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Comparative in vitro Sun Protection Factor (SPF) values of some herbal extracts found in Kinshasa by Ultraviolet Spectrophotometry

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ABSTRACT

The purpose of this study was to determine in vitro the sun protection factor (SPF) of aqueous and ethyl acetate herbal extracts of some commonly used vegetable found in Kinshasa by ultraviolet spectrophotometry. The comparative values of the calculated sun protection factor of four commonly used vegetable were evaluated using Mansur equation. Aqueous and ethyl acetate herbal extracts were obtained and after dilution with ethanol the absorbance were recorded between 290-320 nm using UV-vis spectrophotometer. It was observed that all of the tested herbal extracts showed some UV protection capabilities with carrot ethyl acetate extract / paba in the ratio 2000 ppm/20 ppm showed the highest SPF value of 43.38 while the cream containing 2% of coconut ethyl acetate extract showed the lowest SPF value of 0.64. The proposed spectrophotometric method is fast, simple and cost effective for the in vitro determination of SPF values.

Keywords: Sun Protection Factor, UV-vis spectrophotometry, herbal extracts.

INTRODUCTION

Ultraviolet light has been classified by World Health Organization as carcinogenic and produces several adverse effects including mutagenicity, immune depression of the skin, accelerated skin ageing and photodermatoses**.** Solar ultraviolet radiation (UVR) is divided into three categories UV-C (200-280 nm), UV-B (280-320) and UV-A (320- 400 nm). The most biologically damaging radiation UV-C is filtered out by the ozone layer and it is mainly UV-B that is responsible for causing the adverse effects of the UV radiation. UVB radiation (having energy of 30 to 40 times greater than UVA) may promote a deficit in the immunologic functions of the skin in the addition to the anomalies in the DNA[1-5]**.**

Application of sunscreen to the skin changes the way the body reacts to the sun rays. Sunscreens and sunblocks are chemicals that absorb or block UV rays and show a variety of immunosuppressive effects of sunlight. There are several agents available from both synthetic and natural sources with UV-filtering properties. Given their potential to produce considerable human local and systemic exposure, UV filters have to be safe. Synthetic UV filters are known to have potential toxicity in humans and also showed ability to interfere only in selected pathways of multistage process of carcinogenesis [1, 2].

Synthetic UV filters and physical blocker ingredients have also been increasingly reported for allergic and contact dermatitis, phototoxic and photo-allergic reactions, contact urticaria and even solitary cases of severe anaphylactic reactions [3]. A number of people with sensitive skin, such as those suffering from skin hypersensitivity don't want to use chemical sunscreens due to concern about skin exposure to unknown chemicals. Although a variety of hypoallergenic cosmetic products have been introduced for customers with sensitive skin, there are still limited options in sunscreen agents. Therefore, the researchers have turned their attention towards developing herbal sunscreen agents which are effective with less or no side. Herbal botanical sunscreens are safe, widely accepted by consumers and also work in various ways, playing multiple roles in ameliorating the process of carcinogenesis [1].

The effectiveness of a sunscreen is usually expressed by sun protection factor (SPF) which is the ratio of UV energy required to produce a minimal erythemal dose (MED) in protected skin to unprotected skin. A simple, rapid and reliable in vitro method of calculating the SPF is to screen the absorbance of the product between 290-320 nm at every 5 nm intervals [1]**.**

In this study, the in vitro SPF of four herbal extracts were evaluated by UV-vis spectrophotometry . Although, in-vitro methods present some limits; it gives accurate and precise result and avoid the exposure of human subjects to harmful ultraviolet radiation.

MATERIALS AND METHODS

Analytical grade ethanol, analytical grade ethyl acetate and paraamino benzoic acid (paba) were purchased from Merck product. The seeds of palm walnut (Pmw) and *Cucurbita maxima* (Ccm*)*, the carrot and coconut fruits came from Madimba and Oswe villages, Bas-Congo and Bandundu regions (D.R.Congo) in the dry season and sale in the Kinshasa market. The fruits and seeds were authenticated by Ms. Kiye of Biology Department; University of Kinshasa, Kinshasa, D.R.Congo.

Samples preparation of aqueous extracts

The fruits and seeds were grinded separately and 20 gm from each were taken separately in a beaker and extracted overnight with 200 ml of distilled water, then filtered with whatman filter paper. The filtered extract was suitably diluted with ethanol and the absorbance was measured between 290-320 nm using a Hitachi U-3900 H. UV/Visible spectrophotometer, equipped with 1 cm quartz cell and a computer.

Samples preparation of ethyl acetate extract

The seeds and fruits were dried in shade and then ground to produce coarse powder. Previously defatted with petroleum ether, the samples were extracted with ethyl acetate for 48 hours by soxhlet apparatus. The extracts were obtained using a rotatory evaporator and then concentrated at 50°C. The extracts were kept in sterile bottle and stored under refrigerated condition for further analysis.

Preparation of the extract solution:

0.2gm of extract were weighed, transferred to a 100 ml volumetric flask, diluted to volume with ethanol, and then filtered through cotton, to give 2000 ppm solution. Rejecting the first 10 ml, a 25.0 ml aliquot was transferred to 50 ml volumetric flask and diluted to volume with ethanol to produce 1000 ppm solution. Then a 25.0 ml aliquot was transferred to a 50 ml volumetric flask and the volume completed with ethanol (500 ppm solution).

Preparation of the paba solution:

2mg of paba were weighed, transferred to a 100 ml volumetric flask, diluted to volume with ethanol, and then filtered through cotton, to give 20 ppm solution. Rejecting the first 10 ml, a 25.0 ml aliquot was transferred to 50 ml volumetric flask and diluted to volume with ethanol to produce 10 ppm solution. Then a 25.0 ml aliquot was transferred to a 50 ml volumetric flask and the volume completed with ethanol (5 ppm solution).

Formulation of herbal extracts cream:

The composition of the herbal extracts cream formulation is specified in Table 1[4]**.**

Table 1: Composition of herbal extracts cream

Sr. No	Ingredients	Components (%w/w)
1	Cetostearyl alcohol	5.00
2	Stearic acid	4.00
3	Petroleum Jelly	1.00
4	Glycerin	5.00
5	Potassium hydroxide	1.00
6	Water	81.75
7	Methyl paraben sodium	0.20
8	Propyl paraben sodium	0.05
9	Plant extract	2.00

The procedure for in vitro SPF cream determination was that described by Mishra et al [2].

Spectrophotometric measurement and SPF determination

The absorption spectra of samples in solution were obtained in the range of 290 to 320 nm, every 5 nm. Three determinations were made at each point using ethanol as a blank. SPF values were determined using Mansur equation (equation 1). Indeed, Mansur *et al.* [5]**,** developed a very simple mathematical equation which substitutes the *in vitro* method proposed by Sayre *et al.*, [6]**,** utilizing UV spectrophotometry and the following equation 1[7, 8]**:**

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

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 and predetermined by Sayre *et al.*,[6], and are showed in Table 2.

Table 2: Normalized product function used in the calculation of SPF [7].

Wavelength (nm)	EEXI (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180
Total	1

Legend:
EE: erythemal effect spectrum; I: solar intensity spectrum

RESULTS AND DISCUSSION

The absorbance and the SPF values of the samples calculated through UV Spectrophotometric method are shown in tables 3 to 7.

Table 3: In vitro SPF of carrot extracts (A) in various conditions.

λ	Absorbances							
	Ethyl acetate Extracts							
	2000 ppm	1000 ppm	500 ppm	2000ppm+ 20ppm of paba	1000ppm+ 10ppm of paba	500ppm + 5ppm of paba	Cream/2%	Aqueous Extract
290	2.072±0.076	1.233±0.01	0.739±0.085	5.188±0.061	3.088±0.022	1.051±0.012	1.102±0.006	0.246±0.076
295	2.617±0.059	1.358±0.005	0.803±0.089	5.519±0.065	3.219±0.043	0.959±0.055	1.063±0.006	0.206±0.082
300	2.713±0.093	1.417±0.038	0.717±0.059	5.604±0.025	3.204±0.031	0.875±0.039	1.084±0.008	0.187±0.089
305	2.432±0.092	1.539±0.035	0.613±0.023	4.166±0.011	1.366±0.032	0.779±0.042	1.051±0.004	0.157±0.012
310	2.314±0.035	1.210±0.082	0.684±0.050	2.976±0.015	1.760±0.054	0.596±0.056	0.796±0.032	0.155±0.067
315	1.704±0.012	1.108±0.022	0.501±0.056	2.792±0.014	1.392±0.042	0.487±0.044	0.578±0.016	0.144±0.078
320	1.613±0.050	0.968±0.017	0.447±0.095	2.491±0.123	1.191±0.068	0.388±0.057	0.234±0.015	0.142±0.054
SPF	**24.25±0.71**	**13.77±0.40**	**6.61±0.49**	**43.38±0.23**	**21.44±0.38**	**7.60±0.45**	**9.60±0.12**	**1.69±0.57**

Table 4: In vitro SPF of coconut extracts (B) in various conditions.

λ	Absorbance							
	Ethyl acetate Extracts							
	2000 ppm	1000 ppm	500 ppm	2000ppm + 20ppm of paba	1000ppm + 10ppm of paba	500ppm + 5ppm of paba	Cream/2%	Aqueous Extract
290	0.524±0.040	0.292±0.020	0.131±0.010	1.847±0.013	1.052±0.085	0.549±0.008	0.180±0.017	0.922±0.024
295	0.488±0.020	0.256±0.030	0.109±0.010	1.977±0.010	1.010±0.035	0.477±0.006	0.045±0.072	0.840±0.014
300	0.430±0.023	0.200±0.034	0.092±0.020	2.883±0.012	0.821±0.015	0.419±0.032	0.071±0.024	0.784±0.019
305	0.384±0.013	0,170±0.045	0.075±0.040	1.356±0.024	0.661±0.012	0.331±0.065	0.078±0.008	0.705±0.027
310	0.269±0.029	0.122±0.031	0.042±0.050	0.911±0.043	0.434±0.054	0.219±0.076	0.036±0.005	0.694±0.039
315	0.240±0.015	0.117±0.017	0.048±0.014	0.528±0.033	0.262±0.027	0.137±0.005	0.056±0.003	0.676±0.021
320	0.210±0.003	0.085±0.056	0.030±0.007	0.282±0.021	0.130±0.007	0.082±0.044	0.015±0.001	0.627±0.017
SPF	**3.71±0.20**	**1.75±0.35**	**0.74±0.30**	**16.81±0.23**	**6.56±0.25**	**3.3±0.46**	**0.64±0.17**	**7.36±0.25**

Table 5: In vitro SPF of Ccm extract (C) in various conditions.

λ	Absorbance							
	Ethyl acetate Extracts							
	2000 ppm	1000 ppm	500 ppm	2000ppm + 20ppm of paba	1000ppm + 10ppm of paba	500ppm + 5ppm of paba	Cream/2%	Aqueous Extract
290	1.641±0.099	0.758±0.023	0.401±0.039	3.055±0.026	1.394±0.066	1.594±0.017	0.321±0.059	0.988±0.061
295	1.472±0.037	0.667±0.068	0.337±0.034	4.524±0.061	1.231±0.061	0.907±0.014	0.345±0.04	0.715±0.069
300	1.309±0.059	0.614±0.035	0.299±0.090	6.297±0.071	1.065±0.076	0.620±0.028	0.215±0.057	0.48±0.078
305	1.222±0.075	0.519±0.028	0.267±0.095	2.472±0.047	0.854±0.063	0.461±0.027	0.203±0.064	0.373±0.057
310	1.077±0.058	0.467±0.045	0.254±0.033	1.363±0.049	0.589±0.081	0.29±0.036	0.205±0.073	0.292±0.046
315	0.951±0.029	0.432±0.071	0.208±0.023	0.967±0.045	0.416±0.01	0.199±0.031	0.197±0.088	0.218±0.035
320	0.839±0.034	0.359±0.076	0.182±0.007	0.657±0.065	0.274±0.012	0.137±0.029	0.182±0.05	0.179±0.031
SPF	**12.17±0.60**	**5.42±0.41**	**2.75±0.69**	**33.82±0.55**	**8.57±0.65**	**5.00±0.28**	**2.10±0.63**	**4.09±0.60**

Table 6: In vitro SPF of Pmw extracts (D) in various conditions.

λ	Absorbance							
	Ethyl acetate Extracts							
	2000 ppm	1000 ppm	500 ppm	2000ppm + 20ppm of paba	1000ppm + 10ppm of paba	500ppm + 5ppm of paba	Cream/2%	Aqueous Extract
290	0.808±0.091	0.402±0.077	0.220±0.022	2.995±0.015	1.096±0.070	0.409±0.060	0.091±0.034	0.928±0.038
295	0.698±0.075	0.357±0.066	0.204±0.072	1.820±0.036	1.031±0.043	0.572±0.073	0.099±0.014	0.892±0.036
300	0.644±0.051	0.319±0.061	0.172±0.032	1.869±0.072	0.908±0.052	0.659±0.049	0.116±0.008	0.837±0.024
305	0.579±0.030	0.298±0.056	0.164±0.056	1.414±0.023	0.743±0.043	0.677±0.074	0.124±0.051	0.780±0.013
310	0.506±0.062	0.247±0.049	0.127±0.067	0.944±0.029	0.496±0.036	0.503±0.039	0.093±0.007	0.748±0.055
315	0.460±0.062	0.242±0.071	0.125±0.015	0.593±0.053	0.312±0.018	0.693±0.017	0.081±0.006	0.693±0.006
320	0.431±0.010	0.213±0.060	0.102±0.017	0.347±0.033	0.208±0.009	0.942±0.010	0.077±0.009	0.684±0.005
SPF	**5.85±0.49**	**2.95±0.59**	**1.59±0.48**	**14.26±0.42**	**7.28±0.42**	**6.33±0.54**	**1.09±0.23**	**7.93±0.26**

Table 7: In vitro SPF of ethanolic solution of paba.

λ	Absorbance			
	EExI	20 ppm	10 ppm	5 ppm
290	0.0150	5.137±0.031	1.536±0.010	0.687±0.080

295	0.0817	2.399±0.066	1.489±0.051	0.682±0.069
300	0.2874	5.107±0.027	1.357±0.065	0.589±0.076
305	0.3278	4.893±0.028	0.996±0.076	0.438±0.081
310	0.1864	1.459±0.017	0.600±0.065	0.248±0.055
315	0.0837	0.678±0.013	0.304±0.056	0.116±0.042
320	0.0180	0.268±0.012	0.099±0.045	0.023±0.043
SPF		**36.78±0.27**	**10.00±0.66**	**4.35±0.70**

Four herbal extracts (carrot, coconut, Ccm and Pmw) are used and one chemical filter (paba). The calculated SPF values are in the range of 0.64 to 43.38. The SPF number of the aqueous herbal extracts range between 1.69 in carrot and 7.93 in Pmw. Each Sample had various concentrations and it was alone, with the paba and in the cream. The paba concentration / extracts herbal concentration in their mixture had the ratio 1/100. All samples contained herbal extracts with paba had calculated SPF higher than samples with herbal extracts alone. The calculated SPF decrease when the concentration decreases. The calculated SPF values for carrot have been found to be the highest among the ethyl acetate extracts studied and the coconut had the lower calculated SPF in various concentrations except in the sample 2000ppm+20ppm of paba. The carrot aqueous extracts has the lower calculated SPF (1.69) and the Pmw aqueous extracts the higher SPF (7.93). In general, it can be noticed that the in vitro SPF ethyl acetate extracts are higher than the in vitro SPF aqueous extracts. The carrot and Ccm ethyl acetate extracts gave in vitro SPF higher than the aqueous extracts. But for coconut and Pmw, aqueous extracts gave in vitro SPF higher than ethyl acetate extracts. Figure 1 show different calculated SPF values in various conditions.

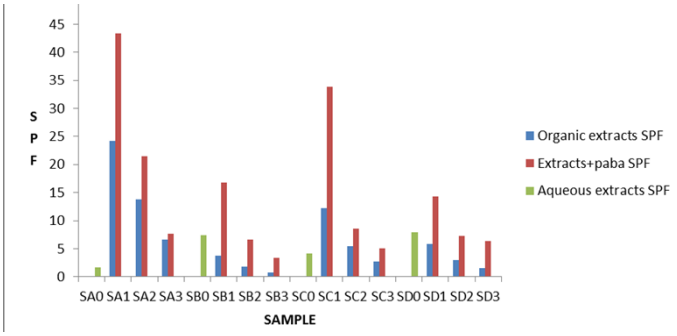

Figure 1 : Comparative SPF values of herbal extracts ethanolic solutions in various conditions.

Legend: Indices:

Indice 0: in water.

Indice 1: 2000 ppm (no paba) and 2000 ppm + 20 ppm (with paba), a mixed of equal volume.

Indice 2: 1000 ppm (no paba) and 1000 ppm + 10 ppm (with paba), a mixed of equal volume.

Indice 3: 500 ppm (no paba) and 500 ppm + 5 ppm (with paba), a mixed of equal volume.

Indice A: carrot extracts

Indice B: coconut extracts

Indice C: Ccm extract

Indice D: Pmw extracts

The figure 2 gives the comparative SPF values of herbal extracts and cream containing herbal extracts.

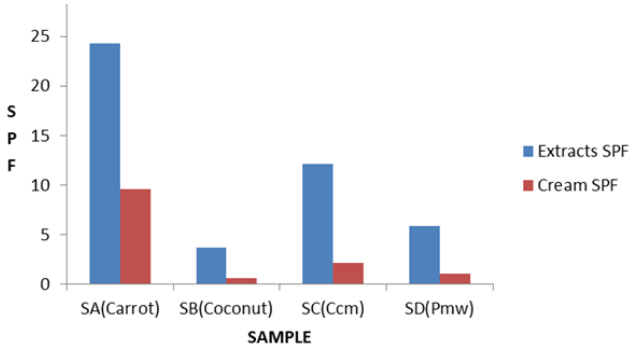

Figure 2: Comparative SPF values of herbal extracts and cream containing herbal extracts.

DISCUSSION

Sunscreens are used to aid the body's natural defense mechanisms to protect against harmful UV radiation from the sun. Its function is based on its ability to absorb, reflect or scatter the sun's rays [7]**.** Measurement of SPF number is ultimate way to determine effectiveness of sunscreen formulation and it became a worldwide standard for measuring the effectiveness of sunscreen products. It gives an idea about how long one can stay in the sun without getting burn by the sun rays. The higher the SPF, the more protection a sunscreen offers against UV-light.

Even though several synthetic sunscreens are available, they have limited applications in cosmetics due to their potential toxicity in humans and ability to interfere only in selected pathways of carcinogenesis. Botanical and herbal agents are known to be safe and have been widely accepted by consumers. They also work in various ways by stimulating the immune response, inducing gene suppression, detoxifying carcinogens, blocking oxidative damage to DNA, initiating selected pathways or by other mechanisms [1]. Thus, these herbal agents play multiple roles in ameliorating the process of carcinogenesis. Therefore, these herbal formulations at optimum concentrations could produce several beneficial effects to the skin apart from functioning as UV filters that is among the aim of this research.

The table 8 gives the summary of the in vitro SPF obtained for the four samples.

As it can be seen in figure 2 and table 8, data of average SPF values of herbal extracts and creams formulated were compared to each other at same concentration indicating herbal extracts in vitro SPF were found to be greater than those of the formulated cream . In their works Shenekar et al [9] and Lokapure [10] obtained similar results.

Among analyzed samples, the carrot extracts exhibits a maximal absorbance higher than that of other samples (figure 1). This is probably due to the fact that the carrot extracts can contain more flavonoids and other phenolics as the most important components

[11, 12] than the other samples, presenting thus, a calculated SPF higher than that of the others. As the creams formulated have the same extracts concentrations, data variation can be due to the composition of those formulations. As it can be seen (table 8), the calculated SPF observed for the mixture paba/herbal extracts in the ratio 1/100 were higher compared to the calculated SPF of herbal extracts alone. The similar results were found by Padhila and Duarte [13] who, showed through their results that after incorporation to a 2% solution of the synthetic sunscreen octhylmethoxycinnamate, the extracts showed intensification in SPF values, suggesting that this can be an interesting method to intensify SPF. It should be therefore a good way to improve the sunscreen UV protection in decreasing sufficiently the synthetic filter proportion.

Table 8: Synthetic table of the in vitro SPF.

	SPF values							
	Ethyl acetate extracts							
Samples	2000 ppm	1000 ppm	500 ppm	2000 ppm+ 20ppm of paba	1000ppm + 10 ppm of paba	500ppm + 5ppm of paba	Cream 2%	Aqueous extract
Carrot (SA)	24.25±0.71	13.77±0.40	6.61±0.49	43.38±0.23	21.44±0.38	7.6±0.45	9.6±0.12	1.69±0.57
Coconut (SB)	3.71±0.20	1.75±0.35	0.74±0.30	16.81±0.23	6.56±0.25	3.3±0.46	0.64±0.17	7.36±0.25
Ccm (SC)	12.17±0.60	5.42±0.41	2.75±0.69	33.82±0.55	8.57±0.65	5±0.28	2.1±0.63	4.09±0.60
Pmw (SD)	5.85±0.49	2.95±0.59	1.59±0.48	14.26±0.42	7.28±0.42	6.33±0.54	1.09±0.23	7.93±0.26

Hence in the present study the spectrophotometric method emphasized on protection against UVB by considering the absorbance in the UVB range i.e. from 290nm-320nm. From the results of the present study, it demonstrated that cream show protection against UVB radiation and indicated as SPF 0.64 to 9.60 for coconut and carrot cream respectively. The in vitro SPF values revealed that the sunscreen formulations can be applicable for different skin type. In fact, different skin type, I-VI requires ideal SPF as shown in Table 9

Table 9: Sun protection factor (SPF) rating, by the way applies only to UVB radiation [3]

Skin type	**Details**	**Ideal SPF**
I	Always burns easily, never tans (Sensitive)	8 or more
II	Always burns easily, tans minimally (Sensitive)	6-7
III	Burns moderately, tans gradually (Light brown, Normal)	4-5
IV	Burns minimally, always tans well (Moderate brown, Normal)	2-3
V	Barely burns, tans profusely (Dark brown, Insensitive)	2
VI	Never burns, deeply pigmented (Insensitive)	Not indicated

Thus, the coconut cream and the Pmw cream can be applicable for skin type VI; carrot cream can be applicable for skin type I to VI. Ccm can be applicable for skin type IV and V. The different creams formulated can be considered as an efficient validated topical product for the most of Kinshasa people.

The result obtained shows that ability of extracts to absorb UV radiation and hence, their UV protection ability. This proved activity of plant, shows its importance and prophylactic utility in anti-solar formulation. This will be a better, cheaper and safe alternative to harmful chemical sunscreens used nowadays in the industry[9].

There are many factors affecting the determination of SPF values, as for example, no applicability of proper methods for evaluation of sunscreen products, the use of different solvents in which the sunscreen are dissolved; the combination and concentration of the sunscreen and herbal extracts; the type of emulsion; the effects and interactions of vehicle components, such as esters, emollients and emulsifiers used in the formulation; the interaction of the vehicle with the skin; the addition of other active ingredients; the pH system, viscosity and the emulsion rheological properties, among other factors, which can increase or decrease UV absorption of each formulation [7].

The effect that different solvents and emollients have upon the wavelength of maximum absorbance and upon the UV absorbance of several sunscreens chemical alone or in combination is well known and documented [1, 3, 6, 14-18]. Active ingredients can produce UV absorption bands, thus interfering with those of UVA and UVB sunscreen. This effect is reflected in the calculated SPF values, especially for the mixture herbal extracts: paba.

Therefore, to develop sunscreens with better safety and high SPF, the formulator must understand the physicochemical principle, not only the UV absorbance of the actives ingredients, but also vehicle components, such as esters, emollients and emulsifiers used in the formulation, since sunscreen can interact with other components of the vehicle, and these interactions can affect formulation efficacy[7]. As it can be seen in figure 2, the in vitro SPF of the extracts are higher than the in vitro SPF of the cream.

CONCLUSIONS

The in vitro SPF values of the ethyl acetate and aqueous extracts of some available herbal found in Kinshasa were evaluated. It showed that most of them have the UV protection capabilities indicating sunscreen activity as well the formulations produced by incorporating herbal extracts. Along with their many beneficial effects and safety, these botanicals could become good, cheap and easily available ingredients for sunscreen formulations.

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