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Article

Development of sunscreen products containing passion fruit seed extract

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Application of sunscreen is the most established method of protecting skin from premature aging and photoaging. In this study, the passion fruit seed extract, enriched with biologically beneficial phenolics, was formulated into sun-protective makeup product. The UVB protection of concealer mousse was found have twofold higher sun protection factor (SPF) than the liquid foundation (15.48 ± 1.60 and 5.88 ± 0.30, respectively). The SPF of concealer mousse as well as the liquid foundation containing 0.1% and 0.3% of the passion fruit seed extract were 18.75 ± 0.28, 18.99 ± 0.71 and 9.32 ± 0.88, 9.77 ± 1.37, respectively. Therefore, the concealers with a similar sun-protective efficacy ($p>0.05$) were included for stability test accordingly. The sun-protective efficacy did not significantly shift ($p>0.05$) because the 0.1% and 0.3% passion fruit extract concealers had SPF of 18.09 ± 1.48 and 18.60 ± 1.21. The concealers exhibited UVA photoprotection with a boot star rating of 4 and a critical wavelength wider than 370 nm. The safety of 0.1% passion fruit extract concealer mousse was assessed. It did not cause skin irritation when assessed in human volunteers. This sunscreen makeup product provides UVA and UVB protection and is therefore suitable for daily application.

Uniterms: Cosmetics/study. Photoprotection/sun protection. Photoprotection/products development. Passion fruit/applications.

INTRODUCTION

Ultraviolet A (320-400 nm) and B (290-320 nm) light can cause irreversible skin damage, including cancer, hyperpigmentation, and aging. Application of sunscreen protects skin from damages; therefore, it is accounted as an important strategy to combat adverse effect of sun exposure. Currently, plant-based actives are receiving increased attention (Cefali *et al*., 2016; Silva *et al*., 2016). Those of fruit residues as high value-added chemical from food supply chain wastes are established and emerging globally. The alternative feedstock for innovative chemical and materials i.e. sustainable consumption by transforming wastes from fruit processing is interesting because of its high volume, chemical richness, and heterogeneity. Therefore, transformation of this waste into active healthy ingredients without harmful effects on food safety will be economically favorable way of managing these agricultural waste products that is often expensive to treat and dispose (Matharu, Melo, Houghton, 2016).

Passion fruit with a purple rind (*Passiflora edulis*) is popularly consumed in the form of beverage to improve physical and mental health and enhance beauty (Gruenwald, 2009). Therefore, this fruit is cultivated as an important agricultural crop in Thailand. However, a large amount of waste is generated from agro-industrial processing of this fruit, i.e. peels and seeds, which could cause environmental pollution (Lourith, Kanlayavattanakul, 2013). The seed of passion fruit has been widely used as a multifunctional ingredient in certain industries (Leão *et al*., 2014) including cosmetics owing to its potent antioxidant activity and UV protection efficacy, which are synergistic in photoaging protection. The antioxidant activity of the seed extract is comparable to that of ascorbic acid, owing to a high content of health beneficial phenolics. A previous investigation in

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our laboratory revealed the sun-protective property of the extract, which is similar to that of benzophenone-3 (BP3), octylmethoxycinnamate (OMC), and ferulic acid at their different sun protecting ranges. Moreover, the passion fruit seed extract has been used as a natural skin-lightening agent owing to its tyrosinase inhibitory effect. The sun-protective property of the extract is supported by quercetin and rosmarinic acid contained in it (Lourith, Kanlayavattanakul, 2013), which had been previously reported as potential ingredients against photooxidative damage (Choquenet *et al*., 2008; Sánchez-Campillo *et al*., 2009; Silva *et al*., 2016).

In this work, the stable and efficient sunscreen formulations containing passion fruit seed extract were developed. Their sun-protective capacities were investigated *in vitro*. The selected products underwent skin irritation test in human volunteers.

MATERIAL AND METHODS

All of the solvents for extraction were reagent grade purchased from Sigma-Aldrich (USA). Standards, reagents and solvents for 2,2′–Azino-*bis*(3-ethylbenzothaiazoline)-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing ability of plasma (FRAP) assays were from Fluka (USA) except absolute EtOH and EtOH were from Merck (Germany).

Those for formulations were of cosmetic grade supplied by Namsiang (Thailand).

Passion fruit seed extract preparation and quality control

Seeds of purple *P. edulis* cultivated in Chiang Rai, the northern province of Thailand, were received from the fruit juice production (2nd Royal Factory at Mae Chan) closed to Mae Fah Luang University. The seeds were treated and extracted by refluxing in 40% methanolic water for 30 min. The whole was filtered and concentrated under *vacuo*, partitioned with *n*-hexane and EtOAc, respectively, giving *n*-hexane, EtOAc and aqueous (Aq.) fractions, accordingly, of which the EtOAc fraction was included for further steps due to its potent activity. Thereafter, quality control by means of antioxidant activities using ABTS, DPPH and ferric reducing ability of plasma (FRAP) assays of the extract were assessed (Lourith, Kanlayavattanakul, 2013).

In brief, the stock solution containing ABTS (7 mM) and potassium persulfate (2.450 mM) was kept under ambient temperature for 16 h in a light protection vessel. Before use, the solution was diluted in EtOH to obtain an absorbance of 0.700 ± 0.200 at 750 nm with the microplate reader (ASYS, UVM340, UK). In the assay, 20 µL of samples were mixed with the ABTS solution (180 µL), individually. The absorbance at 750 nm was determined after 5 min of mixing using the microplate reader. The ability to scavenge $ABTS^{\bullet+}$ was calculated. The inhibitory concentration at 50% (IC_{50}) of a reference compound, ascorbic acid, was used for comparison to the IC_{50} value of the extract.

DPPH radicals (6×10^{-5} M) were prepared in absolute EtOH. The scavenging activity of the extract against $DPPH^{\bullet}$ was monitored at 517 nm using the microplate reader. The IC_{50} of the extract was calculated in comparison with ascorbic acid.

FRAP reagent was prepared in a 2,4,6-tri(2-pyridyl)-*S*-triazine (TPTZ) solution (10 mM, 2.5 mL) in a solution containing 40 mM HCl, $FeCl_3$ (20 mM, 2.5 mL) and acetate buffer, pH 3.6 (0.3 M, 25 mL). The test samples in EtOH were reacted with the FRAP reagent. The absorbencies were recorded at 595 nm using the microplate reader. The reducing power was expressed as an equivalent concentration (EC) to that of 1 mM $FeSO_4$ and ascorbic acid was used as the positive control.

All of the procedures were repeated in triplicates.

Formulation of makeup products

Liquid foundation and concealer mousse, the popular makeup product, with the ingredients as shown in Table I and II were subjectively to be developed. Passion fruit seed extract from the previous study (Lourith, Kanlayavattanakul, 2013) was added into the stable base formulations.

Accelerated stability and *in vitro* photoprotective efficacy assessment

The preparations were firstly assessed by means of a centrifugation assay (3,500 rpm for 30 min). Those that remained homogeneous were further encountered in 7 heat-cool cycles (4 ± 2 °C for 24 h and 45 ± 2 °C for 24 h, each cycle) (Chuarienthong, Lourith, Leelapornpisid, 2010). pH (Satorius Docu-pH+ Meter, Germany) and sun protection ability before and after heat-cool cycle were determined using SPF analyzer (Optometrics LLC/SPF-290F, USA). The sun protection factor (SPF) was assessed by spreading each formulation (2 mg/ cm^2) in an evenly manner over a Transpore tape (70.7 mm^2), the substrate, with a fingertip covered with a vinyl glove. The plate was then kept in the dark for 15 min, and exposed to a xenon arc solar simulator. The measurement was performed by

9 different scanning spots on the substrate at 2 nm interval between 290–400 nm (COLIPA, 2011; Kanlayavattanakul, Lourith, 2012a, 2012b). UVB protection efficacy as SPF, UVA protection efficacy as Boot star rating, and critical wavelength were recorded. All of the measurements were undertaken in triplicates.

Primary skin irritation test

Healthy Thai volunteers aged between 20 and 40 years old with no skin diseases were enrolled in this study. The experimental protocol was conformed to the Helsinki protocol. People with hypersensitive skin or a history of allergies were excluded. Women who were pregnant or lactating and people who were dieting also were excluded from the study. All subjects were informed about the study both in writing and verbally and signed a written consent form, which was approved by the ethical committee of Mae Fah Luang University prior to enrollment.

A preliminary single-application closed patch test was performed in 20 volunteers. Finn chambers (8 mm) obtained from Smartpractice (USA) were used for observation of a skin irritation against the sample (20 μL) for a period of 24 h on the volar forearms of volunteers. Skin irritation severity was graded over a range of 0-4. Data collected were used to calculate MII (Mean Irritation Index). A MII value < 0.2 was interpreted as non-irritation in a compassion with water (negative control) and 0.1% sodium lauryl sulfate (positive control) (Chuarienthong, Lourith, Leelapornpisid, 2010).

Statistical analysis

Data are presented as the mean ± SD. The parameters were compared and analyzed using one sample t test and ANOVA test with a significance level of $p<0.05$ using the SPSS program version 16.0.

RESULTS AND DISCUSSION

The passion fruit seed extract was prepared using the same procedure described in our previous study, and is shown in Table III (Lourith, Kanlayavattanakul, 2013). Assessment of antioxidant activity is one of the general protocols to ensure the quality and safety of the botanical extract used in personal care and cosmetics products. Antioxidant activity may vary depending on geographical condition and harvesting period (Antignac *et al*., 2011). This would help in delineating specifications of the passion fruit seed extract for industrial application.

Thereafter, the extract was further developed into the sunscreen makeup product as shown in Figure 1, and

TABLE I - Composition of the liquid foundation

Ingredient	% w/w		
	F	**F0.1**	**F0.3**
C30-45 alkyldimethicone	1-2	1-2	1-2
PEG-9 dimethicone	1-2	1-2	1-2
Cyclopentasiloxane	6-8	6-8	68
Cyclopentasiolxane and dimethicone cross polymer	0-1	0-1	0-1
TiO_2	10-12	10-12	10-12
Yellow iron oxide treated silicone	0.8-1.2	0.8-1.2	0.8-1.2
Black iron oxide treated silicone	0.05-0.07	0.05-0.07	0.05-0.07
Red iron oxide treated silicone	0.1-0.3	0.1-0.3	0.1-0.3
Polymethylsilsesquioxane	0.5-1.5	0.5-1.5	0.5-1.5
Caprylyl methicone	9-11	9-11	9-11
DI water	53-55	53-55	53-55
Propylene glycol	10-12	8-10	8-10
NaCl	0.5-1	0.5-1	0.5-1
Propylene glycol (and) diazolidinyl urea (and) methylparaben (and) propylparaben	0.4-0.5	0.4-0.5	0.4-0.5
Triethanolamine	0.02-0.03	0.02-0.03	0.02-0.03
Propylene glycol	1-2	1-2	1-2
Passion fruit extract	-	0.1	0.3

TABLE II - Composition of the concealer mousse

Ingredient	% w/w		
	M	M0.1	M0.3
Caprylyl methicone	2-5	2-5	2-5
Squalane	1.8-2.1	1.8-2.1	1.8-2.1
Isostearyl isostearate	0.9-1.4	0.9-1.4	0.9-1.4
Dimethicone	0.9-1.5	0.9-1.5	0.9-1.5
Talcum	6.1-7.7	6.1-7.7	6.1-7.7
Titanium dioxide, hydrated silica, hydrogen dimethicone	2-4	2-4	2-4
Titanium dioxide	12-16	12-16	12-16
Yellow iron oxide treated silicone	2-3	2-3	2-3
Red iron oxide treated silicone	0.4-0.6	0.4-0.6	0.4-0.6
Black iron oxide treated silicone	0.2-0.4	0.2-0.4	0.2-0.4
Dimethicone (and) cetearyl dimethicone crosspolymer	35-38	35-38	35-38
cyclopentasiloxane (and) dimethicone crosspolymer	11.9-12.3	11.9-12.3	11.9-12.3
Jojoba oil	5.1-6.3	5.1-6.3	5.1-6.3
Beeswax	1.1-1.5	1.1-1.5	1.1-1.5
Propylene glycol (and) diazolidinyl urea (and) methylparaben (and) propylparaben	0.1-0.2	0.1-0.2	0.1-0.2
Propylene glycol	0.1-0.3	0.1-0.3	0.1-0.3
Passion fruit extract	-	0.1	0.3

TABLE III - Antioxidant activities of the passion fruit seed extract

Antioxidant	Ascorbic acid	Passion fruit seed extract	
		Present study	Lourith, Kanlayavattanakul (2013)
ABTS (IC_{50}, µg/mL)	3.404 ± 0.025	6.607 ± 0.011	9.0 ± 0.0
DPPH (IC_{50}, µg/mL)	2.660 ± 0.079	4.410 ± 0.020	2.7 ± 0.2
FRAP (EC)	6,214.689 ± 28.249	2,018.942 ± 15.785	2,814.0 ± 11.6

their sun protection factor (SPF) was examined *in vitro* as shown in Figure 2.

SPF is an indicator evaluating UVB protection efficacy of sunscreen product. *In vivo* assay using human volunteers has been conducted (International Organization for Standardization, 2010). However, an alternative method, *in vitro* SPF assessment, has been developed because it is more time efficient, less expensive, and more ethnical (Fujikake *et al*., 2014). Therefore, *in vitro* assessment, i.e., the method described by Mansur *et al*. (1986), is widely

F F0.1 F0.3 M M0.1 M0.3

FIGURE 1 - Foundation and concealer mousse containing passion fruit seed.

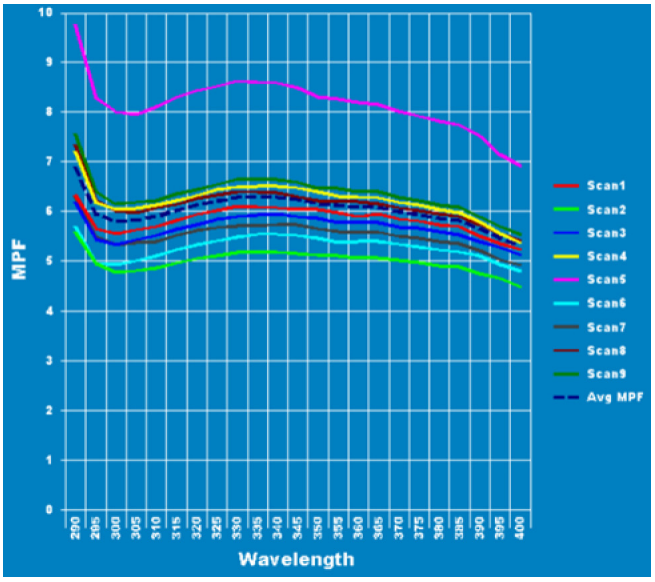

FIGURE 2 - An example of absorbance pattern during *in vitro* SPF analysis.

used along with the optometric SPF analyzer and the Labsphere UV transmittance analyzer, during the research and product development because they are reliable and efficient for the SPF determination before human testing (Chiari *et al*., 2014; Jarzycka *et al*., 2013; Séhédic *et al.,* 2009; Surget *et al*., 2015). In addition, *in vitro* SPF analysis is more feasible with the industrial practice.

Both the base concealer mousse and foundation exhibited similar sun protective activity in terms of boot star rating and critical wavelength greater than 4 and wider than 370 nm, revealing a broad protection against UVB and UVA (Jain, Jain, 2010; Séhédic *et al*., 2009). However, SPF of the base mousse was significantly ($p = 0.000$) better than that of the foundation (Table IV).

Incorporation of the passion fruit seed extract significantly ($p<0.05$) enhanced UVB protective efficacy.

TABLE IV - Sun protection efficacy of the passion fruit makeup products

Formula	UVB protection	UVA protection	
	SPF	Boots star rating	Critical wavelength
F	5.88 ± 0.30	5.00 ± 0.00	389.40 ± 0.16
F0.1	9.32 ± 0.88	5.00 ± 0.00	389.40 ± 0.00
F0.3	9.77 ± 1.37	5.00 ± 0.00	389.40 ± 0.00
M	15.48 ± 1.60	4.00 ± 0.00	388.60 ± 0.22
M0.1	18.75 ± 0.28	4.00 ± 0.00	388.70 ± 0.00
M0.3	18.99 ± 0.71	4.00 ± 0.00	388.70 ± 0.00

UVB protection of passion fruit concealer mousse was significantly ($p>0.05$) better than that of the liquid foundation. Moreover, the synergistic effect of the pigments of the base mousse was 7.34% higher than that of the foundation. However, 0.1% and 0.3% passion fruit concealer mousses insignificantly differed in terms of SPF ($p=0.902$), as shown in Table IV. The passion fruit mousse concealers were therefore selected for a stability evaluation under accelerated conditions of 7 heat-cool cycles. The developed sunscreen makeup products were physicochemically and chemically stable, as shown in Table V.

The concealer mousse containing 0.1% of passion fruit extract was therefore chosen for skin irritation test in the human volunteers owing to its photoprotective efficacy and economic feasibility. Closed patch test was assessed in 20 Thai volunteers. No skin irritation was observed when the base and passion fruit concealer mousses were used, which was similar to the effect of water (MII = 0).

Protection of skin against harmful UV rays is important in the prevention of premature skin aging and

TABLE V - Stability of passion fruit concealer mousse

Condition	Parameter	Formula		
		M	M0.1	M0.3
Initial	pH	6.54 ± 0.03	6.49 ± 0.03	6.09 ± 0.06
	SPF	15.48 ± 1.60	18.75 ± 0.28	18.99 ± 0.71
	Boots star rating	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
	Critical wavelength	388.60 ± 0.22	388.70 ± 0.00	388.70 ± 0.00
Heat-cool	pH	6.48 ± 0.02	6.30 ± 0.50	5.89 ± 0.40
	SPF	15.56 ± 0.53	18.09 ± 1.48	18.60 ± 1.21
	Boots star rating	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
	Critical wavelength	388.80 ± 0.11	388.30 ± 0.00	388.70 ± 0.00

photoaging (Iannacone, Huges, Green, 2014). Therefore, application of sunscreen is the most established method. Particularly, UV filters with antioxidant activity are highly accredited for their superior protection from skin damage (Oresajo *et al*., 2010). Plant-derived sunscreen agents are dramatically popular alternatives to the standard synthetic agents. Natural active ingredients are greatly presumed by the consumers as being safer than the synthetic ones and economically advantageous alternatives (Oliveira *et al*., 2009). In addition, the scientific evidence supports the effectiveness and benefits of the topical botanical therapies, and the proof of safety enables dermatologists and cosmetic chemists to encourage the consumers to use these products (Fisk *et al*., 2014; Iannacone, Huges, Green, 2014).

Makeup products are decorative cosmetics formulated in several dosage forms. Use of natural ingredients in makeup products is gaining popularity among the cosmetic users and industries (Fisk *et al*., 2014). In addition, makeup products such as foundation and mousse having sun-protective effects are widely popular. However, application of titanium dioxide (TiO_2) as sunscreen and pigment in the product may not match the skin color or tone, particularly in Asian skin type. Thus, alternative agents, especially those of botanical sunscreens, are in focus. In addition, UVA and UVB protective cosmetics are appropriate for daily use and *in vitro* evaluation of sun protective effect is recommended to investigate the protecting efficacy (Nash, Tanner, 2014; Séhédic *et al*., 2009). Passion fruit seed extract with an SPF comparable to that of BP3, OMC, and ferulic acid (Lourith, Kanlayavattanakul, 2013) and better than Thanaka (Kanlayavattanakul, Lourith, 2012a; 2012b) is the natural sunscreen widely used in Myanmar and the northern part of Thailand. Makeup products in the form of liquid foundation and concealer mousse are popular sunscreen products, and application of a thick layer additionally contributes to the sun protection efficacy (Petersen, Wulf, 2014).

The UVB protection efficacy of the passion fruit concealer mousses further strengthens its protection against photo-damage of epidermal Langerhans (Lee *et al*., 1999). In addition, a twofold higher SPF of concealer mousses than that of the foundation, with SPF greater than 15, reveals their benefit as a daily topical sunscreen product (Séhédic *et al*., 2009) for skin cancer protection (Skin Cancer Foundation, 2016). Furthermore, the presented sunscreen product containing passion fruit seed extract was found to be better than the products containing 10% of *Helichrysum arenarium*, *Crateaegus monogyna*, and *Sambac nigra*, with SPF of 6.80 ± 0.26, 6.00 ± 0.42, and 9.88 ± 1.66, respectively (Jarzycka *et al*., 2013), as well as 10% green coffee oil cream with SPF of less than 4 (Chiari *et al*., 2014). This presented natural sunscreen products exhibited a comparable SPF (11.14 ± 1.44 and 17.78 ± 2.92) with some of the commercially available tinted creams and better critical wavelengths (378 and 377 nm), as previously reported (Séhédic *et al*., 2009). In a comparison with the SPF 13.57 ± 1.15 of sunscreen product incorporated with ethyl acetate (EtOAc) fraction of *Salicornia ramosissima* (Surget *et al*., 2015), passion fruit seed EtOAc fraction mousse exhibited better sun protection.

CONCLUSION

An efficient and safe sunscreen product in the form of concealer mousse containing passion fruit seed extract was proposed. This makeup product with photoprotective effect is suitable for daily application against photo-damage. This is accounted as one of the evidence-based researches, supporting the active agent for sunscreen products derived from the renewable sources. The prepared extract is compatible with other cosmetic ingredients. Therefore, the non-irritating sunscreen products containing passion fruit seed extract are encouraged for further *in vivo* efficacy evaluation.

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