42571

by 42571 42571

Submission date: 10-Jan-2025 06:05PM (UTC+0700)
Submission ID: 2562019886
File name: 42571-132595-1-RV.doc (191.5K)
Word count: 5202
Character count: 31695

Potential Combination of Cermai (*Phyllanthus acidus*) and Mulberry (*Morus alba*) Fruit Extract as a Candidate for Tyrosinase Inhibitor

Potential Combination of Cermai (*Phyllanthus acidus*) and Mulberry (*Morus alba*) Fruit Extract as a Candidate for Tyrosinase Inhibitor

Abstract

Melanin, pigmen yang berasal dari radiasi UV, sangat penting dalam mencegah kerusakan kulit dan dapat menyebabkan masalah estetika dan dermatologis yang signifikan. Melanogenesis adalah proses kompleks yang melibatkan enzim dan sitokin, dengan UV sebagai kontributor utama. Tirosinase adalah enzim kunci dalam sintesis melanin. Studi ini bertujuan untuk mengevaluasi aktivitas antioksidan, kandungan fenolik total, kandungan flavonoid, dan nilai Sun Protection Factor (SPF) dari kombinasi Ekstrak Buah Cermai (CE) dan Ekstrak Buah Mulberry (ME) sebagai kandidat inhibitor tirosinase. Hasil penelitian menunjukkan bahwa kandungan fenolik total tertinggi diamati pada Ekstrak Buah Cermai (CE), diikuti oleh kombinasi ME:CE dalam rasio 1:3, 1:1, 3:1, dan kemudian Ekstrak Buah Mulberry (ME). Tren serupa diamati pada kandungan flavonoid. Aktivitas antioksidan, seperti yang ditunjukkan oleh nilai IC_{50}, mengikuti urutan berikut ini 418,30 %; 400,49 %; 367,73 %; 358,04 %; dan 344,43 % masing-masing. Nilai Sun Protection Factor (SPF) tertinggi diamati pada Ekstrak Buah Mulberry. Kesimpulannya, kombinasi ekstrak buah cermai dan buah mulberry dalam berbagai rasio menunjukkan potensi menjanjikan sebagai inhibitor tirosinase, yang dapat melindungi dari hiperpigmentasi yang disebabkan oleh UV.

Kata kunci: Buah Mulberry, Cermai, Depigmentasi Kulit, Ekstrak Alami, Inhibitor Tirosinase

Abstract

Melanin, a pigment derived from UV radiation, is crucial in preventing skin damage and can cause significant aesthetic and dermatological issues. Melanogenesis is a complex process involving enzymes and cytokines, with UV being a primary contributor. Tirosinase is a key enzyme in melanin synthesis. This study aims to evaluate the antioxidant activity, total phenolic content, flavonoid content, and Sun Protection Factor (SPF) value of a combination Cermai Extracts (CE) and Mulberry fruit Extracts (ME) as a candidate for tyrosinase inhibitors. The results demonstrated that the highest total phenolic content was observed in Cermai Extract (CE), followed by the combination ME:CE in rations of 1:3, 1:1, 3:1, and then the Mulberry extract (ME). A similar trend was observed in flavonoid content. Antioxidant activity, as indicated by IC50 values, followed this order 418.30; 400.49; 367.73; 358.04; and 344.43 respectively. The highest Sun Protection Factor (SPF) value was observed in Mulberry Extract. In conclusion, the combination of Cermai and Mulberry fruit extracts in various ratios shows promising potential as tyrosinase inhibitors, which could protect against UV-induced hyperpigmentation.

***Keywords**: Cermai, Mulberry fruit, Natural Extract, Skin Depigmentation, Tyrosinase Inhibitor*

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 plays as a rate-limiting enzyme in the synthesis of melanin. Tyrosinase inhibitors are examined in the presence of a monophenolic 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 inhibited tyrosinase through a multi-phase kinetic process and bind to tyrosinase at a single binding site mainly by hydrogen bonds and van der Waals. Furthermore, it was reported that three flavonols including galangin, kaempferol, quercetin inhibit the oxidation of L-DOPA catalysed by mushroom tyrosinase and presumably this inhibitory activity comes from their copper chelating ability. While their corresponding flavones, chrysin, apigenin and luteolin, are not identified as copper chelator, 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 or 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 that penetrates the skin, the greater and 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. 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 which functions to help the substrate bind to the enzyme. 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. 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

Material

The equipment used included measuring cups, test tubes (iwaki), microplates, volumetric flasks, erlenmeyer flasks, micropipettes (Eppendorf), dark bottles, ovens, UV-Vis spectrophotometer (thermoscientific) and cuvettes, analytical scales, blenders, rotary evaporators, hot plates, shakers, filter paper, plastic wrap, aluminum foil, tissue.

Materials used in this study include cermai dan mulberry fruits, 96% ethanol (Merck), distilled water, DPPH (2,2-diphenyl-1-picrylhydrazyl), 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.

Methods

Preparation of cermai dan 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 varieties, ages and harvest time of the fruits is not determined as it is not a variable in this study.

Only ripe cermai fruits, which were pale yellow, firm, and fresh, and fresh mulberries were selected for this study. Both cermai and mulberry fruits underwent a series of preparation steps, including wet sorting, washing, chopping, drying and dry sorting, and determination. Cermai and mulberry fruit that has been thinly sliced is then placed in the oven at 40° C for 24 hours. After drying the samples were sorted to separate impurities and damaged due to the drying process.

Extraction

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

Yield Calculation

The yield was obtained by comparing the weight of cermai and mullbey fruit extract and the weight of simplicia before extraction. The calculation of yield refers to(Widhiana Putra et al., 2020) with the formula:

$$Yield\ (\%) = \frac{Weight\ of\ extract\ (g)}{Weight\ of\ simplisia\ (g)}$$

Antioxidant Activity Determination

The antioxidant activity was tested used the DPPH (2,2-diphenyl-1-picrylhydrazyl) method with UV-Vis spectrophotometry at a maximum wavelength of 400-800nm (Putini et al., 2023). analyzing cermai and mulberry fruit ethanol extracts at varying concentration. 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:

$$\%\,AA = \frac{Abs.DPPH - Abs.Sampel}{Abs.DPPH}\,x\,100\%$$

Total Phenolic Determination

Optimal Wavelength Measurement

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% Na2CO3 solution was added, and the mixture was allowed to rest at room temperature for 90 minutes. The range of 600-850 nm was used to measure the maximum wavelength absorption

Preparation of Gallic Acid Standard Solution

100 mL of distilled water were 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. 1 mL of Folin-Ciocalteau reagent was added to 0.1 milliliters of each concentration, and the mixture was incubated for five 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 a 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 GAE (Gallic acid equivalent) in 1 gram 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 0.8 g of extract was dissolved in ethanol to obtain a solution concentration of 1000 ppm, then serially diluted to 200 ppm, 400 ppm, 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 showed in table 1.

Tabel 1. Yield of ethanolic extract cermai and mulberry fruits

Sampel	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.

Tabel 2. 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, constructed with gallic acid concentrations ranging from 40 to 80 ppm.

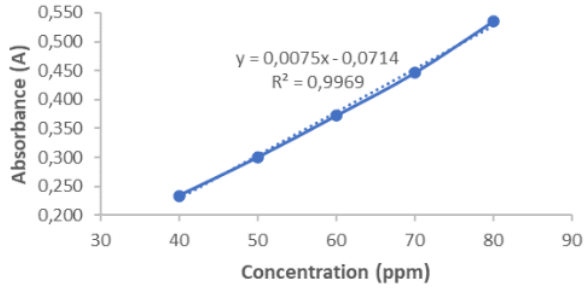

Figure 1. Gallic acid calibration curve

The table 3 shows the result of total, the highest phenolic concentration was observed in the pure cermai extract, yielding a total phenolic content of 4.26%. 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). 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.

Tabel 3. 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 have been obtained with the curve equations y=0.0067x-0.0474 and R^2= 0.9957 shows in figure 2. The quercetin standard curve was constructed with absorbance readings at 429 nm for concentrations ranging from 40 to 120 ppm.

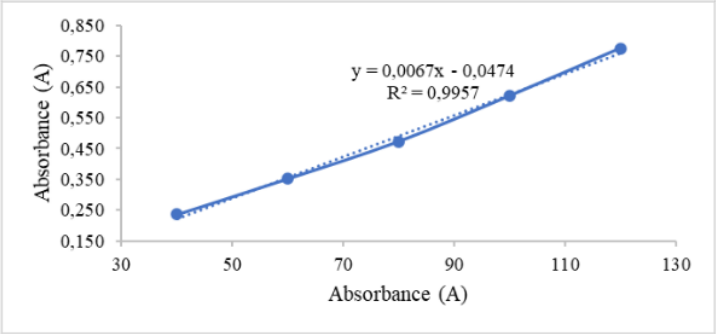

Figure 2. Quercetin calibration curve

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. This moderate flavonoid concentration 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).

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

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).

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 of 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 some other fruits (Nisar et al., 2018; K. 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).

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 (Hidayatun nikmah 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 a 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.,). The IC_{50} results align with findings that *Morus alba* exhibits potent radical-scavenging properties due to these bioactive compounds (Ghosh et al., 2021; Chawansuntati et al., 2024).

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 (Pati & 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 rasio of 1:1 and 3:1 combination 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 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) and their derivatives and several compounds including terpenoid were characterised as potent tyrosinase inhibitors. The appropriate functionalisation of these inhibitors such as C-6 and C-7 hydroxyl groups of the isoflavone skeleton, 4-functionalisation thiophene-2carbaldehyde thiosemicarbazone with a methoxyacetyl group and the aldehyde group and methoxy group on C4 of benzaldehyde derivatives may be improved the inhibitory activity of these inhibitors. 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

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. 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's 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 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 moderate 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. 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 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 AND SUGGESTION

Based on the results of the study obtained the following conclusions:

1. Cermai and Mulberry fruit extract in various combination have the potential as a candidate for tyrosinase inhibitor to protect the hyperpigmentation.
2. The Mulberry extract have shown higher activity antioxidant with IC_{50} 344.43 %.

3. The higher SPF value is Mulberry extract.

ACKNOWLEDMENTS

This research was funded by Health Polytechnic of Putra Indonesia Malang and Department of Biology, Faculty of Mathematic and Natural Sciences, University of Islamic Malang.

REFERENCE

'Aini, D. Q., Sjakoer, N. A. A., & Mubarakati, N. J. (2023). Antioxidant Assay of Endophytic Fungi Extract from Mango Mistletoe (Dendrophthoe pentandra (L.) Miq) Leaves. *JSMARTech*, *4*(1), 09–13. https://doi.org/10.21776/ub.jsmartech.2023.004.01.09

Ali, A., Ashraf, Z., Kumar, N., Rafiq, M., Jabeen, F., Park, J. H., Choi, K. H., Lee, S., Seo, S.-Y., Choi, E. H., & Attri, P. (2016). Influence of plasma-activated compounds on melanogenesis and tyrosinase activity. *Scientific Reports*, *6*(1), 21779. https://doi.org/10.1038/srep21779

Böhm, M. (2020). Disorders of Melanin Pigmentation. In G. Plewig, L. French, T. Ruzicka, R. Kaufmann, & M. Hertl (Eds.), *Braun-Falco´s Dermatology* (pp. 1–35). Springer. https://doi.org/10.1007/978-3-662-58713-3_69-1

Bonesi, M., Leporini, M., Tenuta, M., & Tundis, R. (2019). The Role of Anthocyanins in Drug Discovery: Recent Developments. *Current Drug Discovery Technologies*, *16*. https://doi.org/10.2174/1570163816666190125152931

Chamali, S., Bendaoud, H., Bouajila, J., Camy, S., Saadaoui, E., Condoret, J.-S., & Romdhane, M. (2023). Optimization of accelerated solvent extraction of bioactive compounds from *Eucalyptus intertexta* using response surface methodology and evaluation of its phenolic composition and biological activities. *Journal of Applied Research on Medicinal and Aromatic Plants*, *35*, 100464. https://doi.org/10.1016/j.jarmap.2023.100464

Chawansuntati, K., Hongjaisee, S., Sirita, K., Kingkaew, K., Rattanathammethee, K., Kumrapich, B., Ounjaijean, S., Kongkaew, A., & Lumjuan, N. (2024). Effects of quercetin and extracts from Phyllanthus emblica, Morus alba, and Ginkgo biloba on platelet recovery in a rat model of chemotherapy-induced thrombocytopenia. *Heliyon*, *10*(2). https://doi.org/10.1016/j.heliyon.2024.e25013

El-Nashar, H. A. S., El-Din, M. I. G., Hritcu, L., & Eldahshan, O. A. (2021). Insights on the Inhibitory Power of Flavonoids on Tyrosinase Activity: A Survey from 2016 to 2021. *Molecules*, *26*(24), 7546. https://doi.org/10.3390/molecules26247546

Ghosh, R., Barua, P., Sikder, O., Saha, S., Mojumder, S., & Sikdar, D. (2021). Comparison of phenolic content and antioxidant activity of two common fruits of Bangladesh in solvents of varying polarities. *Food Research*, *5*(6), 187–196. https://doi.org/10.26656/fr.2017.5(6).253

Hassan, F., Arshad, M., Li, M., Rehman, M., Loor, J., & Huang, J. (2020). Potential of Mulberry Leaf Biomass and Its Flavonoids to Improve Production and Health in Ruminants: Mechanistic Insights and Prospects. *Animals*, *10*. https://doi.org/10.3390/ani10112076

He, hailun, Li, anqi, Li, shiqin, Tang, jie, Li, li, & Xiong, lidan. (2021). Natural components in sunscreens: Topical formulations with sun protection factor (SPF). *Biomedicine & Pharmacotherapy*, *134*, 111161. https://doi.org/10.1016/j.biopha.2020.111161

Heckmann, M., Stadlbauer, V., Drotarova, I., Gramatte, T., Feichtinger, M., Arnaut, V., Atzmüller, S., Schwarzinger, B., Röhrl, C., Blank-Landeshammer, B., & Weghuber, J. (2024).

Identification of Oxidative-Stress-Reducing Plant Extracts from a Novel Extract Library—Comparative Analysis of Cell-Free and Cell-Based In Vitro Assays to Quantitate Antioxidant Activity. *Antioxidants*, *13*(3), 297. https://doi.org/10.3390/antiox13030297

Hidayatunnikmah, N., Latifah, A., & Rosyida, D. A. C. (2023). Anthocyanins in Mulberry Leaves (Morus rubra L.) Ethanol Extract as the Inhibitor for the Growth of Candida albicans. *EMBRIO: Jurnal Kebidanan*, *15*(1), Article 1. https://doi.org/10.36456/embrio.v15i1.6346

K., S., C., S., Sampathrajan, V., Nivethadevi, P., Pandian, K., & Elangovan, S. (2023). Identification of Phytochemical Constituents in Phyllanthus acidus L. Leaf through Gas Chromatography-Mass Spectroscopy as Biostimulant. *Asian Journal of Chemistry*, *35*, 673–678. https://doi.org/10.14233/ajchem.2023.27552

Karkoszka, M., Rok, J., & Wrześniok, D. (2024). Melanin Biopolymers in Pharmacology and Medicine—Skin Pigmentation Disorders, Implications for Drug Action, Adverse Effects and Therapy. *Pharmaceuticals*, *17*(4), 521. https://doi.org/10.3390/ph17040521

Kobus-Cisowska, J., Szczepaniak, O., Szymanowska-Powałowska, D., Piechocka, J., Szulc, P., & Dziedziński, M. (2019). Antioxidant potential of various solvent extract from *Morus alba* fruits and its major polyphenols composition. *Ciência Rural*, *50*, e20190371. https://doi.org/10.1590/0103-8478cr20190371

Logesh, R., Prasad, S. R., Chipurupalli, S., Robinson, N., & Mohankumar, S. K. (2023). Natural tyrosinase enzyme inhibitors: A path from melanin to melanoma and its reported pharmacological activities. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, *1878*(6), 188968. https://doi.org/10.1016/j.bbcan.2023.188968

Luna, S. L. R. D., Ramírez-Garza, R. E., & Saldívar, S. O. S. (2020). Environmentally Friendly Methods for Flavonoid Extraction from Plant Material: Impact of Their Operating Conditions on Yield and Antioxidant Properties. *The Scientific World Journal*, *2020*, 6792069. https://doi.org/10.1155/2020/6792069

Mbunde, M., Mdegela, R. H., Laswai, H. S., & Mabiki, F. P. (2018). Quantification of phenolics, flavonoids and antioxidant activity of Tamarindus indica from selected areas in Tanzania. *Biofarmasi Journal of Natural Product Biochemistry*, *16*(1), 22–28. https://doi.org/10.13057/biofar/f160103

Miragliotta, A. M. G., Ojeda, G. A., Gonzalez, R. B., Jara, E. R., Teibler, G. P., Peruchena, N. M., & Torres, A. M. (2024). Extraction of anti-hyperglycaemic bioactive compounds from *Phyllanthus niruri* L. through solvent mixture design*: In vitro* and *in vivo* evaluation. *Phytomedicine Plus*, *4*(3), 100622. https://doi.org/10.1016/j.phyplu.2024.100622

Muflihah, Y. M., Gollavelli, G., & Ling, Y.-C. (2021). Correlation Study of Antioxidant Activity with Phenolic and Flavonoid Compounds in 12 Indonesian Indigenous Herbs. *Antioxidants*, *10*(10), 1530. https://doi.org/10.3390/antiox10101530

Nisar, M. F., He, J., Ahmed, A., Yang, Y., Li, M., & Wan, C. (2018). Chemical Components and Biological Activities of the Genus Phyllanthus: A Review of the Recent Literature. *Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry*, *23*(10), 2567. https://doi.org/10.3390/molecules23102567

Nursid, M., Khatulistiani, T., Noviendri, D., Hapsari, F., & Hardiyati, T. (2020). Total phenolic content, antioxidant activity and tyrosinase inhibitor from marine red algae extract collected from Kupang, East Nusa Tenggara. *IOP Conference Series: Earth and Environmental Science*, *493*, 012013. https://doi.org/10.1088/1755-1315/493/1/012013

Obaid, R. J., Mughal, E. U., Naeem, N., Sadiq, A., Alsantali, R. I., Jassas, R. S., Moussa, Z., & Ahmed, S. A. (2021). Natural and synthetic flavonoid derivatives as new potential tyrosinase inhibitors: A systematic review. *RSC Advances*, *11*(36), 22159. https://doi.org/10.1039/d1ra03196a

Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, *2*(5), 270. https://doi.org/10.4161/oxim.2.5.9498

Putri, R. R., & Khonsa, K. (2022). FORMULASI DAN UJI AKTIVITAS ANTIOKSIDAN EKSTRAK BUAH CERMAI (Phyllanthus acidus L.) DENGAN METODE DPPH. *JIFMI : Jurnal Ilmiah Fitomedika Indonesia*, *1*(1). https://ojs.stikesylpp.ac.id/index.php/JIFMI/article/view/570

Rosa, G. P., Palmeira, A., Resende, D. I. S. P., Almeida, I. F., Kane-Pagès, A., Barreto, M. C., Sousa, E., & Pinto, M. M. M. (2021). Xanthones for melanogenesis inhibition: Molecular docking and QSAR studies to understand their anti-tyrosinase activity. *Bioorganic & Medicinal Chemistry*, *29*, 115873. https://doi.org/10.1016/j.bmc.2020.115873

Royani, A., Mubarak, N. M., Hanafi, M., Verma, C., Lotulung, P. D. N., Prastya, M. E., Alfantazi, A., & Manaf, A. (n.d.). Effect of solvent polarity on yield extract, antioxidant and antibacterial activities of phytochemicals from Andrographis paniculata leaves. *Indian Chemical Engineer*, *0*(0), 1–15. https://doi.org/10.1080/00194506.2024.2409260

Villanueva-Bermejo, D., Siles-Sánchez, M. de las N., Martín Hernández, D., Rodríguez García-Risco, M., Jaime, L., Santoyo, S., & Fornari, T. (2024). Theoretical framework to evaluate antioxidant synergistic effects from the coextraction of marjoram, rosemary and parsley. *Food Chemistry*, *437*, 137919. https://doi.org/10.1016/j.foodchem.2023.137919

Widhiana Putra, I. K., Ganda Putra, G. P., & Wrasiati, L. P. (2020). Pengaruh Perbandingan Bahan dengan Pelarut dan Waktu Maserasi terhadap Ekstrak Kulit Biji Kakao (Theobroma cacao L.) sebagai Sumber Antioksidan. *JURNAL REKAYASA DAN MANAJEMEN AGROINDUSTRI*, *8*(2), 167. https://doi.org/10.24843/JRMA.2020.v08.i02.p02

Xiang, Z., Liu, L., Xu, Z., Kong, Q., Feng, S., Chen, T., Zhou, L., Yang, H., Xiao, Y., & Ding, C. (2024). Solvent Effects on the Phenolic Compounds and Antioxidant Activity Associated with Camellia polyodonta Flower Extracts. *ACS Omega*, *9*(25), 27192–27203. https://doi.org/10.1021/acsomega.4c01321

Yang, C.-Y., Guo, Y., Wu, W.-J., Man, M.-Q., Tu, Y., & He, L. (2022). UVB-Induced Secretion of IL-1β Promotes Melanogenesis by Upregulating TYR/TRP-1 Expression In Vitro. *BioMed Research International*, *2022*, 8230646. https://doi.org/10.1155/2022/8230646

Zolghadri, S., Bahrami, A., Khan, M. T. H., Munoz-Munoz, J., Garcia-Molina, F., Garcia-Canovas, F., & Saboury, A. A. (2019). A comprehensive review on tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *34*(1), 279. https://doi.org/10.1080/14756366.2018.1545767

42571

ORIGINALITY REPORT

17%	%	17%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Samaneh Zolghadri, Asieh Bahrami, Mahmud Tareq Hassan Khan, J. Munoz-Munoz et al. "A comprehensive review on tyrosinase inhibitors", Journal of Enzyme Inhibition and Medicinal Chemistry, 2019 Publication	6%
2	"Biomass-based Cosmetics", Springer Science and Business Media LLC, 2024 Publication	2%
3	Budiono Budiono, Sumirah Budi Pertami, Kasiati, Siti Nur Arifah, Mochammad Fitri Atho'illah. "Lactogenic effect of Polyscias scutellaria extract to maintain postpartum prolactin and oxytocin in lactating rats", Journal of Ayurveda and Integrative Medicine, 2022 Publication	1%
4	Darasia, M Mahendradatta, A Hasizah, Rahmaniar. "Comparison of soxhletation and Microwave Assisted Extraction method for extracting polyphenols in cacao pod husks	1%

(Theobroma cacao L.)", IOP Conference Series: Earth and Environmental Science, 2023
Publication

---

5 Adila Salih ElObeid, Afaf Kamal-Eldin, Mohamed Anwar K. Abdelhalim, Adil M. Haseeb. "Pharmacological Properties of Melanin and its Function in Health", Basic & Clinical Pharmacology & Toxicology, 2017 — 1%
Publication

---

6 Nafise Nabizade, Amanollah Zarei-Ahmady, Mohammad Reza Shushizadeh, Ebrahim RajabZadehGhatrami, Fereshteh Golfakhrabadi. "Comparison study of Sargassum Vulgare and Padina Pavonica of Persian Gulf extracts for their bioactive compounds, antioxidant activity, and sun protection factor to improve UV absorption", Springer Science and Business Media LLC, 2024 — 1%
Publication

---

7 Bund, R.K.. "Rapid lactose recovery from buffalo whey by use of 'anti-solvent, ethanol'", Journal of Food Engineering, 200710 — <1%
Publication

---

8 Yetri Elisya, Harpolia Cartika, Adin Hakim. "Sunscreen Cream Formulation from Corn Cob Extract (*Zea maysM/em> L.) and Robusta* — <1%

Coffee Bean Extract (Coffea canephora Pierre ex A. Froehner)", Asian Journal of Applied Sciences, 2020
Publication

---

9 Loizzo, M.R.. "Radical scavenging, antioxidant and metal chelating activities of Annona cherimola Mill. (cherimoya) peel and pulp in relation to their total phenolic and total flavonoid contents", Journal of Food Composition and Analysis, 201203 <1%
Publication

---

10 Ayşegül Bozdemir, Eda Şensu, Emine Şükran Okudan, Beraat Özçelik, Aysun Yucetepe. " Ultrasound-assisted enzymatic extraction of proteins from : Investigation of antioxidant activity and techno-functional properties ", Journal of Food Processing and Preservation, 2022 <1%
Publication

---

11 Tiantian Xiu, Peng Liu, Shuangling Zhang, Dehong Du et al. "Polyphenol Nanoparticles of Millet, Rice and Wheat: Extraction, Identification, Functional and Morphological Characteristics", International Journal of Food Science & Technology, 2022 <1%
Publication

---

12 Kalidas Shetty, Dipayan Sarkar. "Functional Foods and Biotechnology - Biotransformation <1%

and Analysis of Functional Foods and Ingredients", CRC Press, 2020
Publication

---

13 Xiaoyong Chang, Yanyan Feng, Tiantian Dong, Qingguo Wang. "Inhibitory effect and the involved mechanism of furaneol on enzymatic browning of potatoes", Postharvest Biology and Technology, 2024 <1%
Publication

---

14 Berrougui, H.. "Marrubium vulgare extract inhibits human-LDL oxidation and enhances HDL-mediated cholesterol efflux in THP-1 macrophage", Life Sciences, 20061214 <1%
Publication

---

15 Markhali, Fereshteh Safarzadeh. "Optimization of Polyphenol Extraction from Olive Leaves Using Ohmic Heating (Emerging Processing) and Particle Size Reduction (Pre-Processing)", Universidade do Minho (Portugal), 2024 <1%
Publication

---

16 Mohammed, Diaa Mamdouh Ahmed. "Mass Production of Phenolic Compounds Through Plant in vitro Cultures", Technische Universitaet Berlin (Germany), 2024 <1%
Publication

---

17 Ning Xia, Jie Wang, Yanzhuo Jia, Jiawei Duan et al. "Optimization of the process of extracting <1%

essential oil of rosemary by hydro distillation with different auxiliary methods", LWT, 2024
Publication

---

18 Ramkrishna Sen, Susmita Mukherjee, Rajashree Paul, Rajiv Narula. "Biotechnology and Biological Sciences", Routledge, 2019 <1%
Publication

---

19 Aslınur Doğan, Suleyman Akocak. "Natural products as tyrosinase inhibitors", Elsevier BV, 2024 <1%
Publication

---

20 Hasene Keskin Çavdar, Sultan Avşar. "Ultrasonic extraction of Inula viscosa: Enhancing antioxidant bioactivity and its application in sunflower oil as an antioxidant", Ultrasonics Sonochemistry, 2024 <1%
Publication

---

21 Xiaoyan Zhang, Wenbo Meng, Yilun Chen, Yong Peng. "Browning inhibition of plant extracts on fresh-cut fruits and vegetables—A review", Journal of Food Processing and Preservation, 2022 <1%
Publication

---

22 Ayu Nala El Muna Haerussana, Angreni Ayuhastuti, Siti Fira Yuniar, Hana Alifah Bustami, Widyastiwi Widyastiwi. "Taro (Colosia esculenta) Leaves Extract Inhibits Streptococcus mutans ATCC 31987", Borneo Journal of Pharmacy, 2022 <1%

Publication

23	Cheng Tang, Tingting Bao, Qiqi Zhang, Hongyu Qi, Yishan Huang, Boxun Zhang, Linhua Zhao, Xiaolin Tong. "Clinical potential and mechanistic insights of mulberry (Morus alba L.) leaves in managing type 2 diabetes mellitus: Focusing on gut microbiota, inflammation, and metabolism", Journal of Ethnopharmacology, 2023 Publication	<1%
24	Linda Octavia Kusumawardhani, Taufik Setyadi, Risqi Firdaus Setiawan. "Supply Chain Analysis of Tofu Products in The FR Tofu Agroindustry Sepande Village", Jurnal Ilmiah Membangun Desa dan Pertanian, 2024 Publication	<1%
25	Anh C. Ha, Tan M. Le. "Improvement of the anti-tyrosinase activities by a combination of herb extractions and removing its tannin content as potential use in whitening cosmetics", South African Journal of Botany, 2023 Publication	<1%
26	Clara Muñoz-Bas, Rita Vedor, Daniela Machado, Joana Cristina Barbosa et al. "In vitro evaluation of biological properties of high-added value ingredients (date juice and date powder) obtained from date co-products", Applied Food Research, 2024 Publication	<1%

27	*Fang Peng, Hongchen Liu, Songnan Hu, Fengxia Yue, Dongdong Xiao, Lei Guo, Haisong Qi. "High throughput preparation of antioxidant polysaccharide-based polymers with UV-resistant and antibacterial performance", Food Hydrocolloids, 2022* Publication	<1 %
28	*Joanna Kobus-Cisowska, Marcin Dziedziński, Daria Szymanowska, Oskar Szczepaniak et al. "The Effects of Morus alba L. Fortification on the Quality, Functional Properties and Sensory Attributes of Bread Stored under Refrigerated Conditions", Sustainability, 2020* Publication	<1 %
29	*Mai Sayed Fouad, Mosad A. Ghareeb, Ahmed A. Hamed, Esraa A. Aidy et al. "Exploring the antioxidant, anticancer and antimicrobial potential of Amaranthus viridis L. collected from Fayoum depression: Phytochemical, and biological aspects", South African Journal of Botany, 2024* Publication	<1 %
30	*Parimelazhagan Thangaraj. "Phytomedicine - Research and Development", CRC Press, 2020* Publication	<1 %
31	*T. K. Lim. "Edible Medicinal And Non Medicinal Plants", Springer Science and Business Media LLC, 2012*	<1 %

Publication

32 Amr Hafez, Ahmed Elwahy, Khaled Radwan, Nourhan Fouad, Ehab Mahdy, Ahmed Fouad. "DNA Barcoding for Taxonomic Resolution in Lamiaceae: Evaluating rbcL, matK, and trnH-psbA Markers for Species Identification", Springer Science and Business Media LLC, 2024 <1%
Publication

33 Maria Dyah Nur Meinita, Dicky Harwanto, Amron, Md. Abdul Hannan, Gwi-Taek Jeong, Il Soo Moon, Jae-Suk Choi. "A concise review of the potential utilization based on bioactivity and pharmacological properties of the genus Gelidium (Gelidiales, Rhodophyta)", Journal of Applied Phycology, 2023 <1%
Publication

34 Mariarosaria Leporini, Rosa Tundis, Vincenzo Sicari, Teresa Maria Pellicanò et al. "Impact of extraction processes on phytochemicals content and biological activity of Citrus×clementina Hort. Ex Tan. leaves: New opportunity for under-utilized food by-products", Food Research International, 2020 <1%
Publication

35 Meta Safitri, Sufyan Sauri, Banu Kuncoro, Arini Aprilliani. "FORMULATION AND ANTIOXIDANT ACTIVITY TEST OF FACE TONER EXTRACT PREPARATION FROM 70% ETHANOL OF <1%

CUCUMBER FRUIT (Cucumis sativus L.)", Jurnal Farmasi Sains dan Praktis, 2024
Publication

---

36 *Beata Gąsowska-Bajger, Hubert Wojtasek. " Reactions of Flavonoids with -Quinones Interfere with the Spectrophotometric Assay of Tyrosinase Activity ", Journal of Agricultural and Food Chemistry, 2016* <1%
Publication

---

37 *Cristóbal Noé Aguilar, A. K. Haghi. "Functional Foods and Nutraceuticals for Human Health - Advancements in Natural Wellness and Disease Prevention", CRC Press, 2021* <1%
Publication

Exclude quotes	*On*	*Exclude matches*	*Off*
Exclude bibliography	*On*		