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Research Article

IN-VITRO EVALUATION OF SUN PROTECTION FACTOR OF A CREAM FORMULATION PREPARED FROM EXTRACTS OF *MUSA ACCUMINATA (L.), PSIDIUM GUJAVA* (L.) AND *PYRUS COMMUNIS* (L.)

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ABSTRACT

Objectives: Use of phytoconstituents, especially obtained from fruits extract with high content of flavonoids has gained considerable importance in personal care products such as creams and lotions. Finding new results and data through experiments will be helpful for both researchers and industry on the subject. The purpose of this study was to evaluate *in-vitro* sunscreen activity of a cream formulation containing the fruit extract of *Musa accuminata*, *Psidium gujava* and *Pyrus* communis based on their flavonoid contents.

Methods: Extraction of fruits to include maximum quantity of flavonoids was carried out using solvent system comprising of methanol (35%), ethanol (35%), and distilled water (30%). The cream was formulated and tested for the physicochemical parameters such as color, odor, pH and spreadability. While total flavonoid content was determined by aluminum chloride colorimetric method. The *in-vitro* sun protection factor (SPF) of cream formulation and commercially available sunscreen was determined by ultraviolet spectrophotometric method.

Results: The total flavonoid content of cream formulation was found to be 45.81±8.49 and expressed in terms of standard quercetin equivalent µg/g. The SPF value for the cream formulation was recorded as 3.90, whereas commercially available sunscreen it was 12.26, indicating that cream formulation has photoprotective activity and may be used to develop a good cosmetic formulation and to explore its commercial viability.

Conclusion: Use of phytoconstituents, especially those obtained from fruits extract with high content of flavonoids has gained considerable importance in personal care products such as creams and lotions. Finding new results and data through experiments will be helpful for both researchers and industry on the subject. The proposed spectrophotometric method is simple and rapid for SPF determination. Due to the high cost and time consumption relating to *in vivo* SPF determination andsome ethical issues for the volunteers, the *in vitro* method is gaining more importance.

Keywords: Antioxidants, Formulation, *In-vitro* sun protection factor, Photoprotection, Fruit extract, Ultraviolet-visible spectroscopy.

INTRODUCTION

Skin is the outermost and the largest part of the body and it is most sensitive to photodamage because it directly exposed to solar radiation and other environmental factors. The harmful effects of solar radiation are usually caused by the ultraviolet (UV) region of the electromagnetic spectrum, which can be divided into three regions: UVA (320-400 nm), UVB (290-320 nm) and UVC (200-290 nm). UVC radiation is filtered out by the ozone layer before reaching earth. UVA and UVB radiation is not completely filtered out by the ozone layer and is responsible for the damage due to sunburn and premature aging of the skin [1]. It may cause several harmful effects to the eye, skin and immune system. Prolong exposure of UV radiations may initiate the production of reactive oxygen species, which causes oxidative injury and impairment of the antioxidant system. These injuries impaired the metabolic pathways of the skin, which leads to photoaging, erythema, edema, sunburn, lines and wrinkles, photosensitivity, immunosuppression, DNA damage as well as skin cancer in most severe conditions [2]. Therefore sunscreen compounds are generally incorporated in many cosmetic formulations such as creams, lotions, moisturizers and other skin care products [3]. The main purpose of sunscreen is to protect the skin against UVA and UVB rays and to conserve the moisture content of skin and its own natural oils, which may be lost during the exposure of solar radiation [4]. The sunscreen should be protective, chemically inert, non-irritating, non-toxic and photo stable [5].

The skin's natural sunscreens such as squalane, proteins, absorbing lipids and nucleotides have been used from several years. The squalane protects the skin's sensitive lipids. Allantoin, a product of purine nucleotide catabolism [6] that naturally occurs in the body, absorbs the spectrum of UV radiation therefore, used in sun care products and clarifying lotions because of its ability to heal minor wounds and promote healthy skin [7]. The phenolics (an aromatic ring bearing at least one hydroxyl substituent) exhibit a wide variety of beneficial biological activities, including antiviral, antibacterial, immune-stimulating, antioxidants, anti-allergic, anti-hypertensive, anti-arrhytmic, anti-thrombotic, hepatoprotective, hypocholesterolemic, anti-inflammatory and anti-carcinogenic actions. Flavonoids such as quercetin, luteolin and catechins are better antioxidants as compared to other antioxidants, such as vitamin C, vitamin E and β-carotene, used in various cosmetic formulations. Quercetin, apigenin and rutin were reported to be effective in UVA and UVB range. Therefore, the phenolics may be good in preventing UV-induced oxygen free radical generation and lipid peroxidation, which involved in photoaging and skin cancer [8]. The most common herbs used in cosmetology are aloe vera, basil, green tea, almond, olive, jojoba and cucumber etc. The photo protection against UV radiation can be determined *in-vivo* or *in-vitro*. The *in-vivo* determined by photo testing in human volunteers has been used from several years. It is complicated, time consuming as well as costly technique. Due to this, scientists have developed an *in-vitro* technique to measure the efficiency of sunscreen [9]. The *in-vitro* test is quick, inexpensive screening methodology. The sunscreen protection factor (SPF) is defined, as the UV energy required producing a minimal erythema dose (MED) on protecting skin, divided by the UV energy required to produce a MED on unprotected skin.

$$SPF = \frac{\text{MED in sunscreen} - \text{Protected skin}}{\text{MED in non-sunscreen} - \text{Protected skin}}$$

The MED is defined as the lowest time interval or dosage of UV light irradiation sufficient to produce a minimal, perceptible erythema on unprotected skin [10].

In-vitro SPF can be determined using spectrophotometric analysis or UV-2000S UV transmittance analyzer. The spectrophotometric analysis encompasses absorption characteristics of the sunscreens agents, while UV-2000S UV transmittance analyzer involves the measurement of absorption or the transmission of UV radiation through sunscreen product films in quartz plates or bio membranes over a wavelength range from 290 nm to 400 nm [11].

In present study the cream formulation containing fruits extract were evaluated for total flavonoid content and *in-vitro* SPF was determined according to the method described [12]. The absorbance values were calculated by following equation:

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

Where, CF=Correction factor (10), EE (λ)=Erythmogenic effect of radiation with wavelength λ, Abs (λ)=spectrophotometric absorbance values at wavelength λ. The values of EE×I [13] are constant (Table 1).

METHODS

Cream formulation and its preparation

A cream formulation was prepared and its ingredients are shown in Table 2.

The oil phase of cream was prepared by heating the ingredients (cetostearyl alcohol, stearic acid, cetomacrogol-100, lanolin and glycerin) at 75°C±2 with constant stirring using hot plate. While, for the preparation of aqueous phase purified water was heated separately in 2000 ml capacity beaker at 80°C±2. To this methyl and propyl parabens were dissolved with occasional stirring and temperature was brought to 75°C±2. The two phases (oil and aqueous) were mixed together with vigorous stirring for about 1-2 minutes. Finally the fruit extracts were added with constant stirring till a thick cream is formed. The temperature was further reduced to around 45°C using cold-water bath and stirring was discontinued. The cream was stored in wide mouth air tight amber colored glass container and stored in cool dry place.

Reagents

All chemicals and solvents used in the study were of analytical grade and they were purchased locally. Aluminum chloride ($AlCl_3$), potassium acetate (BDH), quercetin (Sunrise Nutrachem), methanol, ethanol (Merck), commercially available sunscreen (labeled SPF 15) and distilled water was used. It is reported that maximum 50% of ethanol could be used in cosmetics [14]. The maximum solubility was observed in 40% ethanol and 60% distilled water solution.

Table 1: Physical parameters of formulated cream

Parameters	Observations
Color	White
Odour	Odourless
pH	6.69
Spreadability	17.25±0.35

Table 2: Composition of cream formulation

S. No.	Ingredients	Uses	Components (%w/w)
1	Cetostearyl alcohol	Emulsifier	35
2	Stearic acid	Emollient, Co-emulsifier	40
3	Cetomacrogol-100	Emulsifier	9
4	Lanolin	Emollient	50
5	Glycerin	Humectant	156.6
6	Methyl paraben	Preservative	4
7	Propyl paraben	Preservative	0.4
8	*M. accuminata, P. gujava* and *P. communis* hydro-alcoholic extract	Active ingredient	10
9	Distilled water	Vehicle	695

M. accuminata: Musa accuminata, P. gujava: Psidium gujava, P. communis: Pyrus communis

Instruments

Double beam Shimadzu UV-visible spectrophotometer equipped with 1 cm quartz cell, pH meter (Systronics), ultrasonicator, weighing balance (Sartorius).

Evaluation of physicochemical parameters

The physicochemical properties such as color, odor, pH and spreadability of the cream were evaluated.

Color

The color was observed visually against dark background.

pH measurement

1 g of cream was dispersed in 9 ml of distilled water to determine the pH at 27°C using the pH meter.

Spreadability

The parallel plate method is most widely used method for determining the spreadability of semisolid preparations. A modified laboratory apparatus was used to evaluate spreadability. The setup consists of two glass slides placed on a tripod stand on which excess of cream (3 g) was applied in between two glass slides. The upper slide is movable and the lower slide was firmly fixed to the stand. 100 g weight was placed on them for 5 minutes to compress the cream to uniform thickness and the excess cream was scrapped off from the edges. Then 50 g weight was added to one side of the slide and the slide is pulled till it covers a distance of 10 cm. The time in seconds required to separate two glass slides by 10 cm was taken as a measure of spreadability. A shorter interval indicates better spreadability [15]. The spreadability was calculated by using the formula:

$$S=m.l/t$$

Where, S=Spreadability, m=Weight tied to upper glass slide, l=Length of glass slide, t=Time taken to separate them.

Estimation of total flavonoid content $AlCl_3$ colorimetric method

The total flavonoid content was determined using $AlCl_3$ colorimetric method [16].

Preparation of stock solution

10 mg of the cream formulation was accurately weighed and transferred to 50 ml volumetric flask and the volume was adjusted with methanol (80%) and then diluted to 25, 50 and 100 µg/ml.

Determination of total flavonoid content

A volume of 0.5 ml of cream formulation of various concentration 100, 50, 25 µg/ml were separately mixed with 1.5 ml methanol (95%), 0.1 ml of $AlCl_3$ (10%), 0.1 ml potassium acetate solution (1 M) and 2.8 ml distilled water and incubate at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm. Blank was prepared in similar way by replacing $AlCl_3$ with distilled water. Total flavonoid contents were calculated as quercetin standard calibration curve. The calibration curve was prepared by preparing quercetin solution at concentrations 6.25-125 µg/ml in methanol (80%). The total flavonoid content of the cream was expressed in terms of standard quercetin equivalent (QE) µg/g [17].

Determination of SPF value

1.0 g of cream formulation and commercial cream was weighed, transferred to 100 ml volumetric flask, diluted to volume with ethanol and water (40:60) then ultrasonication for 5 minutes after that filtered through Whatman No. 1 filter paper and collect the filtrate by rejecting the first 10 ml of filtrate. 5.0 ml of aliquot was taken in 50 ml volumetric

flask and diluted to volume with ethanol and water (40:60). Subsequently 5.0 ml of aliquot was transferred to 25 ml volumetric flask and the volume completed with ethanol and water (40:60). The absorbance values of each aliquot prepared were determined from 290 nm to 320 nm at 5 nm interval, using ethanol and distilled water (40:60) solution as a blank. The readings were taken in triplicate and the determinations were made at each point. The obtained absorbance values between 290 and 320 nm were multiplied with the respective EE (λ) values. Their summation was taken and multiplied with the correction factor (10) to obtain the SPF values. Data were expressed as ± standard error mean.

RESULTS AND DISCUSSION

The results of physicochemical analysis of cream formulation are shown in Table 1.

Monitoring the pH value is important for determining the stability of pharmaceuticals and cosmaceuticals. Any change in pH of the product indicates a possible interaction or occurrence of chemical reactions which may provide an idea on the quality of the final product [18]. The pH of human skin normally ranges from 4.5 to 6.0. Due to frequent washing and used of soap, the acidity of the skin is lost. Therefore, moisturizer has an acidic range should be used to normalize the skin. Acceptable pH range of moisturizers should be 5-8 range [19]. The cream formulation had a pH value of 6.69 (Table 1), which is an acceptable and non-skin irritating pH value.

Spreadability is a term expressed to denote the extent of the area to which the topical application spreads on affected parts of the skin. The therapeutic efficacy of the formulation depends on its spreading value. Thus, determination of spreadability is important for evaluating the topical application. The nature of cream formulation was homogenous, uniformly spreadable and emollient. The total flavonoid content of cream formulation [17] was 45.81±8.49 QE µg/g (Table 3) by reference to a standard curve (Fig. 1) (y=0.0009x+0.001, r^2=0.9189).

Table 3: Total flavonoid content of cream formulation

S. No.	Concentration µg/ml	Total flavonoid content QE µg/g
1	25	36.56
2	50	47.67
3	100	53.22
4	Mean	45.81±8.49

QE: Quercetin equivalent

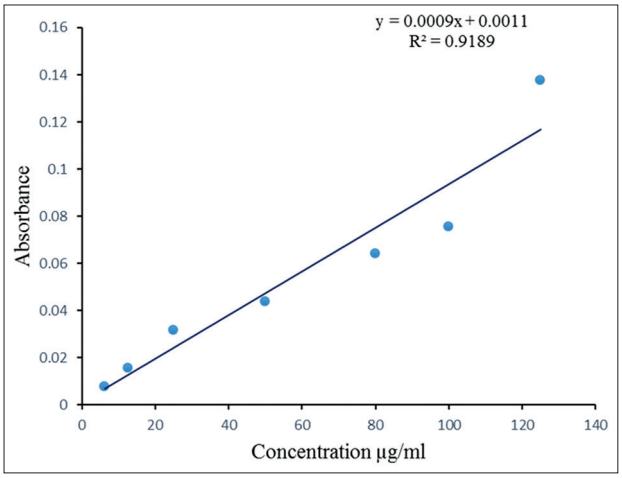

Fig. 1: Quercetin standard calibration curve

Flavonoids don't seem to be simply detectable therefore, $AlCl_3$ was used as a complexing reagent. The method is based on the formation of a stable complex between $AlCl_3$ and keto and hydroxyl groups of flavones and flavonoids. It is one of the most frequently used analytical procedures applied to the flavonoid content determination in various plants. Phenols and polyphenolic compounds, like flavonoids, are widely found in food products derived from plant sources. Studies have shown that increasing levels of flavonoids in the diet might decrease the certain type of diseases [20].

The SPF is a quantitative measurement of the effectiveness of a sunscreen formulation. In this study the cream formulation containing fruits extract of, *Musa accuminata*, *Psidium gujava* and *Pyrus communis* was evaluated for sunscreen activity using *in-vitro* SPF method. The SPF value is shown in Tables 4 and 5. The reported SPF values of broad spectrum sunscreens [21] are shown in Table 6.

By comparing the SPF value obtained from cream formulation with the values given in Table 6, it is evident that the cream will have the property to block around 73% of UV radiation, ultimately reflecting the overall sunscreen activity of the cream. This may be due to the presence of flavonoids, flavones, phenolics acid as well as other phytoconstituents of the fruit extracts used in the formulation. Flavonoids and phenolic compounds have been reported as functional components in plants and fruits, which play important role in the management of inflammation and erythema, due to solar radiations. The antioxidant property of the flavonoids and phenolic compounds further potentiates the UV protection or photo-protection activity. Antioxidants provide endogenous photo-protection and are essential for the protection and maintenance of skin health. Further, flavonoids can also modulate enzyme activity and effect cell division [22].

There are several aspects affecting the determination of SPF values, for example, the use of different solvents in which the sunscreens are dissolved; the combination and concentration of the ingredient; the nature of emulsion, the effect and interactions of diluents, such as esters, emollients and emulsifiers used in the formulations; the interaction of vehicle with the skin; addition of other active ingredients; the pH system and many other factors that may increase or decrease the UV absorption of sunscreen. Excipients and other active ingredients can also produce UV absorption bands, thus interfering with those of UVA and UVB sunscreens. It reflected in a finished formulation mostly for lotions if the SPF value is more than 15. To be effective in preventing sunburn as well as other skin damage, a sunscreen product should have a wide range of absorbance between 290 nm and 400 nm. The proposed UV spectrophotometric method is simple, rapid, utilizes low cost reagents and can be applied for *in-vitro* determination of SPF values in many cosmetic formulations. Although, efficiency of a sunscreen formulation has been evaluated

Table 4: *In-vitro* SPF value of cream formulation measured under different wavelength

S. No.	Wavelength (λ nm)	EE×I (normalized)	Absorbance×CF×EE×I	SPF=∑EE (λ)×I (λ)×Absorbance (A)×10
1	290	0.0150	0.06405±0.002	3.909257
2	295	0.0817	0.339055±0.002	
3	300	0.2874	1.155348±0.001	
4	305	0.3278	1.27842±0.003	
5	310	0.1864	0.702728±0.001	
6	315	0.0839	0.305396±0.002	
7	320	0.018	0.062640±0.002	

SPF: Sun protection factor

Table 5: *In-vitro* SPF value of commercial cream measured under different wavelength

S. No.	Wavelength (λnm)	EE×I (normalized)	Absorbance×CF×EE×I	SPF=∑EE (λ)×I (λ)×Absorbance (A)×10	Labelled SPF
1	290	0.0150	0.1593±0.002	12.258103	15
2	295	0.0817	0.933831±0.001		
3	300	0.2874	3.428682±0.001		
4	305	0.3278	4.061442±0.002		
5	310	0.1864	2.393376±0.003		
6	315	0.0839	1.063852±0.003		
7	320	0.018	0.21762±0.001		

SPF: Sun protection factor

Table 6: SPF values and corresponding percentage of UV blockage

SPF	Percent of UV blocked
2	50
4	75
5	80
10	90
15	93
25	96

SPF: Sun protection factor, UV: Ultraviolet

through *in-vivo* test, it is not only tedious but also involves some ethical issues [9]. SPF value for sunscreen above 2 is considered as having good sunscreen activity [4].

In the present study, cream formulation was found good sunscreen activity 3.909 and hence may be considered as good candidate for sunscreen or cosmaceuticals purposes. The fruit extracts rich in phenolic compounds possess an excellent property to reduce the oxidative damage. In view of this, the use of phytoconstituents, especially containing flavonoids in formulation has gained more importance as they are safe, effective and useful against the harmful effects of UV rays. Therefore, it can be used for the protection of photo induced intrinsic oxidative stress as well as structural alterations in the skin. Hence, to develop sunscreens with high SPF, the formulator must know the physicochemical properties, not just the UV absorbance of the actives, but also diluents, such as esters, emollients and emulsifiers used in the formulations.

CONCLUSION

The study provided reasonable data to conclude that seasonal fruits possess antioxidant property, which is capable of protecting the skin from the harmful effect of various physico-chemical factors. Therefore, it can be employed in cream and lotion formulations to obtain sunscreen activity. Plants, fruits, and vegetables contain various substances, especially the flavonoids, which are often good for skin care, having no harmful effects. Therefore, it is necessary to find out a good combination of phytoconstituents, which can produce best possible effects to the skin. The method used in this work is simple, fast, economical and also easy-to-used for the evaluation of creams and lotions to observe the sun protective effect on the skin.

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