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Original Research Article

Effect of Solvent Extraction on Antityrosinase and Sun Protection Factor of Mulberry (*Morus alba* L.) Cultivated in Wajo, Indonesia

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ABSTRACT

Mulberry (*Morus alba* L.) is commonly used as a silkworm feed and a medicinal herb in Indonesia. There has not been much research on mulberry leaf pharmacological activities influenced by different planting places, whether because of different soil characteristics or other environmental factors such as ecology. This study aimed to investigate the effect of solvent extraction on antityrosinase and sun protection factor (SPF) of the mulberry leaves grown in Wajo, South Sulawesi, Indonesia. This study separately used different solvent extraction methods (96% ethanol, hexane, ethyl acetate, chloroform, and butanol). Antityrosinase was investigated *in vitro* assays, while UV spectrophotometry was used to measure the UV absorption, and the Mansur equation was applied to obtain the final SPF. The result showed that 96% ethanol extract presented the most potent to inhibit tyrosinase (IC_{50} 35.03±0.16 µg/mL) followed by butanol (IC_{50} 159.26±2.19 µg/mL), chloroform (IC_{50} 234.51±22.14 µg/mL), ethyl acetate (IC_{50} 283.76±3.65 µg/mL) and hexane (523.97±54.73 µg/mL). Our finding also suggested that butanol extract has the highest SPF value of 12. 84±0. 55 (high protection category) at 750 µg/mL. Overall, the experimental results revealed that mulberry significantly inhibits hyperpigmentation-related tyrosinase and sun protection, indicating that they might be used as bioactive metabolites in cosmetic and medicinal formulations to combat skin hyperpigmentation.

***Keywords*:** Mulberry, Tyrosinase, Solvent, Sun Protection Factor.

Introduction

The cause of skin blackness is overexpression of melanogenesis induced by ultraviolet (UV) light, hormones, or conditions like melasma. Melanocytes in the basal layer of the epidermis produce most of the melanin in human skin. Many people experience hyperpigmentation or dark skin blemishes, especially in tropical climates.[1,2] A crucial regulatory enzyme significantly affecting melanogenesis in melanocytes is tyrosinase, also known as polyphenol oxidase. [3] The biosynthesis of the pigment melanin is continued using a typical quinone precursor catalyzed by tyrosinase. [4]

It has been demonstrated that wearing sunscreen lowers the risk of skin erythema, hyperpigmentation, and nonmelanoma skin malignancies. UV radiation, or light with wavelengths shorter than visible light, is blocked by chemical or physical components included in sunscreens. Sunscreen filters actively block UVA and UVB radiation, while UVC is blocked by ozone from reaching the earth. [5] In general, the likelihood that light radiation will harm living things increases with wavelength. [6,7]

A perfect skin cosmetic would shield the skin from UVA and UVB rays while also being secure, inert, non-toxic, and photostable. One of the natural photo protectants includes mulberry (*Morus alba* L.). The mulberry tree is the most significant member of the family *Moraceae*.

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According to earlier research, mulberry leaves significantly reduced free radical formation and increased antityrosinase activity. In order to explain the probable oxidation capability of the ethanolic extract of mulberry twigs, two phenolic components, including maclurin and morin, have been found. Of these, maclurin and morin have demonstrated superiority over the other two. [8,9]

Mulberry ethanol extracts also suppressed tyrosinase activity and expression in SK-MEL-2 melanoma cells, which decreased melanin production. [10] A new active compound, mulberroside F, a polyphenol group, was identified from mulberry leaves that inhibit melanin formation *in vitro*. [11] In addition, mulberry stilbene glycosides' inhibitory impact on tyrosinase allows them to reduce skin hyperpigmentation. [12]

Therefore, designing an extraction method to improve this biological activity connected to cosmeceuticals is desirable and worthwhile. The best solvents are aqueous solutions comprising ethanol, hexane, butanol, and ethyl acetate. But ethanol has long been acknowledged as an excellent, safe solvent for extracting. [13] On the other hand, the content and activity of active components may require influent by environmental factors in their growing locations. [14] This study investigated the antityrosinase and sun protection factor (SPF) of mulberry growing locations in Wajo, South Sulawesi, Indonesia.

Materials and Methods

Plant collection and extract preparation

The leaf of mulberry, family Moraceae, was collected from the Kawasan Home Industry Kain Sutra Garden in Walennae, district of Sabbangparu, the regency of Wajo, South Sulawesi, Indonesia (-4.2189823613252475, 120.0041752082022), in the second week of July 2022. Plant materials were authentically identified by Ms. Marwati, Department of Biological Pharmacy, Sekolah Tinggi Ilmu Farmasi Makassar, a voucher specimen No. A1827888. For analysis, 400 g of

air-dried pulverized mulberry was extracted by cold macerated with 96% ethanol (3 × 800 mL) for 72 h. The crude extract was filtered, and the residual leaves were re-macerated thrice. The crude extract was collected and evaporated using a rotary evaporator under reflux (BÜCHI, Switzerland). The same procedure is done to make each hexane, ethyl acetate, chloroform, and butanol extract. All the extracts were kept in a desiccator until use. Extractable compounds were expressed as yield and said in percentage. The yield of extraction was calculated from the following equation:

$$\text{Yield (\%)} = \frac{W1}{W2}\ x\ 100$$

Where W1 is the quantity of the crude extract obtained after solvent evaporation, and W2 is the quantity of air-dried pulverized mulberry.

Tyrosinase-Inhibiting activity

According to the reference of Indrisari *et al*., the tyrosinase activity was measured using L-tyrosine as a substrate with some modifications. [15] First, all the extracts were dissolved in dimethyl sulfoxide (DMSO) and diluted to six different concentrations (15.63; 31.25; 62.50; 125.00; 250.00; and 500.00 µg/mL). In a 96-well plate, 30 µL of each sample and 100 µL of L-tyrosine (100 mg/L) were added. A 20 µL of mushroom tyrosinase (350 units/mL) was added to the plate after pre-incubation of 10 min at 37 °C, and the plate was then incubated for an additional 20 min at 37 °C. In order to create a negative control, 30 µL of the sample was replaced with 30 µL of buffer, while 50 µL of buffer and 100 mL of L-tyrosine (100 mg/L) were used as the blank. The absorbance of dopachrome was measured at 490 nm using an ELISA microplate reader (Biorad). Kojic acid in the six different concentrations was used as the positive control at 1.0; 2.0; 4.0; 8.0; 16.0; and 32.0 µg/mL. The following equation was used to compute the inhibitory rate of tyrosinase:

$$\text{Tyrosinase inhibition (\%)} = \frac{OD_2 - OD_1}{OD_1 - OD_0}\ x\ 100$$

where OD_2 is the negative control; OD_1 is the reaction with the sample; and OD_0 is the blank.

Determination of SPF

The SPF value for mulberry extracts was assessed as per the method described earlier.[16] Briefly, 100.0 mg of all samples were weighed and transferred to a 100 mL volumetric flask, diluted to volume with 96% ethanol, followed by ultrasonication for 5 min, and then filtered through cotton. A 2.5; 5.0; and 7.5 mL aliquot was transferred to a 10 mL volumetric flask and diluted with 96% ethanol (250.00; 500.00; and 750.00 µg/mL). Using a 1 cm quartz cell and ethanol as a blank, the absorption spectra of the samples in solution were acquired in the range of 290 to 320 nm, every 5 nm. The following equation was used to compute the SPF value:

$$SPF = CF \times \sum_{320}^{290} x\ EE_{(\lambda)} \times I_{(\lambda)} \times OD_{(\lambda)}$$

where CF=10 (Correction Factor), $EE_{(\lambda)}$=erythemogenic effect of radiation at wavelength, $I_{(\lambda)}$=Intensity of solar light at wavelength, and $OD_{(\lambda)}$= optical density of wavelength by extracts. The values for the term EE x I are constants, which are shown in Table 1.

Table 1: A normalized product function is used to determine SPF.[16]

Wavelength (λ nm)	EE x I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	03278
310	0.1864
315	0.0180
320	1.000

Statistical analysis

Three parallel replicates, mean and standard deviations (mean ± SD), were used to express the results. By employing Tukey's multiple range tests and one-way analysis of variance (ANOVA) with a confidence level of $p < 0.05$, the means were separated using the statistical program SPSS 19.0.

Results and Discussion

The leaves were obtained from the mulberry extract and assessed antityrosinase and SPF using different solvents such as 96% ethanol, hexane, chloroform, ethyl acetate, and butanol. The effectiveness of the solvent in extracting compounds is seen based on the yield value. Table 2 shows the yield of extract effect by solvent. The highest yield was 96% ethanol extract, followed by ethyl acetate, chloroform, hexane, and butanol extracts. Significant differences were observed among extraction yields obtained by 96% ethanol compared to other solvents ($p < 0.05$). This result showed that 96% ethanol could extract more polar and semi-polar compounds.

The results revealed that 96% ethanol obtained the highest yield extracts. Ethanol or ethyl alcohol has two different substituents: the polar group could solve polar materials, and the hydrocarbyl tail could solvate nonpolar materials. It causes ethanol called the semipolar solvent. Therefore, this result confirmed the effect of solvent extraction on the yield extraction and consequently confirmed the richness of this plant in polar and nonpolar substances. [17,18]

The polarity of the solvents used, which also significantly increases the solubility of phytochemical substances, may cause variation in extract yields. [19,20] Five different solvents with varying polarities were used, and they can be grouped as follows (starting from lower dielectric constant values): hexane (2.02), chloroform (4.81), ethyl acetate (6.02), butanol (17.68), 96% ethanol (24.5). [21] This variation appears to be related to the different polarities of the component extracts, which significantly influence the solubility of phytochemical substances and the solvents used.

Plant for disease treatment has been known to their ancestors for cancer, inflammation, and hyperpigmentation. The most prevalent facial pigmentary condition affecting young and older people is hyperpigmentation. It has been noted that patches or localized hyperpigmentation might result from an increase in melanin synthesis locally or from an imbalance in the distribution of melanin. As active ingredients, whitening agents like hydroquinone, arbutin, kojic acid, or azelaic acid are frequently utilized in cosmetic goods. [22,23] In this research, mulberry leaves were evaluated to inhibit tyrosinase activity. Tyrosine is converted to dihydroxy-phenylalanine (DOPA) and then to DOPA quinone by the enzyme tyrosinase, which also plays a role in melanogenesis. Tyrosinase is a metalloenzyme with copper at its active site that is known to catalyze these reactions by changing the copper atoms' oxidative sites. [10,24]

Figure 1 shows the antityrosinase activities of mulberry with various solvents. It was elucidated that 96% ethanol shows the highest inhibition of tyrosinase. In addition, Fig 1 also indicates that the higher the concentration used, the higher the activity. The antityrosinase for different extraction solvents was 96% ethanol > ethyl acetate > chloroform > hexane > butanol.

Table 2: Effect of solvent on yield from mulberry extracts. Data expressed as means ± standard deviations of three independent extractions (n = 3)

Extracts	Yield (%)
96% Ethanol	4.03 ± 0.02*
Ethyl acetate	1.54 ± 0.04
Butanol	1.15 ± 0.04
Chloroform	1.31 ± 0.05
Hexane	1.16 ± 0.06

Note: *Significant difference for $p < 0.05$ with all extracts

The IC_{50} of the extract is comparably related to its yield compounds richness (Lower IC_{50} values indicate a higher antityrosinase activity). Similar to the extract yield, 96% ethanol extracts showed the highest antityrosinase activities. The IC_{50} obtained from the 96% ethanol extract was 35.03±0.16 µg/mL and was significant to all extracts ($p < 0.05$), while kojic acid as a positive control was 6.65±0.21 µg/mL. The antityrosinase activities with regards to different used solvents were as follows: 96% ethanol > ethyl acetate > chloroform > hexane > butanol (Table 3).

This found that 96% ethanol extract shows the highest antityrosinase activity. It has previously been demonstrated that mulberry leaf extract in ethanol has a potent tyrosinase inhibitory action. [24] Recent studies have confirmed the role of morin's metal ion chelation in the tyrosinase inhibition processes. Morin was discovered to be connected to tyrosinase at a single binding site mostly by hydrogen bonds and van der Waals forces. Through a multiphase kinetic process, the binding type was eversible and inhibited tyrosinase competitively. [25] Mulberry contains kaempferol and quercetin, which in silico study has binding energies of -7.1 and -8.4 kcal/mol, inhibitory effect on tyrosinase, respectively. [26] By chelating the copper in the active tyrosinase site, kaempferol and quercetin competitively decrease tyrosinase activity. Nevertheless, polyphenolic substances like flavonoids may create complexes with metal ions and display antioxidative activity. [27,28] Similar to Faizatun *et al.* (2017), the 96% ethanol extract exhibited the highest radical scavenging and tyrosinase inhibitory activity than the extract with 50% ethanol, 70% ethanol, 60% methanol, 85% methanol, and 100% methanol. [29] Furthermore, the efficacy of 96% ethanol was found to be a well-known depigmentation agent.

In addition, inhibiting tyrosine is not enough. Cosmetic candidates are also expected to have a protective effect against UV rays (SPF). SPF measures how much UV radiation is required to produce sunburn in the presence of mulberry extract. Table 4 presents the effect of different solvent extraction on the SPF value of mulberry. Generally, the highest SPF value was shown by butanol extract (8.69±0.57 till 12.84±0.55), followed by ethanol extract (5.07±0.09 till 10.79±0.47), and ethyl acetate extract (5.83±0.11 till 5.36±0.11). Hexane and chloroform extract showed the lowest SPF value. At a concentration of 250 to 750 µg/mL, butanol extract showed high protection category, and contras with ethanol extract at a concentration of 250 to 500 µg/mL showed a moderate protection category. Ethyl acetate extract showed a moderate protection category at all concentrations used, while hexane and chloroform extract showed a low protection category at all concentrations used.

Table 3: IC_{50} of antityrosinase inhibition of different solvent extracts of mulberry. Data expressed as means ± standard deviations of three independent extractions (n = 3)

Sample	IC_{50} (µg/mL)
Ethanol	35.03 ± 0.16*
Hexane	523.97 ± 54.73
Chloroform	234.51 ± 22.14
butanol	159.26 ± 2.19
Ethyl acetate	283.76 ± 3.65
Kojic acid	6.65 ± 0.21

Note: *Significant difference for $P < 0.05$ with all extracts

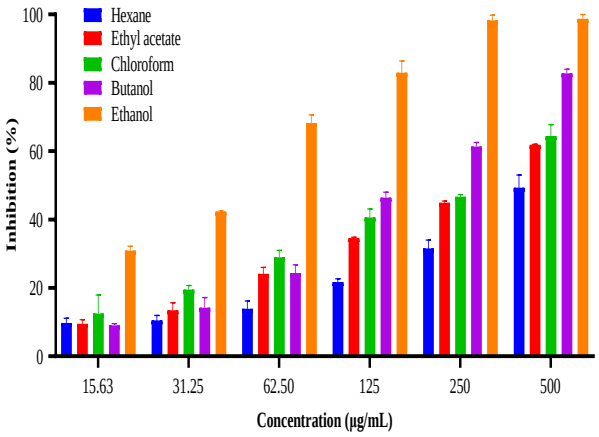

Figure 1: Effect of different solvents on antityrosinase activities of mulberry. Data expressed as means ± standard deviations of three independent extractions (n = 3).

Table 4: The effect of solvent extraction on the SPF value of mulberry. Data expressed as means ± standard deviations of three independent extractions (n = 3)

Extracts	**Concentration (µg/mL)**	**SPF value**	**Protection categories**
Ethanol	250	5.07 ± 0.09	Moderate
	500	6.65 ± 0.41	Moderate
	750	10.79 ± 0.47	High
Ethyl acetate	250	5.83 ± 0.11	Moderate
	500	5.07 ± 0.07	Moderate
	750	5.36 ± 0.11	Moderate
Butanol	250	8.69 ± 0.57	High
	500	9.74 ± 0.66	High
	750	12.84 ± 0.55	High
Hexane	250	1.30 ± 0.23	Low
	500	1.28 ± 0.23	Low
	750	1.33 ± 0.27	Low
Chloroform	250	1.51 ± 0.06	Low
	500	1.56 ± 0.15	Low
	750	2.11 ± 0.32	Low

Mulberry from Rumah Sutera, Bogor, Indonesia, with different concentrations of 400 and 500 µg/mL, could meet the SPF values of ≥15. [30] SPF 15 is 15 times longer with SPF 15 than you could without it. Additionally, it's crucial to understand that SPF only covers UVB rays. There is no UVA SPF equivalent. Future needs evaluation to measure the ability of mulberry to protect skin from UVA.

Conclusion

These results suggest that the aqueous extract of a diabetic folklore recipe did not exhibit any acute toxicity signs or symptoms. Further, isolation, identification, chemical compositions, and the major active compounds of the recipe responsible for the hypoglycemic effect should be undertaken in order to confirm and clarify the mechanism behind this activity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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