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Antioxidant, Anti-Melanogenesis, and Cytotoxic Effects of *Clitoria ternatea* (Butterfly Pea) Flower Extract on B16 Melanoma Cells

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ABSTRACT

Butterfly pea (*Clitoria ternatea*) flowers contain high levels of anthocyanins and flavonoids, functioning as natural antioxidants to combat free radicals. The antioxidant activity can prevent cell damage and inhibit melanogenesis, contributing to skin whitening by reducing melanin production. The present study investigated the antioxidant, anti-melanogenesis, and cytotoxic effects of butterfly pea flower (BPF) extract on B16 melanoma cells, aiming to explore its potential as a safer, natural alternative for skin whitening. Antioxidant properties and anti-melanogenesis potential of BPF extract were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and tyrosinase inhibition assays, respectively. The cytotoxicity of BPF extract on B16 melanoma cells was further examined using the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay. The results of the DPPH assay indicated that BPF extract exhibited an IC_{50} value of 165.10±7.78 ppm, compared to quercetin (the reference compound) with an IC_{50} value of 3.53±0.08, indicating moderate antioxidant potential. Butterfly pea flower extract showed an IC_{50} of 130.90±3.52 ppm in tyrosinase inhibition assay, indicating a weaker anti-melanogenesis effect than hydroquinone (IC_{50} value of 17.04±0.14 ppm). The MTT technique revealed high biocompatibility with 85% B16 melanoma cell viability at 250 ppm for BPF, compared to only 24.45% for doxorubicin at 30 ppm. These findings underscore the potential of BPF extract as a bioactive ingredient for skin care, offering moderate antioxidant activity with safe, natural anti-melanogenesis effects. The present study supports BPF's application in cosmetic formulations aimed at skin protection and whitening, with minimized toxicity for prolonged use.

***Keywords*:** Antioxidant, Butterfly pea flowers, *Clitoria ternatea*, IC_{50}, Melanin, L-tyrosin, Tyrosinase.

Introduction

Antioxidants play a key role in skin whitening by combating oxidative damage, which can exacerbate skin conditions such as hyperpigmentation and dark spots. Free radicals produced by exposure to sunlight, pollution, and other environmental factors can harm skin cells and enhance melanin production.[1,2] Melanin, a natural pigment in the body's epidermis, is crucial in protecting the skin from the sun's harmful ultraviolet radiation. When overproduced, however, melanin can lead to hyperpigmentation. The enzyme tyrosinase is essential in melanin production, and its increased activity can result in hyperpigmentation.[3,4] Antioxidants help to reduce inflammation and prevent excessive melanin formation by neutralizing free radicals and reducing oxidative stress.[5,6]

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The butterfly pea (*Clitoria ternatea*) is a flowering plant in the *Fabaceae* family, prized for its striking blue or purple blossoms. The plant flourishes abundantly in tropical regions, particularly in Southeast Asia, including Indonesia, where it is widely utilized in several culinary and medicinal contexts.[7] Historically, butterfly pea flowers (BPF) have been employed in traditional medicine to address many health conditions, including stress, inflammation, and infections. Chemical analysis reveals the robust presence of bioactive compounds in BPFs, notably anthocyanins, particularly ternatin, responsible for the flowers' distinct blue colour.[8] Moreover, BPFs harbour flavonoids such as quercetin, kaempferol, saponins, and tannins, further enriching their therapeutic potential.[9–11] The BPF extract is widely recognized for its potent antioxidant properties, primarily due to the presence of bioactive compounds like anthocyanins, flavonoids, and phenolics. These compounds neutralize free radicals, the primary drivers of cellular oxidative stress, by donating electrons or hydrogen atoms, thereby reducing oxidative damage to DNA, proteins, and lipids. This antioxidant activity also shields the skin, preventing premature aging, inflammation, and damage caused by exposure to ultraviolet (UV) light.[12,13]

Moreover, the antioxidant properties of the BPF extract also suggest its potential as an anti-melanogenesis agent. Antioxidants can promote skin whitening by inhibiting the tyrosinase enzyme, thereby reducing melanin production at the cellular level. Tyrosinase catalyzes the conversion of tyrosine to dihydroxyphenylalanine (DOPA) and then to dopaquinone, which is the first step in melanin production. Antioxidants like quercetin and anthocyanins can inhibit tyrosinase

activity by binding to the enzyme's active site or reducing the oxidation of tyrosine and DOPA. The reduction in melanin formation leads to a decrease in skin pigmentation. Therefore, BPF extract shows potential as an active ingredient in cosmetics for skin-lightening applications.[14–16] Safety testing is essential to confirm that skin-whitening agents are safe for prolonged use. The 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay on B16 melanoma cells is a commonly used method to assess the cellular toxicity of these compounds. The method assesses cell viability based on the ability of live cells to reduce MTT to formazan, which produces a colour change that can be measured quantitatively. This test is crucial to confirm the effectiveness of the whitening agent in inhibiting melanogenesis, ensuring its safety and non-toxicity to skin cells. It is also vital to confirm that the skin-whitening compound is safe for prolonged topical application.[17] The present study aimed to evaluate the antioxidant properties, anti-melanogenesis potential, and cytotoxic effects of BPF extract on B16 melanoma cells.

Materials and Methods

Sources of chemical reagents

The chemical reagents used in this study included BPF aqueous extract, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Merck), analytical-grade ethanol (Smartlab), HPLC-grade methanol (Merck), quercetin (Sigma Aldrich), Aqua Bides (Ikapharmindo), hydroquinone (Merck), tyrosinase enzyme (Merck), L-tyrosine (Merck), phosphate buffer solution (Merck), dimethyl sulfoxide (Merck), and formazan (Sigma).

Evaluation of the antioxidant activity of butterfly pea flower extract

Standard solutions of quercetin were prepared at concentrations of 1, 2, 3, 4, and 5 ppm, while BPF extract solutions were prepared at concentrations of 25, 50, 200, 400, and 1000 ppm using ethanol as the solvent. From each concentration series, 2 mL was pipetted and added to 2 mL of 50 ppm DPPH reagent solution in a test tube wrapped in aluminum foil. The mixture was vortexed for 1 minute, incubated for 60 minutes in a dark place, and then checked for absorbance at a wavelength of 514 nm with a UV-vis spectrophotometer (Shimadzu UV-1780). The absorbance values were used to compute the inhibition percentage and IC_{50}.[11.]

Assessment of the anti-melanogenesis potential of butterfly pea flower extract

Phosphate buffer solution (2 mL, pH 6.8) was added to the vial, followed by the addition of 5 mL of 6.6 mM L-tyrosine substrate. Standard hydroquinone solutions with concentrations of 12, 14, 16, 18, 20, and 22 ppm, along with standard BPF extract solutions at concentrations of 25, 75, 200, 400, 600, and 1000 ppm were added to each vial. The contents were then homogenized. Subsequently, 100 units of tyrosinase enzyme were added and homogenized. The mixture was then incubated for 2.5 hours at room temperature, after which the absorbance was measured at 480 nm using a UV-vis spectrophotometer (Shimadzu UV-1780). The absorbance values obtained at each concentration were processed to determine the IC_{50} value.[18]

Determination of the cytotoxic effect of butterfly pea flower extract

The B16 murine melanoma cells were seeded in a 96-well plate and left overnight. After that, the cells were treated with different concentrations (15.625, 31.25, 62.5, 125, 250, 500, and 1000) of BPF extract. After treatment, MTT solution was added to each well, and the plates were incubated for an additional 4 hours at 37°C. The media was removed, and dimethyl sulfoxide was added to dissolve the formazan crystals in each well. After that, the crystals were incubated for 10 minutes at 37°C, and their absorbance was measured at 540 nm using a microplate reader (Biobase BK-EL 10A).[19]

Results and Discussion

Antioxidant activity of butterfly pea extract

Quercetin is a flavonoid, well-regarded for its potent antioxidant properties, capable of inhibiting lipid oxidation and preventing DNA damage caused by free radicals. Research by Boots *et al*. (2008) has shown that quercetin enhances the activity of crucial antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, which are essential for cellular protection against oxidative stress.[20] The DPPH method, a validated and widely accepted technique for evaluating antioxidant activity, was used in this study to assess the antioxidant potential of BPF extract. This method measures a compound's ability to scavenge free radicals, observed as a colour change from purple to yellow as the compound interacts with DPPH radicals.[21,22] Quercetin, renowned for its potent antioxidant activity, was used as a reference standard in this study to validate the DPPH method, as it consistently demonstrates strong radical-scavenging activity, ensuring an accurate comparison of antioxidant potential.

The antioxidant activity of BPF extract, with an IC_{50} value of 165.10±7.78 ppm, was compared to quercetin, which showed a significantly lower IC_{50} of 3.53±0.08 ppm, indicating quercetin's higher antioxidant capacity. This difference reflects the distinct antioxidant potential of each compound. As a pure flavonoid, quercetin has an optimized molecular structure that efficiently scavenges free radicals, evident in its low IC_{50} value. In contrast, BPF extract, while containing flavonoids, also includes other active compounds like anthocyanins that contribute to its antioxidant activity but do not exhibit the same potency as pure quercetin. Thus, while quercetin is a more potent antioxidant, BPF extract remains a valuable natural antioxidant source, suitable for applications where moderate antioxidant activity is desired. The antioxidant activity is essential for protecting cells from oxidative damage, which can contribute to preventing premature aging and improving overall skin health.[23,24] The IC_{50} values of DPPH radical scavenging activities of quercetin and butterfly pea flower extract was presented at Table 1 and figure 1.

Table 1: IC_{50} values of DPPH radical scavenging activities of quercetin and butterfly pea flower extract

DPPH Scavenging of Quercetin				**DPPH Scavenging of BPF Extract**			
Concentration	**% Inhibition**			**Concentration**	**% Inhibition**		
(ppm)	**1**	**2**	**3**	**(ppm)**	**1**	**2**	**3**
1	10.40	11.40	13.24	25	15.11	14.98	13.09
2	28.32	28.49	28.65	50	26.32	25.24	26.86
3	39.37	39.71	40.21	200	54.79	55.33	55.33
4	53.11	53.61	54.11	400	66.40	63.83	65.86
5	67.51	68.51	69.01	1000	87.45	88.66	87.31
IC_{50} (ppm) Mean ± SD	3.53 ± 0.08			IC_{50} (ppm) Mean ± SD	165.10 ± 7.78		

DPPH: 2,2-diphenyl-1-picrylhydrazyl

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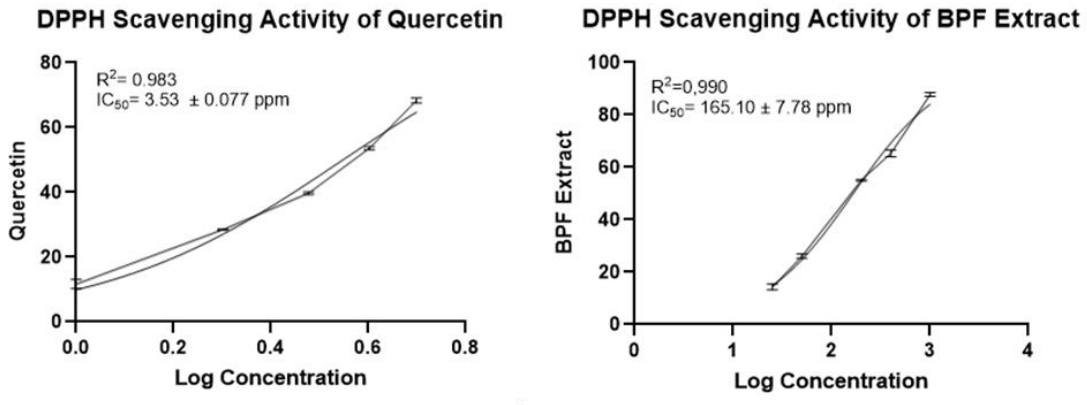

Figure 1. Non linier regression curve of DPPH scavenging activity of quercetin and BPF extract

Anti-melanogenesis activity of butterfly pea extract

Tests for anti-melanogenesis activity can be conducted using various methods to evaluate the impact of compounds on melanin production and related enzymes. *In vitro* methods, such as B16-F10 cells, measure melanin production following compound treatment. In contrast, *in vivo* assays involve using animal models with induced hyperpigmentation to assess the overall effects of the compound. Enzymatic assays measure changes in enzyme activity to assess the inhibition of tyrosinase, a key enzyme involved in melanogenesis. Additionally, expression analysis of melanogenesis-related genes, such as tyrosinase, TRP-1, and TRP-2, can be used to evaluate the impact of compounds on gene regulation. Recent research indicates that combining these methods provides a more comprehensive understanding of the potential of compounds in treating pigmentation disorders.[25]

The anti-melanogenesis activity of BPF extract was assessed using the tyrosinase enzyme inhibition method with the substrate L-tyrosine. Hydroquinone was employed for comparison. The tyrosinase enzyme plays a key role in melanin biosynthesis, which affects skin pigmentation. In the present study, BPF extract inhibited the activity of the tyrosinase enzyme, with an IC_{50} value of 130.90±3.52 ppm. However, this value was higher than that of hydroquinone, which had a significantly lower IC_{50}. Previous research has shown hydroquinone is effective as a tyrosinase inhibitory agent through a direct competitive mechanism. On the other hand, the tyrosinase inhibitory activity of BPF extract may be influenced by its anthocyanin and flavonoid content, which are known to possess enzyme-inhibitory properties and antioxidant activity. Although BPF extract demonstrated lower inhibitory effectiveness than hydroquinone, it shows potential as a safer, natural alternative for skin whitening formulations. Its reduced risk of side effects, along with antioxidant properties that help protect the skin from oxidative stress, make it a compelling option.[26,27] IC_{50} values of tyrosinase inhibition by hydroquinone and butterfly pea flower extract was presented at Table 2 and figure 2.

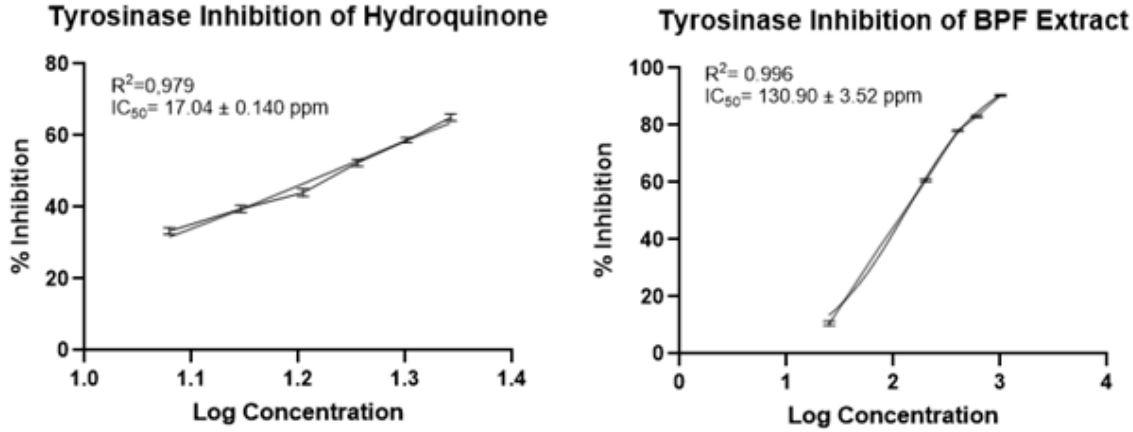

Figure 2: Non-linear regression curve of tyrosinase inhibition activity of hydroquinone and butterfly pea flower extract.

Effect of butterfly pea flower extract on B16 melanoma cell viability

The cytotoxic potential of BPF extract was evaluated against B16 melanoma cells using the MTT assay. This method allowed the measurement of mitochondrial activity. The results indicated that the extract at a concentration of 250 ppm did not cause a significant reduction in cell viability, suggesting that it is safe for the cells. The BPF extract contains quercetin, a flavonoid compound known for its antioxidant and anti-inflammatory properties, which may help protect cells by combating free radicals and reducing oxidative stress. To confirm the assay, doxorubicin, a compound with known cytotoxic effects, was used as a control. The viability test with doxorubicin showed a cell viability percentage of 24.45±2.2 at a concentration of 30 ppm, demonstrating its strong cytotoxicity on B16 melanoma cells. Furthermore, the extract's lowered toxicity profile supports its safety as a skin-whitening agent, making it a preferable choice for long-term use in cosmetic products. In some researches quercetin-containing natural extracts brighten the skin by inhibiting tyrosinase activity. They are also safe and have minimal side effects compared to synthetic chemicals.[28–31] Percentage viability of B16 melanoma cells following treatment with BPF extract and doxorubicin was presented in Table 3 and Table 4.

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Table 2: IC_{50} values of tyrosinase inhibition by hydroquinone and butterfly pea flower extract

Tyrosinase Inhibition of Hydroquinone				Tyrosinase Inhibition of BPF Extract			
Concentration (ppm)	% Inhibition			Concentration (ppm)	% Inhibition		
	1	2	3		1	2	3
12	32.56	33.03	34.44	25	10.53	11.53	10.06
14	40.55	38.44	39.61	75	37.80	37.80	37.77
16	44.31	42.90	45.01	200	60.63	61.25	60.19
18	52.77	51.36	53.24	400	78.19	78.35	78.08
20	59.58	58.64	58.17	600	83.14	83.44	82.82
22	64.52	64.52	66.16	1000	90.55	90.73	90.14
IC_{50} (ppm) Mean ± SD	17.04 ± 0.14			IC_{50} (ppm) Mean ± SD	130.90 ± 3.52		

Table 3: Percentage viability of B16 melanoma cells following treatment with butterfly pea flower extract

Replication	Concentration (ppm)						
	1000	500	250	125	62.5	31.25	15.625
1	79.23	83.06	92.99	93.34	93.86	99.8	91.57
2	78.66	82.20	94.41	97.73	97.64	92.09	90.94
3	78.69	85.84	95.77	96.49	100,00	99.69	96.38
mean	78.86	83.70	94.39	95.86	97.17	97.19	92.97
SD	2.20	2.40	3.20	2.40	3.00	5.40	2.50

Table 4: Percentage viability of B16 melanoma cells following treatment with doxorubicin

Replication	Concentration (ppm)						
	30	15	7.5	3.75	1.875	0.9375	0.46875
1	25.1	26.4	33.1	39.2	49.1	65.9	71.6
2	22.0	30.6	33.6	37.6	47.3	55.8	67.1
3	26.3	30.6	38.8	42.2	53.1	57.7	67.4
mean	24.45	29.19	35.14	39.64	49.80	59.82	68.69
SD	2.2	2.4	3.2	2.4	3.0	5.4	2.5

Conclusion

Butterfly pea flower extract demonstrated promising antioxidant, anti-melanogenesis, and low cytotoxic properties on B16 melanoma cells. With moderate antioxidant activity and tyrosinase inhibition, the extract offers a natural, safer alternative to synthetic skin-whitening agents. Its low cytotoxicity further supports its potential for long-term cosmetic applications, providing skin protection while reducing pigmentation.

Conflict of Interest

There is no conflict of interest.

Authors' Declaration

The author hereby declares that the work presented in this article is original and that any responsibility for claims relating to the content of this article shall be borne by them.

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References

1. Neha K, Haider MR, Pathak A, Yar MS. Medicinal prospects of antioxidants: A review. Eur J Med Chem. 2019; 178: 687–704. doi: 10.1016/j.ejmech.2019.06.010
2. Addor FAS. Antioxidants in dermatology. A Bras Dermatol. 2017; 92(3):356–362. doi: 10.1590/abd1806-4841.20175697
3. Wenas DM, Elya B, Sutriyo, Setiwan H. Antioxidant and Tyrosinase Inhibitory Activities of Unripe and Ripe Fruit and Seed Extracts of *Eugenia uniflora*. Trop J of Nat Prod Res. 2024; 8(7): 7734-7739. doi: 10.26538/tjnpr/v8i7.16.
4. Thawabteh AM, Jibreen A, Karaman D, Thawabteh A, Karaman R. Skin pigmentation types, causes and treatment—

A review. Molecules. 2023; 28(12):4839-4867. doi: 10.3390/molecules28124839
5. Masaki H. Role of antioxidants in the skin: Anti-aging effects. J Dermatol Sci. 2010; 58(2):85–90. doi: 10.1016/j.jdermsci.2010.03.003
6. Xekardakis D, Krueger-Krasagakis S, Krasagakis K. Hyperpigmentation. 1st ed. (Rigopoulos D, Katoulis AC. eds). CRC Press: Boca Raton; 2017; 18-20. doi: 10.1201/9781315162478.
7. Sarma DSK, Kumar D, Yamini C, Santhalahari C, Lahari C, Kumar GC. Review on *Clitoria ternatea*. Int J Pharm Sci Med. 2023; 8(9):43–58. doi. 10.47760/ijpsm.2023.v08i09.004
8. Ashraf K, Adlin NF, Basri AN, Ahmad W, Sultan S. The traditional uses, phytochemistry, and pharmacological effects of *Clitoria ternatea*: A review. Indian J Pharm Edu Res. 2023; 58(1):1–14. doi: 10.5530/ijper.58.1.1
9. Multisona RR, Shirodkar S, Arnold M, Gramza-Michalowska A. *Clitoria ternatea* flower and its bioactive compounds: Potential use as microencapsulated ingredient for functional foods. Applied Sci. 2023; 13(4):2134-2158. doi: 10.3390/app13042134
10. Kazuma K. Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. Phytochemistry. 2003; 62(2):229–237. doi: 10.1016/S0031-9422(02)00486-7.
11. Nurhayati R, Shoviantari F, Erawati MT, Yuwono M. Butterfly pea (*Clitoria ternatea* L.) flower water and ethanol extract: Phytochemical screening, FTIR analysis, and antioxidant activity estimation using comparison of ABTS, DPPH, and FRAP assays. Res J Pharm Technol. 2024; 17(5):1973–1982. doi: 10.52711/0974-360X.2024.00313
12. Mukherjee PK, Maity N, Nema NK, Sharkar BK. Bioactive compounds from natural resources against skin aging. Phytomedicine. 2011; 19(1):64–73. doi: 10.1016/j.phymed.2011.10.003
13. Chakraborthy GS, Kumar V, Gupta S, Kumar A, Gautam N, Kumari L. Phytochemical and pharmacological aspects of *Clitoria ternatea* - A review. J Applied Pharm Sci Res. 2018; 1(2): 3–9. doi: 10.31069/japsr.v1i2.13061.
14. Hearing VJ. Determination of melanin synthetic pathways. J Inves Derma. 2011; 131:E8–E11. doi: 10.1038/skinbio.2011.4.
15. Briganti S, Camera E, Picardo M. Chemical and instrumental approaches to treat hyperpigmentation. Pigment Cell Res. 2003; 16(2):101–110. doi: 10.1034/j.1600-0749.2003.00029.x
16. Brenner M, Hearing VJ. The protective role of melanin against uv damage in human skin. Photochem Photobiol. 2008; 84(3):539–549. doi: 10.1111/j.1751-1097.2007.00226.x.
17. Hashemi-Shahri SH, Golshan A, Mohajeri SA, Baharaa J, Amini E, Salek F, Sahebkar A, Tayarani-Najaran Z. ROS-scavenging and anti-tyrosinase properties of crocetin on B16F10 murine melanoma cells. Anticancer Agents Med Chem. 2018; 18(7):1064–1069. doi: 10.2174/1871520618666171213143455.
18. Abidin Z, Khaeriah U, Pratama M, Baits M. Tyrosinase inhibitor activity measurement of crude and purified extract of moringa leaves (*Moringa oleifera* L.). Indo J Pharm Sci Tech. 2019; Supp (1)1: 52-58. doi: 10.24198/ijpst.v1i1.19152
19. Kumar P, Nagarajan A, Uchil PD. Analysis of cell viability by the MTT assay. Cold Spring Harb Protoc. 2018; 2018(6). doi: 10.1101/pdb.prot095505.
20. Boots AW, Haenen GRMM, Bast A. Health effects of quercetin: From antioxidant to nutraceutical. Eur J Pharmacol. 2008; 585(2–3):325–337. doi: 10.1016/j.ejphar.2008.03.008.
21. Gulcin İ, Alwasel SH. DPPH radical scavenging assay. Processes. 2023; 11(8):2248-2268. doi: 10.3390/pr11082248.
22. Reveny J, Maha HL, Laila L. A Comparative study of phytochemical screening and DPPH radical scavenging activity of *Ficus carica* Linn. leaves extracts. Trop J Nat Prod Res. 2023; 7(2):2337–2340. doi: 10.26538/tjnpr/v7i2.5.
23. Rudzińska A, Juchaniuk P, Oberda J, Wiśniewska J, Wojdan W, Szklener K, Mańdziuk S. Phytochemicals in cancer treatment and cancer prevention—Review on epidemiological data and clinical trials. Nutrients. 2023; 15(8):1896-1930. doi: 10.3390/nu15081896.
24. Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochemistry. 2000; 55(6):481–504. doi: 10.1016/S0031-9422(00)00235-1.
25. Dej-adisai S, Koyphokaisawan N, Wattanapiromsakul C, Nuankaew W, Kang T, Pitakbut T. *In vitro*, *in vivo*, and *in silico* analyses of molecular anti-pigmentation mechanisms of selected Thai rejuvenating remedy and bioactive metabolites. Molecules. 2023; 28(3):958. doi: 10.3390/molecules28030958.
26. Chang T-S. An updated review of tyrosinase inhibitors. Int J Mol Sci. 2009; 10(6):2440–2475. doi: 10.3390/ijms10062440.
27. Zolghadri S, Bahrami A, Hassan Khan MT, Munoz-Munoz J, Garcia-Molina F, Garcia-Canovas F, Saboury A. A comprehensive review on tyrosinase inhibitors. J Enzyme Inhib Med Chem. 2019; 34(1):279–309. doi: 10.1080/14756366.2018.1545767
28. Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT assay: Utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. Int J Mol Sci. 2021; 22(23):12827-12857. doi: 10.3390/ijms222312827
29. Tang SM, Deng XT, Zhou J, Li QP, Ge XX, Miao L. Pharmacological basis and new insights of quercetin action in respect to its anti-cancer effects. Biomed Pharm. 2020; 121:109604-109611. doi: 10.1016/j.biopha.2019.109604
30. Choi MH, Yang SH, Kim DS, Kim N, Shin H, Liu K. Novel quercetin derivative of 3,7-dioleylquercetin shows less toxicity and highly potent tyrosinase inhibition activity. Int J Mol Sci. 2021; 22(8):4264-4284. doi: 10.3390/ijms22084264
31. Akindele A, Palmer E. Effects of hydroethanolic leaf extract of *Ipomoea asarifolia* (Convolvulaceae) in doxorubicin and isoproterenol-induced toxicity in rats. Trop J Nat Prod Res. 2018; 2(2):59–66. doi: 10.26538/tjnpr/v2i2.2