Pharmacogn. Res., 2023; 15(3):537-543.
https://www.phcogres.com

Original Article

In vitro Cytoprotective Activity Study of Cocoa (*Theobroma cacao* L.) Pod Husk Ethanolic Extract against Blue Light Irradiation

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ABSTRACT

Background: Blue light exposure has been shown to induce ROS generation and subsequent inflammatory pathways that lead to cell death, in which antioxidants could counteract this effect. Although regarded as waste, cocoa (*Theobroma cacao* L.) Pod Husk (CPH) possesses a high polyphenolic contents, which could serve as antioxidants. **Objectives:** To characterize CPH ethanolic extract based on its antioxidant capacity and observe the cytoprotective ability of CPH *in vitro* upon blue light exposure. **Materials and Methods:** CPH ethanolic extract was characterized through total phenolic content, total flavonoid content, and three antioxidant assays, then treated on HaCaT cells, in which cell viability was measured through MTS assay. **Results:** CPH extract showed high phenolic and flavonoid contents and high antioxidant capacity through DPPH and FRAP assay. CPH extract started to exert cytotoxicity from concentrations of 400 µg/mL and above, while 100 µg/mL and above in AA. Furthermore, CPH extract showed significant cytoprotective effect at 50 µg/mL at 11.92 ± 0.83% cell viability increase, wherein AA failed to protect HaCaT cells at the same concentration at 15.79 ± 0.72% cell viability decrease. **Conclusion:** CPH could serve as an alternative as blue light protection agent as it was safe to be used at higher concentrations and was able to protect HaCaT cells from blue light irradiation better than AA.

Keywords: Antioxidant, Blue light, Cell culture, Cocoa pod husk, HaCaT, Cocoa pod husk cytoprotectivity against bluelight.

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Received: 01-03-2023;
Revised: 29-03-2023;
Accepted: 13-05-2023.

INTRODUCTION

Sun exposure has been widely known to negatively affect human skin. This negative effect is caused by the component of sunlight, which includes 5%-7% of Ultraviolet (UV) radiation, 54% of infrared radiation, and 44% of visible light. However, it is not until recently that the impact of visible light was discovered to be detrimental to the skin.[1] Shorter wavelengths from 400 to 500 nm has been shown to contribute to skin photoaging by decreasing skin laxity, increasing wrinkles, and especially causing hyperpigmentation.[1]

The source of this shorter wavelength High Energy Visible (HEV) light does not only come from sunlight, but also from artificial light sources. The intensity of HEV light increases from cell phones, computers, TV and the sun. Exposure to HEV light could induce the formation of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) in the skin, which are associated with Extracellular Matrix (ECM) degradation, collagen and elastin fibers degradation and hyperpigmentation, which contribute to premature skin aging.[1,2] On molecular levels, ROS induces the activation of inflammatory pathways, lipid peroxidation, DNA damage and strand breaks.[3,4] Inflammatory reactions include activation of Matrix Metalloproteinases (MMP) that degrade ECM, causing damage to the skin physiology.[5] In lipid peroxidation, ROS triggers the production of malondialdehyde, an oxidant that causes damage to the cells.[6]

Administration of exogenous antioxidants was proven to benefit the skin protection against ROS. Blue light damages the skin

DOI: 10.5530/pres.15.3.056

by not directly overwhelming the cellular antioxidant defense through the formation of ROS, but also through the continual production of a low level of radicals which could evade the defense mechanism, causing permanent DNA damage.[2] This leads to a need for ingredients that could have antioxidant and/or blue light-absorbing properties. Antioxidants such as vitamin C has pro-oxidant effects at high concentrations, while using UV-absorbing compounds have limited to no data in regards to its protection in attenuating blue light.[1] To address the issue, Cocoa Pod Husk (CPH) (*Theobroma cacao* L.) is presented as a potential ingredient that could protect the skin against blue light damage, as it contains polyphenols such as phenolic compounds and flavonoids.[7] Phenolic acids and flavonoids have the potential to protect the skin against blue light exposure, as they could scavenge and interact with ROS/RNS, terminating the chain reaction before the cell is seriously damaged.[8] Flavonoids are also suggested to have a blue light-absorbing capacity.[9] CPH ethanolic extract has been shown to contain a high level of polyphenol.[10,11] The utilization of CPH as a material in skin care products could also contribute to the environment, as CPH is the by-product of the cocoa industry, which builds up 70-75% of the whole cocoa fruit.[11]

This research aims to conduct a characterization of CPH ethanolic extract, along with analyzing the skin protection ability of different concentrations of CPH ethanolic extract against blue light irradiation *in vitro*.

MATERIALS AND METHODS

Extraction of plant material

The plant was obtained from National Research and Innovation Agency (BRIN), Indonesia, obtained from Pesawaran, Lampung, Indonesia var. *Forastero*. Twenty grams of CPH was extracted with 100 mL of 70% ethanol. The sample was shaken using an incubator shaker (Taitec BR-43FL) at 170 rpm and 25°C for 24 hr. The mixture was centrifuged (Tomy KITMAN-T24) at 5000 G and 4°C for 10 min. The extraction was repeated several times and the collected filtrate was combined. The solvent was evaporated using a rotary evaporator (IKA RV 10 Digital), followed by drying the sample with a freeze dryer (Alpha 1-2 LD Plus Christ).

Total Phenolic Content (TPC)

The total phenolic content was characterized by utilizing the Folin-Ciocalteu assay. Ten grams of CPH extract was mixed with 10 mL of methanol and then filtered. Five mL of 7.5% Folin-Ciocalteu reagent was then added and incubated at room temperature for 8 min. Subsequently, 4 mL of 1% NaOH was added to the mixture, and the absorbance was measured with UV-vis Spectrometer (Shimadzu 1280) at 730 nm. The result was expressed as milligrams of gallic acid equivalent (mg GAE/g extract).

Total Flavonoid Content (TFC)

The total flavonoid content was done using the aluminium chloride method. Briefly, 10 mg of CPH extract with ethanol was dissolved in 10 mL ethanol. Afterwards, 2 mL of the solution was mixed with 0.1 mL 10% $AlCl_3$, 0.1 mL 1 M CH_3COOH, and 2.8 mL of water. The mixture was then incubated in the dark at room temperature for 30 min. Then, the absorbance was measured using UV-vis Spectrometer (Shimadzu 1280) at 415 nm. The results were expressed as milligrams of quercetin equivalent (mg QE/g extract).

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay

The antioxidant contained in the plant extract reduces DPPH into 1,1-diphenyl-2-picrylhydrazyl. Briefly, 100 µM DPPH was prepared in methanol. Afterwards, 1:1 ratio v/v of DPPH solution and varying concentrations of CPH extract were mixed. The same was done with ascorbic acid as standard. The mixtures were incubated in the dark at room temperature for 30 min. Then, the absorbance was measured using a UV-vis spectrophotometer (Shimadzu 1280) at 517 nm. Each concentration was measured in triplicates. The free radical scavenging ability was calculated using the following formula.

Free radical scavenging ability (%) = [Absorbance (blank) – Absorbance (sample)]/Absorbance (blank) × 100%

Equation 1. Free radical scavenging ability (%) for DPPH assay

Ferric reducing antioxidant power (FRAP) assay

Prior to the assay, the reagent was prepared by mixing 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3$ solution, and 300 mM, pH 3.6 acetate buffer solution at a ratio of 1:1:10 v/v. A concentration of 1000 µg/mL CPH ethanolic extract was dissolved. Meanwhile, a series of AA as standard was dissolved. Then, 100 µL of each sample was mixed with 3 mL of FRAP reagent, incubated in a dark condition at room temperature for 10 min. Absorbance was measured using UV-vis Spectrophotometer (Shimadzu 1280) at 593 nm. The results were expressed as milligrams of ascorbic acid equivalent (mg AAE/g extract).

2,2′-azino-bis(3-ethylbenzene- thiazoline-6-sulfonic acid (ABTS) assay

The ABTS reagent was first made by mixing a 1:1 ratio of 2.45 mM Ammonium persulfate and 7 mM ABTS. The solution was incubated for 16 hr prior in a dark condition, at room temperature. Then, ABTS radical reagent was diluted until it reached an absorbance of 0.700 when measured at 734 nm, using UV-vis Spectrophotometer (Shimadzu 1280). A concentration of 1000 µg/mL CPH ethanolic extract was dissolved. Meanwhile, a series of AA as standard was dissolved. 1:1 v/v ratio of sampel and ABTS reagent was mixed and incubated for 6 min in the dark at room temperature. The absorbance was measured by UV-vis

Table 1: Antioxidant assay results of cocoa pod husk (CPH) extract and ascorbic acid (AA) through DPPH, FRAP and ABTS assays.

Assay	Parameter	CPH	AA
DPPH	IC_{50}	52.32 ± 3.63 µg/mL	5.53 ± 3.88 µg/mL
FRAP	AA equivalent	33.03 ± 0.13 mg AAE/g extract	–
ABTS	AA equivalent	3.086 ± 0.003 mg AAE/g extract	–

Spectrophotometer (Shimadzu 1280) at 734 nm. The results were expressed as milligrams of ascorbic acid equivalent (mg AAE/g extract).

HaCaT cell culture and harvesting

HaCaT cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing L-glutamine and pyridoxine hydrochloride, supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum (Gibco, USA), incubated in 5% CO_2 at 37°C (ThermoScientific, USA). The cells were cultured and passaged once the confluency reached 60-80%. Cells were harvested by using 0.25% trypsin-EDTA (Gibco, USA).

Cytocompatibility assay of CPH and AA

HaCaT cells were seeded at a density of 1 x 10^4 for each well in the 96-well plate, then incubated for 24 hr, in a CO_2 incubator. At around 90% confluency, the cells were treated with the CPH extract and AA separately, using concentrations from 200 to 6.25 µg/mL. Cell viability was measured by adding 15 µL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) reagent (CellTiter 96® AQ_{ueous} One Solution Assay, Promega, US) to each well, followed by three hours of incubation in 5% CO_2, at 37°C. The absorbance was measured at 490 nm wavelength using a plate reader (The Infinite®M200 NanoQuant, TECAN, Switzerland). The assay was done in triplicate and the cell viability was calculated using Equation 2.

$$\text{Cell viability (\%)} = \frac{[\text{Absorbance(treatment)} - \text{Absorbance(blank)}]}{[\text{Absorbance (negative control)} - \text{Absorbance(blank)}]} \times 100\%$$

Equation 2. Cell viability (%) for MTS assay.

Cytoprotective assay upon blue light irradiation

HaCaT cells were seeded at a density of 1 x 10^4 for each well in a 96-well plate, then was incubated for 24 hr. HaCaT cells were treated with CPH ethanolic extract and AA in two concentrations, 25 and 50 µg/mL in triplicates. The wells were irradiated under blue light for 6 hr at a distance of 15 cm. A control plate (external control) was not exposed to blue light radiation. The cell viability was assessed using MTS assay as previously mentioned.

Statistical analysis

The data obtained were presented as mean ± standard deviation. Statistical analysis was done using GraphPad Prism 8. The nomallity of data distribution was analyzed using Shapiro-Wilk test. The normally-distributed data were anayzed with one-way ANOVA, accompanied with Dunnett's *post-hoc* test. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

TPC, TFC and antioxidant assays

The extraction process yielded 9% w/w of CPH extract. The characterization was performed through extrapolation of standard curve. CPH ethanolic extract contains a total phenolic content of 133.47 ± 34.10 mg GAE/g extract (R=0.999) and a total flavonoid content of 10.71 ± 0.32 mg QCE/g extract (R=0.998).

Subsequently, three antioxidant assays were conducted in this study, which were DPPH, FRAP, and ABTS assay and shown in Table 1.

The IC_{50} of ascorbic acid was analyzed and found to be 5.53 ± 3.88 µg/mL, while the IC_{50} of cocoa pod husk ethanolic extract was found to be 52.32 ± 3.63 µg/mL. In addition, based on the FRAP assay, the CPH ethanolic extract contains an antioxidant content of 33.03 ± 0.13 mg AAE/g. While based on the ABTS assay, the CPH ethanolic extract contains an antioxidant content of 3.086 ± 0.003 mg AAE/g.

Cytotoxicity assay of CPH (*Theobroma cacao* L.) ethanolic extract and AA

Several concentrations were tested on HaCaT cells for the CPH ethanolic extract (Figure 1). CPH ethanolic extract was significantly cytotoxic to HaCaT cells starting at concentrations of 400 and 800 µg/mL. In addition, the IC_{50} of CPH ethanolic extract was found to be 430.36 µg/mL. Meanwhile, AA showed significant cytotoxic effects starting at concentrations of 100 and 200 µg/mL. As such, concentrations of 25 and 50 µg/mL were chosen for the cytoprotective assay.

Cytoprotective assay against blue light irradiation

Two controls were used for this experiment, an external and internal control. The external control was not irradiated with blue light, while the internal control was. CPH ethanolic extract at a concentration of 50 µg/mL showed a significant increase of cell viability at 11.92±0.83% in comparison with the internal control (Figure 2). In this experiment, the ascorbic acid did not show any

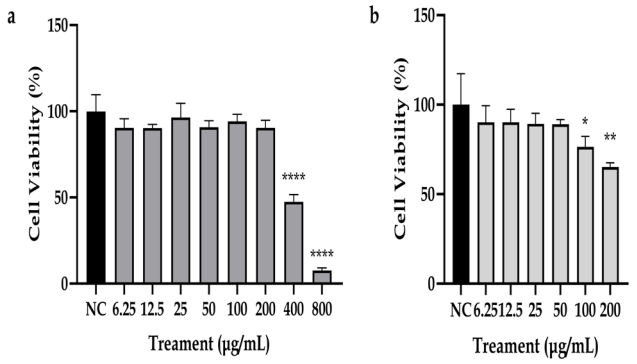

Figure 1: HaCaT cell viability following cytotoxicity assay of (a) CPH ethanolic extract and (b) Ascorbic acid for 6 hr of incubation time. NC = Negative Control. Values represent mean ± SD (*n*=3). Statistical analysis was done using one-way ANOVA and Dunnett *post hoc* test (*p = 0.220, **p = 0.0011, ****p < 0.0001).

cytoprotective ability against the blue light irradiation, shown at the decreased cell viability at 15.79±0.72% compared to the internal control.

Upon analysis of the cell photograph, Figure 3 shows the cell morphology of between internal control and 50 µg/mL of CPH ethanolic extract.

DISCUSSION

Cocoa pod husk, available in abundance as a waste product of chocolate production, was usually discarded after the production process. As a by-product, it exhibits great potential for cosmetic and skincare application due to its high antioxidant properties. In this study, TPC was measured as CPH ethanolic extract was reported to contain polyphenols, including phenolic acids such as salicylic acid, p-hydroxybenzoic acid, protocatechuic acid, and flavonoid, mainly catechin and (epi)gallocatechin.[12] These polyphenol compounds were reported for its ROS scavenging

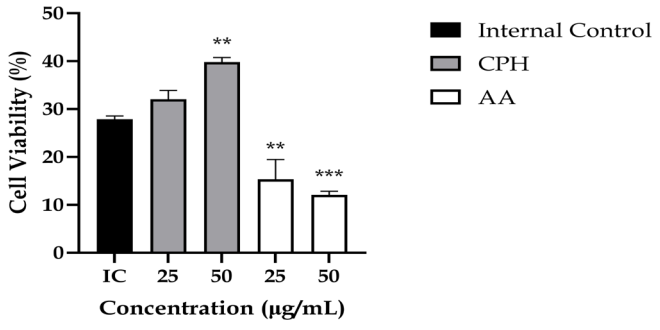

Figure 2: HaCaT's cell viability based on the cytoprotective study (MTS assay) of CPH ethanolic extract (CPH) and ascorbic acid (AA). IC = Internal Control (untreated cells under bluelight). Values represent mean ± SD (*n*=3). Statistical analysis was done using one-way ANOVA and Dunnett *post hoc* (**p < 0.005, ***p = 0.0027).

activities, which could interact with the ROS produced by blue light, although the exact mechanism has not been elucidated.[13]

In a previous study, TPC value of CPH extract was estimated to be 49.54 ± 3.39 mg GAE/g extract.[14] Our CPH extract was shown to have an approximately 2.69-fold higher TPC content at 133.47 ± 34.10 mg GAE/g extract. In another study, the highest TFC value to CPH extract was estimated to be 4.34 mg QE/g extract.[15] Our CPH extract was shown to have an appoximately 2.47-fold higher TFC content at 10.71 ± 0.32 mg QCE/g extract. Thus, we conclude that our CPH extract showed higher TPC and TFC values than previous studies. The difference in TPC and TFC could be affected by the type of solvent and solvent ratio.[16]

In addition, the flavonoid contained in CPH was reported to be mainly catechin and (epi)gallocatechin.[12] As flavonoids are included as polyphenols, it also has protection ability against blue light, in which it has ROS scavenging activity, antioxidant activity, and ROS production inhibition.[17] A higher ethanol percentage could result in a higher TFC value, while a longer extraction process increases the TFC value but decrease if the optimum extraction time is exceeded, as the cell wall in the CPH will break continuously due to the initiation and diffusion of the solvent.[18]

Beside TPC and TFC measurement, three different antioxidant capacity assays were also performed to confirm its antixodant potency. As there is no universal antioxidant assay that can give a definitive elucidation to an extract's antioxidant capacity, it is advised to perform at least three different antioxidant assays to give a wider perspective on the antioxidant capacity.[19] Herein, DPPH, FRAP and ABTS assays were performed. DPPH assay measures the antioxidant capacity based on both Hydrogen Atom Transfer (HAT) and Single-Electron Transfer (SET), while ABTS is a HAT-based assay, and FRAP is a SET-based assay.[20] The antioxidant capacity of the extract was measured as bluelight irradiation could cause the formation of free radicals.[21] Thus, we hypothesized that one of the cytoprotection mechanisms was through radical-scavenging activities, hence the antioxidant capacity assays were performed. The extract showed varying results of antioxidant potency. Through DPPH assay, the result showed that AA possessed a higher antioxidant potential than CPH ethanolic extract (Table 1). An IC_{50} below 100 µg/mL can be regarded as high antioxidant potential.[22] Thus, as the IC_{50} of CPH ethanolic extract was 52.32 ±3.63 µg/mL, the extract showed a high antioxidant potential. Currently, there is limited data on the antioxidant capacity of CPH ethanolic extract through FRAP assay. Compared to other extracts such as *Boesenbergia pandurata* rhizome ethanolic extract, known for its potent use as a photoprotectant with an antioxidant activity of 37.59 mg AAE/g,[23] CPH ethanolic extract showed comparable results at 33.03 ± 0.1333 mg AAE/g through FRAP assay. In comparison with another type of plant extract that could be utilized in cosmetics products, for example, in a study, *Lavandula pedunculata* extracts possessed a high antioxidant capacity of 165.4 ±10.7 mg AAE/g

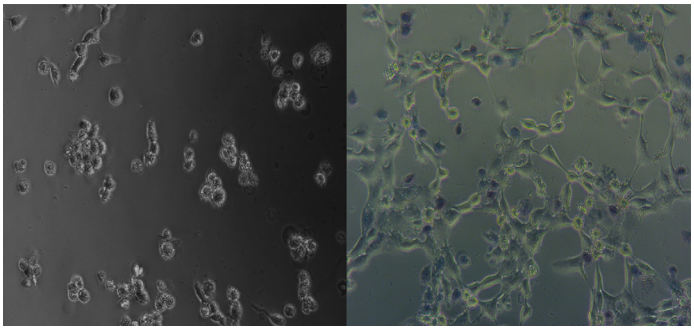

Figure 3: Cell photograph after 6 hr of incubation. Left: internal control with no treatment, Right: treated with 50 µg/mL of CPH ethanolic extract.

through ABTS assay.[24] Thus, in comparison to these extracts, the antioxidant activity of CPH ethanolic extract from this experiment, 3.09 ± 0.003 mg AAE/g, could be regarded as low. As such, we conclude that our extract showed a high antioxidant capacity through DPPH and FRAP assay, while a low antioxidant capacity through ABTS assay. Comparing these assays through the mechanisms of action, we postulate that the mechanism of antioxidant capacity leaned towads SET-based reactions.

Cytotoxicity assay of CPH (*Theobroma cacao* L.) ethanolic extract and AA

Prior to the cytoprotective assay, cytocompatibility assay of CPH ethanolic extract and AA were conducted. This was done to determine the cytocompatible concentrations that are safe for the cells. AA is often utilized as a control positive in many studies as it generally has a fundamental job in maintaining the function of the cells.[25] As the mechanism of bluelight-induced cytotoxicity is through the production of ROS, we used AA, a gold standard of antioxidant, as the control positive.[26] Though in a higher concentration, starting at 100 µg/mL, it showed decreasing cell viability, meaning the ascorbic acid has some extent of cytotoxicity towards the HaCaT cells. This could be due to the pro-oxidant properties of AA. In high concentrations, AA reacts with iron or copper in the body in a reaction called the Fenton reaction, which produces oxygen radicals as a result, leading to cytotoxicity in the cells.[27,28] As such, we postulate that CPH extract can serve as a safer alternative than AA, as it showed a safer cytotoxicity profile in higher concentrations than AA.

In general, CPH extract can be deemed as non-toxic toward HaCaT cells, as a previous study identifying the active compounds in CPH concluded that it possessed procyanidin type B, especially procyanidin B2, which consist of the dimer structures of epicatechin that with little toxicity towards the HaCaT cells.[25]

Cytoprotective assay against blue light irradiation

The cytoprotective assay was done to assess whether CPH ethanolic extract has the ability to protect the HaCaT cells against ROS caused by blue light irradiation. Blue light irradiation induces oxidative stress due to the accumulation of ROS produced.[29] This is due to cytochrome c oxidase, a biological fluorophore in the mitochondria, that produces ROS when affected by blue light.[30] Subsequently, the increased ROS production also significantly increases caspase-3/7 activity, which later induces cell apoptosis.[31] Thus, the presence of exogenous antioxidant here was hypothesized to help the skin in scavenging for the ROS species, and thus protect the cells from the negative effects of blue light. This protection capability is speculated to come from the polyphenols content of CPH ethanolic extract, which includes the phenolic compounds and flavonoids and also highly correlated with antioxidant capacity.[12]

The cytoprotective study of the CPH ethanolic extract showed an increased cell viability at 11.92±0.83% at a concentration of 50 µg/mL. Meanwhile, illustrations of the cells treated with 50 µg/mL of CPH ethanolic extract compared to internal control was shown in Figure 3. Interestingly, AA showed a decreased cell viability at 15.79±0.72% at the same concentration. This showed that CPH ethanolic extract showed better cytoprotective capabilities in protecting HaCaT cells against bluelight irradiation than AA within the same treatment concentration. Thus, CPH ethanolic extract could be used as a better alternative to protect against bluelight due to its superior cytocompatibility and higher cytoprotective capability. The cytotoxic effect of AA has been correlated with the formation of H_2O_2 in the extracellular surroundings of the cells, which was suggested to be caused by the aerobic 'auto-oxidation' of the ascorbate whem the AA concentration is too high.[27]

These results of cytoprotective action may be linked to the total phenolic and flavonoid, along with antioxidant capacity. Recent studies eludicated polyphenols present in CPH, including catechin, quercetin, epicatechin, gallic acid, coumaric acid, protocatechin, and many more.[7,32,33] Catechin, the most abundant polyphenol in CPH, possesses potent antioxidant capacity through both direct (scavenging ROS, chelating metal ions) and indirect mechanisms (inducing antioxidant enzymes, inhibiting pro-oxidant enzymes, inducible nitric oxide synthase, suppessing stress-realted signaling pathways such as Activator Protein 1 (AP-1) and nuclear factor κB (NF-κB).[34]

Similarly, ascorbic acid exerts its antioxidant capacity through direct and indirect mechanisms.[35] The antioxidant capacity of a polyphenol is highly and positively correlated with the number of hydroxyl groups.[36] As polyphenols donate hydrogen atoms to neutralize free radicals, their structure remains stable due to the presence of resonance-stabilized rings. These polyphenols contained in the extract may exert antioxidant activity which includes the interaction with ROS/RNS, scavenging of radicals, metal ion chelation, repression of ROS production by restriction of enzymes, upregulating the antioxidant defenses, and many other mechanisms.[8] As such, the polyphenols present in CPH can act as safer and better alternative of antioxidants than AA and against blue light. Further analysis on the mechanisms of action based on gene expression could be done in future studies in

order to elucidate the molecular mechanisms and gain a deeper understanding.

CONCLUSION

This research provides the data on the characterization and the skin protection ability of the ethanolic extract of Cocoa Pod Husk (CPH) (*Theobroma cacao* L.), which is otherwise regarded as waste, against blue light irradiation *in vitro* on HaCaT cells. Upon characterization, CPH ethanolic extract has TPC value of 133.47 ±34.10 mg GAE/g extract and a TFC value of 10.71 ± 0.32 mg QCE/g extract. In addition, CPH extract showed high antioxidant capacity through DPPH and FRAP assay, while low capacity through ABTS assay. As such, we speculate that the mechanism of antioxidant capacity of CPH extract leaned towards SET-based reactions. Furthermore, CPH showed a better safety profile than ascorbic acid (AA), as it was cytocompatible with the cells at higher concentrations than AA. Cytoprotective assay of CPH extract showed significantly increased cell viability at 11.92 ± 0.83%, while AA showed significantly reduced cell viability at the same concentration of 50 µg/mL at 15.79 ± 0.72%. This showed that CPH could serve as a potential protective agent against blue light, with better safety profile than AA, and thus are prospective as an alternative active ingredients in cosmetics or skincare dedicated for free-radicals protection coming from blue light exposure.

ACKNOWLEDGEMENT

This research was partially funded by Research Program (DIPA Rumah Program) at the Research Organization for Life Sciences and Environment, the National Research and Innovation Agency (BRIN).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AA: Ascorbic acid; **ABTS**: 2,2'-azino-bis(3-ethylbenzene-thiazoline-6-sulfonic acid; **AP-1:**Activator protein 1; **CPH**: Cocoa pod husk; **DMEM:** Dulbecco's Modified Eagle's Medium; **DPPH**: 2,2-diphenyl-1-picryl-hydrazyl-hydrate; **ECM**:Extracellular matrix; **FRAP:** Ferric reducing antioxidant power; **HAT**:Hydrogen atom transfer; **HEV**: High energy visible; **MMP**:Metalloproteinase; **MTS**:3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; **NF-κB**: Nuclear factor κB; **RNS:** Reactive nitrogen species; **ROS**: Reactive oxygen species; **SET**: Single-electron transfer; **TFC**:Total flavonoid content; **TPC:** Total phenolic content; **UV:** Ultraviolet.

SUMMARY

This research observes the cytoprotective effects of ethanolic extract of cocoa pod husk (CPH) (*Theobroma cacao* L.), which is otherwise regarded as waste, against blue light irradiation *in vitro* on HaCaT cells. CPH showed a better safety profile than ascorbic acid (AA), and better effects in protecting CPH against blue light irradiation than AA. This showed that CPH could serve as a potential protective agent against blue light.

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Cite this article: Adiyanto SA, Chriscensia E, Wibowo EC, Nathanael J, Crystalia AA, Putra ABN, *et al*. *In vitro* Cytoprotective Activity Study of Cocoa (*Theobroma cacao* L.) Pod Husk Ethanolic Extract against Blue Light Irradiation. Pharmacog Res. 2023;15(3):537-43.