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POTENTIAL COMBINATION OF CERMAI (*Phyllanthus acidus*) AND MULBERRY (*Morus alba*) FRUIT EXTRACT AS A CANDIDATE FOR TYROSINASE INHIBITOR

KOMBINASI POTENSIAL EKSTRAK BUAH CERMAI (*Phyllanthus acidus*) DAN MURBEI (*Morus alba*) SEBAGAI KANDIDAT INHIBITOR TIROSINASE

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Abstract

Melanin, a pigment derived from UV radiation, is crucial in preventing skin damage and can cause aesthetic and dermatological problems such as hyperpigmentation or hypopigmentation. Melanogenesis is a complex process involving enzymes and cytokines, with UV being a primary contributor. Tyrosinase is a key enzyme in melanin synthesis. This study aims to test the potential of combining cermai fruit extract (CE) and mulberry fruit (ME) as a tyrosinase inhibitor. The tests included antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, total phenolic content using the Folin Ciocalteu method, flavonoid content using the $AlCl_3$ colorimetric method, and sun protection factor (SPF) value using UV-Vis spectrophotometric method. The results showed that the highest total phenolic content was observed in (CE), followed by the combination of ME: CE in the ratio of 1:3, 1:1, 3:1, and then ME. The same pattern was seen in the flavonoid content assay results. The antioxidant activity, as indicated by the IC_{50} values, followed the following order CE 418.30%; ME: CE (1:3) 400.49%; ME: CE (1:1) 367.73%; ME: CE (3:1) 358.04%; and ME 344.43%. The highest SPF value was observed in ME. It can be concluded that this study shows that the combination of CE and ME extracts has potential as a tyrosinase inhibitor and skin protective agent from hyperpigmentation due to UV exposure.

Keywords*:* Cermai; Extract; Hyperpigmentation; Mulberry fruit; Tyrosinase inhibitor

Abstrak

Melanin, pigmen yang berasal dari radiasi UV, sangat penting dalam mencegah kerusakan kulit, dapat menyebabkan masalah estetika dan dermatologis seperti hiperpigmentasi atau hipopigmentasi. Melanogenesis adalah proses kompleks yang melibatkan enzim dan sitokin, dengan UV sebagai kontributor utama. Tirosinase adalah enzim kunci dalam sintesis melanin. Penelitian ini bertujuan untuk menguji potensi kombinasi ekstrak buah cermai (CE) dan buah mulberry (ME) sebagai penghambat tirosinase. Pengujian meliputi aktivitas antioksidan menggunakan metode 2,2-diphenyl-1-picrylhydrazyl (DPPH), kandungan fenolik total dengan metode Folin Ciocalteu, kandungan flavonoid dengan metode kolorimetri $AlCl_3$, dan nilai sun protection factor (SPF) dengan metode spektrofotometri UV-Vis. Hasil penelitian menunjukkan bahwa kandungan fenolik total tertinggi diamati pada (CE), diikuti oleh kombinasi ME : CE dalam rasio 1:3, 1:1, 3:1, dan kemudian ME. Pola yang sama terlihat pada hasil uji kandungan flavonoid. Aktivitas antioksidan, seperti yang ditunjukkan oleh nilai IC_{50}, mengikuti urutan berikut ini CE 418,30%; ME : CE (1:3) 400,49%; ME : CE (1:1) 367,73%; ME : CE (3:1) 358,04%; dan ME 344,43%. Nilai SPF tertinggi diamati pada ME. Dapat disimpulkan pada penelitian ini menunjukkan bahwa kombinasi ekstrak CE dan ME berpotensi sebagai inhibitor tirosinase dan agen pelindung kulit dari hiperpigmentasi akibat paparan sinar UV.

Kata kunci*: Buah mulberry; Cermai; Ekstrak; Hyperpigmentasi; Inhibitor tirosinase*

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INTRODUCTION

Exposure to UV rays from the sun activates tyrosinase enzymatic activity, which leads to melanin production. In the melanin biosynthetic pathway, tyrosine will be hydroxylated to form catecholamine 3,4 dihydroxyphenylalanine (DOPA), which is then oxidized to 3,4 dioxyphenylalanine (dopaquinone) before cyclization to 5,6 indole quinone and further polymerization to form melanin. Melanin, the primary pigment responsible for various types of pigmentation found in animal and human skin, hair, and eyes, serves a protective function against UV radiation. However, when the number of melanocytes produced is uncontrolled or abnormal, it will cause an abnormal amount of melanin, which can trigger hyperpigmentation, a condition where melanin synthesis or distribution becomes uneven, forming dark patches on the skin (Böhm, 2020; Karkoszka et al., 2024).

Hyperpigmentation is commonly addressed by inhibiting melanogenesis, specifically targeting tyrosinase, which is a copper-containing enzyme crucial in pigmentation control (Logesh et al., 2023). One of the efforts to prevent hyperpigmentation is to inhibit the process of forming new melanin synthesis called melanogenesis. Where inhibition is carried out on tyrosinase, tyrosinase is the most important enzyme in controlling pigmentation. Tyrosinase is a multifunctional copper-containing metalloenzyme with dinuclear copper ions, which acts as a rate-limiting enzyme in the synthesis of melanin. Tyrosinase inhibitors are examined in the presence of a mono phenolic substrate such as tyrosine or a diphenolic substrate such as L-dopa, and activity is assessed based on dopachrome formation. Based on kinetics studies, morin reversibly inhibits tyrosinase through a multi-phase kinetic process and binds to tyrosinase at a single binding site mainly by hydrogen bonds and van der Waals. Furthermore, it was reported that three flavonols, including galanin, kaempferol, and quercetin, inhibit the oxidation of L-DOPA catalyzed by mushroom tyrosinase. Presumably, this inhibitory activity comes from their copper-chelating ability. While their corresponding flavones, chrysin, apigenin, and luteolin, are not identified as copper chelators, it is believed that the chelation mechanism by flavonols may be attributed to the free 3-hydroxyl group (Yang et al., 2022).

Studies have shown that UV intensity directly influences tyrosinase activity: the greater the UV exposure, the more active the enzyme becomes, accelerating melanin production and increasing hyperpigmentation risk (Ali et al., 2016). UV rays can increase melanin synthesis in the skin and cause hyperpigmentation. Hyperpigmentation is a pigment disorder in human skin. Increased melanin synthesis or uneven distribution of melanin can cause local pigmentation and cause aesthetic skin problems. Melanin is a pigment that protects the skin from exposure to UV rays. The process of melanin formation occurs with the help of the enzyme tyrosinase and UV light. Tyrosinase is an enzyme that plays a role in the formation of skin pigment, known as the process of melanogenesis. In the process of melanogenesis, the enzyme tyrosinase will regulate melanin biosynthesis by hydroxylating L-tyrosine into L-DOPA and then oxidizing L-DOPA into dopaquinone. Dopaquinone is converted into dopachrome through an autooxidation reaction to become dihydroxy-indole (DHI) or dihydroxy-indole-carboxyclic-acid (DHICA) to form melanin (Rosa et al., 2021).

The activity of the tyrosinase enzyme depends on the intensity of the incoming UV light. The more UV light penetrating the skin, the faster the tyrosinase enzyme works so that more melanin can form. Melanin formation can be inhibited in several ways, one of which is inhibiting the activity of the tyrosinase enzyme so that it cannot produce melanin and reducing the occurrence of hyperpigmentation. While various synthetic tyrosinase inhibitors, such as kojic acid, hydroquinone, and mercury-based compounds, are effective, they often carry significant side effects, including potential carcinogenic and mutagenic risks when used frequently (Zolghadri et al., 2019). Therefore, exploration of tyrosinase enzyme inhibitor compounds with strong and safe inhibitory power is urgently needed. Natural compounds such as flavonoids and phenolics have shown promising tyrosinase inhibitory effects due to their antioxidant properties and metal-chelating abilities (Obaid et al., 2021).

Flavonoids are reported to inhibit tyrosinase activity through competitive inhibition, directly affecting melanogenesis. Flavonoid compounds inhibit the tyrosinase enzyme by a competitive inhibitor mechanism with its substrate. This aligns with previous research demonstrating that certain

flavonoids, such as quercetin (Liang, 2024), compete with the natural substrate, L-tyrosine, for the enzyme's active site. Meanwhile, phenolic compounds are known to have the ability to chelate copper ions (Cu^{2+}). Cu^{2+} in the tyrosinase enzyme acts as a cofactor that helps the substrate bind to the enzyme. Studies have shown that phenolic, like kojic acid (Peng et al., 2023), can effectively chelate Cu^{2+} within the tyrosinase enzyme, thereby disrupting its catalytic activity. This chelation mechanism prevents the proper binding of the substrate, effectively inhibiting tyrosinase activity. The loss of the cofactor in the enzyme reduces the enzyme's ability to bind its substrate so that melanin is not formed. Additionally, antioxidants as free radical scavengers help prevent the oxidative reactions necessary for tyrosinase activation (Nursid et al., 2020; El-Nashar et al., 2021). The cermai and mulberry fruits are notable sources of flavonoids and phenolics, making them potential candidates for tyrosinase inhibition.

This study aims to evaluate the antioxidant and total phenolic content in a combination of cermai and mulberry fruit extracts. Cermai (*Phyllanthus acidus*) and mulberry (*Morus alba* or other *Morus* species) fruits have both been individually studied for their antioxidant properties and phenolic content. Several studies have reported significant antioxidant activity in cermai extracts, attributed to their rich content of phenolic compounds, including flavonoid, alkaloid, phenolic, terpenoid, and saponin (Andrianto et al., 2017). These compounds scavenge free radicals through various mechanisms, such as hydrogen atom transfer and single electron transfer (Hassanpour & Doroudi, 2023). For example, one study was conducted by Putri and Khonsa (2022), who extracted cermai fruit using the maceration method with 70% ethanol solvent. The extract was then tested for antioxidant activity using the DPPH method at concentrations of 0.6, 1.24, and 2.5%. The results showed that the extract had weak antioxidant potential, with IC_{50} values of 320.64, 225.18, and 174.82 ppm, respectively (Putri & Khonsa, 2022).

Similarly, mulberry fruits are known for their high antioxidant capacity, primarily due to the presence of chlorogenic acid and rutin (Arfan et al., 2012). These compounds contribute to the observed radical scavenging activity and reduce the power of mulberry. The combination of these two fruits, each with a distinct profile of bioactive compounds, may lead to synergistic antioxidant effects. Therefore, this current research study aims to investigate the antioxidant and total phenolic content of combined cermai and mulberry extracts to explore this potential synergy. By exploring their inhibitory potential against tyrosinase, this research seeks to contribute to the development of natural compounds that could effectively prevent hyperpigmentation through melanogenesis inhibition. This experimental research focuses on optimizing the antioxidant and phenolic profiles of cermai and mulberry extracts as tyrosinase inhibitor candidates.

MATERIALS AND METHODS

The equipment used included measuring cups, test tubes (Iwaki), microplates, volumetric flasks, Erlenmeyer flasks, micropipettes (Eppendorf), dark bottles, ovens, UV-Vis spectrophotometer (Thermo scientific), cuvettes, analytical scales, blenders, rotary evaporators, hot plates, shakers, filter paper, plastic wrap, aluminum foil, and tissue. Materials used in this study included cermai dan mulberry fruits, 96% ethanol (Merck), distilled water, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin Ciocalteu reagent (Merck), gallic acid (Merck), sodium carbonate (Na_2CO_3) 7.5% (Merck), 2% $AlCl_3$ solution, and quercetin, used as a standard solution with a specified concentration.

Preparation of Cermai and Mulberry Fruits

The cermai fruit used in this study was harvested from a tree in Probolinggo, East Java and the mulberry fruits were obtained from Malang, East Java. The authenticity of both fruits was verified at UPT Laboratorium Herbal Materia Medica Batu, East Java. Specific information on the fruits' varieties, ages, and harvest time was not determined as it was not a variable in this study.

Only ripe, pale yellow, firm, and fresh cermai fruits and ripe, dark-purplish-black color and fresh mulberries were selected in this current study. Both cermai and mulberry fruits underwent a series of preparation steps, including wet sorting, washing, chopping, drying, dry sorting, and determination. The cermai and mulberry fruits that had been thinly sliced were then placed in the

oven at 40 °C for 24 hours. After drying, the samples were sorted to separate impurities and damage due to the drying process.

Extraction

The extraction was carried out through the maceration process. Dried cermai and mulberry fruits weighing 200 g and 57.6970 g were soaked in 96% ethanol solvent in a ratio of 1:5 for 24 hours. After soaking, the mixture was filtered. Filtrates were evaporated using a rotary evaporator to remove the solvent and concentrate the extract at a temperature of 70–80 °C and 100 rpm so that a thick ethanol cermai and mulberry fruit extract was obtained.

Yield Calculation

The yield was obtained by comparing the weight of cermai and mulberry fruit extract and the weight of Simplicia before extraction. The calculation of yield refers to Putra et al. (2020) with the formula yield (%)= weight of extract (g)/ weight of simplisia (g).

Antioxidant Activity Determination

The antioxidant activity was tested using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method with UV-Vis spectrophotometry at a maximum 400–800 nm wavelength (‘Aini et al., 2023) and analyzing cermai and mulberry fruit ethanol extracts at varying concentrations. A 0.5 mL aliquot of the sample was mixed with 2.7 mL of 20 ppm DPPH solution, shaken, and incubated in the dark for 30 minutes. The absorbance was measured at 517 nm, with DPPH as a negative control. Antioxidant activity (%AA) was calculated using the formula, %AA= abs DPPH - abs sample/ abs DPPH × 100%.

Total Phenolic Determination

Optimal Wavelength Measurement

A 1 mL of Folin-Ciocalteau reagent was added to 0.1 mL of gallic acid solution at a concentration of 100 ppm, and the mixture was incubated for five minutes. After that, 1 mL of a 7.5% Na_2CO_3 solution was added, and the mixture was allowed to rest at room temperature for 90 minutes. The 600–850 nm range was used to measure the maximum wavelength absorption.

Preparation of Gallic Acid Standard Solution

100 mL of distilled water was used to dissolve 10 mg of gallic acid. Concentrations of 40, 50, 60, 70, and 80 ppm were obtained by pipetting as much as 4, 5, 6, 7, and 8 mL, then diluting it with distilled water to 10 mL. A 1 mL of Folin-Ciocalteau reagent was added to 0.1 mL of each concentration, and the mixture was incubated for 5 minutes. After homogenizing the mixture with 1 mL of a 7.5% Na_2CO_3 solution, it was allowed to rest at room temperature in the dark for 90 minutes. A maximum wavelength of 766 nm was used to measure the sample.

Determination of Total Phenolics of Extract

The sample solution was prepared by pipetting 0.1 mL plus 1 mL of Folin-Ciocalteu reagent (1:10 v/v water), left for 5 minutes, then added 0.8 mL of Na_2CO_3 (75 g/L water) and left for 30 minutes at room temperature. Absorbance measurements were performed. Total polyphenol content is expressed in mg gallic acid equivalent (GAE) in 1 g of extract. The total phenolic content test was carried out using the Folin-Ciocalteu method using UV-Vis spectrophotometry at 760.5 nm.

Total Flavonoid Content Determination

Flavonoid content was measured using the $AlCl_3$ colorimetric method with quercetin as a standard. First, a calibration curve of standard quercetin solution was prepared. The sample's absorbance was then compared to this calibration. In brief, to determine sample absorbance, 4 mL of methanol was added to the sample, followed by 1 mL of 2% $AlCl_3$ solution. The mixture was incubated at room temperature for 30 minutes before measuring absorbance at 430 nm.

Sun Protection Factor Value Determination

The SPF value was determined in vitro using UV-Vis spectrophotometry. A total of 0.1 g of extract was dissolved in ethanol to obtain a solution concentration of 1,000 ppm, then serially diluted to 200, 400, and 600 ppm. The absorbance of each concentration was measured in the 290–320 nm range at 5-minute intervals.

RESULTS

Yield Calculation

The yield of ethanolic extracts is a crucial indicator of extraction efficiency and the potential bioactive compound content in plant-based samples. In this study, the ethanolic extraction of cermai and mulberry fruits yielded shown in Table 1.

Table 1. Yield of ethanolic extract cermai and mulberry fruits

Sample	Extract result (g)	Yield (%)
Cermai fruit	20	10
Mulberry fruit	13.5777	23.53

Antioxidant Activity Determination

The antioxidant activity test on cermai, mulberry, and their various combinations revealed differences in IC_{50} values, indicating varying levels of antioxidant potential. An IC_{50} value represents the concentration required to inhibit 50% of free radicals; lower IC_{50} values denote higher antioxidant capacity. The antioxidant test data is shown in Table 2.

Table 2. The result of antioxidant activity

Group	Antioxidant activity IC_{50} (%)
Mulberry extract (ME)	344.43
Cermai extract (CE)	418.30
ME: CE (1:3)	400.49
ME: CE (1:1)	367.73
ME: CE (3:1)	358.04

Total Phenolic Determination

The total phenolic content (TPC) in various samples of cermai (*Phyllanthus acidus*) and mulberry (*Morus alba*) extracts, as well as their combinations, was measured using the gallic acid equivalent method with spectrophotometry at a wavelength of 715 nm. Figure 1 shows that the total phenol content of gallic acid produces the equation y= 0.0075x-0.07144 with R^2= 0.9969. The standard curve is constructed with gallic acid concentrations ranging from 40 to 80 ppm.

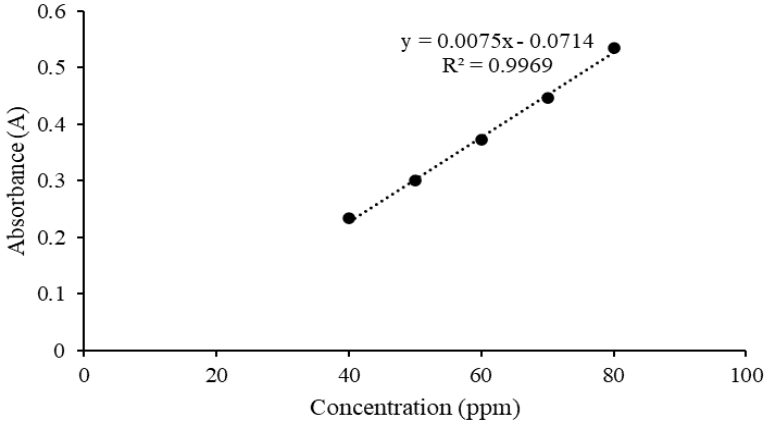

Figure 1. Gallic acid calibration curve

Table 3 shows the total results. The highest phenolic concentration was observed in the pure cermai extract, yielding a total phenolic content of 4.26%. In comparison, the mulberry extract exhibited a phenolic concentration of 3.56%, slightly lower than that of cermai. Mixed samples with varying ratios of mulberry and cermai showed intermediate phenolic levels. The 1:3 ratio (25% mulberry: 75% cermai) resulted in a TPC of 4.02%, suggesting a synergistic increase in phenolic

content due to the higher proportion of cermai. Conversely, the 3:1 mixture (75% mulberry: 25% cermai) yielded a TPC of 3.65%, aligning more closely with the phenolic content of the mulberry extract alone.

Table 3. The result of phenolic total

Group	Phenolic total (%)
Mulberry extract (ME)	3.56
Cermai extract (CE)	4.26
ME: CE (1:3)	4.02
ME: CE (1:1)	3.98
ME: CE (3:1)	3.65

Total Flavonoid Content Determination

The total flavonoid content in various ratios of cermai and mulberry extracts, tested using quercetin as a standard, revealed a concentration-dependent increase in absorbance. Quercetin standard curves were obtained using the curve equations y= 0.0067x-0.0474 and R2= 0.9957, as shown in Figure 2. The quercetin standard curve was constructed with absorbance readings at 429 nm for concentrations ranging from 40 to 120 ppm.

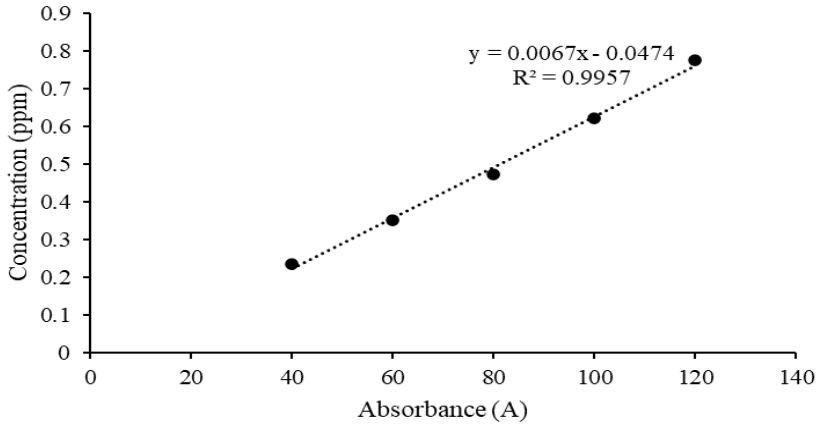

Figure 2. Quercetin calibration curve

After the absorbance of cermai and mulberry fruit extract was plotted in the equation, the total flavonoid content was obtained and it is presented in Table 4. The mulberry extract alone yielded a flavonoid concentration of 23.04 ppm, corresponding to a 0.23% total flavonoid content.

Table 4. Result of flavonoid content

Group	Flavonoid content (%)
Mulberry extract (ME)	0.23
Cermai extract (CE)	0.30
ME: CE (1:3)	0.27
ME: CE (1:1)	0.26
ME: CE (3:1)	0.24

Sun Protection Factor Value Determination

The SPF values of various formulations containing mulberry and cermai extracts were evaluated at different concentrations and ratios, indicating their effectiveness as natural UV protectants. SPF values were calculated based on absorbance readings at specific wavelengths (290–320 nm) to assess each sample's photoprotective properties. The results of determining the SPF value are presented in Table 5.

Table 5. Result of sun protection factor

Group	Sun protection factor
Mulberry extract (ME)	7.16
Cermai extract (CE)	4.09
ME: CE (1:3)	6.55
ME: CE (1:1)	6.49
ME: CE (3:1)	7.11

DISCUSSION

Yield Calculation

The yield of cermai extract was 10%, which is relatively moderate and aligns with previous findings suggesting that cermai contains bioactive compounds, albeit in lower concentrations than Malaka fruits (*Phyllanthus emblica*) (Nisar et al., 2018; Sangeetha et al., 2023). This result may reflect the influence of the cermai fruit's cellular structure and its content of phenolic compounds, which can affect extraction efficiency. Studies have indicated that cermai's lower extraction yield may also result from its limited soluble compounds in ethanol, as ethanol predominantly extracts moderately polar to non-polar phytochemicals (Miragliotta et al., 2024). Ethanol is most effective at extracting moderately polar to non-polar compounds, including flavonoids, tannins, and certain alkaloids. However, if cermai fruit contains a higher proportion of water-soluble phenolics, such as specific polyphenols or glycosides, ethanol alone may not be sufficient for efficient extraction. Additionally, the resistance of cell walls to breakdown can further hinder ethanol's ability to penetrate the plant material, resulting in a lower extraction yield.

The yield for the mulberry extract was significantly higher at 23.53%, which is consistent with the known high polyphenol and anthocyanin content of *Morus alba*, making it more readily extractable by ethanol. This higher yield may also be attributed to mulberry's soft cellular structure and a rich composition of soluble compounds that ethanol can efficiently extract (Bonesi et al., 2019). Recent research has shown that mulberry fruit yields well with ethanol due to its anthocyanins and flavonoids, compounds that are highly soluble in ethanol and contribute to both the yield and antioxidant activity of the extract (Hidayatunnikmah et al., 2023).

The difference in yield between cermai and mulberry extracts suggests that mulberry is a more efficient source of bioactive compounds for ethanol-based extractions. This difference aligns with findings in extraction efficiency studies where high polyphenolic and anthocyanin-rich fruits generally produce higher yields in ethanol, a solvent known for effectively extracting medium-to-non-polar compounds (Xiang et al., 2024). Furthermore, the higher yield in mulberry may imply better economic feasibility for large-scale production in industries that focus on natural antioxidants and nutraceuticals.

Antioxidant Activity Determination

The IC_{50} of mulberry extract was 344.43 ppm, demonstrating a relatively high antioxidant activity. Previous studies have shown that mulberry is rich in polyphenols and anthocyanins, which contribute to its significant antioxidant capacity (Royani et al., 2024). The IC_{50} results align with findings that *Morus alba* exhibits potent radical-scavenging properties due to these bioactive compounds (Chawansuntati et al., 2024; Ghosh et al., 2021).

The cermai extract exhibited a higher IC_{50} of 418.30 ppm, indicating lower antioxidant activity compared to mulberry. This result suggests that cermai's phenolic content may be less effective or present in lower concentrations. Research showed that although cermai contains bioactive compounds with antioxidant properties, its effectiveness may vary depending on the compound concentration and extraction method used (Putri & Khonsa, 2022).

Among the combinations, the 1:1 ratio exhibited the highest antioxidant activity with an IC_{50} of 367.73 ppm. This result aligns with research indicating that combining antioxidants from different sources can create a synergistic effect, enhancing overall antioxidant activity (Chamali et al., 2023). The 1:1 and 3:1 combination ratio yielded better results than cermai alone, suggesting that the mulberry's higher polyphenol content likely contributed to increased radical scavenging (Kobus-Cisowska et al., 2019).

The variations in IC_{50} values across combinations reflect that the proportions of active compounds impact the total antioxidant capacity. Studies have documented similar effects, where combining extracts with differing antioxidant profiles enhances efficacy, as seen in combinations of anthocyanin-rich fruits. The findings suggest that a 1:1 combination could be optimal for developing antioxidant-rich formulations.

Total Phenolic Determination

The differences in TPC across samples can be attributed to the varying phenolic compositions of the fruits involved. Cermai is known for its robust antioxidant activity, which may be related to its higher total phenolic compounds. This result is consistent with existing literature, which notes that cermai fruits contain high levels of polyphenolic compounds, contributing to their antioxidant properties (Pandey & Rizvi, 2009). This property is valuable for applications requiring antioxidant potency, including food preservation and medicinal formulations. Meanwhile, the slightly lower TPC in mulberry extracts is compensated by its flavonoid and anthocyanin content, which also contribute to its health benefits (Muflihah et al., 2021).

Based on the results, phenolic compounds (simple phenols and polyphenols), their derivatives, and several compounds, including terpenoids, were characterized as potent tyrosinase inhibitors. The appropriate functionalization of these inhibitors, such as C-6 and C-7 hydroxyl groups of the isoflavone skeleton, 4-functionalization thiophene-2carbaldehyde thiosemicarbazone with a methoxyacetyl group, and the aldehyde group and methoxy group in C4 of benzaldehyde derivatives, may be improved the inhibitory activity of these inhibitors. A synergistic strategy for tyrosinase inhibitors is a useful strategy for the improvement of their inhibitory activities. The mixtures of glabridin: resveratrol, glabridin: oxyresveratrol, resveratrol: oxyresveratrol, acid: l-ascorbic with arbutin, 1-phenyl-2-thiourea or kojic acid have shown synergistic effect on tyrosinase. These studies may provide a scientific strategy for screening effective tyrosinase inhibitors.

Total Flavonoid Content Determination

Cermai extract showed a slightly higher flavonoid content of 30.36 ppm or 0.30%. Studies suggest that cermai contains robust antioxidant compounds, including quercetin and other flavonoids that contribute to its higher flavonoid content, supporting its potential as a bioactive extract (Mbunde et al., 2018). The moderate flavonoid concentration of mulberry aligns with previous studies reporting the presence of significant flavonoid content in mulberry, attributed to compounds such as quercetin and kaempferol, which have antioxidant potential (Muflihah et al., 2021). This mixture 1:3 yielded a flavonoid content of 27.67 ppm, translating to a 0.27% concentration, demonstrating the synergistic effect of combining both extracts. The mixture's slightly elevated flavonoid content compared to mulberry alone suggests an enhancement in flavonoid presence, possibly due to the combined bioactive components in both extracts. The 1:1 mulberry to cermai ratio resulted in a flavonoid concentration of 26.33 ppm (0.26%), slightly lower than the 1:3 mixture. The slight decrease in flavonoid concentration in the 1:1 mixture compared to the 1:3 mixture could be due to differences in polyphenol contributions from each fruit, extraction efficiencies, and potential interactions affecting solubility. The 1:3 ratio likely favors a higher release of flavonoids from the dominant fruit component, leading to a slightly greater total flavonoid yield. This reduction may indicate an interaction effect where specific flavonoid compounds from cermai could be competitively inhibiting the flavonoid content absorption. This ratio showed a flavonoid concentration of 24.39 ppm (0.24%), indicating a declining trend in flavonoid concentration with a higher mulberry proportion. This result is consistent with findings that mulberry flavonoid concentration tends to stabilize or decrease when in mixtures with higher cermai content (Luna et al., 2020; Villanueva-Bermejo et al., 2024).

Sun Protection Factor Value Determination

The higher SPF value is for mulberry extract. The SPF value is in the range 7–15, where this result is included in the maximum protection category for a substance to be used as an active sunscreen agent to protect the skin. The mulberry extract demonstrated an SPF value of 7.16, suggesting maximum UVB protection. This SPF level corresponds with prior research highlighting the UV-absorbing capabilities of flavonoid and polyphenolic compounds in mulberry, which are known to provide natural sunscreen benefits (Ghazi, 2022). The SPF value of cermai extract was 4.09, lower than that of mulberry, indicating a lesser degree of UVB protection. Despite its lower SPF, cermai extract is valued for its antioxidant properties, which can reduce UV-induced oxidative stress (Hassan et al., 2020; Heckmann et al., 2024). The 1:3 mulberry to cermai mixture achieved an SPF of 6.55, suggesting a slightly enhanced UV protection relative to cermai alone. This mixture

benefits from the combined UV-protective properties of both extracts, aligning with studies that show synergistic effects of mixed botanical extracts. At a 1:1 ratio, the mixture reached an SPF of 6.49, indicating comparable UV protection to the 1:3 mixture. This result may imply that beyond a certain concentration, the combination of active compounds in cermai and mulberry reaches a plateau effect in SPF efficiency. The slight increase in SPF with the 1:3 ratio suggests a marginal improvement in UV protection, but the near-identical values imply a plateau effect where increasing cermai content does not drastically enhance SPF. This is likely due to the saturation of UV-absorbing compounds, synergistic optimization at the 1:1 ratio, and potential stabilization effects that prevent further SPF enhancement. The highest SPF value of 7.11 was obtained from the 3:1 mulberry to cermai mixture. This finding aligns with evidence that Mulberry's flavonoids and phenolic content play a significant role in UV absorption, enhancing the photoprotective effect when present in higher ratios (He et al., 2021).

CONCLUSION

The study results indicate that cermai and mulberry fruit extracts, in various combinations, have promising potential as tyrosinase inhibitors for protecting against hyperpigmentation. Among the tested extracts, mulberry demonstrated the highest antioxidant activity, with an IC_{50} value of 344.43%, suggesting its strong free radical scavenging capability. Additionally, the mulberry extract exhibited the highest SPF value, highlighting its superior UV protection properties. These findings support the potential application of mulberry and cermai extracts in cosmetic and dermatological formulations aimed at skin protection and pigmentation control.

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