Malays. Appl. Biol. (2022) 51(4): 201-211
https://doi.org/10.55230/mabjournal.v51i4.29

IN-VITRO MELANOGENESIS, CYTOTOXICITY, AND ANTIOXIDANT ACTIVITIES OF *Peltophorum pterocarpum* LEAF EXTRACTS

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Accepted 3 October 2022, Published online 31 October 2022

ABSTRACT

Melanin is a protective pigment against cellular damage and skin cancer. *Peltophorum pterocarpum* has been used for centuries to treat skin diseases like eczema and psoriasis. However, whether *P. pterocarpum* leaf extract can affect melanin synthesis with the advantage of antioxidant defense against ultraviolet (UV) radiation remains to be investigated. We aimed to examine the melanogenesis, cytotoxicity, and antioxidant activities of *P. pterocarpum* leaf extracts. A two-dimensional (2D) cell culture model was employed to demonstrate the effect of *P. pterocarpum* extracts on melanin synthesis. The cell viability of B16-F1 melanoma cells was measured by Neutral Red Uptake (NRU) assay. Antioxidant activity was accessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) assays. The ethanol extract of *P. pterocarpum* dose-dependently increased the melanin content and displayed cytotoxicity to B16-F1 melanoma cells at the highest concentration. In contrast, the equal amounts of the aqueous extract significantly inhibited melanin synthesis and did not show any cytotoxic effects on B16-F1 melanoma cells. The *P. pterocarpum* ethanol extract had a significantly higher total phenolic and flavonoid content than the aqueous extract and was more effective at scavenging DPPH free radicals and intracellular ROS induced by UVB, with IC_{50} of 519.24 ± 122.57 vs 1798.45 ± 143.09 µg/mL and 878.00 ± 23.50 vs 1379.00 ± 21.81 µg/mL, respectively. These findings suggest that the pro-melanogenic and anti-melanogenic activities of *P. pterocarpum* extract with antioxidant capacity against UVB-induced cellular damage are affected by the total phenolic and flavonoid contents.

Key words: B16-F1 melanoma cells, melanin, *Peltophorum pterocarpum*, total flavonoids, total phenolics

INTRODUCTION

Melanin is a biological pigment that is required for hair, skin, and eyes. It is also expressed in the ears and the neurological system (Palareti *et al*., 2016). Melanogenesis is primarily determined by genetic factors and is strongly impacted by various intrinsic factors, such as oxidation and hormonal imbalance (Bellei & Picardo, 2020), as well as extrinsic factors like UV radiation and pollution (Tuerxuntayi *et al*., 2014; Umar *et al*., 2022). Skin pigmentation disorders include a broad spectrum of manifestations, comprising hypopigmentation and hyperpigmentation in both benign and malignant situations. These anomalies can result in major cosmetic deformity, low self-esteem, personality development, and social connections. (Dabas *et al*., 2020). Therefore, regulating normal skin melanin synthesis and distribution is vital in maintaining health and expressing beauty.

Topical steroids (corticosteroids), surgery (skin grafts), laser treatment, and phototherapy are commonly applied to treat skin pigmentation disorders (Zubair *et al*., 2019). Although the use of steroids is deemed practical, the medicine is associated with side effects and necessitates a lengthy period of recurrent therapy (Ohguchi *et al*., 2019). Excessive and prolonged usage of steroids can result in skin damage such as redness, thinning, itching, acne, and burning (Passeron, 2017; Visuvanathan *et al*., 2018). Although laser therapy and phototherapy are considered non-invasive procedures, they are pricey and primarily available in large cities.

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Peltophorum pterocarpum (Jemerlang laut) is an ornamental tree that belongs to the Fabaceae (Leguminosae) family. It is traditionally used as an anti-inflammatory in the treatment of sprains, swellings, and bruises, as an astringent for relief of post-natal intestinal disorders, and as a cream for muscle aches, sores, and problems in the eyes (Jha *et al*., 2018). *Peltophorum pterocarpum* extract has a variety of biological activities, such as anti glycemic (Manaharan *et al*., 2011), antibacterial (Sukumaran *et al*., 2011), antioxidant (Vajpai *et al*., 2021), and cytotoxic activities (Kim *et al*., 2020). Quercetin derivatives were discovered as the active ingredient in *P. pterocarpum* extract (Manaharan *et al*., 2011; Li *et al*., 2019). Nagata *et al*. (2004) first reported that the addition of quercetin to cultured melanoma cells improves melanin synthesis. However, Fujii and Saito (2009) and Arung *et al*. (2011) have demonstrated the anti-melanogenesis effect of quercetin in human malignant cell lines and B16 mouse melanoma cells. These findings indicate that extrinsic regulatory cues can influence melanogenesis in cells. Therefore, our study was primarily designed to examine whether *P. pterocarpum* extract can enhance or reduce melanin content in B16-F1 mouse melanoma. We further investigated the antioxidant capacity of *P. pterocarpum* extracts to reduce ROS generation induced by UVB.

MATERIALS AND METHODS

Plant extraction

Peltophorum pterocarpum leaves were collected from SIRIM Berhad, Shah Alam, Malaysia in October 2019. The plant was authenticated and taxonomically identified by a botanist in the Herbarium of Universiti of Putra Malaysia. A voucher specimen (SK 3187/17) was deposited at the same institute for further reference. The extract of *P. pterocarpum* leaves was prepared according to Manaharan *et al*. (2011). The leaves were washed with tap water, and shade dried at room temperature, followed by oven drying at 40 °C. The dried leaves were ground using a dry miller (Fritsch, Idar-Oberstein, Germany). 100 g of powdered leaves were dissolved in different solvents (distilled water & ethanol) at a sample: solvent ratio of 1:10 (w/v). The extraction using distilled water was carried out at 40°C while the ethanol extraction was conducted at ambient temperature. The mixtures were placed on an orbital shaker for 24 h. The ethanol extract was filtered and concentrated using a rotary evaporator (Eyela N-1200A, Tokyo, Japan) while the aqueous extract was lyophilized using a freeze dryer (Labconco Freezone 18, Missouri, USA). The residues were collected, and the percentage of extraction yield was calculated as follows:

$$\text{Extraction yield (\%)} = \frac{W_A}{W_0} \times 100\%$$

where W_A is the weight of the extract after evaporating solvent or freeze-drying and W_0 is the dry weight of the sample.

Maintenance of cell culture

B16-F1 murine melanoma cells and human keratinocytes HaCat cells were obtained from the American Type Culture Collection (ATCC), Manassas, Virginia, United States, and cultured in Dulbecco's Modified Eagle Medium (DMEM) (buffered with sodium bicarbonate), supplemented by 10% (v/v) new-born calf serum and fetal bovine serum, respectively. Penicillin (100 units/mL) and streptomycin (100 mg/mL) were added to both cultures. All cell cultures were kept in a humidified incubator at 37 °C and 5% CO_2. The cells were removed from the flask by treatment with Trypsin-EDTA once the monolayer had reached 70–80% confluence.

Melanogenesis assay

Cell viability assay

The effect of *P. pterocarpum* leaf extracts on the viability of B16-F1 cells was evaluated using an NR-based assay as described by Üstün Alkan *et al*. (2014). Each well of 24-well culture plates was seeded with 0.1 mL of cell suspension with an approximate concentration of 1.0×10^5 cells/mL. The plate was incubated at 37 °C for 24 h in a humidified atmosphere of 5% CO_2 and 95% air to obtain subconfluent cell monolayers (70-90% confluency) before use. The cells were treated for 48 h with ethanol and aqueous extracts with concentrations ranging from 250 ng/mL to 8000 ng/mL. The treatment medium was removed and 500 µL of neutral red medium (50 µg/mL) was added to each well. The plate was incubated at 37 °C with 5% CO_2 for 3 h in the dark to allow the uptake of dye into the cells. The neutral red medium was removed, and the cells were then rinsed with PBS. The supernatant was discarded, and a 500 µL fixative solution (1% w/v $CaCl_2$ in 0.5% v/v formaldehyde) was added to the wells, and the plate was incubated at room temperature for 1 min to fix the cells. The fixative solution was removed and replaced with 1 mL of NR extract solution containing 49% of water, 50% of ethanol, and 1% of acetic acid. The plate was shaken on a microtiter plate shaker for 15 min at room temperature and the absorbance was read at 540 ± 10 nm using a microplate spectrophotometer (FLUOstar Omega, BMG LabTech, Germany). The percentage of B16-F1 viability was calculated using the following equation:

$$\text{(\% cell viability)} = \frac{[X_{\text{treated cells}} - X_{\text{blank}}]}{[X_{\text{tcontrol}} - X_{\text{blank}}]} \times 100\%$$

where X is the absorbance of viable cells.

Melanin content

The melanin synthesis was assessed on B16-F1 cultured cells as described previously by Junlatat *et al*. (2018) with some modifications. The cells were seeded at a density of 1.0×10^5 cells/mL in the 24-well culture plates and incubated at 37 °C with 5% CO_2 in a humidified incubator until 80% confluent. B16-F1 cells were treated with six concentrations varying from 250 ng/mL to 8000 ng/mL for another 48 h at 37 °C with 5% CO_2. For extracellular melanin content, 75 µL of the cultured medium was collected and incubated with 75 µL of 5% trichloroacetic acid (TCA) at room temperature. The solution was mixed and centrifuged at 2000 rpm. The pellet was dissolved with 100 µL NaOH (1N) and shaken for 15 min at room temperature. The absorbance was read at 475 nm and melanin content was determined in comparison with the standard curve of 5 mg/mL synthetic melanin. The alpha-melanocyte-stimulating hormone (α-MSH) (100 nM) was used as a positive control, and the untreated cells represent a reference. The percentage of melanin stimulation was calculated as follows:

$$\%\ \text{melanin stimulation} = \frac{[Y_{sample} - Y_{reference}]}{Y_{reference}} \times 100\%$$

where Y is the absorbance of melanin content.

Determination of polyphenol content

Total phenolic content

The total phenolic (TPC) content was determined using the Folin-Ciocalteu assay as described by Choo *et al*. (2011). 1 mg/mL of crude samples (aqueous and ethanol) were sonicated and vortexed to solubilize them before transferring 20 µL of each dilution into a 96-well microplate. 100 µL of Folin-Ciocalteu reagent (10%) was added and the mixture was agitated at room temperature for 10 min. 80 µL of sodium carbonate solution (75 mg/mL) was added to each well and allowed to incubate for 60 min at room temperature. Gallic acid was used as a standard and was prepared freshly before use. The absorbance of samples and standard was measured at 765 nm using a microplate reader (FLUOstar Omega, BMG LabTech, Germany). The results were expressed in gallic acid equivalents, GAE, in mg/mL of a liquid sample. A minimum of three independent experiments were performed with a minimum of three internal replicates.

Total flavonoid content

The aluminum chloride assay described by Zhishen *et al*. (1999) was employed to quantify the total flavonoid content (TFC) of crude samples. 1 mL of each crude sample of 1 mg/mL (ethanolic & aqueous) and 5% sodium nitrite (0.3 mL) were added into a separate glass test tube. The mixture was incubated at room temperature for 5 min. 10% of aluminum chloride solution (0.3 mL) was then added to the reaction mixture. The mixture was continued to incubate at room temperature for another 5 min. Two mL of 1 M sodium hydroxide was added at the end of the incubation period. Distilled water was used as a blank. The reaction mixture was allowed to stand at room temperature for 10 min before being measured for absorbance. The absorbances were measured at 510 nm using a microplate reader (FLUOstar Omega, BMG LabTech, Germany). Results were expressed as mg of rutin equivalent (RE) per g of extract, using the following equation:

$$\text{TFC, mg RE/g} = \frac{V_s}{m_s} \times c$$

where C is the rutin standard value obtained from the standard calibration graph in mg/mL, V_s is the volume of sample in mL, and m_s is the mass of the sample in g.

Determination of antioxidant activity

DPPH radical scavenging assay

DPPH radical scavenging assay for plant extracts was performed as described in Ling *et al*. (2008). A total of 190 µL of 0.004% DPPH ethanolic solution was added to the 10 µL sample with varying concentrations (250, 500, 1000, & 2000 µg /mL) in a 96-well plate. The mixture was mixed and agitated using a microplate shaker and later allowed to incubate at 37 °C for 10 min. The absorbance was measured at 515 nm using a microplate reader (FLUOstar Omega, BMG LabTech, Germany). Ethanol served as a control, while L-ascorbic acid, a well-known standard with strong antioxidant activity, was used as a standard. L-ascorbic acid was prepared freshly before use. The percentage of free radical scavenging activity was calculated using the following equation, where A is the absorbance.

$$\frac{[A_{Negative\ control} - A_{sample}]}{A_{Negative\ control}} \times 100\%$$

The DPPH assay was performed in triplicates. The antioxidant activity of the extracts was expressed as half-maximal inhibitory concentration (IC_{50}).

Cellular ROS inhibition activity

The cellular ROS inhibition activity was evaluated as described in Pygmalion *et al*. (2010) and Oh *et al*. (2017) with slight modification. Human keratinocyte cell line, HaCaT has seeded at concentration 1.0×10^5 cells/mL into 24 well plates and cultured until they reach 70-80% confluence. The growth media from all wells were removed and discarded carefully upon reaching the confluence. Cells were gently washed two times with phosphate buffer saline (PBS). The cells were pre-treated with 500 µL of filtered test extract at six concentrations varying from 125 to 4000 µg/mL and standard (0.5 mg/mL of L ascorbic acid), for 24 hr and further incubated with 20 µM of DCFH-DA

for 30 min in the dark at 37 °C and 5% CO_2. The cells were rinsed and exposed to 200 µL of PBS for UVB irradiation at 312 nm (70 mJ/cm^2) using a filtered light source apparatus (UVP, Upland, California, USA) for approximately 2 min, whereas control plates were kept in the dark. Fluorescence intensity was measured at $\lambda_{excitation}$ = 485 ± 20 nm and $\lambda_{emission}$ = 528 ± 20 nm using a multimode microplate reader (FLUOstar Omega, BMG LabTech, Germany). The capacity of crude samples to reduce the oxidative cell environment was evaluated as expressed in CAA%, where Y represents the fluorescent intensity of exposed cells. Results were expressed in comparison with exposed cells, which were not pre-treated with test compounds.

$$CAA\ \% = \frac{[Y_{reference} - Y_{sample}]}{Y_{reference}} \times 100\%$$

Experiments were performed at least three times along with a minimum of three internal replicates. IC_{50} of the extracts was calculated to identify the inhibitory effects of *P. pterocarpum* extracts on the oxidation of DCFH-DA.

Statistical analysis

All experiments were performed in triplicate. Results were presented as mean ± standard deviation (SD). The IC_{50} concentrations were determined by performing a nonlinear regression of the inhibition (%) against the extract concentration plot using Prism version 9.0 (GraphPad Software Inc., San Diego, CA). A student's paired t-test was employed to evaluate significant means among two groups, while a one-way analysis of variance (ANOVA) followed by the Tukey test was employed for multiple comparisons. All analyses were performed at a 95% confidence level.

RESULTS

Extraction yields, total phenolic, and total flavonoid contents of *P. pterocarpum* extracts

The effect of solvents on the percentage extraction yield, TPC, and TFC of *P. pterocarpum* extracts is shown in Table 1. The results demonstrated that the ethanol extract of *P. pterocarpum* leaf exhibits a significantly higher content of total phenolic and total flavonoid than the aqueous extract. However, the extract yield with ethanol was about 29.47% lower than the aqueous extract.

Cell viability of B16-F1

The NR-based assay was employed to determine the B16-F1 mouse melanoma cell viability after treatment with ethanol and aqueous *P. pterocarpum* leaf extracts. Figure 1 shows that there was no significant difference in the viability of B16-F1 mouse melanocyte cells between untreated control and aqueous extract of *P. pterocarpum* leaves at a concentration ranging from 250 ng/mL to 8000 ng/mL. The viability of B16-F1 mouse melanocyte cells was consistently higher than 90% (91.03 ± 2.45) % after treatment at all concentrations of aqueous extract of *P. pterocarpum* leaves. This finding suggests that the aqueous extract did not have cytotoxic effects on B16-F1 cells. Ethanol extracts exhibit a high percentage of viability in the range between 72.35 ± 4.43 and 98.05 ± 2.10%, following the same incubation period and treatment concentrations up to 4000 ng/mL. However, treatment with ethanol extract of *P. pterocarpum* leaves significantly reduced the percentage of B16-F1 mouse melanocyte cell viability to 54.21 ± 4.36% at 8000 ng/ mL as compared to the untreated control.

Melanin content

The amounts of melanin secreted into the cell-cultured medium in comparison with the untreated cells were determined to observe the capacity of *P. pterocarpum* leaf extracts to stimulate melanogenesis. As shown in Figure 2, the ethanol extract of *P. pterocarpum* leaves increased the percentage of melanin content in B16-F1 mouse melanocyte cells significantly as compared to aqueous extract in a concentration-dependent manner. Melanin production increased from 12.75 ± 2.90% to 58.20 ± 1.08% at concentrations of 250 ng/mL and 8000 ng/mL of *P. Pterocarpum* ethanol extract. The treatment of 100 nM α-MSH increased melanin production by 27.47 ± 0.37% in B16-F1 cells, indicating that the ethanol extract of *P. pterocarpum* leaves increased the melanin content of the cells 2-fold higher than the positive control (α-MSH). However, treatment of 250 to 4000 ng/mL of aqueous extract of *P. pterocarpum* inhibited melanin content by 7.15 ± 1.82% and 15.76 ± 2.88%, respectively. Treatment at 8000 ng/mL of *P.*

Table 1. Effect of solvents on the percentage extraction yield, TPC, and TFC of *P. pterocarpum*

Extracts	Yield (%)	TPC (mg GAE/g)	TFC (mg RE/g)
Aqueous	10.01 ± 0.53^b	236.01 ± 5.32^a	51.01 ± 0.48^a
Ethanol	7.06 ± 0.64^a	460.80 ± 4.88^b	108.99 ± 1.77^b

Data are expressed as mean ± SD of three independent repetitions and three technical replications. Superscript letters within the same column indicate significant (p<0.05) differences in means. GAE = gallic acid equivalent; RE = rutin; TPC = total phenol content; TFC = total flavonoid content.

pterocarpum aqueous extract increased the production of melanin by only 5.06 ± 0.39% as compared to the untreated control.

DPPH radical scavenging activity

Figure 3 shows the DPPH radical scavenging activity of *P. pterocarpum* extracts and the positive control. Ascorbic acid had the highest scavenging capacity as compared to the aqueous and ethanol extracts. There was a significant difference in DPPH radical scavenging activity between the aqueous and ethanol extracts. As illustrated in Table 2, the ethanol extract had significantly lower IC_{50} values than the aqueous extract. A lower IC_{50} indicates a higher scavenging activity. This finding demonstrated that the ethanol extract exhibits stronger DPPH radical activity than the aqueous extract and is in line with the findings on TPC and TFC.

Cellular ROS inhibition activity

A DCFH-DA cell-based assay was used to examine the effect of preconditioning of HaCaT cells with ethanol and aqueous extracts of *P. pterocarpum* on intracellular UVB-induced ROS levels. We found that *P. pterocarpum* leaf extracts showed significant inhibitory activity even at low concentrations (Figure 4). The ethanol and aqueous extracts inhibited intracellular ROS generation by 64.46 ± 2.15% and 58.93 ± 0.25%, respectively at 2000 µg/mL. Meanwhile, 500 µg/mL of ascorbic acid inhibits 68.58 ± 0.83% of UVB-induced ROS production.

Table 3 shows the IC_{50} values of aqueous and ethanol extracts of *P. pterocarpum*. The ethanol extract significantly inhibited intracellular ROS levels, approximately 1.6-fold lower than the aqueous extract.

DISCUSSION

This study was mainly conducted to investigate whether *P. pterocarpum* leaf extracts impact melanin production and further explore its cytotoxicity and antioxidant capabilities. We demonstrated herein that the polyphenol content and bioactivities of *P. pterocarpum* leaf extracts were affected by the polarity of the extraction solvent. Our results showed for the first time that the ethanol extract of *P. pterocarpum* increased the production of melanin, had stronger DPPH radical scavenging activity, inhibited UVB-induced ROS intracellular formation, but displayed cytotoxicity to B16-F1 cells at the highest concentration. Meanwhile, the aqueous extract reduced melanin formation, exhibited lower free radical scavenging activity, and did not show any cytotoxic effects on B16-F1 mouse melanocyte cells. These observations support the hypothesis that

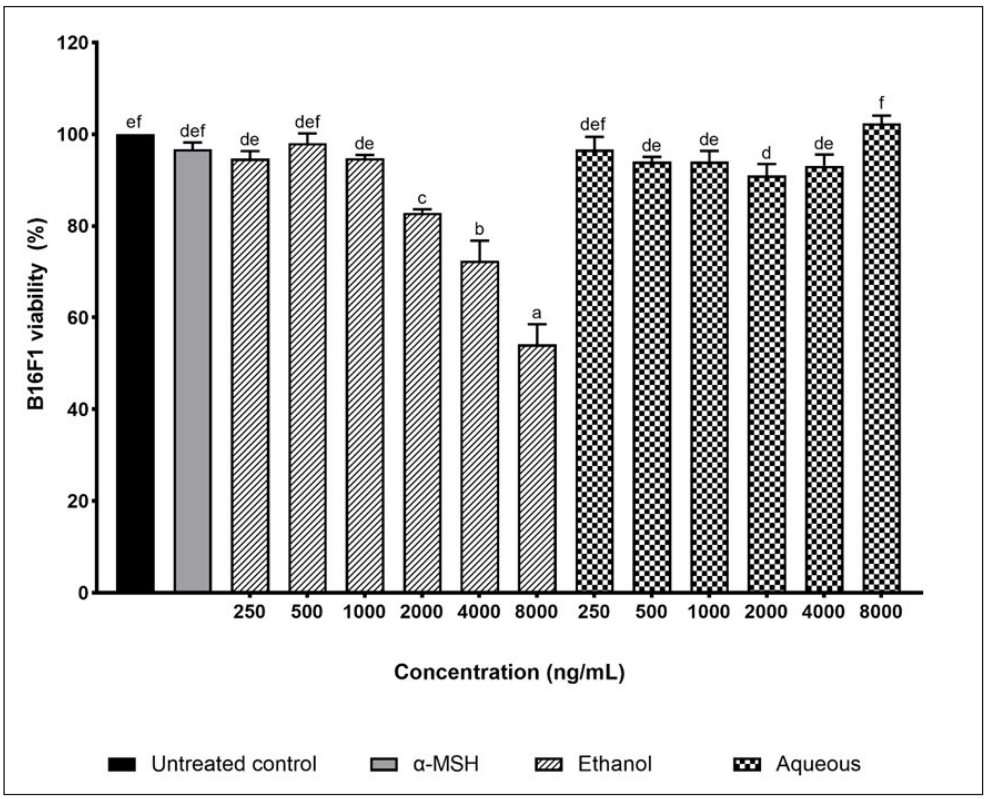

Fig. 1. Effect of *P. pterocarpum* extracts on melanocyte cell viability. NR stained was performed on cells treated with six different concentrations. Values are mean ± SD derived from three independent experiments; different lowercase letters on the histograms indicate a significant difference between treatments ($p \leq 0.05$) (ANOVA Tukey's test).

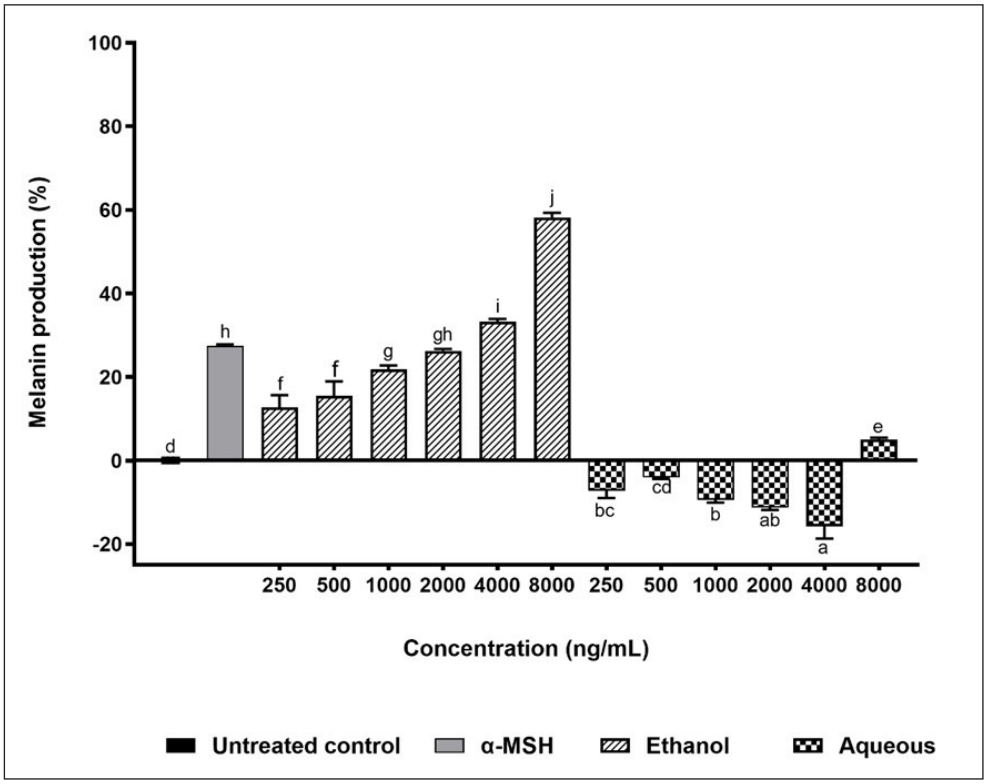

Fig. 2. Production of melanin by aqueous extract and ethanol extracts of *P. pterocarpum* in B16-F1 cells. Values are mean ± SD derived from three independent experiments; different lowercase letters on the histograms indicate a significant difference between treatments ($p \leq 0.05$) (ANOVA Tukey's test).

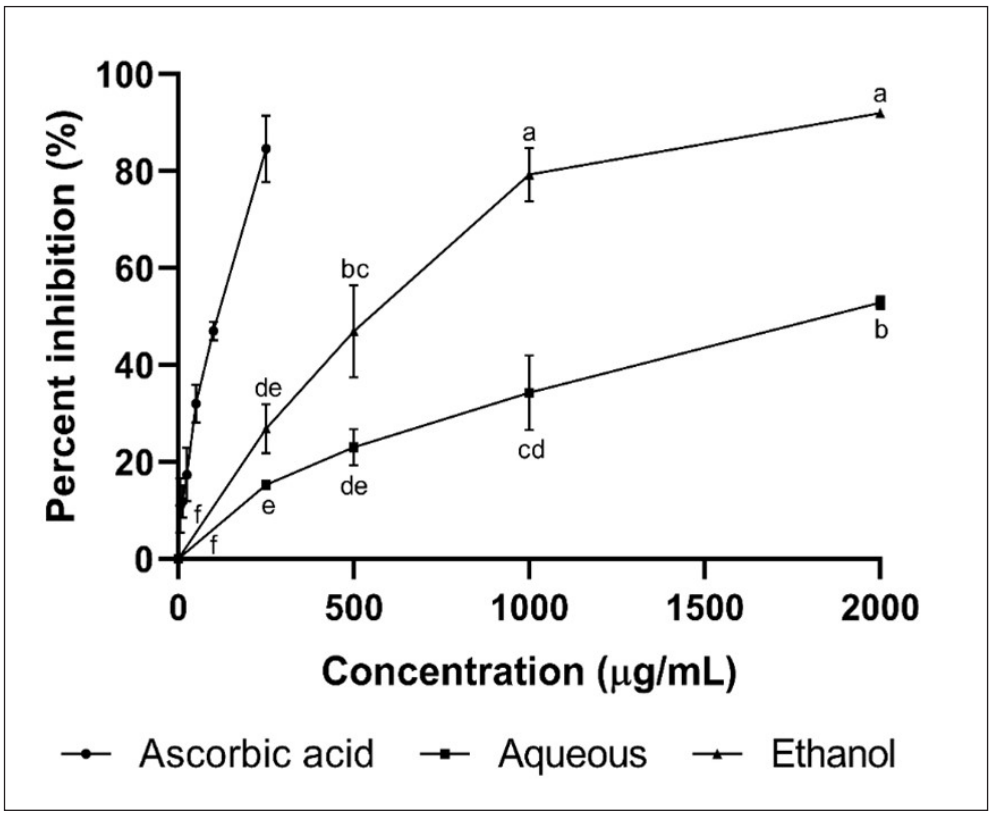

Fig. 3. DPPH radical scavenging capacities of aqueous and ethanol extracts of *P. pterocarpum* and positive control (ascorbic acid). Different superscript letters indicate significant ($p<0.05$) differences in means between extracts at a similar concentration. Results are means ± SD ($n=3$).

Table 2. IC_{50} values of DPPH scavenging activity of *P. pterocarpum* extracts

Extracts	IC_{50} value (µg/mL)
Aqueous	1798.45 ± 143.09[c]
Ethanol	519.24 ± 122.57[b]
Ascorbic acid	98.19 ± 14.09[a]

Data are presented as mean ± SD of triplicate in three independent experiments. Different superscripted letters indicate significant differences from one another (p>0.05).

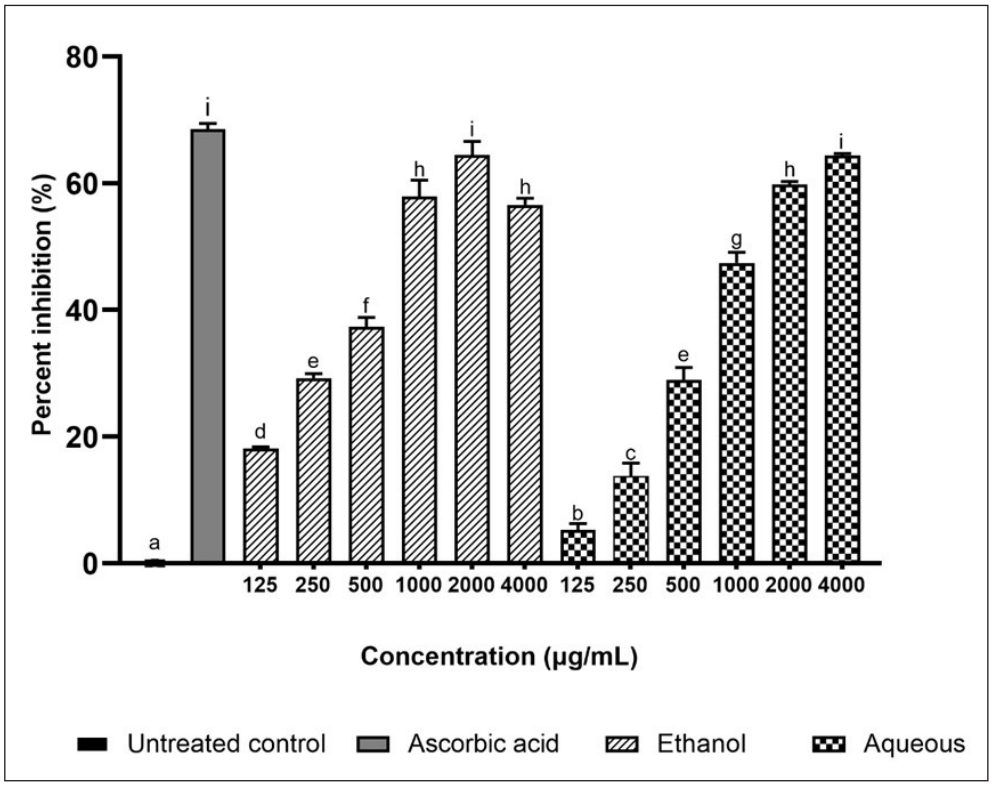

Fig. 4. Inhibition of peroxyl radical-induced DCFH oxidation by ethanol and aqueous extracts of *P. pterocarpum*. Values are mean ± SD derived from three independent experiments; different lowercase letters on the histograms indicate a significant difference between treatments ($p \leq 0.05$) (ANOVA Tukey's test).

Table 3. IC_{50} values for the inhibition of DCFH-DA oxidation by *P. pterocarpum* extracts

Extracts	IC_{50} value (µg/mL)
Aqueous	1379.00 ± 21.81[a]
Ethanol	878.00 ± 23.50[b]

Data are presented as mean ± SD of triplicate in three independent experiments.

the polarity of the extraction solvent can influence the phytochemical constituents and biological activities of plant extracts. Organic solvents such as methanol or ethanol are shown to reach inner cavities or pores of the plant matrix and are thus more powerful for extracting flavonoids (Rodríguez De Luna *et al*., 2020). The biological activity is directly influenced by the type of flavonoid extracted (Chávez-González *et al*., 2020). It was demonstrated by Netcharoensirisuk and colleagues (2021) that polar flavonoids enhanced melanin production by blocking two-pore channel 2 (TPC2) in endolysosomal membranes.

Higher TPC and TFC values but lower extract yield were observed in the ethanol leaf extract than in the aqueous extract of *P. pterocarpum* (Table 1). In agreement with previous studies analyzing the effect of solvent polarity on extraction yield and phytochemical content, these data suggest that ethanol is more efficient than pure water for extracting polyphenols in *P. pterocarpum* leaf. It has been demonstrated that the plant extract obtained by water was associated with higher extract yields, but lower phenolic and flavonoid content as compared to ethanol extract (Nawaz *et al*., 2020). The differences in the polarity of the extraction solvents have also been shown can affect the toxicity and biological properties (Wakeel *et al*. 2019; Rostagno *et al*. 2020).

B16 cells are extensively used to examine melanogenesis, depigmentation, tumor metastasis, and cytotoxicity measurements of various substances in skin models. We first examine the cytotoxic effect of *P. pterocarpum* leaf extracts against B16-F1 mouse melanocyte cells. According to ISO 10993-5, any substance is regarded to have cytotoxic potential if the cell viability percentage is reduced to less than 70% of the untreated control (Ilieva *et al*., 2021; Nogueira *et al*., 2021). In this study, we found that both *P. pterocarpum* extracts did not exhibit any cytotoxic effect against B16-F1 mouse melanocyte cells (Figure 1) up to 4000 ng/mL. However, treatment with the ethanol extract of *P. pterocarpum* at the highest concentration showed cytotoxic activity in B16-F1 melanoma cells. This finding suggests that *P. pterocarpum* ethanol leaf extract has a potential melanogenesis-promoting effect. It is because cells that are actively synthesizing melanin were reported to be more vulnerable to self-destruction (Pawelek *et al*., 1986). The melanocytotoxicity effects have been demonstrated in melanogenesis potentiators such as α-MSH and anisaldehyde (Nitoda *et al*., 2007; Loser *et al*., 2010).

We provide the first evidence that the ethanol extract of *P. pterocarpum* leaf stimulates melanin formation in a concentration-dependent manner, whereas the non-cytotoxic aqueous extract inhibits melanin content up to 15% (Figure 2). We notice *P. pterocarpum* ethanol extract increased melanin production 2-fold higher than α-MSH at 8000 ng/mL that exhibiting a cytotoxicity effect. These results are consistent with previous findings that reported melanin synthesis generates ROS inside pigment-forming cells, thus, potentially cytotoxic to cells through activation of the p38 signaling pathway (Kim *et al*., 2015). Our finding also supports the hypothesis that inhibition of melanin synthesis reduces intracellular ROS in melanocytes (Jenkins & Grossman, 2013). Therefore, elucidating a novel pro-melanogenic agent with antioxidant properties is a great approach to preventing radical-induced damage inside pigment-forming cells.

DPPH and DCFH-DA assays were performed to evaluate the *in vitro* antioxidant activity of *P. pterocarpum* extracts. We showed that *P. pterocarpum* ethanol leaf extract had higher DPPH radical scavenging capacities than the aqueous extract (Table 2). The higher DPPH radical scavenging ability of the ethanol extracts could be attributed to the presence of a higher content of polyphenols and flavonoids (Priyanthi & Sivakanesan, 2021). Intracellular ROS generation was monitored to further investigate the protective effects of *P. pterocarpum* leaf extracts on UVB-induced oxidative stress in HaCaT cells. Consistent with the DPPH assay, we found that the ethanol extract had higher inhibition of UVB-induced intracellular ROS with an IC_{50} value two times lower than the aqueous extract (Table 3). Similar observations have been reported by Hong *et al*. (2016) and Bourhim *et al*. (2021) who demonstrated that extracts with the highest TFC and antioxidant activity displayed the strongest melanogenesis-promoting effects.

CONCLUSION

Results from this study demonstrated that the phenolic and flavonoid content of *P. pterocarpum* extract influences melanogenesis, cytotoxicity, and antioxidant activities. We suggest the ethanol extracts that had stronger antioxidant activity exert a pro-melanogenesis effect, whereas the *P. pterocarpum* aqueous leaf extract has an anti-melanogenesis function. *P. pterocarpum* extract merits further study to confirm its inhibitory and stimulatory potential mechanisms.

ACKNOWLEDGMENT

We thank the SIRIM Berhad and Faculty of Applied Sciences, Universiti Teknologi MARA for the facilities.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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