ORIGINAL ARTICLE

Photoprotective Measures of Selected Sunscreens and Their Antioxidant Adjuvantion Effects in the Malaysian Climate

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ABSTRAK

Industri penjagaan kulit Malaysia sedang berkembang maju dengan memperlihatkan peningkatan bilangan krim pelindung cahaya matahari yang dihasilkan setiap tahun. Kekurangan peraturan dan kawalan dalam aspek penjagaan kesihatan kulit menyebabkan ketidaktentuan kualiti krim pelindung cahaya matahari. Objektif kajian ini adalah untuk menentukan keberkesanan dan keselamatan krim pelindung cahaya matahari tempatan berbanding dengan krim antarabangsa serta kesan penambahan antioksidan asli ke dalam krim-krim tersebut. Tiga krim tempatan dan tiga krim luar negara telah dikaji untuk menentukan faktor pencegahan ultraviolet A (UVAPF), faktor pencegahan cahaya (SPF) dan fotostabiliti secara in vitro. Sampel krim disapu pada plat polimetilmetakrilat yang kasar di mana penyerapan cahaya dibaca dengan spektrofotometer sebelum dan selepas dua jam pendedahan kepada radiasi cahaya. Kaedah yang sama diulangi dengan menambahkan krim pekat vitamin C dan E yang terdapat dalam pasaran. Krim dari luar negara didapati mempunyai label SPF yang jitu (p=0.009) manakala krim tempatan adalah lebih stabil terhadap cahaya (p=0.003). Walau bagaimanapun, kedua-dua krim tidak mempunyai UVAPF yang cukup (p=0.471). Penambahan vitamin C meningkatkan nilai SPF (p=0.04) kedua-dua krim manakala vitamin E meningkatkan fotostabiliti (p=0.000) mereka. Penambahan kombinasi vitamin C dan E kepada krim pelindung cahaya matahari dengan menariknya tidak mengubah nilai SPF dan UVAPF berbanding dengan penambahan satu vitamin secara berasingan. Yang pentingnya, kombinasi vitamin menurunkan fotostabiliti (p=0.002) krim berbanding dengan penambahan vitamin E sahaja. Kesimpulannya, pelindungan terhadap cahaya terbaik dicapai melalui penggunaan krim pelindung cahaya bersama dengan krim yang mengandungi hanya salah satu vitamin C atau E sahaja.

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Kata kunci: *krim pelindung cahaya matahari, faktor pencegahan ultraviolet A, faktor pencegahan cahaya, fotostabiliti, antioksidan*

ABSTRACT

The Malaysian skincare industry is growing rapidly with a vast number of new sunscreens being produced annually. Inadequate skincare regulations and lack of enforcement have resulted in the overrating of sunscreens' quality. The objectives of this study were to evaluate the efficacy and safety of the local and international sunscreens and to determine the effects of adding concentrated antioxidants into them. Three local and three internationally manufactured sunscreens were tested for the *in vitro* Ultraviolet A protection factor (UVAPF), Sun Protection Factor (SPF) and photostability. The creams were spread onto roughened polymethylmethacrylate plates where the absorbance was measured using a spectrophotometer before and after two hours of sunlight irradiation. The procedure was repeated combining available concentrated vitamin C and E creams. International sunscreens were found to have more accurate SPF labels (p=0.009) while local sunscreens were found to be more photostable (p=0.003). However, both sunscreens had inadequate UVAPF (p=0.471). Vitamin C enhanced the SPF values (p= 0.04) of both groups of sunscreens while vitamin E enhanced their photostability (p=0.000). Interestingly, combining vitamins C and E rich creams with the sunscreens had no effect on the SPF and UVAPF values as compared to the use of a single vitamin. More importantly, the combination of vitamins decreased the photostability (p=0.002) of sunscreens as compared to the addition of vitamin E alone. In conclusion, photoprotection is best achieved when sunscreens are used together with creams containing either vitamin C or E alone.

Keywords: sunscreen, sun protection factor (SPF), ultraviolet A Protection Factor (UVA PF), photostability, antioxidant

INTRODUCTION

Once an unprotected skin is exposed to the sunlight, ultraviolet (UV) radiation which is absorbed by the skin chromophores interacts with oxygen molecules to generate reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radical anion, hydroxyl radical and singlet oxygen. Eventually, these hazardous ROS damages the neighbouring deoxyribonucleic acid (DNA), proteins, and lipids in the skin (Bastuji-Garin et al. 2002). Therefore, prolonged and repeated exposures to sunlight would cause skin problems such as photodamage, photoaging and skin cancer (Gilchrest 2008).

Sunscreen is claimed to be the gold standard in protecting our skin from photodamage as it contains UV filters that may be present in the form of organic compounds, inorganic molecules or organic particulates (Seite

2000). When the skin is exposed to UV light, UV filters in sunscreen absorb, reflect and scatter the UV irradiation thus preventing it from reaching the skin. Antioxidants have been used as an adjuvant to sunscreens because of their ability to neutralize ROS generated by UV radiation (Seite 2000). Hence, a vast number of sunscreens containing antioxidant additives have been introduced in the market.

Sun Protection Factor (SPF) which measures the amount of protection against UVB (290-320 nm), is a multiple of UV irradiance required to cause erythema on a sunscreen applied skin as compared to a bare skin. A high SPF value indicates a better protection from sun burning of the skin. On the other hand, Ultraviolet A protection factor (UVAPF) measures the amount of protection to UVA (320-400 nm) radiation afforded by sunscreens where a high value indicate a better protection from persistent pigment darkening of the skin when exposed to sunlight (Diffey et al. 2000). In our search for sunscreen samples for this study, most sunscreens were found to have no information regarding this value.

Photostability is defined as the ability of a product to retain its integrity upon exposure to light. A product is considered photostable when the reduction of SPF and UVAPF values are less than 20% after exposure to light (Garoli et al. 2008). A research conducted by Herzog and Sommer (2000) found that chemical compounds in sunscreens that are unstable with exposure to sunlight may serve as another source of free radicals upon degradation.

Steven et al. (2010) found that antioxidant additives in some sunscreens had no effect in naturalizing ROS developed in photodamaged skin. Various reasons were postulated as the cause of this finding such as low antioxidant power, low biological activity, insufficient concentration of antioxidant as well as the inability of antioxidant to penetrate the skin. Thus, more effective methods and means of adding antioxidants to improve photoprotection are much needed.

Therefore, the aim of this study is to compare the photoprotective measures of some commercially available sunscreens in Malaysia. The efficacy and safety of international and local branded sunscreens is compared in terms of their SPF label accuracy, relative UVAPF level and their photostability. The adjuvant effects of adding concentrated vitamin C and E creams in improving photoprotection is also assessed since these vitamins have been tested to protect against skin cancer and photoaging though their efficacy towards SPF and UVAPF values have yet to be determined to date (Lin et al. 2003).

MATERIALS AND METHODS

Sample Size

The sample size was calculated based on Mead's Resource Equations 1988. The error of degree of freedom was set in between 10-20. A total of three local and three international manufactured sunscreens were analysed in triplicates in this study, based on the European Cosmetic Products Trade Association

(COLIPA) guideline for UVAPF 2009 determination (Table 1).

Operational Definitions

The international and local sunscreens were selected from a range of commercially available sunscreens that were manufactured outside and in Malaysia respectively.

Inclusion Criteria

The selected sunscreens contained no antioxidants and had details of the country from where they were manufactured. The antioxidant creams contained mainly vitamins C or E as the active ingredient. Table 2 shows the details of sunscreens and antioxidant creams used in the experiment.

Table 1: Sample Size and Combination of the Test Samples

Manufacture Place	Sunscreen Type	Combination of Test Samples (unit)			
		Sunscreens Only	Vitamin C	Vitamin E	Vitamin C+E
International	Sunscreen 1	3	3	3	3
	Sunscreen 2	3	3	3	3
	Sunscreen 3	3	3	3	3
Local	Sunscreen 4	3	3	3	3
	Sunscreen 5	3	3	3	3
	Sunscreen 6	3	3	3	3
Total		18	18	18	18

Table 2: Details of the Sunscreen and Antioxidant Creams

Product	SPF Label	Major Ingredients
International Sunscreens		
Sunscreen 1	50+	Ethylhexyl Methoxycinnamate, Zinc Oxide, Diethylamino Hydroxybezoyl Hexyl Benzoate
Sunscreen 2	50+	Zinc Oxide, Ethyhexyl Methocinnamate, CL 77891
Sunscreen 3	50+	ButylMethoxydibenzoylmethane, Octocrylene, Benzophenone-3, Ethylhexyl Salicyclate
Local Sunscreens		
Sunscreen 4	20+	Titanium Dioxide, Octyl Methoxyccinnamate, Butyl Methoxydibenzoylmethana
Sunscreen 5	25	Titanium Dioxide, Ethylhexyl Methoxycinnamate
Sunscreen 6	25	Titanium Dioxide, Hydroxystearic Acid
Antioxidant Creams		
Vitamin E cream (Concentration: 10000 iu/ 200 ml)		Tocopheryl Acetate Butylated hydroxytoluene (BHT)
Vitamin C cream (Concentration: 50x)		Glycyrrhiza Glabra Root Extract Myrarciacia Dubia Fruit Juice Malpighia Glabra Fruit Juice

Table 3:UV Index at 31st of May 2012 by hour

Time	10:23	10:58	11:32	12:07	12:42	13:14	13:26	14:04	14:44	15:22	16:02
UV Index	4.55	5.91	3.84	4.44	4.33	6.96	4.08	4.16	7.27	5.43	3.78

Method

Roughened polymethylmethacrylate (PMMA) plates with measurement of 40mm x 40mm x 2.5mm from NIC Advertising, Ampang, Selangor, Malaysia were used. An amount of 2mg/cm^2 of sunscreens alone and 4mg/cm^2 of the combinations of sunscreens with Vitamin C or/and E creams was added onto the plate with the aid of a weighing scale with variations 5% of weight. The test samples were applied onto the plate as multiple small droplets and then spread as even as possible. The test sample was then allowed to stabilize for 15 minutes in a dark, air conditioned room.

The absorbance profile of the test samples were measured by a two-beam UV/VIS Spectrophotometer Shimadzu UV-2450 spectrophotometer.

Polymethylmethacrylate (PMMA) plate covered with test samples was placed in a plate holder of the spectrophotometer. The UV absorbance of each test sample was measured from 290-400 nm at 1 nm intervals at five different places per plate. The data was read using UVProbe 2.33. The average of five readings was taken for analysis. Since consumers are commonly advised to reapply sunscreens every two hours (Diffey 2001), the test samples were also exposed under the sun for two hours (12:00 pm-14:00 pm). Table 3 shows the UV index of the day of the experiment provided by the Malaysian Meteorological Department, Petaling Jaya, Selangor, Malaysia. Hence, the average UV index for two hours of irradiation was 4.74.

The absorbance profile of the test samples after irradiation was taken again. All data collected was analysed using Statistical Product and Service Solutions (SPSS) version.20.

RESULTS

Figure 1 shows examples of absorbance profiles of test samples. The shape and height of the curves varied depending on the type and concentration of UV filters used in the test samples. The in vitro SPF and UVAPF values as well as photostability were calculated based on the listed formulas.

Formulae Calculating In vitro SPF, In vitro UVAPF and Photostability

$$SPF\ in\ vitro = \frac{\int_{290\ nm}^{400\ nm} E(\lambda)*I(\lambda)*d\lambda}{\int_{290\ nm}^{400\ nm} E(\lambda)*I(\lambda)*10^{-A(\lambda)}*d\lambda}$$

where,

E (λ) = Erythema Action Spectrum at wavelength λ

I (λ) = Spectral Irradiance of UV source at wavelength λ

d λ = Wavelength step = 1nm

A (λ) =Absorbance of the test samples at wavelength λ

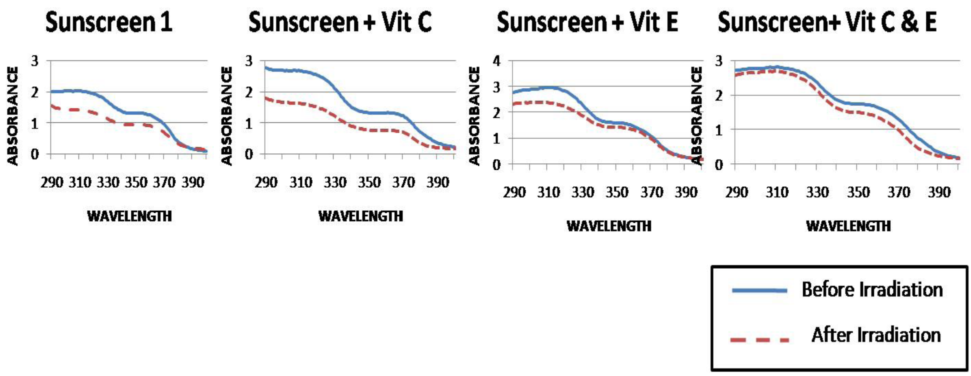

Figure 1: Example of Absorbance Profiles for Various Combinations of Test sample

$$\mathrm{UVA-PF} = \frac{\int_{290\ nm}^{400\ nm} P(\lambda) * I(\lambda) * d\lambda}{\int_{290\ nm}^{400\ nm} P(\lambda) * I(\lambda) * 10^{-A(\lambda)} * d\lambda}$$

P (λ) = Persistent Pigment Darkening Action Spectrum at wavelength λ
I (λ) = Spectral Irradiance of UV source at wavelength λ
dλ = Wavelength step = 1nm
A (λ) = Absorbance of the test samples at wavelength λ

$$Photostability = \frac{SPF\ (before\ exposure)}{SPF\ (after\ exposure)}\ x\ 100\%$$

Local vs International Sunscreens

SPF Label Accuracy

Coefficient of adjustment (C) was used as a standard to measure the accuracy of SPF label of the sunscreen. It was calculated by using formula 4. Values between 0.8-1.2 meant the SPF label was accurate. Figure 2 shows SPF label and in vitro SPF of various sunscreens.

Formula 4: Calculation for Coefficient of Adjustment

$$SPF\ label = \frac{\int_{290\ nm}^{400\ nm} E(\lambda) * I(\lambda) * d\lambda}{\int_{290\ nm}^{400\ nm} E(\lambda) * I(\lambda) * 10^{-A(\lambda) * C} * d\lambda}$$

where,

E (λ) = Erythema Action Spectrum at wavelength λ
I (λ) = Spectral Irradiance of UV source at wavelength λ
d λ = Wavelength step = 1nm
A (λ) = Absorbance of the test samples at wavelength λ
C = Coefficient of adjustment

A total of nine (100%) international sample plates had accurate SPF label while only three (33.3%) local sample plates had C values that fell between 0.8-1.2. A Fisher's exact test was computed to test the association between manufacturing places and the SPF label accuracy. The result revealed that there was significant association between the manufacturing place and the SPF label accuracy. The international sunscreens had more accurate SPF label than local

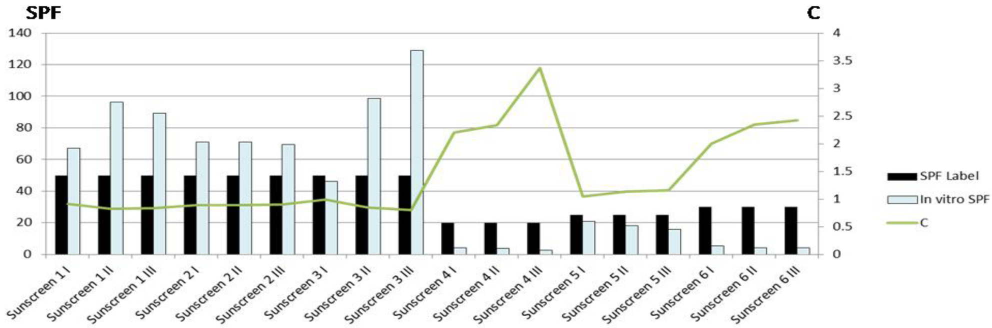

Figure 2: Comparison between Local and International Sunscreens in terms of SPF Label Accuracy

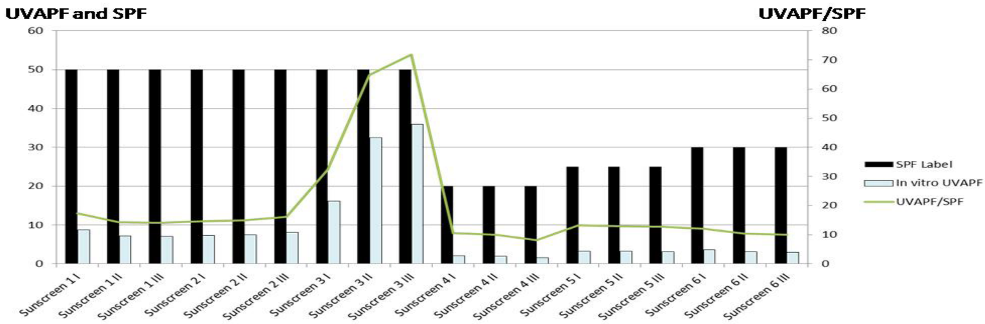

Figure 3: Comparison of Relative UVAPF Level between Local and International Sunscreens

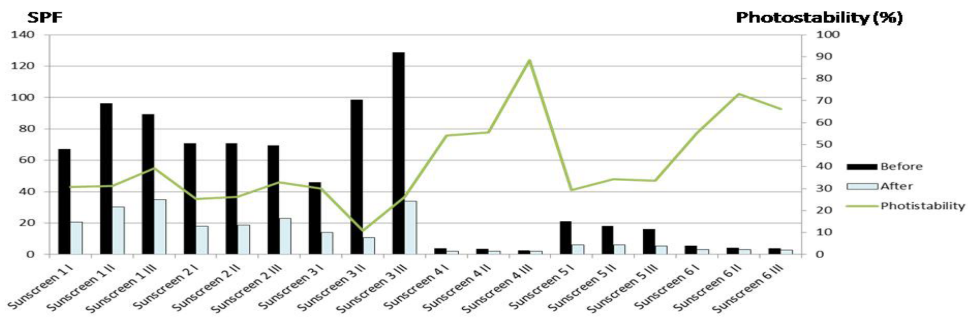

Figure 4: Comparison of Photostability between Local and International Sunscreens

sunscreens (p=0.009). The details of the analysis are shown in Table 4.

Relative UVAPF Level

According to the European Commission 2006, sunscreens should provide UVAPF of at least one third of the SPF label to ensure balanced and adequate protection against both UVA and UVB radiation. Figure 3 shows the comparison of relative UVAPF level between local and international sunscreens. There were only two (22.2%)

Table 4: Analysis of the Comparison between Local and International Sunscreens in terms of SPF Label Accuracy

	SPF Accuracy		Total	Test	p
	Acceptable	Not Acceptable			
International	9 (100.0%)	0 (0.0%)	9 (100.0%)	Fisher's Exact Test	0.009
Local	3 (33.3%)	6 (66.7%)	9 (100.0%)		
Total	12	6	18		

Table 5: Analysis for the Comparison of Relative UVAPF Level between Local And International Sunscreens

	Relative UVAPF Level		Total	Test	p
	Acceptable	Not Acceptable			
International	2(22.2%)	7 (77.8%)	9 (100.0%)	Fisher's Exact Test	0.471
Local	0 (0.0%)	9 (100.0%)	9 (100.0%)		
Total	2	16	18		

Table 6: Analysis for the Comparison of Photostability between Local and International Sunscreens

Manufacture Place	N	Median	Interquatile range	Test	Mean Rank	p
International	9	30.13	6.31	Mann-Whitney U	5.78	p =0.003
Local	9	55.4	35.72		13.22	
Total	18					

international sunscreens with acceptable UVAPF to SPF label ratio. Fisher's exact test proved that most of the sunscreens regardless of the manufacturing place were not providing enough UVA protection (p=0.471) as shown in Table 5.

Photostability

Figure 4 displays the photostability of international and local sunscreens. Local sunscreens scored a mean rank of 13.22 while the mean rank for international sunscreens was 5.78 only in Mann-Whitney U test (Table 6). Hence, local sunscreens were more photostable than international sunscreens (p=0.003).

Roles of Antioxidant Creams in Photoprotection

Differences Before and After Adding Antioxidants onto Sunscreens

A comparison between in vitro SPF, in vitro UVAPF and photostability of sunscreens alone, sunscreens added with Vitamin C cream, sunscreens added with Vitamin E cream and sunscreens added with a combination of Vitamins C and E cream was performed. Table 7 shows the differences before and after adding antioxidants into sunscreens according to the above-mentioned combination. With reference to the Kruskal-Wallis

Table 7: Differences Before and After Adding Antioxidants onto Sunscreens

Parameter	Combination	N	Median	Interquatile Range	Test	Mean Rank	p
In vitro SPF	Sunscreen only	18	33.56	71.38	Kruskal Wallis	25.17	p =0.009
	Sunscreen + Vitamin C	18	115.9	287		45.67	
	Sunscreen + Vitamin E	18	77.82	199.5		31.72	
	Sunscreen + Vitamin C and E	18	67.77	239.2		43.44	
In vitro UVAPF	Sunscreen only	18	5.35	5.13	Kruskal Wallis	25.72	p =0.007
	Sunscreen + Vitamin C	18	10.63	15.41		45.78	
	Sunscreen + Vitamin E	18	5.98	12.06		30.44	
	Sunscreen + Vitamin C and E	18	9.63	12.26		44.06	
Photostability	Sunscreen only	18	33.22	26.9	Kruskal Wallis	36.5	p =0.002
	Sunscreen + Vitamin C	18	22.46	12.51		21.22	
	Sunscreen + Vitamin E	18	54.66	29.8		47.06	
	Sunscreen + Vitamin C and E	18	60.04	66.44		41.22	

tests, addition of either Vitamin C or E creams with sunscreens improved the in vitro SPF (p=0.009), in vitro UVAPF (p=0.007), and photostability (p=0.002).

Differences between the Photoprotection Levels of Vitamins C and E Cream

Vitamin C cream enhanced the SPF of the sunscreens (p=0.040) while Vitamin E made the sunscreens more photostable (p=0.000). However, there is no significant differences between Vitamin C and E in improving UVAPF (p=0.054) of the sunscreens (Table 8).

The Synergistic Effects of Combining both Vitamins C and E Cream

We observed that combining both Vitamins C and E cream into a sunscreen did not significantly improve the SPF and UVAPF (p>0.005) compared to the use of a single antioxidant cream. More importantly, the above combination also showed lower photostability (p=0.002) compared to adding Vitamin E alone (Table 9).

Table 8: Effects of Adding Vitamin C or Vitamin E Creams nto Sunscreens

Parameter	Combination	N	Median	Interquatile range	Test	Mean Rank	p
In vitro SPF	Sunscreen + Vitamin C	18	115.9	287	Mann-Whitney U = 97.00	22.11	p =0.040
	Sunscreen + Vitamin E	18	77.82	199.5		14.89	
In vitro UVAPF	Sunscreen + Vitamin C	18	10.63	15.41	Mann-Whitney U =101.00	21.89	p =0.054
	Sunscreen + Vitamin E	18	5.98	12.06		15.11	
Photostability	Sunscreen + Vitamin C	18	22.46	12.51	Mann-Whitney U =38.00	11.61	p =0.000
	Sunscreen + Vitamin E	18	54.66	29.8		25.39	

DISCUSSION

Local vs International Sunscreens

SPF Label Accuracy

Results of analysis showed that local sunscreens had less accurate SPF label than international sunscreens probably because the selected locally produced sunscreens did not comply with the Good Manufacturing Practice (GMP) guidelines which recommend products to be tested in terms of quality, packaging, safety, toxicology and labeling before being awarded with the certificate.

Relative UVAPF Level

Our result is consistent with the finding from Environmental Work Group (EWG) 6th Annual Sunscreen Guide 2012 where most of the sunscreens were found to provide inadequate UVA protection. However information regarding UVAPF provided by the sunscreens was not a requirement in passing the Safety Assessment of Cosmetic Product in any country. Most sunscreens were found to screen out UVB only. Thus, prolonged exposure to sunlight does increase the risk of melanoma despite the use of sunscreens (Gorham 2007).

Photostability

After two hours of irradiation under the sun, all sunscreen samples had decreased in vitro SPF values. This might be due to degradation of UV filters with exposure to irradiation which decreases the photostability of sunscreens thus leading to lower SPF values.

According to the EWG Skin Deep Sunscreen Guideline 2011, low-SPF products contained fewer amounts of UV filters than high-SPF sunscreens. Consumers are not recommended to use sunscreens with high SPF values because degradation of excessive UV filters during exposure to sunlight produces ROS which damages the skin (XX). Sunscreens with high SPF values are thus suitable only for short duration of sun exposure. In our study, SPF

Table 9: Synergistic Effects of Combining Both Vitamins C And E Cream

Parameter	Combination	N	Median	Interquatile Range	Test	Mean rank	p
In vitro SPF	Sunscreen + Vitamin C	18	115.9	287	Kruskal Wallis	31.39	p =0.100
	Sunscreen + Vitamin E	18	77.82	199.5		21.06	
	Sunscreen + Vitamin C + E	18	67.77	239.2		30.06	
In vitro UVAPF	Sunscreen + Vitamin C	18	10.63	15.41	Kruskal Wallis	31.44	p =0.115
	Sunscreen + Vitamin E	18	5.98	12.06		21.28	
	Sunscreen + Vitamin C + E	18	9.63	12.26		29.78	
Photostability	Sunscreen + Vitamin C	18	22.46	12.51	Kruskal Wallis	17.22	p =0.02
	Sunscreen + Vitamin E	18	54.66	29.8		34.5	
	Sunscreen + Vitamin C + E	18	60.04	66.44		30.78	

values for locally produced sunscreens were 50% lower than the international sunscreens. Hence, locally produced sunscreens are suggested to be more photostable than international sunscreens.

Roles of Antioxidant Creams in Photoprotection

Using vitamin C, vitamin E or the combination of both creams together with sunscreens are found to improve the function of sunscreens in terms of SPF, UVAPF and photostability.

According to Dominique Lutz et al. (1999), SPF and UVAPF values are affected by the concentration of UV filters and the thickness of sunscreens applied. This is in line with our study which found that the increased thickness of applied sunscreens upon application of antioxidant creams showed increased values of SPF and UVAPF. In other words, antioxidant creams are able to enhance the photoprotection of sunscreens without increasing the concentration of UV filters.

In addition, photostability of the sunscreens is also improved with the use of antioxidant creams. This may be due to the capability of antioxidants to neutralize free radicals generated from UV light and degraded UV filters (Edlich et al. 2004). This in turn prevents subsequent generation of ROS which disrupts the integrity of sunscreen leading to increased photostability.

Vitamin C cream increased the in vitro SPF level more than vitamin E cream. Both vitamins C and E have been shown to have no UVB filtering functions (John et al. 2008). The increase in SPF value could have been contributed by minor additives in the Vitamin C cream such as Ethylhexyl Methoxycinnamate and

Butyl Methoxydibenzolmethane which are UVB filters.

There is no significant difference between vitamins C and E in improving UVAPF of the sunscreens. As mentioned before, antioxidant creams act as a supplement to the sunscreen since they have no UV filtering properties.

Meanwhile, vitamin E cream showed higher efficacy in improving the photostability of the sunscreen compared to vitamin C. α-Tocopherol acetate (Vitamin E) is stable and will not be converted to its unstable free form α-tocopherol if applied topically (Alberts et al. 1996). On the other hand, L- ascorbic acid (Vitamin C) will always be metabolized to its unstable counterpart by the skin (Sheldon et al. 2001). Though effort had been put in esterifying L-ascorbic acid to make it more stable in topical cream, unfortunately it is still less stable than α-tocopherol.

The Synergistic Effects of Combining Both Vitamins C and E Cream

The synergistic effects of combining both vitamins C and E cream were not observed in our experiment. This combination improved the SPF and UVAPF values insignificantly compared to adding Vitamin C or E alone. This is contradictory to findings by other researchers. The explanation for this insignificance is probably due to the same thickness that we applied for the three combinations.

Combining both Vitamin C and E onto sunscreens demonstrated lower photostability in comparison to adding Vitamin E alone. We postulated that portion of degraded Vitamin C reduces the stability of this combination.

CONCLUSION

The use of sunscreen alone is not effective regardless of the place of manufacture. Concentrated antioxidant creams should be used as adjuvant for photoprotection. As concurrent use of vitamins C and E does not improve the quality of sunscreen, it is best to apply a layer of sunscreen together with vitamin C rich cream for intense sun exposure or Vitamin E rich cream for longer duration of sun exposure.

ACKNOWLEDGEMENT

- Department of Biochemistry and Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Raja Muda Aziz, 50300 Kuala Lumpur, Malaysia
- Malaysian Meteorological Department, Petaling Jaya, Selangor, Malaysia
- This research was approved by the UKM Research Ethics committee and funded by Dana Fundamental UKM (FF-100-2012)

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