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Original Article

Photoprotective Activity of *Citrus sinensis* Peel Crude Extract: *In vitro* Analytical Method and Formulation

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ABSTRACT

Background: Peel extract of *Citrus sinensis* is rich in beneficial phytochemicals that can protect the skin from the harmful effects of UV radiation. This study evaluates the suitability of *C. sinensis peel* waste as the primary active ingredient in a photoprotective formulation. **Materials and Methods:** Freeze-dried powder of *C. sinensis* peel was extracted using Soxhlet extract, with ethyl acetate as the solvent, and formulated into sunscreen formulation. **Results:** Phytochemical evaluation of the crude extract revealed that the flavonoid, phenolic and terpenoid contents were 35.21±2.3 mg quercetin equivalent (QE)/g, 59.40±3.0 mg gallic acid equivalent (GAE)/g and 3.33±0.1 mg linalool/g, respectively. The photoprotective properties and free radical scavenging activity of the formulation were investigated by Sun Protection Factor (SPF) and 2,2′-Diphenyl-1-picrylhydrazyl (DPPH) assay. The sunscreen formulation exhibited photoprotective activity with SPF value of 2.41±0.01 and a strong scavenging activity with IC_{50} value of 37.3±2.26 ppm. The physical evaluations of the formulation (organoleptic evaluation, pH, viscosity, spreadability and the type of emulsion) were performed and showed a stable profile for 5 weeks of storage. **Conclusion:** The findings indicate that the sunscreen formulation containing ethyl acetate peel extract of *C. sinensis* may provide indirect photoprotection by reducing UV-induced oxidative stress, a critical contributor to skin inflammation and photoaging. Moreover, improved and valuable antioxidant effect is expected when applying of the developed formulation topically.

Keywords: *Citrus sinensis*, Soxhlet, Sunscreen Formulation, Sun Protection Factor, 2,2′-diphenyl-1-Pierylhydrazyl.

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INTRODUCTION

Over-exposure to UV radiation causes high level production of Reactive Oxygen Species (ROS) in skin, which results in oxidative stress that leads to cellular damages.[1] Sunscreens are used to reduce the damaging effect of the UV radiation. Current synthetic sunscreens may cause undesirable side effects such as allergic reactions and environmental issues.[2] This has led to a new approach where natural sunscreens have become a new trend of photoprotection. Plant-derived antioxidants such as terpenoids, flavonoids and phenolic compounds are used as sunscreen agents, due to their protective effects against sun damage.[3] Flavonoids and phenolics can absorb UV radiation due to chromophores, such as conjugated systems, that are often found in molecules with aromatic rings.[4,5] Moreover, terpenoids and specifically carotenoids are effective radical scavengers, which neutralize the free radicals induced by UV.[6] Previous studies demonstrated that a sunscreen formulation that incorporates extracts of *Heteropogon contortus*,[7] *Solanum lycopersicum*,[8] *Vitis* vinifera,[9] and *Citrus aurantifolia*[5] showed excellent photoprotective properties with SPF value ranged from 6 to 36.

Citrus sinensis is well-known as a good source of antioxidant due to the presence of bioactive metabolites including polyphenols, flavonoids, steroids, hydroxyamides, carotenoids, and thus can potentially prove helpful to protect against UV damage.[7] The peels of citrus fruits account for approximately 50-60% of the fruit's total weight, but only about 33% of citrus fruits

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are processed, leaving tons of peels as agro-industrial waste.[8] Therefore, it is crucial to figure out an appropriate approach to manage, reuse, or dispose this waste. The literatures revealed that citrus peels have the potential to be utilized as ingredients in cosmetics and pharmaceutical products.[9] Recently, our research group also revealed that ethyl acetate extract of *C. sinensis* peel has the potential to be formulated into effective sunscreens, due to its promising photoprotective and antioxidants free radical scavenging activities.[10] Fruit peel extracts have been shown in numerous studies to have photoprotective properties, but their incorporation into conventional sunscreen formulations is still limited, especially when it comes to substituting synthetic sunscreen.[11] Moreover, a thorough evaluation of such formulation's physicochemical stability is lacking in current studies. The ease of obtaining discarded citrus peels along with their bioactive potential, makes them valuable natural sources to be incorporated into cosmetic products, while also reducing environmental impacts through the promotion of sustainable reuse of agro-industrial waste. Therefore, this study aimed to evaluate the suitability of *C. sinensis* peel waste as the primary active ingredient in a photoprotective formulation through *in vitro* analytical methods, with the objective to develop a value-added and sustainable photoprotective formulation.

MATERIALS AND METHODS

Plant material and extraction

The *C. sinensis* fruits were purchased at fruit market in Giant, Shah Alam, Selangor, Malaysia. To prepare ethyl acetate extract, the freeze-dried powder of *C. sinensis* fruit peels undergo the same treatment as described in our previous study and the obtained extract was concentrated under vacuum at 45°C to ensure complete solvent elimination.[10]

Total Flavonoid Content (TFC)

TFC was determined by aluminum chloride colorimetric assay as described by previous study with slight modifications.[12] The 50 mg/mL stock solution of extract was used to prepare various sample concentrations ranging from 5.0, 10.0, 15.0, 20.0, 25.0 mg/mL. About 1.0 mL extract of each concentration was added into 4 mL of distilled water. Then, 0.3 mL of 5% $NaNO_2$ was added followed by 0.3 mL of 10% $AlCl_3$ after 5 min and 2.0 mL of 1M NaOH after 6 min. Then, the volume of the mixture was topped up to 10.0 mL. The absorbance was measured by UV-VIS spectrophotometer at 510 nm. The data were expressed as mg Quercetin Equivalents (QE) to 1.0 g of sample in dry weight.

Total Phenolic Content (TPC)

TPC was determined by Folin-Ciocalteu colorimetric method as previously explained with slight modifications.[12] Sample concentrations of 0.2, 0.4, 0.6, 0.8, 1.0 mg/mL were prepared from the 2.0 mg/mL extract stock solution. About 5.0 mL of 10% Folin-Ciocalteu's Reagent (FCR) and 4.0 mL of 7% Sodium Bicarbonate (Na_2CO_3) was added to 1.0 mL of sample to make a final volume of 10.0 mL. After incubation for 30 min at 40°C in the water bath, the absorbance was measured at 760 nm against blank by using a UV-VIS spectrophotometer (Modal: HACH DR6000, United States). The data were expressed as mg Gallic Acid Equivalents (GAE) to 1.0 gram of sample in dry weight. The TPC were determined by using following formula:

$$C = c\frac{V}{m}$$

Where C=total phenolic content mg GAE/g dry extract, c=concentration of gallic acid obtained from calibration curve in mg/mL, V=volume of extract in mL, and m=mass of extract in gram.

Total Terpenoids Content (TTC)

TTC was determined by the method adapted from a previous study.[13] About 2.0 mL of chloroform was added into 1.0 mL of 50 mg/mL extract solution. The mixture was vortexed thoroughly then left for 3 min. Subsequently, 200 µL of concentrated Sulfuric Acid (H_2SO_4) was added into the mixture, followed by incubation at room temperature for 1.5 to 2 hours in the dark. After that, the supernatant was carefully decanted without disturbing the reddish-brown precipitate, and 3.0 mL of absolute methanol was added and vortexed well until the precipitate completely dissolved. Absorbance was read at 538 nm using UV-vis spectrophotometer. The data were expressed as mg linalool equivalents to 1.0 gram of sample in dry weight.

Preparation of Sunscreen Formulation

The Sunscreen Formulation (SF) was prepared by adding the 1.50% (w/v) of the peel extract bit by bit into the formulation base which consisted of 3.00% (w/v) Tefose 63, 18.00% (w/v) paraffin liquid, 3.00% (w/v) cetyl alcohol, 0.05% (w/v) methyl paraben, 0.05% (w/v) propyl paraben and added aquadest up to 100% (w/v).[14] The formulation was stirred and mixed homogeneously. The same formulation was prepared without the extract to serve as a blank formulation (BF).

Photoprotective Efficacy Assessment

The SF and ethyl acetate at a ratio of 1:16 were mixed and heated over a stirred bath until a homogeneous mixture was obtained, based on the method adapted from previous study with slight modifications.[15] The mixture was centrifuged for 10 min at 3000 rpm, and supernatant was diluted with ethyl acetate at a ratio of 1:10. The absorbance was measured using UV-vis spectrophotometry from 290-320 nm at 5 nm intervals. The BF was prepared with the same treatment and served as blank. The photoprotective efficacy of SF was determined through the measurement of SPF value based on Mansur's equation:[16]

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

where: EE (λ) is the Erythmogenic Effect of radiation with wavelength λ, I (λ) is the solar intensity spectrum, EE (λ) x I (λ) are constants, Abs (λ) is the absorbance values at wavelength λ of test sample and CF is the correction factor (=10).

DPPH Free Radical Scavenging Activity Assessment

The free radical scavenging activity of SF was measured by using 2,2-diphenyl-2-picylhydrazyl (DPPH) with some modifications.[17] A concentration of 1000 ppm formulation stock was prepared. Then, diluted to a series of concentrations of 60-20 ppm and the volume was completed with DMSO to 10.0 mL. About 1.0 mL of each diluted solution was added to 1.0 mL of 0.05 mM DPPH solution and 2.0 mL of methanol. The test tube was then vortexed and incubated in dark condition for 15 min. The absorbance was measured at 517 nm. The BF was served as negative control. All experiments were performed in triplicate and the percentage of inhibition of DPPH by SF was calculated by using the following formula:

$$\text{DPPH Radical Scavenging Activity \%} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100\%$$

Where: $\text{Abs}_{\text{control}}$ is the absorbance of DPPH solution without sample and $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH solution with sample.

The concentration of test sample required to scavenge 50% DPPH solution (IC_{50}) is obtained from linear regression plot of average inhibition % versus concentrations.

Physical Characterization for Sunscreen Formulation

Physical Properties Evaluations

The Sunscreen Formulation (SF) and Blank Formulation (BF) were evaluated for their organoleptic and physical properties including color, odor, organoleptic shape, pH, and viscosity up to 5 weeks of storage with measurements made at regular interval of each week. The color, odor, and shape in organoleptic (liquid/ semisolid/ solid) were observed using sense of organ. The pH of the formulation was measured using digital pH meter and viscosity of the formulation was measured using Brookfield R/S Plus Rheometer with the concentric cylinder -14 under rpm of 40. Both tests were performed at room temperature.[14]

Spreadability

The spreadability of formulation was evaluated by the parallel plate method. About 1 g of formulation was placed over one of the slides, while the other slide was placed upon the top of the formulation. A 125 g weight was placed upon the upper slide, uniformly pressed so pressure was applied on the slides to form a thin layer of formulation between two slides. The weight was removed, and the spread diameter was measured.[18]

Types of emulsion

The emulsion system of both BF and SF was determined by using methylene blue staining test.[19] A drop of methylene blue solution was added in formulation and mixed well following observation under microscope. The o/w emulsion system showed blue background and globule appeared colourless.

Microbiological test limit

The microbial analysis of the SF was performed by PERMULAB SDN BHD (392059-X) (a BVAQ Joint Venture Company) (BVAQ Reference: 23-195734) using the standard method of analysis according to the method: BP 2019 Appendix XVI. The tests included the detection of specific microorganisms such as *Pseudomonas aeruginosa* (0.1 g), *Staphylococcus aureus* (0.1 g) and *Candida albicans* (0.1 g); and total microbial count including total aerobic microbial count (CFU/g) and total combined yeast and mold count (CFU/g). The limit for total viable aerobic count (bacteria, yeast, and mold) should not exceed 1000 CFU/g according to the test limit set by the National Pharmaceutical Regulatory Agency (NPRA, 2022) under the Annex I, Part 14 cosmetics guideline.[20]

Heavy metal test limit

Four heavy metals including arsenic as As (mg/kg), lead as Pb (mg/kg), cadmium as Cd (mg/kg), and mercury as Hg (mg/kg) in the SF were analyzed by PERMULAB SDN BHD (392059-X) (a BVAQ Joint Venture Company) (BVAQ Reference: 23-195734) using method: USP 233 (2013)/ICP MS.[21] The National Pharmaceutical Regulatory Agency (NPRA, 2022) established the maximum limit of heavy metal content in cosmetics according to Annex I, Part 14 of the guidelines. Mercury content must not exceed 1 ppm, lead content must not surpass 20 ppm, arsenic content is restricted to not more than 5 ppm, and cadmium should not exceed 5 ppm.[20]

RESULTS

Total Flavonoid Content (TFC)

The TFC content was determined by comparing absorbance of each extract concentration to the quercetin standard. The calibration demonstrated linear behavior over a concentration range from 0.2 - 1.0 mg/mL. Statistical analysis of the data resulted in a high correlation coefficient (r^2) value of 0.9883. Additionally, the determined intercept and slope were found to be 0.1064 and 0.5115, respectively. The TFC measures 35.21±2.3 mg of Quercetin Equivalent per gram of extract (mg QE/g). Refer to Figure 1 for the visualisation of the calibration curve.

Total Phenolic Content (TPC)

The TPC content were derived from a standard calibration curve of gallic acid in the range of 0.02-0.10 mg/mL with a high correlation coefficient (r^2) value of 0.9987. The determined intercept and

slope were found to be 0.0005 and 0.0102, respectively. The TPC is recorded as 59.40±3.0 mg of Gallic Acid Equivalent per gram of extract (mg GAE/g). Figure 2 shows the calibration curve of gallic acid.

Total Terpenoid Content (TTC)

The TTC content was determined based on the obtained absorbance which was compared to the standard calibration curve of linalool in the range of 50 - 300 µg/mL. Regression equation of the calibration curve shows a correlation coefficient (r^2) value of 0.9219 with determined intercept value of -0.3575 and slope value of 0.0048. The extract presented TTC of 3.33±0.1 mg of linalool per gram of extract. The calibration plot of linalool is shown in Figure 3.

Photoprotective efficacy assessment for sunscreen formulation

Mansur equation with the pre-defined constants (EE (λ) x I (λ)) for wavelength from 290-320 nm was used to calculate the SPF value of SF.[22] The photoprotective assessment of SF was done and detected with a SPF value of 2.41±0.01 (Table 1).

DPPH free radical scavenging activity assessment for sunscreen formulation

The DPPH free radical scavenging activity assessment of SF with concentration of 20 to 60 ppm reported percentage of inhibition ranging from 22.34% to 76.71% (Figure 4). Linear regression plot of average inhibition % versus concentrations showed a coefficient (r^2) value of 0.9690 with determined intercept value of -1.8700 and slope value of 1.3890. The IC_{50} value of SF was calculated as 37.3±2.26 ppm.

Physical characterization for sunscreen formulation

Physical evaluations of formulations reported a stable profile for both BF and SF in observation of color, odor, organoleptic shape in 5 weeks of storage (Table 2). BF shown white in color while SF was yellow in color. There were no odours presented, and organoleptic shape remained as semi-solid in both formulations. There was no change in color, odor, and organoleptic shape of the formulations across the 5 weeks of storage. The pH of formulations was shown in Table 3 and viscosity was represented by Figure 5. The spreadability of SF (5.97±0.00 cm) was relatively higher than BF (5.50±0.00 cm) and SF presented o/w emulsion system while BF presented w/o emulsion system in the methylene blue test (Figure 6).

In terms of microbiological and heavy metal tests (Table 4), SF showed less than 1000 CFU/g for the total viable aerobic count; *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Candida albicans* were not detected in the sample. The heavy metal content of mercury, lead, arsenic and cadmium were all within the limit.

DISCUSSION

Studies reported the predominant compound in *C. sinensis* peel is D-Limonene.[23-25] Meanwhile, polyphenols, flavonoids, limonoids, carotenoids, and vitamin C are also remarkable phytochemicals that were found in *C. sinensis* peel. These stated phytochemicals contribute to the antioxidant properties to act as free radical scavengers.[26] The quantitative evaluations such as TFC, TPC and TTC give a good guide to the concentration of antioxidants present in *C. sinensis* peel. Similar result for TPC and TFC on extract of *C. sinensis* peel was reported in literature.[23,24] TPC value was in the range of 67.35±2.9 to 160.30±3.0 mg GAE/g and TFC ranging from 4.36±1.17 to 22.20±0.5 mg QE/g. These allow us to validate the richness of flavonoids and phenolics compounds in the peel of *C. sinensis*. Numerous studies have explored the phytochemistry of *C. sinensis* peel. Detected flavonoids include naringin, hesperetin, hesperidin, nobiletin, and tangeritin.[26-29] Moreover, study conducted to quantify the phenolics from *C. sinensis* peel reported several phenolic acids such as gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, caffeic acid, and

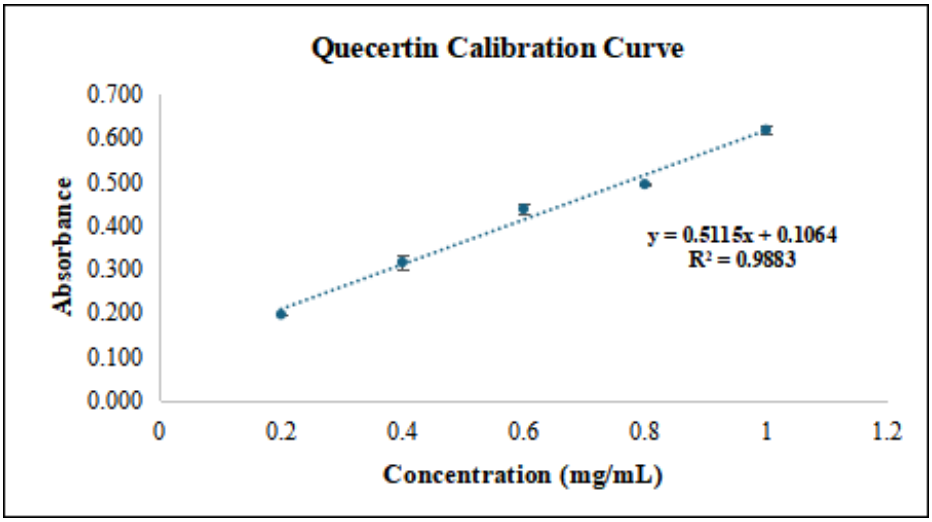


Figure 1: Calibration curve of quercetin standard.

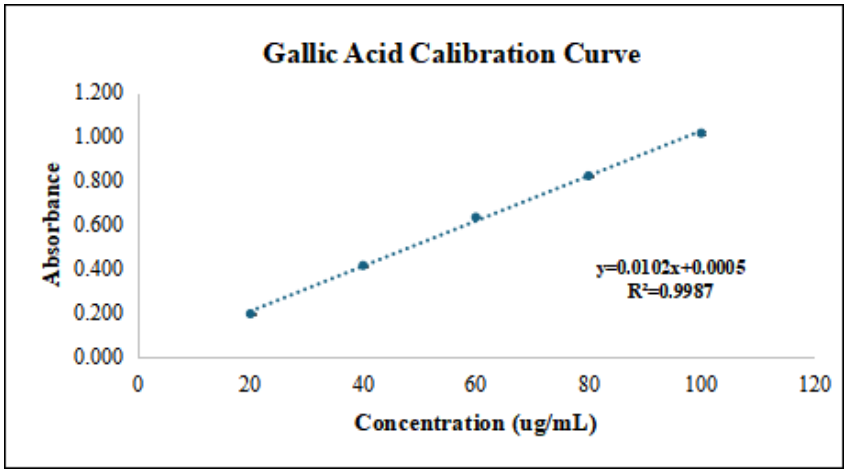


Figure 2: Calibration curve of gallic acid standard.

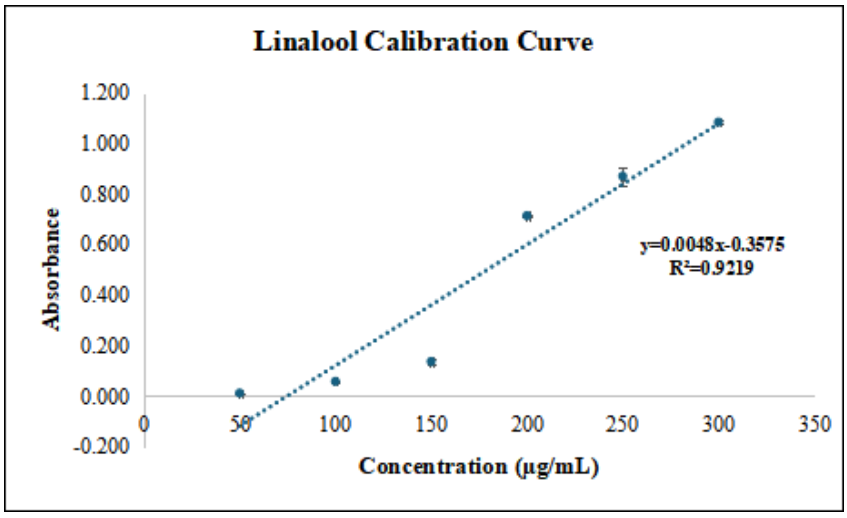


Figure 3: Calibration curve of linalool standard.

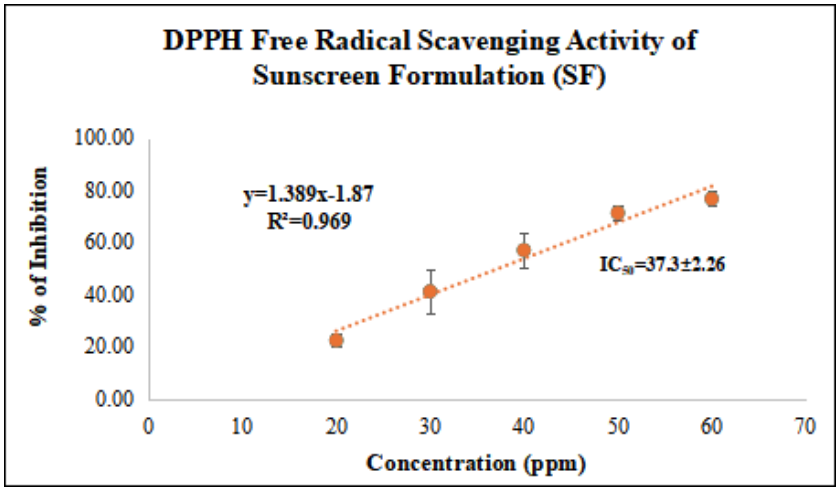


Figure 4: DPPH free radical scavenging activity of SF.

ferulic acid.[30] These identified phytochemicals significantly contribute to the antioxidant capacity of *C. sinensis* peel which potentially preventing the photodamage induced by UV radiation through scavenging the Reactive Oxygen Species (ROS) in the skin. So far, there is a lack of comprehensive scientific study has been conducted on the total terpenoid content of *C. sinensis* peels using the stated method. Previous study performed TTC analysis by similar treatment on *Serevenia buxifolia* (453.70 to 842.58 mg

linalool/g) and *Enicostemma litorrale* (70.00 mg linalool/g).[13,25] This shows that our sample contain relatively lower concentration of terpenoids. D-limonene is the most reported predominant compound detected in *C. sinensis* peel.[23-24,30] It is a type of terpenoid, specifically monocyclic monoterpene. It is a promising antioxidants compound that can reduce ROS formation by different mechanisms.[32] Linalool, myrcene and heptanal are also common terpenoids, isolated from *C. sinensis* peel.[23,30]

SPF measures how much UV radiation is needed to cause sunburn on sunscreen-protected skin relative to the UV radiation amount required to cause sunburn on unprotected skin. As the SPF value increases, sunburn protection increases. SPF can be assessed using UV-Vis spectrophotometry as it can measure absorbance at different wavelengths hence SPF value can be calculated using the obtained absorbance in Mansur's equation.[26] When sunlight is not absorbed or reflected by the sunscreen, it transmits through the solution, and this indicates skin penetration occurs. The higher the amount of light a sunscreen absorbs or reflects, the lesser the light is transmitted through the sunscreen, and the lesser the UV radiation reaches the skin. This demonstrated that sunscreen has higher efficiency in blocking UVB radiation. The SPF value obtained from Mansur's equation is supposed to show a direct correlation to the efficiency of sunscreen protection towards UVB radiation. Most sunscreens absorb UVB, and SPF only measures how well a sunscreen protects against UVB rays and UVA protection is not rated.[27] This explains why SPF UV-vis spectrophotometry involves wavelengths of 290-320 nm, which refers to the wavelength for UVB.

The SPF value for ethyl acetate extract of *C. sinensis* peels (100 ppm) that reported by our previous work was 7.34±0.05.[10] There is a decrease of photo protectant activity after the same amount of extract (100 ppm) was formulated as SF. A similar result was reported for a formulation containing mareme leaf and meniran herb (2:1) at a concentration of 600 ppm, which exhibited a lower SPF value of 2.55. However, the same 600 ppm combination extract of mareme leaf and meniran herb (2:1), when tested as is, showed an SPF value of 17.32. This indicates a significant decline in SPF value with approximately 85.27% after extracts were formulated into a lotion.[28] Similar result was reported in tomato formulation study where the 0.5% of tomato extract exhibited SPF value of 17.74 however 1% tomato extract formulation showed SPF value of 18.84.[8] The SPF value of tomato extract can be said to decline after it was prepared as a formulation. The SPF value of tomato extract can be said to decline after it was prepared as a

Table 1: *In vitro* **Sunscreen Protection Factor (SPF) of SF. Each value represents a mean±SD (*n*=3).**

nm	EE (λ) x I (λ)	Average absorbance	Mean±SD
290	0.0150	0.2164	0.03±0.12
295	0.0817	0.2211	0.18±0.03
300	0.2874	0.2310	0.66±0.02
305	0.3278	0.2410	0.79±0.01
310	0.1864	0.2564	0.48±0.01
315	0.0839	0.2707	0.23±0.00
320	0.0180	0.2741	0.05±0.01
SPF	1.0000	0.2410	2.41±0.01

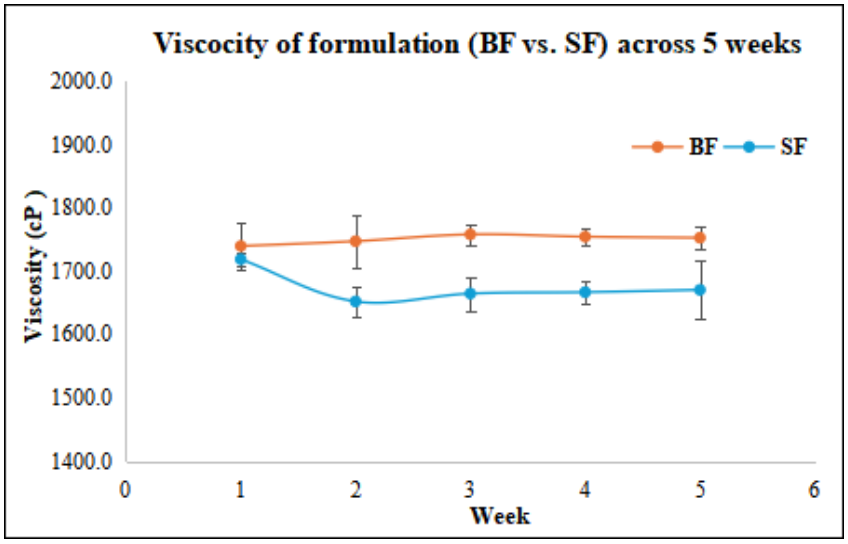
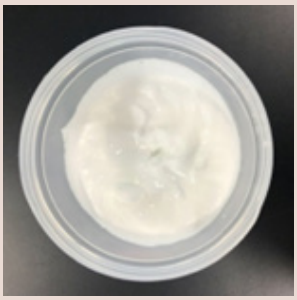
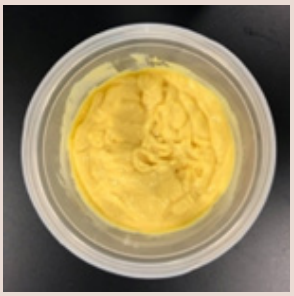
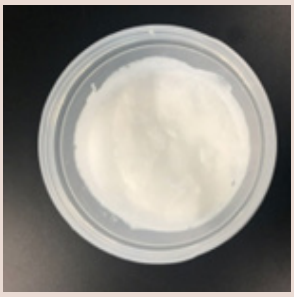
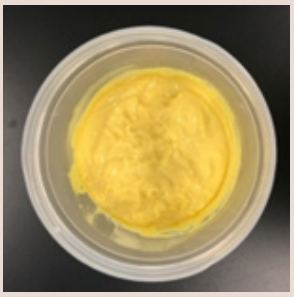
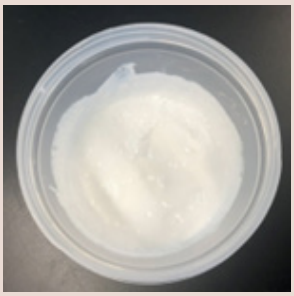
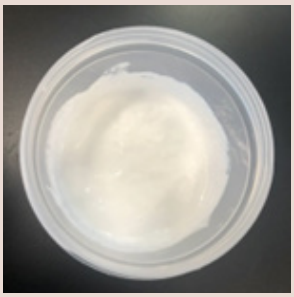
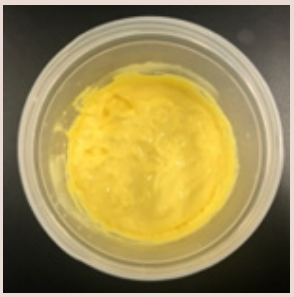
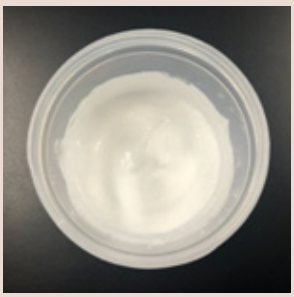
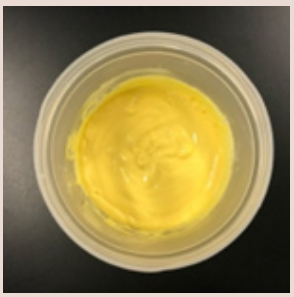


Figure 5: Viscosity of BF and SF across 5 weeks.

Table 2: Color, odor and organoleptic shape of BF and SF in 5 weeks of storage.

Week	Blank Formulation (BF)			Sunscreen Formulation (SF)		
	Color	Odor	Shape	Color	Odor	Shape
1		No	Semi-solid		No	Semi-solid
2		No	Semi-solid		No	Semi-solid
3		No	Semi-solid		No	Semi-solid
4		No	Semi-solid		No	Semi-solid
5		No	Semi-solid		No	Semi-solid

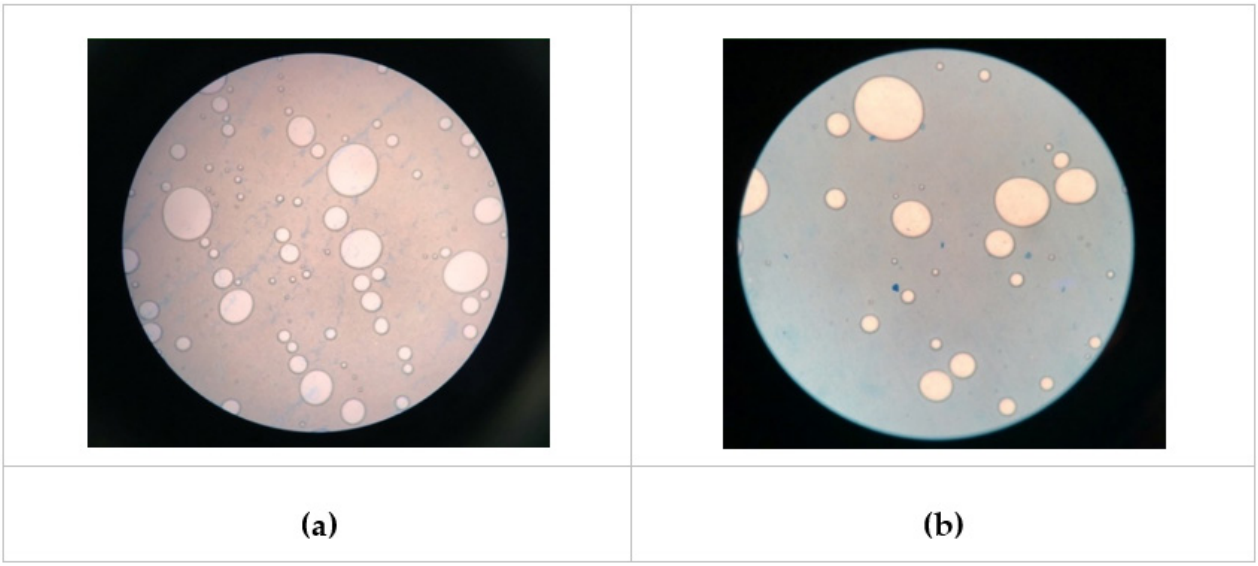


Figure 6: Type of emulsion of (a) BF and (b) SF.

Table 3: The pH of BF and SF over 5 weeks.

Week	BF	SF
1	6.37±0.0	5.96±0.0
2	6.39±0.0	6.03±0.0
3	6.47±0.0	6.10±0.0
4	5.82±0.0	6.09±0.0
5	5.73±0.0	5.81±0.0

formulation. This concluded that more concentration of extracts was required in formulation of sunscreen lotion to achieve the same SPF value of extracts. The decline of SPF value may be due to the interference of the base formulation which affects the absorption of UV light. Furthermore, treatment of sample for SPF measurements can also cause a decrease of SPF value in formulation.[15] A finished sunscreen product that contains a single active ingredient must provide a minimum SPF value of 2 and it is classed as minimal sunscreen product (SPF 2 to 12).[29] The SPF value of SF was in the range of minimal sunscreen products. However, a recommended SPF value for a sunscreen formulation has to be above 15. This is because the minimum SPF value of 15 is required to effectively hold as much as 95% of UVB ray's radiation from reaching the skin.[30] Therefore, more concentration of extract has to be incorporated in order for SF to achieve the desired photoprotection of SPF 15.

Free radicals cause oxidative stress on the skin, resulting in various skin problems such as photoaging, hyper-pigmentation, inflammation and even skin cancer.[31] An antioxidant is a compound that scavenges free radical generation. Antioxidant activity refers to the relative radical-scavenging abilities of compounds through their properties as electron or proton plus electron donating agents.[32] The antioxidant assay can be done by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method. It is based on the electron-transfer principle. When antioxidants reduce the DPPH solution through the transfer of a proton plus an electron, this results in a color change in the DPPH solution from violet to yellow (diphenylpicryl hydrazine). The DPPH method requires a UV-Vis spectrophotometer to obtain the absorbance. DPPH is a stable free radical that possesses a deep purple color and has a strong absorption around 517 nm.[33] The absorbance will decrease in the presence of proton donor molecules such as antioxidants. The DPPH free scavenging activity (%) of the Sunscreen Formulation (SF) was determined based on the absorbance assessed by UV-vis and calculated based on the formula stated in methodology. The IC_{50} refers to inhibitory concentration, which was the minimal effective concentration of the SF that can inhibit 50% of the absorbance of the DPPH, meanwhile the data on percent inhibition of the test is required to calculate IC_{50} value.[17] The smaller the IC_{50} value, the stronger the antioxidant free radical scavenging power.

The DPPH IC_{50} value presented by *C. sinensis* peels extracts in our previous work was much higher as compared to our findings which was 33.14 x 10^3 ppm.[10] SF showed increase in antioxidant free radical scavenging activity after it was formulated. Comparable outcome has also been reported in the study carried on gel formulation containing extract showed higher antioxidant activity when compared to extract.[34] The antioxidants sample that reported scavenging activity on DPPH with IC_{50} values below 50 ppm are classified as very strong antioxidants, 50 -100 ppm are classified as strong antioxidants, and 100 -150 ppm are classified as weak antioxidants.[35] SF presented a DPPH scavenging activity IC_{50} value that lower than 50 ppm therefore it is considered as a very strong antioxidant. Furthermore, topical antioxidants have potential to supplement body's innate defence to neutralize free radicals in order to provide photoprotection.[36] Since oxidative stress is a key contributor to UV-mediated skin damage, the strong

Table 4: Microbiological and heavy metal tests for SF.

Test	Result	Unit	Method Reference
Total Combined Yeasts and Moulds Count	<10	cfu/g	BP 2019 Appendix XVI B.5
Pseudomonas aeruginosa	Absent	/0.1g	In-house M36 (Based on BP2019) (Detection)
Staphylococcus aureus	Absent	/0.1g	In-house M37 (Based on BP2019) (Detection)
Total Aerobic Microbial Count	<10	cfu/g	BP 2019 Appendix XVI B.5
Candida albican	Absent	/0.1g	In-house M38 (Based on BP2019) (Detection)
Arsenic	<0.0100	mg/kg	USP 233 (2013)/ ICP MS
Cadmium	<0.0100	mg/kg	USP 233 (2013)/ ICP MS
Lead	0.121	mg/kg	USP 233 (2013)/ ICP MS
Mercury	<0.0100	mg/kg	USP 233 (2013)/ ICP MS

Results that are prefixed with '<' indicate the lowest level at which the analyte can be reported, and that in this case the analyte was not observed above this limit; CFU = Colony Forming Unit.

DPPH free radical scavenging activity observed in SF exhibited promising antioxidant capability and minimal photoprotection. Hence it is potential to be formulated into topical antioxidant formulation with photoprotection as an added value may still offer a degree of photoprotection through mitigating oxidative stress induced by UV exposure, thereby contributing to its overall photoprotective efficacy. Furthermore, previous study reported the addition of botanical extracts or botanical antioxidants to a broad-spectrum sunscreen may further decrease UV-induced damage compared with sunscreen alone which contribute to non-sunscreen photoprotective benefits.[37] In brief, antioxidant formulation capable of protecting skin cells against the damaging effects of UV-induced Reactive Oxygen Species (ROS): photodamage, skin aging, and cancer.

Unstable formulation can have significant impacts upon the end-user's protection from solar radiation, hence physical evaluations for color, odor, organoleptic shape, pH, and viscosity of formulations were performed for 5 weeks with measurements taken on a weekly basis. The pH of SF was slightly lower than BF which might be due to the acidity of *C. sinensis* peel extract. However, pH of both formulations still falls into the preferred range of pH for cosmetic preparations which is between pH 4 to 7.5.[38] Viscosity of SF is slightly lower than BF due to the addition of extract lowered the viscosity of formulation which contributes to higher spreadability of SF compared to BF.[14]

Hydrophilic surfactants promote o/w emulsions whereas lipophilic surfactants promote w/o emulsions.[39] Tefose 63 used in the formulation is lipophilic surfactant hence it promotes for w/o emulsion. However, the emulsification technique including applied shear and oil-water ratio used can be crucial in determining the final emulsion type than the surfactants themselves.[40] The formulations were made under the same condition with same applied shear however different emulsion systems were presented in BF and SF. The addition of *C. sinensis* peels extracts is believed to affect the oil-water ratio in the system therefore SF formed in o/w system. This can be attributed to catastrophic phase inversion phenomena. Catastrophic phase inversion in emulsions is induced by changing the ratio of oil/water.[41]

Sunscreens can be formulated in the form of o/w and w/o emulsion.[2] Generally, sunscreens made in the form of w/o emulsions.[42] The w/o emulsion systems provide longer UV protection on skin due to their contribution to improve water resistance properties, but it also leaves a greasy feeling after being applied.[43,44] However, o/w emulsion has light and moisturizing texture, but their UV protection tends to wear off faster. Due to low oil content, o/w emulsions are absorbed faster, and the formulation can be easily washed from the skin when compared to w/o emulsions.[45] Therefore, w/o formulation will be more desired for sunscreen as it will not easily wash away. Nevertheless, w/o emulsions are more suitable for dry skin while o/w emulsion are more suitable for oily skin.[46] Since SF is o/w emulsion, thus it is more recommended to be developed as daily care product for oily-skin users due to its light textual property.

To ensure that cosmetics do not contain any prohibited or harmful substances, all restricted ingredients should be used within the allowable limits and conditions of use. These procedures are parts of the Post Market Surveillance (PMS) programme which is usually monitored by NPRA to ensure the cosmetic products compliance.[20] Both microbiological and heavy metal tests adhered to the requirement of Annex I, Part 14 cosmetics guideline.

CONCLUSION

Sunscreen Formulation (SF) containing *C. sinensis* peel extract has a stable physical profile throughout the 5 weeks of storage, with an SPF value of 2.41±0.01 and satisfying antioxidant activity with DPPH IC_{50} value of 37.3±2.26 ppm. These results

indicate SF is classed under minimal sun protection product and concurrently show a promising antioxidant activity. To ensure SF achieve desired photoprotection of SPF 15, higher concentration of extract is required to be incorporated in the formulation. The observed antioxidant activity indicates that SF still provides a form of photoprotection through free radical scavenging mechanisms, despite the fact that the SPF value is low and approaches the threshold of ineffectual UV protection according to standard classifications. This antioxidant defence may serve as an adjunct to conventional UV filtration by mitigating oxidative stress caused by UV exposure. To enhance its photoprotective efficacy and meet the minimum SPF threshold of 15, further research is required. This comprises the optimization of the concentration of *C. sinensis* peels extract and the exploration of formulation variables including pH, emulsion type, particle dispersion and carrier systems. The synergistic potential with conventional UV filters and the photostability studies should also be examined to elucidate its full protective potential. It is anticipated that the SPF value of SF can be substantially enhanced without compromising the formulation's antioxidant capacity by addressing these formulation factors.

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ABBREVIATIONS

UV: Ultra-Violet; **SPF:** Sun Protection Factor; **DPPH:** 2,2'-diphenyl-1-picrylhydrazyl; **ROS:** Reactive Oxygen Species; **TFC:** Total Flavonoid Content; **TPC:** Total phenolic content; **TTC:** Total Terpenoid Content; **QE:** Quercetin Equivalents; **GAE:** Gallic Acid Equivalents; **FCR:** Folin-Ciocalteu's Reagent; **SF:** Sunscreen Formulation; **BF:** Blank Formulation; **NPRA:** National Pharmaceutical Regulatory Agency; **w/v:** Weight/Volume; **o/w:** Oil in Water; **w/o:** Water in Oil; **$AlCl_3$:** Aluminium Chloride; **$NaNO_2$:** Sodium Nitrite; **Na_2CO_3:** Sodium Bicarbonate; **H_2SO_4:** Sulfuric Acid; **DMSO:** Dimethyl Sulfoxide; **As:** Arsenic; **Pb:** Lead; **Cd:** Cadmium; **Hg:** Mercury.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest. The copyright of this work is granted by Intellectual Properties Corporation of Malaysia (MyIPO) with notification number of CRLY2023W00870 under the category of work of literacy which have been recorded in the Register of Copyright, in accordance with section 26B of the Copyright Act 1987 [Act332].

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ETHICAL APPROVAL

This study was reviewed by the University Ethics Committee (UEC) of Management and Science University, Malaysia and exempted from ethical review (EA-L1-01-SPH-2024-06-0001X). There were no ethical issues to be considered as study does not involve any human or animals.

SUMMARY

The present study confirmed and quantified the amounts of plant-derived antioxidants in crude extract of *C. sinensis*. The amount of antioxidant was decreased in the following order: phenolic (59.40 mg GAE/g) > flavonoids (35.21 mg QE/g) > terpenoids (3.33 mg linalool/g). These preliminary scientific findings revealed that SF would be effective to be developed as topical antioxidant non-sunscreen photoprotective formulation which can neutralize UVB-induced ROS and provide additional photoprotective benefits protect skin cells to prevent for skin aging and cancer.

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