc due to two compounds, ascorbic acid and caffeic acid which prevent water retention. The present work deals with the preparation and evaluation of cucumber gel for antioxidant and sunscreen activity.

MATERIALS AND METHODS

Plant Material

Fresh cucumbers were obtained from the local market of Meerut. DPPH was obtained from Sigma Aldrich chemical ltd Germany. Carbopol 940 LR was purchased from SD Fine Chemical Ltd. Mumbai. Benzophenone, ferric chloride and trichloroacetic acid were purchased from Central Drug House, New Delhi. Potassium ferricyanide was purchased from RFCL Ltd New Delhi. All other chemicals used were of analytical grade.

PREPARATION OF EXTRACT

For preparation of cucumber extract, cucumbers were properly peeled, washed and chopped finely. The chopped material was then crushed in a grinder and the slurry obtained was passed through the muslin cloth in order to obtain the cucumber juice. The juice was then lyophilized at a temperature of -70^0C so that a freeze dried extract in the solid form can be obtained. The extracts were stored by keeping in refrigerator till further use.

PREPARATION OF GEL

For preparing the gel carbopol- 940 LR was soaked in water for 5-6 hours to obtain a homogenous mass. The extract was then incorporated in carbopol and triturated well. All the other ingredients were added and mixed well.[2]

TABLE I: Formulation of Gel

S.No.	Ingredient	Quantity
1	Carbopol	1
2	Herbal extract	2.5gm
3	Triethanolamine	To pH 6-7
4	Glycerine	5%
5	Distilled water	Upto 25 ml

EVALUATION OF SUNSCREEN ACTIVITY

1. Benzophenone method[3]

For the measurement of SPF (Sun Protection Factor) the effectiveness of sunscreen gels were analysed using the reaction to produce benzopinacol. Underlying principle is that reaction of

benzophenone with isopropanol depends on the absorption of UV light. The conversion products include benzopinacol and acetone. The benzopinacol is the insoluble product that is removed when the solution is filtered. The more UV light that reaches the solution of benzophenone and isopropyl alcohol, the greater the amount of benzopinacol precipitate formed. Therefore, the difference in yield between a vial coated with sunscreen gel and one without will be proportional to the reduction in absorbed UV radiation caused by the sunscreen gel.

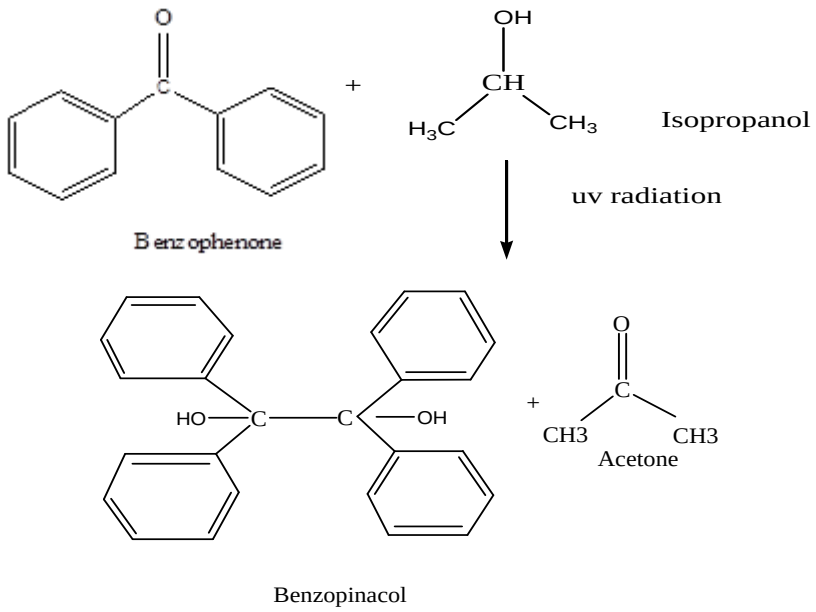

Fig 1: Mechanism of Benzophenone Method for determination of SPF

Benzophenone solution

20% w/v solution of benzophenone was prepared by dissolving 4gms of benzophenone in 20 ml of isopropanol.

Study Design

For the analysis of sunscreen activity of the laboratory prepared gel the product was compared to the commercial preparations of varying SPF 15, 20, 30 and a control in which no preparation is applied. Five Petri plates were taken and 10ml of solution was added to each of them. After adding the solvent the petri plates were covered properly and uniform layer of different preparations were applied. One petri plate which does not contain any preparation was considered as control. The petri plates were kept in sunlight for a period of 6 hours and thereafter kept in dark for overnight and readings were recorded as the amount of crystals of benzopinacol.

2. UV spectrophotometric method[4]

The *in vitro* methods are in general of two types. a) Methods which involve the measurement of absorption or the transmission of UV radiation through sunscreen product films in quartz plates or biomembranes. b) Methods in which the absorption characteristics of the sunscreens agents are determined based on spectrophotometric analysis of dilute solutions. Mansur *et al.* (1986) developed a very simple mathematical equation utilizing UV spectrophotometry. The following equation was given by Mansur

$$SPF_{spectrophotometric} = CF \times {}^{320}\sum_{290} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where EE – erythemal effect spectrum, I – solar intensity spectrum, Abs - absorbance of sunscreen product; CF – correction factor (= 10). The values of EE x I are constants. They were determined by Sayre *et al.* (1979) and are showed in Table II. The aim of this research was to determine the SPF values of sunscreens gel containing natural plant extracts by UV spectrophotometry applying Mansur mathematical equation (Equation 1).

TABLE II: Normalized product function used in the calculation of SPF (Sayre *et al.*, 1979)

Wavelength(λ)nm	EE X I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Method

1.0 g of all samples was weighed, transferred to a 100 ml volumetric flask, diluted to volume with water, followed by ultrasonication for 5 min and then filtered through cotton, rejecting the ten first ml. A 5.0 ml aliquot was transferred to 50 ml volumetric flask and diluted to volume with water.

The absorption spectra of samples in solution were obtained in the range of 290 to 450 nm using 1 cm quartz cell and water as a blank. The absorption data were obtained in the range of 290 to 320, every 5 nm and 3 determinations were made at each point, followed by the application of Mansur equation.

3. BOOT STAR rating system[5]

The UVA: UVB ratio used in this UVA labelling system is calculated as an indicator of the UVA absorbance properties of a sunscreen product relative to UVB, rather than a ratio of protection factors. It gives an indication of the physical properties of the sunscreen. UVA: UVB ratios determined using absorbance measurements rather than MPF values will be less affected by either "in use" application rate or variations in application rate during the *in-vitro* UVA evaluation technique. This is because absorbance values are directly proportional to film thickness and concentration of active ingredient whereas protection factors are not. (An increase in product application rate does not necessarily result in the same factor increase in SPF value.

The index of UVA protection is calculated from a plot of wavelength versus log MPF (ie absorbance). It is defined as the area (per unit wavelength) under the UVA portion of the plot, divided by the area (per unit wavelength) under the UVB portion of the same curve.

$$\text{UVA: UVB ratio} = \frac{\text{UVA absorbance area per unit wavelength}}{\text{UVB absorbance area per unit wavelength}}$$

$$\text{UVA area per unit } \lambda = \frac{\int_{320}^{400} a_\lambda x\, d_\lambda}{\int_{320}^{400} d_\lambda}$$

$$\text{UVA area per unit } \lambda = \frac{\int_{290}^{320} a_\lambda x\, d_\lambda}{\int_{290}^{320} d_\lambda}$$

The integrals in the above formulae can be approximated by using Simpson's Rule for irregular areas which states:-

Area = 1/3h x $[(Y_0 + Y_{2m}) + 4(Y_1 + Y_3 + Y_5 \ldots + Y_{2m-1}) + 2(Y_2 + Y_4 + Y_6 \ldots + Y_{2m-2})]$.

Where Y_0, Y_1, Y_2, Y_3…..Y_{2m} are the lengths of a number, ‘2m’ of parallel lines drawn vertically to divide the area under the curve of a graph into ‘2m – 1’ segments of equal width, ‘h’. In practice, the values of Y_0, Y_1, Y_2, Y_3…..Y_{2m} are the absorbance values (a_λ) measured for the sunscreen product and ‘h’ is the wavelength interval at which the absorbance values or MPFs are determined - eg 5 nm.

UVA area per unit wavelength (aUVA/λ)

$aUVA/\lambda = 1/3 \times 5 \times [(a_{320} + a_{400}) + 4 (a_{325} + a_{330} + a_{335} \ldots\ldots + a_{395}) + 2 (a_{330} + a_{340} + a_{350} \ldots\ldots + a_{390})] / 80.$

UVB area per unit wavelength (aUVB/λ)

aUVB/λ= 1/3 x 5 x [(a290 + a320) + 4 (a295 + a305 + a315) + 2 (a300 + a 310)] / 30.

The UVA: UVB ratio is then calculated as:-

$$\text{UVA: UVB ratio} = \frac{\text{aUVA}/\lambda}{\text{aUVB}/\lambda}$$

The calculation of the UVA: UVB absorbance ratio will typically yield values from zero (equal to no UVA absorbance) up to 1.0 (UVA absorbance equal to UVB). Once a UVA: UVB ratio has been determined for a sunscreen product according to the above procedures, the sunscreen can be categorised according to the Boots UVA Star Rating System below.

TABLE III: BOOT STAR rating system

Mean UVA: UVB Ratio		Star Rating Category	Category Descriptor
2nm or greater than 2nm increment measurement	Less than 2nm increment measurement		
0.0 to 0.19	0.0 to 0.2	-	No claim
0.2 to 0.39	0.21 to 0.4	*	Minimum
0.4 to 0.59	0.41 to 0.6	**	Moderate
0.6 to 0.79	0.61 to 0.8	***	Good
0.8 to 0.89	0.81 to 0.9	****	Superior
0.9and above	0.91 and above	*****	Ultra

MEASUREMENT OF ANTIOXIDANT ACTIVITY

1. Determination of reducing power[6,7]

The antioxidant activity of cucumber extract was determined using UV spectrophotometric method and ascorbic acid as a standard. Extract and ascorbic acid were dissolved separately in 1ml of double distilled water in a volumetric flask. To each of the volumetric flask 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of potassium ferricyanide was added.

The mixture was incubated in oven for 20 min at 50^0c. To the incubated solutions 2.5ml of 10% trichloroacetic acid was added and centrifuged for 10 min at 3000 rpm. From the above solution 2.5ml of solution was taken out without disturbing and to it 2.5ml of distilled water and 0.5ml of 1% freshly prepared $Fecl_3$ was added. Absorbance was recorded at 700nm.

2. Determination of DPPH radical scavenging activity[8]

The free radical scavenging activity of the *Cucumis sativus* extract and ascorbic acid was measured. 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was

added to 3.0 ml of aqueous solution of extract at different concentrations (1-9 mg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\%\text{DPPH scavenging activity} = \frac{(\text{Acont} - \text{Atest})\ \text{X}\ 100}{\text{Acont}}$$

Where Acont = absorbance of the control reaction and Atest = absorbance in the presence of the sample of the extract. The antioxidant activity of the extract was expressed as IC50. The IC50 value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%.

pH[9]

The pH of the prepared gel formulation was determined by using digital pH meter.

Spreadability[9]

Spreadability was observed by spreading 1 g of formulation on a clean even glass surface.

Homogeneity[10]

The prepared gel was tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

Swelling Index Study of Topical Gel[10]

Swelling of the polymer depends on the concentration of the polymer, ionic strength and the presence of water. To determine the swelling index of prepared topical gel, 1 gm of gel was taken on porous aluminum foil and then placed separately in a 50 ml beaker containing 10 ml water. Then samples were removed from beakers at different time intervals and put it on dry place for some time after it reweighed. Swelling index was calculated as follows:

Swelling Index (SW) % = [(Wt – Wo) / Wo] × 100.

Wt = final weight of swollen gel at time t, Wo = initial weight of gel.

RESULTS AND DISCUSSION

Sunscreen Activity

1. Benzophenone method

This method compares the ability of different products to block UV radiations. Less the amount of UV reaching to the benzophenone-isopropanol solution less will be the amount of

crystal formed and more will be the sunscreen activity of product. The ability of the prepared cucumber gel was compared with that of various marketed sunscreen preparations having different SPF. The results obtained are shown in the Table IV. From the observations the SPF of cucumber gel was found to be 3.191465.

TABLE IV: Determination of SPF by benzophenone method

S.No.	Formulation	Yield (in gm)	SPF
1	Control	1.39	0
2	SPF 15	0.79	15
3	SPF 20	0.64	20
4	SPF 30	0.39	30
5	Cucumber gel	1.25	**3.191465**

2. UV spectrophotometric method

The value of sun protection factor of the cucumber gel was also calculated by the UV spectrophotometric method. The absorbance of the sample was determined in range from 290-400nm at 5nm wavelength interval. The SPF is mainly the measure of protection against UVB rays having range between 290-320 nm so we calculated the SPF value over this range. The SPF of the formulation by this method was found to be 2.95.

TABLE V: Determination of SPF by UV spectrophotometric method

S.No.	Wavelength(λ)nm	EE X I (normalized)	Absorbance	SPF
1	290	0.0150	0.015	0.05175
2	295	0.0817	0.0817	0.265525
3	300	0.2874	0.2874	0.885192
4	305	0.3278	0.3278	0.957176
5	310	0.1864	0.1864	0.520056
6	315	0.0839	0.0839	0.222335
7	320	0.0180	0.018	0.04572
	Total	1		**2.947754**

3. BOOT STAR RATING System

As the boot star rating system specifies the category of the formulation with reference to the protection against UVA rays in comparison to UVB rays, the value of UVA/UVB ratio determines the protection against UVA rays and it was observed that the formulation has the UVA/UVB ratio value of 0.666 [Table VI] and thus have a 3 star rating and good protection.

TABLE VI: BOOT STAR rating for cucumber gel

S.No.	UVA area per unit wave length	UVB area per unit wave length	UVA:UVB ratio	BOOT STAR rating	Category descriptor
1	0.196	0.294	0.666	***	Good

ANTIOXIDANT ACTIVITY

1. Determination of reducing power

The antioxidant activity of cucumber gel was measured in terms of reducing power using ascorbic acid as a standard. Antioxidant activity of ascorbic acid and cucumber gel was found to be 100% and 30.83% respectively [Table VII].

TABLE VII: Determination of reducing power

S.No.	Sample	Absorbance (700nm)	Antioxidant activity
1	Ascorbic acid	2.4	100%
2	Cucumber extract	0.74	30.83%

2. Determination of DPPH radical scavenging activity

The gel formulation showed concentration dependent anti-radical activity by inhibiting DPPH radical. Table VIII exhibits % DPPH scavenging activity and IC50 value (concentration in µg/ml of gel formulations that inhibits the formation of DPPH radical by 50%) of the gel formulation was found to be 6.44. Figure 1 shows the %DPPH scavenging activity.

TABLE VIII: Determination of DPPH radical scavenging activity.

S.No.	Concentration (mg/ml)	% DPPH scavenging activity	
		Cucumber gel	Ascorbic acid
1	1	15.17094	33.54701
2	2	19.65812	38.88889
3	3	29.27350	47.00855
4	4	37.17949	57.05128
5	5	41.66667	62.39316
6	6	48.07692	66.66667
7	7	54.91453	72.00855
8	8	58.33333	77.99145
9	9	64.10256	81.41026
Result	**IC_{50} (mg/ml)**	**6.441273**	**3.451255**

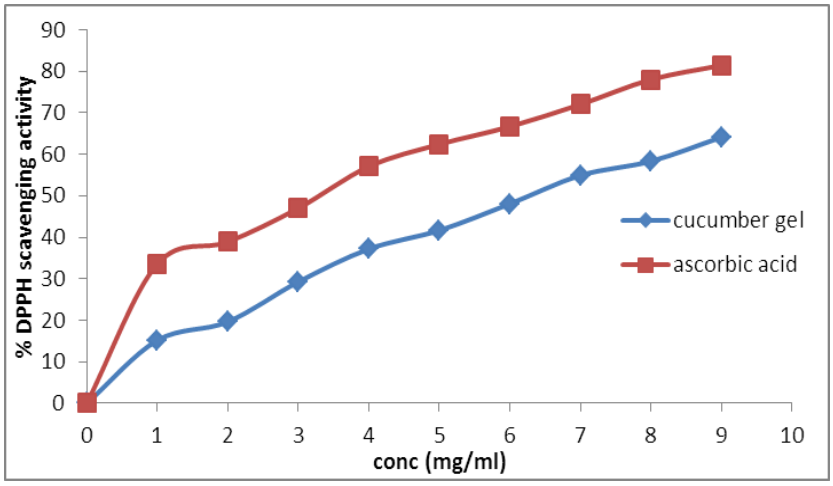

FIGURE 2: DPPH scavenging activity of cucumber gel

SWELLING INDEX

From the results obtained by the study of swelling index it can be concluded that the gel has good swelling capacity. The figure 3 represents the graph showing the swelling index.

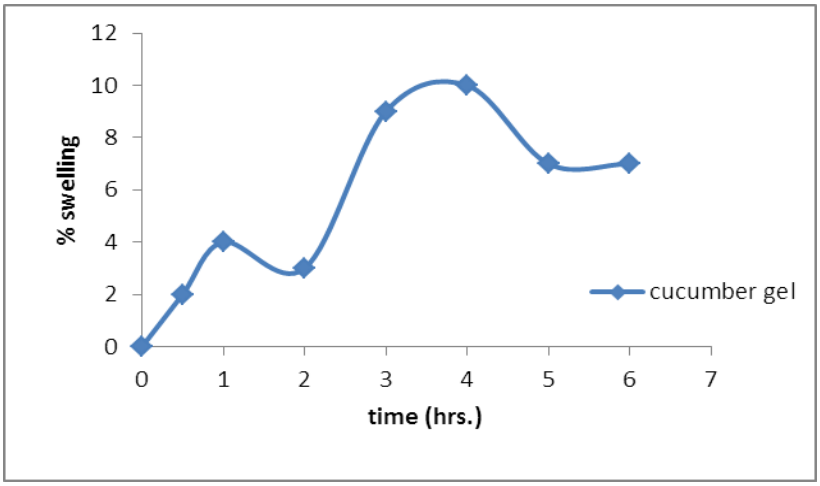

FIGURE 3: Swelling index of cucumber gel

OTHER PARAMETERS

pH- The pH of the formulation was found to be 6.4 which was suitable to use on skin as it comes within the range of skin pH.

Spreadability The gel formulation spreads smoothly on a clean even glass plate with minimum pressure without the presence of any solid or gritty particles**.**

Homogenicity The prepared gel formulation was homogenous as on visual inspection no aggregates were observed in the formulation.

CONCLUSION

From the above study it can be concluded that the formulated cucumber gel is effective in protecting skin against harmful UV radiations to some extent. The gel has the potential to prevent against both the UVB and UVA rays as it has been observed that it has significant SPF value and UVA/UVB ratio respectively. As the SPF obtained with a single natural ingredient is not sufficient enough to prevent the body from harmful UV rays that is why mostly all the commercial herbal sunscreen preparations include physical sun protecting agents (such as titanium dioxide or zinc oxide etc.) in order to provide extra protection. The formulation was also found to possess significant antioxidant activity which may contribute to its sunscreen activity. In addition to the above mentioned parameters the sunscreen gel was found to have good swelling capacity and homogenecity along with good spreadability and pH in the range of skin. Thus the cucumber extract can be used topically not only as moisturizing and skin rejuvenating agent but also has sunscreen effect.

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