

significant (Stahl & Sies, 2012). An additional benefit of topical application over dietary β -carotene is that β -carotene supplements have been shown to increase the incidence of lung cancer in individuals with high risk of cancer (Stahl & Sies, 2012). However, in general it is recommended that both dietary and topical photoprotection are complementary to each other.

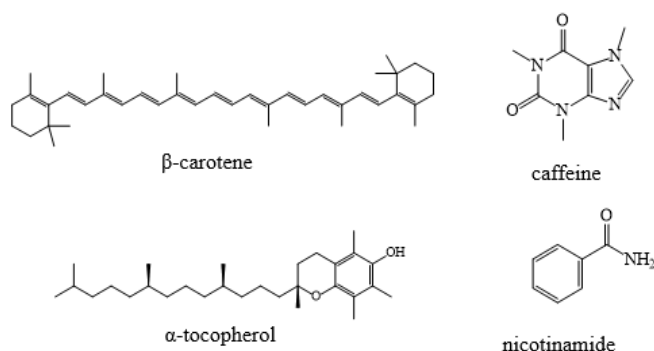


Fig. 1 The antioxidants studied in this work.

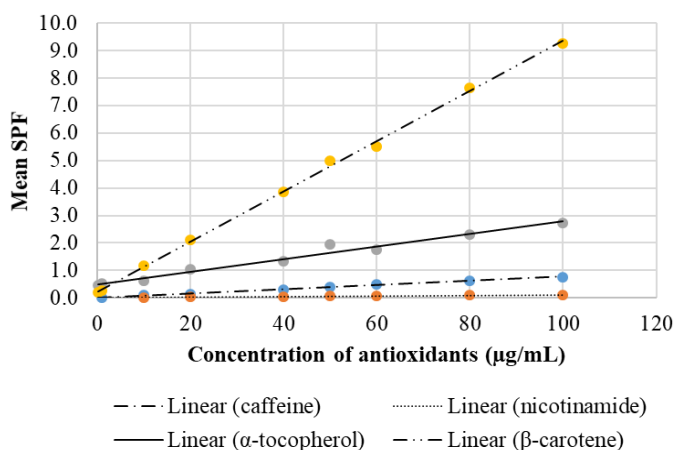


Fig. 2 The mean SPF (n=3) values of antioxidant solutions from concentration 0.1-100 µg/mL in 95% ethanol.

It is worth to note that the SPF of L-ascorbic acid solution was also investigated (Fig. 3). However, the R^2 value of the concentration curve of L-ascorbic acid was found to be 0.2593, indicating that there is no linear relationship between SPF and concentration. The inconsistency in the SPF observed for L-ascorbic solutions may be caused by the degradation of L-ascorbic acid in ethanol solution. Ascorbic acid has been shown to degrade in ethanolic solutions during storage, and that the degradation rates increase with the increase of ethanol concentration (Yuan & Chuan, 1998). Therefore, we suggest that in topical formulations containing L-ascorbic acid, high concentrations of ethanol should be avoided. As ascorbic acid is highly sensitive to air and light, various solutions have been proposed to achieve stabilization of ascorbic acid in cosmetic and pharmaceutical applications (M. Sheraz et al., 2011; M. A. Sheraz, Khan, Ahmed, Kazi, & Ahmad, 2015). Among the reported method is to use ascorbic acid in combination with other vitamins, such as α -tocopherol, to retard its oxidative degradation. The stability of ascorbic acid in oil-water creams were found to be highest in the presence of α -tocopherol compared to other vitamins including riboflavin and nicotinamide (Sheraz et al., 2015). Therefore, we were interested to investigate the SPF property of mixtures containing both L-ascorbic acid and α -tocopherol. L-ascorbic acid (20 µg/mL) solution in varying concentrations of α -tocopherol (0.1-100 µg/mL) was prepared in 95% ethanol, and the SPF were calculated from the absorbance values (Fig. 4).

We found that the SPF of the mixture solution increased proportionally to the concentration of α -tocopherol, with R^2 value of 0.994. However, the SPF of the mixture solutions were found to be

lower compared to the solution of α -tocopherol alone. This suggest that the addition of L-ascorbic acid may have decreased the UV absorbing properties of α -tocopherol. Interestingly, Khamsiah et al. (2012) reported that photoprotection was best achieved when sunscreens are used together with creams containing either vitamin C or E alone. The authors found that combining vitamins C and E rich creams with sunscreens had no effect on the SPF compared to the use of a single vitamin (Khamsiah et al., 2012). Nevertheless, *in vivo* studies have suggested the benefits of combining topical vitamins C and E to protect the skin against UV irradiation and photoprotection (Ba, Hu, & Wang, 2012; Lin et al., 2003). However, it has been suggested that protection by the combination of these two antioxidants is not by their sunscreen effect, but mainly as a consequence of the potent antioxidant properties of both of the vitamins (Dreher, Gabard, Schwindt, & Maibach, 1998). Ebrahimzadeh et al. (2014) found no correlation between antioxidant activity and SPF of some medicinal plants. Hence, in a mixture of various antioxidants, the antioxidant property may not correlate with SPF values. Therefore, although we have shown that L-ascorbic acid may reduce the SPF property of α -tocopherol, the photoprotection in terms of increased antioxidant activity obtained with the combination mixture may outweigh the reduction in SPF. To compensate for the reduction in SPF, we suggest that formulations containing both of the antioxidants should contain additional photoprotective agents with potent UVB absorbing properties.

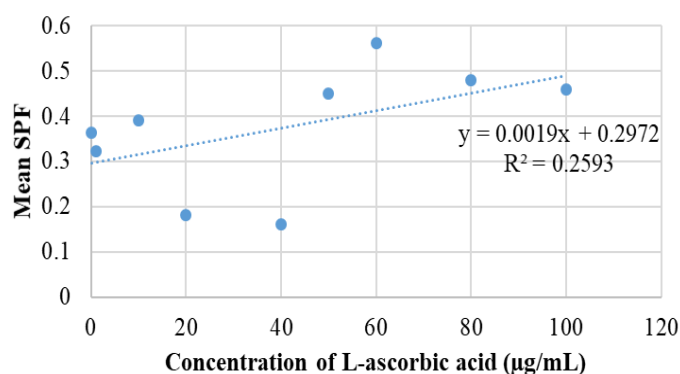


Fig. 3 The mean SPF (n=3) values of L-ascorbic acid from concentration 0.1-100 µg/mL in 95% ethanol.

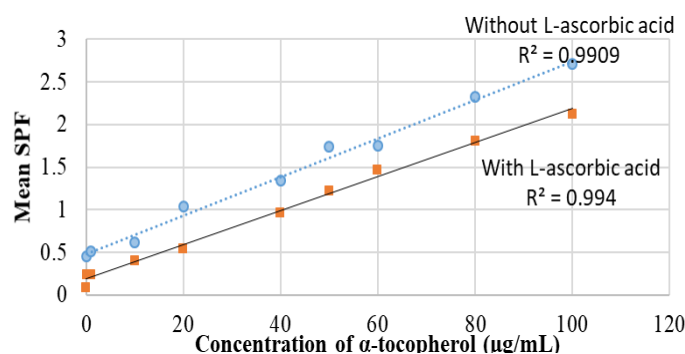


Fig. 4 Normalized mean SPF (n=3) values of α -tocopherol solution (0.1-100 µg/mL), with and without the addition of L-ascorbic acid (20 µg/mL).

Effect of antioxidants on the SPF of sunscreen agents

To study the effect of antioxidants on the SPF of the sunscreen agents, solution of sunscreen agents in 95% ethanol with various concentration of antioxidant (0.1-100 µg/mL) were prepared. The absorbance of these solutions at 290-310 nm were measured with UV/VIS spectrophotometer. The SPF of the solutions were then determined using the Mansur equation. In general, addition of the antioxidants was found to significantly increase the protective capability of the sunscreen agents.

β -carotene was found to have the largest effect on the SPF of octocrylene solution, while α -tocopherol had the largest effect on the

SPF of oxybenzone solution (Figs. 5 and 6). This results supports that incorporating antioxidants with sunscreen agents can contribute to SPF value. In human models, topical application of sunscreen formulations with mixture of antioxidants including caffeine, tocopherol acetate and vitamin E have been shown to further decrease UV-induced damage compared with sunscreen alone (Matsui et al., 2009; Wu et al., 2011). Interestingly, Wu et al. (2011) reported that protection against induction of MMP-9, a marker of photoaging, was not significant when either sunscreen formulation or antioxidant formulation were applied separately. However, complete protection against MMP-9 induction was observed when the two were combined. The mechanism of the added photoprotection by the antioxidants have been attributed to their ability to boost the body's natural reserve and neutralize reactive oxygen species (ROS) from intrinsic sources (e.g. cellular metabolism) and extrinsic factors (e.g. UV damage). UVA is particularly efficient at inducing ROS and is considered to be a major contributor to the process of cutaneous photodamage and photoaging (Wang, Osterwalder, & Jung, 2011). However, our results suggested that in addition to quenching ROS, the antioxidants tested in this study were also capable of enhancing the photoprotection of sunscreen agents by absorption of the UV radiation, indicated by the increase in the SPF. The antioxidants studied in this project are natural compounds and are relatively safe to be included into topical skincare products. Vitamins C, E, and β -carotene are well-characterized vitamins which are commonly added into commercial sunscreen preparations (Pinnell, 2003), with tocopherol being the most predominant vitamin found in sunscreens (Monico et al., 2015). The normal content of the antioxidants studied in this project in commercially available topical formulations as well as their reported benefit as topical application is listed in Table 2.

Table 2 The typical content of the antioxidants used in this study in topical formulations.

| Antioxidants | Typical content | Beneficial properties as topical application |
|-------------------|----------------------------------|---|
| caffeine | Up to 3% (Herman & Herman, 2012) | Photo aging, anticellulite, antioxidant, hair growth promoter, increases the microcirculation of blood in the skin (Herman & Herman, 2012). |
| nicotinamide | Up to 4% (Damian, 2010) | Anti-inflammatory, antipruritic, lightening, antimicrobial, photoprotective (Wohlrab & Kreft, 2014). |
| vitamin E | 0.5 to 1% (Keen & Hassan, 2016) | Antioxidant, skin aging, treatment of granuloma annulare, reducing dark under-eye circles (Keen & Hassan, 2016). |
| β -carotene | Not available | UVA/VIS protection (J. V. Freitas & Gaspar, 2016), infra-red protection (Darvin, Fluhr, Meinke, et al., 2011), improves cutaneous penetration of UV filters (J. V. Freitas, Praça, Bentley, & Gaspar, 2015), improves photostability of sunscreen agent (J. V. Freitas, Lopes, & Gaspar, 2015). |

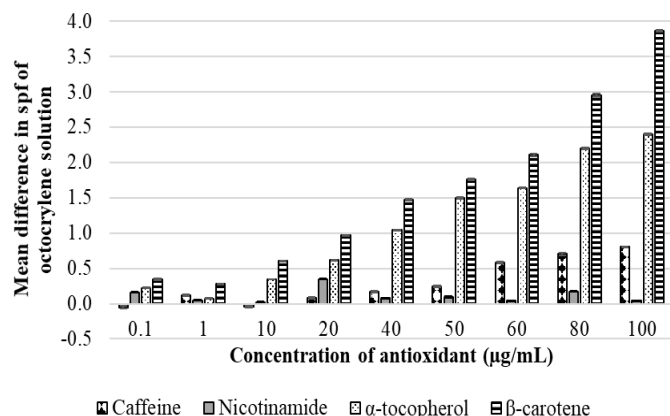


Fig. 5 Difference in mean SPF (n=3) of octocrylene solution (5 µg/mL) with antioxidants compared to without antioxidants. The values are obtained by subtracting the mean SPF value of solutions containing antioxidants with the mean SPF without any antioxidants.

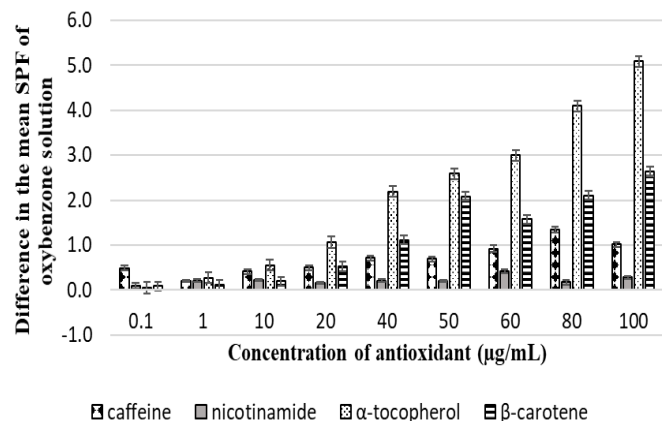


Fig. 6 Difference in mean SPF (n=3) of oxybenzone solution (20 µg/mL) with antioxidants compared to without antioxidants. The values are obtained by subtracting the mean SPF value of solutions containing antioxidants with the mean SPF without any antioxidants.

The mechanism of the SPF enhancing properties of the antioxidants observed in this work remains to be investigated. If the increased in UV absorbance of the sunscreen solutions with the antioxidants were caused by the additive effect of the SPF of the antioxidants, then we would have expected the SPF of the sunscreen solutions to be affected to the similar extend by the same antioxidants. However, this was not what we observed. For octocrylene solution, increase in SPF was most prominent with β -carotene, followed by α -tocopherol, caffeine, and lastly nicotinamide. In the case of oxybenzone, increase in SPF was most prominent with α -tocopherol, followed by β -carotene, caffeine and lastly nicotinamide.

Determination of the SPF of cactus extract

The mean SPF of the cactus extract solution of concentration 3.78 mg/mL and 75.52 mg/mL were calculated as 10.72 ± 0.06 and 5.32 ± 0.01 , respectively. The observed result suggested that the cactus extract contains compounds capable of absorbing UV radiation between the region 290-310 nm, as also indicated by the absorbance spectrum of the cactus extract (Fig. 7). The SPF of the cactus extract was found to be concentration dependent (Fig. 8).

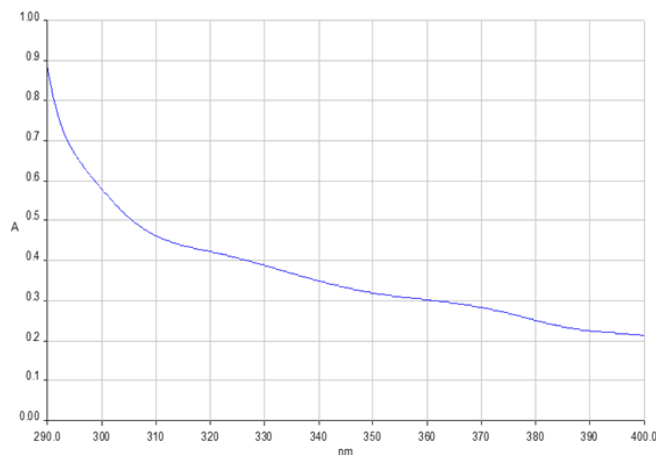


Fig. 7 UV absorbance spectrum of the cactus extract (3.78 mg/mL). The absorbance spectrum was measured with a Perkin Elmer Lambda 25 UV-VIS Spectrometer.

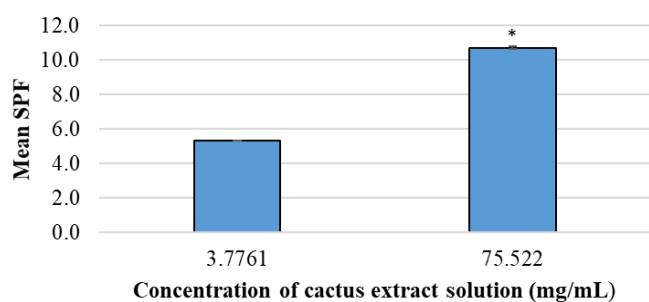


Fig. 8 Mean SPF values of cactus extract solutions (n=3). Significance difference ($p < 0.05$) in SPF of 75.52 mg/mL cactus solution compared to 3.77 mg/mL cactus solution is indicated by the asterisk sign.

Naturally derived compounds are attractive sources of photoprotection, as they are considered safer to human health compared to synthetic sunscreens. The cactus extract could be potentially used as natural sunscreen agent to replace or reduce the concentration of synthetic sunscreen agents. The observed SPF property of the cactus extract may be due to the presence of UV absorbing compounds present in plants such as flavonoids. Flavonoid is an ubiquitous group of naturally occurring polyphenolic compounds characterized by the flavan nucleus. The amount of flavonoids produced by a plant is considered an important factor in the protection of the plant against ultraviolet radiation (Saewan & Jimtaisong, 2013). Infrared analysis was performed on the cactus extract which showed absorption bands at 3364cm^{-1} (broad) and 1614cm^{-1} , which possibly corresponded to O-H stretch and C=O (conjugated) stretch of the flavonoids (Fig. 9). However, it cannot be determined at this stage if flavonoids are present in the cactus extract, and further work is required to characterize the components in the cactus extract. Separation of the cactus extract components can be achieved via preparative HPLC, followed by characterization of the separated compounds by nuclear magnetic resonance (NMR) or tandem mass spectrometry. Alternatively, separation and characterization of the cactus extract components can be performed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Preliminary work done by HPLC suggest that the cactus extract contains several components, with the main compound eluting at 2.204 minutes (Fig. 10).

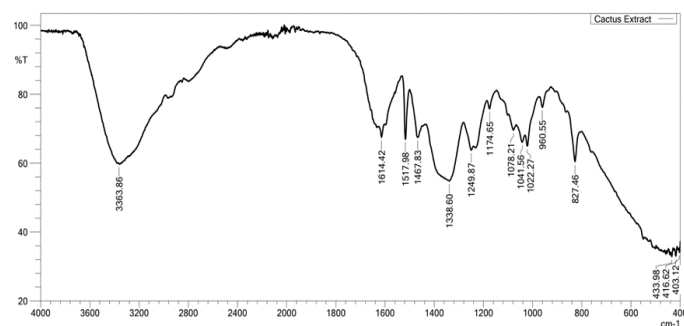


Fig. 9 The IR spectrum of cactus extract. The spectrum was recorded using a Perkin Elmer Lambda 2 FTIR.

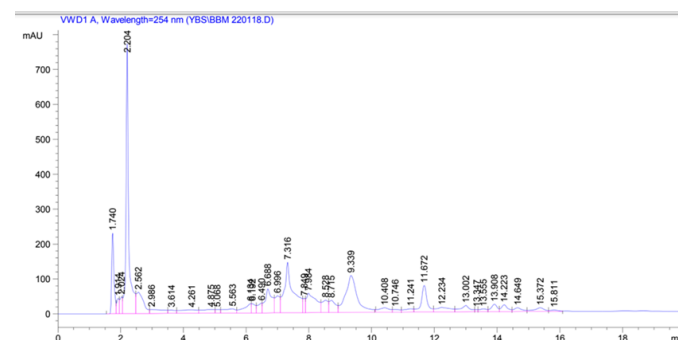


Fig. 10 HPLC chromatogram of cactus extract (3.78 mg/mL) recorded at the wavelength of 254nm.

The effect of cactus extract on the SPF of octocrylene and oxybenzone

The cactus extract was mixed with each of the sunscreen agent in 95% ethanol. The final concentration of cactus extract in each of the sunscreen solutions was 3.78 mg/mL. The concentration of oxybenzone was $20\ \mu\text{g/mL}$ and the concentration of octocrylene was $5\ \mu\text{g/mL}$. The cactus extract was shown to significantly increase the SPF of oxybenzone and octocrylene solutions (Figs. 11 and 12). However, the mechanism of the SPF-enhancing properties of the cactus extract on the SPF of octocrylene and oxybenzone remains to be investigated.

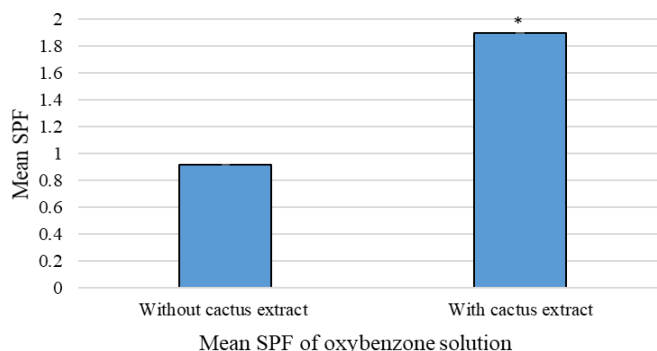


Fig. 11 The mean SPF (n=3) of oxybenzone solution ($20\ \mu\text{g/mL}$), with and without the cactus extract (3.78 mg/mL). Significant difference ($p < 0.05$) in SPF of oxybenzone solution with cactus extract, compared to oxybenzone solution only is indicated by the asterisk sign.

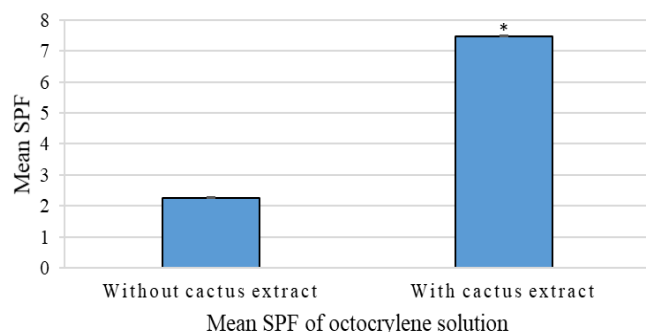


Fig. 12 Mean SPF (n=3) of octocrylene solution ($5\ \mu\text{g/mL}$), with and without cactus extract (3.78 mg/mL). Significance difference ($p < 0.05$) in SPF of octocrylene solution with cactus extract, compared to octocrylene solution only is indicated by the asterisk sign.

CONCLUSION

Antioxidants are frequently added into sunscreen formulations to enhance the photo protection benefit of the product due to their radical quenching abilities, and to maintain stability of the product. The results from this work suggests that antioxidants may also increase the photo protection benefit of sunscreen formulations by increasing the SPF. α -Tocopherol and β -carotene were found to increase the SPF of octocrylene and oxybenzone solutions the most. To the best of our knowledge, the present work is the first to demonstrate the SPF enhancing properties of caffeine, nicotinamide, α -tocopherol, and β -carotene in octocrylene and oxybenzone solutions. Although the exact mechanism is not clear, this work suggests that additional photoprotection may be achieved by appropriate mixtures of sunscreen agents and antioxidants. In addition to the antioxidants, cactus extract was also shown to increase the SPF of the sunscreen agents.

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