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EVALUATION OF PHYTOCHEMICAL AND SPF COMPARISON WITH MARKET SUNSCREEN AND HERBAL EXTRACT FORMULATIONS

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ABSTRACT

Greece, Rome and ancient Egypt have been using *Matricaria Chamomilla L.* (MC) herbs for thousands of years. Application of phytoconstituents. Punica Granatum, or P. granatum, is a native of Iran and a member of the Punicaceae family. Particularly derived from flower and leaf extract, which has a high flavonoid concentration, has become more important in personal care products like lotions and creams. Researchers and industry on the subject will benefit from new data and results obtained through tests. The aim of this study was to assess the *in-vitro* sunscreen activity of a cream formulation that contained Punica Granatum leaf extract and *Matricaria Chamomilla* flower extract according to their flavonoid concentrations. The highest possible amount of flavonoids were extracted from the flower and leaf using a solvent solution consisting of 35% methanol, 35% ethanol, and 30% distilled water. Colour, odour, pH, and other physicochemical properties were examined once the cream was created. Tests for phytochemistry were determined. Using an ultraviolet spectrophotometric technique, the in-vitro sun protection factor (SPF) of cream formulations and commercial sunscreens was ascertained. The utilisation of phytoconstituents in personal care products, particularly in the form of extracts from the leaves of Punica Granatum and *Matricaria Chamomilla* flowers, which have a high flavonoid content, has become increasingly important. Researchers and industry on the subject will benefit from new data and results obtained through tests. The suggested spectrophotometric approach for determining SPF is quick and easy to use. The *in vitro* approach is becoming more and more important because of the significant expense and time involved in determining the SPF *in vivo* as well as certain ethical concerns for the volunteers.

KEYWORDS

Antioxidants, Formulation, *In-vitro* sun protection factor, Flower, Leaves extract and Ultraviolet-visible spectroscopy.

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INTRODUCTION

Natural remedies for common ailments like colds, allergies, upset stomachs and toothaches have been used for centuries, and this trend is continuing. As a result, there has been a shift in the global trend from synthetic to herbal medicines, or what we can call a "Return to Nature" for the prevention of diseases

and ailments. Since nature is a source of medicinal plants, herbal medicine is widely used worldwide. According to the World Health Organisation (WHO), 4 billion people, or 80% of the world's population, use herbal medicines for some basic healthcare needs. The World Health Organisation has acknowledged herbal medicine as a crucial part of primary healthcare and around 11% of the 252 medications are plant-based (Fabricant and Farnsworth, 2001)[1]. Developments in chemistry, isolation, purification, and characterisation of plant active chemicals marked the beginning of the creation of plant drugs. Compared to allopathic medication, herbal medicine is more cost-efficient, less prone to side effects and effective (Mansur *et al*, 1986)[2]. Herbs, plant materials, herbal preparations and herbal products with various plant parts or other plant materials as active components are all considered herbal medicines. The significance of herbal plants and their derivatives in contemporary drug development has been widely reported. The natural resources used to create new medications include medicinal plants (Heinrich, 2000)[3]. Despite the previous three to five years of achievement in the field of medicinal plant drug development research, there are still many obstacles to overcome in the years to come. There are concerns about the quality of herbal products and standardising raw materials becomes a significant problem for the herbal business (Martinez, 2008)[4].

Damage to cellular DNA and proteins causes a continuous degradation process that leads to skin ageing. There are two main categories of ageing processes: "photo-aging" and "sequential skin ageing." Each category has unique historical and clinical characteristics. A universal and predictable process, sequential skin ageing is defined by changes in skin function at the physiological level. Dry, pale skin with wrinkles is the result of ageing because keratinocytes are unable to generate a functional stratum corneum and the rate of production from neutral lipids slows down. On the other hand, excessive exposure to UV radiation from sunshine results in photoaging. Dry, pale and shallow skin with deep furrows and fine wrinkles brought on by the disarray of dermal and epidermal components linked to heliodermatitis and elastosis are its defining characteristics. As a tool in alternative therapy, herbs and plants have already shown promise (Kaur *et al*, 2007[5], Watson *et al*, 2009[6]).

Cosmetics are applied to the skin to improve its appearance and protect it from external and internal hazardous substances (Saraf *et al*, 2010)[7]. By lowering skin problems, cosmetics use contributes to both maintaining long-term excellent health and creating an appealing outward look (Hema *et al*, 2010)[8]. The synthetic or natural components included in skin care products that preserve the skin's elasticity, health, texture and integrity as well as provide moisturization, photoprotection and other benefits This cosmetic's long-lasting skin property management and reduction of free radical formation in the skin are attributed to the components included in skin care formulations. The greatest option for reducing skin conditions including hyperpigmentation, ageing, wrinkles, and rough skin texture, among others, is to use cosmetic items. Herbal plants are easily polluted as they are being grown, processed, and harvested. The two main issues with herbal medicines that have been observed are adulteration and heavy metal contamination (Matsumura, 2004)[9]. Therefore, in order to produce novel herbal drugs and stay up to date with other drug development activities, it is vital to increase the quality and quantity of bioactive components. Many therapeutic plants are used today and researchers have assessed the bioactive components of these plants both scientifically and clinically (Patwardhan *et al*, 2004)[10].

MATERIAL AND METHODS

Plant material

Punica granatum (leaves) from the village of Dhamtaridistric and *Matricaria chamomilla* (flower) from the Organic India Shop in Raipur were obtained, and they were authenticated at the Central Laboratory Facility of the Chhattisgarh

Council of Science and Technology in Raipur, Chhattisgarh.

Chemicals and Reagent samples

The greatest purity (>99.95%) reagents were obtained from Sigma Chemical Co. (Germany) and other suppliers. The absorbances of the samples were measured with a Varian Lambda 532nm UV Spectrometer.

Preparation of extract

Matricaria chamomilla flower and Punica granatum leaf powder (10g) were dried and ground into a powder, which was then continuously mixed in 100ml of 50% methanol for 24 hours at room temperature. Following the filtration procedure, methanol was evaporated over a water bath at a temperature of 60-70^{o}C until only water was left. The last extract was stored in an airtight container.

Phytochemical Test

Test for Flavonoids

Put a few drops of concentrated H2SO4 into a test tube containing a tiny amount of plant extract. Afterwards, the orange tint changes to a cream colour, signifying the presence of flavonoids.

Test for Alkaloids

Take a tiny amount of the separately stirred methanolic plant extract, add a few drops of 10% diluted hydrochloric acid to a test tube and filter it. Mayer's reagent was then applied to the filtrate. There was orange precipitate, indicating the presence of alkaloid.

Test for Tannin

Put a little amount of plant extract in a test tube, then thoroughly mix in 5 millilitres of double-distilled water. After two minutes of boiling in a water bath, filter it. To the filtrate solution, add 1 millilitre of 1% diluted FeCl3. Tannin is indicated by the colours brown and green.

Test for Terpenoid

Put a tiny amount of extract and two millilitres of CHCl3 in a test tube. Next, pour 3 millilitres of concentrated H2SO4 from the test tube's sides. The presence of terpenoids is indicated by the reddish-brown hue.

Test for Phenols

Extract from an aqueous solution needs to be taken. A few drops of a 5% ferric chloride solution are then added. A dark green, blue, or black hue emerges.

Test for Saponin

Put a small amount of plant extract in a test tube and fill it with five millilitres of distilled water. Next, give it a good shake. The presence of saponin is shown by the production of 1 cm of foam.

Test for Coumarins, (NaOH test)

Put 10% NaOH into a test tube containing a plant extract. Next, pour chloroform over it. The presence of coumarin is indicated by a yellow hue.

Test for Glycosides

In a test tube, take a little amount of plant extract and hydrolyse it with 10% HCl. After that, a hydrolysed mixture was cooked for a few hours in a water bath. Next, mix 1 millilitre each of Fehling solutions A and B into the boiling extract. The presence of glycosides is indicated by the production of a crimson precipitate.

Test for Steroids and triterpenoids

Plant sample was mixed with 10ml of benzene, let to soak for 10 minutes and then filtered. Pour in 3 millilitres of concentrated sulphuric acid. Give it a good shake and let it remain. The lower layer displays the colour red.

Test for Quinones and Anthraquinones

Soluble 10mg of extract in isopropyl alcohol. Put a little sulphuric acid in it. The colour red emerges.

Sun Protection Factor (SPF) Test

Instruments

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

Determination of SPF value

The absorbance values of each aliquot prepared were determined from 290nm to 320nm at 5nm interval, using 10ml ethanol and 15ml 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 320nm 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. Using a blank of 10 millilitres of ethanol and 15 millilitres of distilled water (40:60) solution, the absorbance values of each produced aliquot were measured at 5-nm intervals between 290 and 320nm. Three copies of the readings were obtained, and decisions were made at each stage. The corresponding EE (λ) values were multiplied by the absorbance values between 290 and 320nm that were obtained. To get the SPF values, their summation was obtained and multiplied by the correction factor (= 10). The data were presented as mean ± standard error.

RESULTS AND DISCUSSION

It's critical to keep an eye on the pH level while assessing the stability of medications and cosmetics. Any variation in the product's pH suggests that chemical processes may have occurred or interacted, which could provide insight into the final product's quality. Human skin typically has a pH between 4.5 and 6.0. Frequent washing and soap use cause the skin's acidity to disappear. In order to restore skin to normal, moisturiser with an acidic range ought to be applied. A moisturizer's acceptable pH range is between 5-8 (Saraf *et al*, 2010)[11].

Which the skin's afflicted areas absorb after topical treatment. The formulation's spreading value determines how effective it is therapeutically. Therefore, assessing the topical application requires determining the spread ability. The cream's composition was emollient, homogenous, and evenly spreadable (Imam *et al*, 2015)[12].

One of the most popular analytical techniques for determining the flavonoid content of different plants is the analysis of flavonoids. Flavonoids and other phenolic chemicals are common in food products made from plant sources. According to studies, eating a diet higher in flavonoids may help prevent several types of diseases (2013) Hassan *et al*[13].

The SPF is a numerical indicator of how efficient a sunscreen product is. Using the *in-vitro* SPF method, the sunscreen activity of a cream formulation containing floral extract of *Matricaria chamomilla L*. and leaf extract of Punica granatumcommunis was assessed in this work. Tables No.1-2 and Table No.3 display the SPF value.

It is clear that the cream will have the ability to block approximately 90% of UV radiation for chamomile, 94% of UV radiation for pomegranate, and 62% of UV radiation for Lotus Sunscreen when comparing the SPF value obtained from the cream formulation with the values given in Tables No.1-2 and Table No.3. This will ultimately reflect the cream's overall sunscreen activity. The existence of flavonoids, flavones, phenolic acid, and other phytoconstituents in the fruit extracts utilised in the formulation could be the cause of this. According to reports, flavonoids and phenolic compounds are useful components of fruits and plants that are crucial in the treatment of erythema and inflammation brought on by sun exposure. The flavonoid and phenolic compound antioxidant properties enhance the UV protection or photo-protective properties even further. Antioxidants are necessary for the preservation and protection of skin health because they offer endogenous photo-protection. Moreover, flavonoids have the ability to control cell division and enzyme activity (Saewam and Jimtaisong, 2013)[14].

The use of various solvents in which the sunscreens are dissolved, the combination and concentration of ingredients, the nature of the emulsion, the impact and interactions of diluents, such as esters, emollients and emulsifiers used in the formulations, the interaction of the vehicle with the skin, the addition of additional active ingredients, the pH system, and many other factors that may increase or decrease the UV absorption of sunscreen are just a few of the factors that affect the determination of SPF values. UVA and UVB sunscreens' UV absorption bands may be interfered with by excipients and other active components.

If the SPF value is greater than 15, it is reflected in the final formulation primarily for lotions (Emran *et al*, 2015)[15]. A broad spectrum of absorption between 290nm and 400nm is what makes a sunscreen lotion useful in avoiding sunburn and other skin damage. The suggested UV spectrophotometric approach is easy to use, quick, requires inexpensive reagents and may be used to determine the SPF values of numerous cosmetic compositions *in vitro*. While in-vivo testing has been used to assess the efficacy of sunscreen formulations, this process is time-consuming and fraught with ethical concerns (Bambal and Mishra, 2014)[16]. According to Kale *et al*, (2011)[17], sunscreen with an SPF rating of more than 2 is thought to have good sunscreen activity.

The cream formulation used in this investigation demonstrated good sunscreen activity, making it a promising option for use in cosmeceuticals or sunscreen applications. Rich in phenolic components, flower and leaf extracts have a great ability to lessen oxidative damage. Given that they are safe, efficient and helpful in preventing the negative effects of UV radiation, the use of phytoconstituents-especially those containing flavonoids-in formulation has become more significant (Lemberkovics *et al*, 1998)[18]. As such, it can be applied to prevent both structural changes in the skin and photo-induced intrinsic oxidative stress. Therefore, in addition to the UV absorbance of the active ingredients, the formulator must be aware of the physicochemical properties of the diluents-such as esters, emollients, and emulsifiers-used in the formulations in order to create sunscreens with high SPF.

SUMMARY

The study's findings were sufficient to draw the conclusion that antioxidants found in chamomile flowers and pomegranate leaves can shield the skin from the damaging effects of a variety of physiochemical variables. According to these research, the base and extract composition of creams are safer and more stable for up to a year. Since the cream contains a variety of natural ingredients, it may have a synergistic impact without causing any negative side effects. Consequently, it can be utilised in lotion and cream compositions to achieve sunscreen properties. Many chemicals, especially flavonoids, which are frequently beneficial for skin care and have no negative effects, are found in plants, fruits, leaves and flowers. Consequently, it's critical to identify a potent phytoconstituent combination that will have the most impact on skin health. This study employs an easy-to-use, quick, affordable and straightforward method for evaluating creams and lotions in order to assess their ability to protect skin from the sun.

Table No.1: Physical parameters of extract and cream

S.No	Sample	Color	Odour	pH
1	*Matricaria Chamomilla*	Brown	Sweet Hurb	7.6
2	*Punica granatum*	Yellow	Sweet Aroma	6.6
3	Sunscreen	Pinkish	Like Strawberry	8.2

Table No.2: Phytochemical Test of *Matricaria Chamomilla* and *Punica granatum* Extract

S.No	Test	*Chamomile*	Pomegranate
1	Flavonoids Test	+	+
2	Alkaloids Test	+	-
3	Tannin Test	+	+
4	Terpenoid Test	+	+
5	Phenols Test	-	+
6	Saponin Test	+	-
7	Coumarins (NaOH) Test	+	+
8	Glycosides Test	+	+
9	Steroids	-	+
10	Triterpenoids	+	-
11	Quinones	+	+
12	Anthraquinones	+	-

Table No.3: *In-vitro* SPF value of extract and commercial cream measured under different Wavelength

S.No	Wavelength (λ nm)	*Matricaria chamomilla* (25%)	*Punica granatum* (25%)	Sunscreen (25%)
1	Blank	0.137	0.137	0.137
2	290	0.897	1.945	0.191
3	295	0.935	2.002	0.241
4	300	0.946	1.781	0.262
5	305	0.952	1.537	0.269
6	310	0.946	1.298	0.271
7	315	0.925	1.079	0.271
8	320	0.886	0.899	0.264

Table No.4: *In-vitro* SPF value of extract and commercial cream measured under different wavelength

S.No	Wavelength (λ nm)	*Matricaria chamomilla* (25%)	*Punica granatum* (25%)	Sunscreen (25%)
1	Blank	0.163	0.163	0.163
2	290	1.243	2.133	0.503
3	295	1.215	2.457	0.484
4	300	1.175	2.235	0.453
5	305	1.138	1.928	0.428
6	310	1.102	1.620	0.417
7	315	1.059	1.341	0.406
8	320	1.011	1.109	0.395

Table No.5: *In-vitro* SPF value of extract and commercial cream measured under different wavelength

S.No	Wavelength (λ nm)	*Matricaria chamomilla* (25%)	*Punica granatum* (25%)	Sunscreen (25%)
1	Blank	0.171	0.171	0.171
2	290	1.140	2.190	0.491
3	295	1.099	2.681	0.483
4	300	1.057	2.507	0.456
5	305	1.020	2.167	0.428
6	310	0.989	1.822	0.401
7	315	0.956	1.502	0.374
8	320	0.921	1.241	0.348

Table No.6: SPF Activity of Louts Sunscreen and *Matricaria chamomilla*, *Punica granatum* extract

S.No	Wavelength (λ nm)	Louts Sunscreen (Mean+SE)	*M. chamomilla* (Mean+SE)	*P. granatum* (Mean+SE)
1	290	0.44± 0.02	1.09± 0.102	2.08± 0.073
2	295	0.43 ± 0.027	1.08± 0.081	2.38 ± 0.199
3	300	0.40± 0.026	1.05± 0.066	2.17± 0.211
4	305	0.38± 0.024	1.03± 0.054	1.87± 0.183
5	310	0.36± 0.021	1.01± 0.046	1.58± 0.152
6	315	0.33 ± 0.019	0.98± 0.040	1.30± 0.123
7	320	0.316± 0.016	0.94± 0.037	1.08± 0.099

Table No.7: *In-vitro* SPF value of cream formulation measured under different wavelength (*Matricaria chamomilla*)

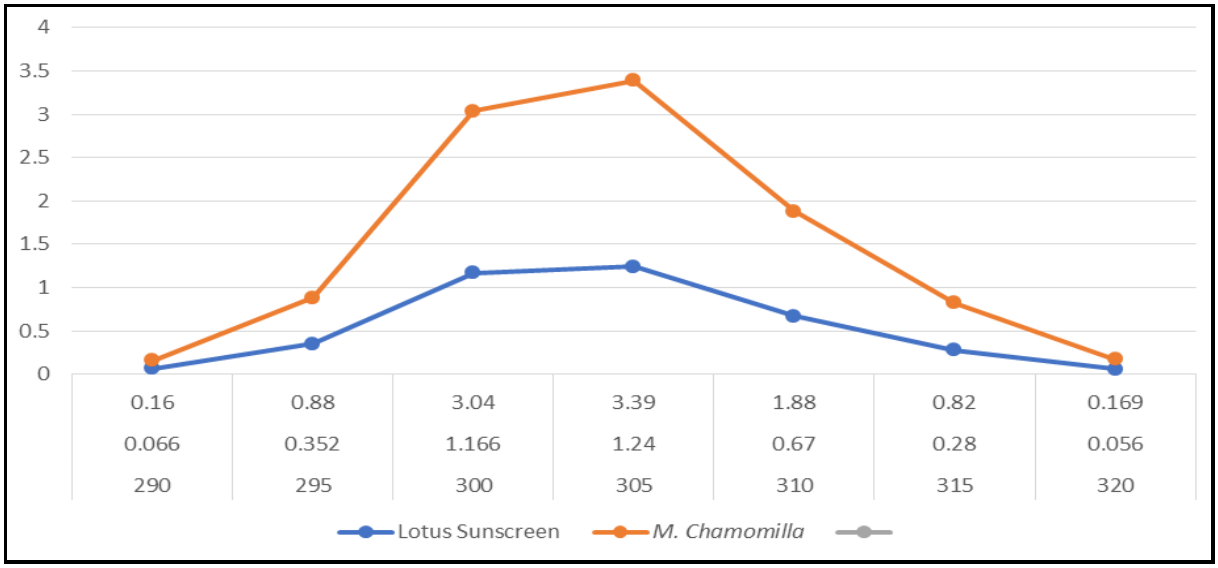
S.No	Wavelength (λ nm)	EE x I (normalized)	Absorbance	Absorbance x CF x EE x I	SPF
1	290	0.0150	1.094	0.16±0.002	10.36
2	295	0.0817	1.083	0.88±0.002	
3	300	0.2874	1.059	3.04±0.001	
4	305	0.3278	1.036	3.39±0.003	
5	310	0.1864	1.012	1.88±0.002	
6	315	0.0839	0.980	0.82±0.001	
7	320	0.018	0.940	0.16±0.002	

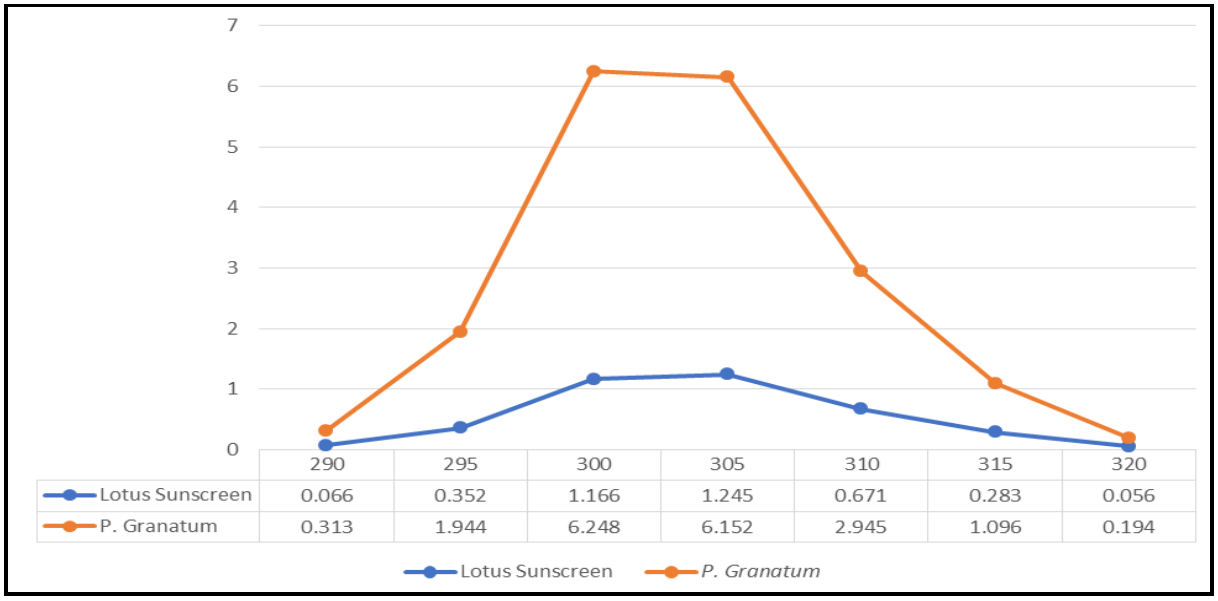
Table No.8: *In-vitro* SPF value of cream formulation measured under different wavelength (*Punica granatum*)

S.No	Wavelength (λ nm)	EE x I (normalized)	Absorbance	Absorbance x CF x EE x I	SPF
1	290	0.0150	2.089	0.31±0.002	18.89
2	295	0.0817	2.380	1.94±0.002	
3	300	0.2874	2.174	6.24±0.001	
4	305	0.3278	1.877	6.15±0.003	
5	310	0.1864	1.580	2.94 ±0.002	
6	315	0.0839	1.307	1.09±0.001	
7	320	0.018	1.083	0.19 ±0.002	

Table No.9: *In-vitro* SPF value of cream formulation measured under different wavelength (Lotus Sunscreen)

S.No	Wavelength (λ nm)	EE x I (normalized)	Absorbance	Absorbance x CF x EE x I	SPF
1	290	0.0150	0.440	0.06±0.002	3.84
2	295	0.0817	0.431	0.35±0.002	
3	300	0.2874	0.406	1.16±0.001	
4	305	0.3278	0.380	1.24 ±0.003	
5	310	0.1864	0.360	0.67±0.002	
6	315	0.0839	0.338	0.28±0.001	
7	320	0.018	0.316	0.05±0.002	





CONCLUSION

The study provided reasonable data to conclude that *Chamomile* flower and pomegranate leaves possess antioxidant property, which is capable of protecting the skin from the harmful effect of various physiochemical factors. These studies suggest that composition of extracts and base of creams are more stable up to 12 months and safe; it may produce synergistic action without side effects as this cream comprising of many natural substances. Therefore, it can be employed in cream and lotion formulations to obtain sunscreen activity. Plants, fruits, leaves and flower 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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CONFLICT OF INTEREST

I declare that I have no conflict of interest.

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