Original Article

Pharmaceutical Sciences and Research (PSR), 11(1), 2024, 27-33

Antioxidant, Tyrosinase Inhibition Activity, and *In Vitro* SPF Evaluation of Pepino Fruit Extract (*Solanum muricatum* Aiton) in Different Solvent Types and Concentrations

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ARTICLE HISTORY

Received: April 2023
Revised: April 2024
Accepted: April 2024

ABSTRACT

Solvent is a key factor that affects the effectiveness of active compound extraction from plant materials (*simplisia*). This study aimed to determine the optimal type and concentration of solvent used in the extraction of pepino fruit based on the parameter of antioxidant and tyrosinase inhibition activity, as well as Sun Protection Factor (SPF) value. The extraction was carried out using the maceration method with ethanol or ethyl acetate as the solvent, each at concentrations of 50%, 70%, and 96%, respectively. The antioxidant activity of the extracts was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The inhibition of tyrosinase and the determination of the SPF value were carried out using *in vitro* test. The results showed that the ethyl acetate extract was better than that of the ethanol extract in terms of antioxidant activity, tyrosinase inhibition, and SPF value. In the ethyl acetate solvent, a concentration of 96% provided the strongest antioxidant, tyrosinase inhibition activity, and the second highest in SPF test. It can be concluded that the optimal solvent for extracting pepino fruit as promising compound for sunscreen formulation is 96% ethyl acetate.

***Keywords**: Solanum muricatum Aiton; antioxidant; tyrosinase; SPF; solvent*

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INTRODUCTION

The pepino fruit (*Solanum muricatum* Aiton) is an Indonesian plant that has the potential as an antioxidant, tyrosinase enzyme inhibitor, and sunscreen. A study conducted by Husnah et al. (2012) on the antioxidant activity of pepino fruit using various solvents, including aquadest, ethanol, ethyl acetate, chloroform, petroleum ether, and n-hexane, showed that the highest antioxidant activity was obtained using 70% ethanol or ethyl acetate solvents, with respective effective concentration 50 (EC_{50}) values of 22.11 µg/mL and 23.81 µg/mL, respectively.

Sudha et al. (2012a) reported that ethyl acetate extract of ripe pepino fruit had antioxidant activity with an IC50 value of 0.16 mg/mL. Sudha et al. (2011) also reported that the ethyl acetate extract of raw pepino fruit showed antioxidant activity of 0.44 mg/mL. Based on the findings of Sinulingga et al. (2022), the anti-tyrosinase activity increases with the increase in antioxidant activity. Antioxidant function to scavenge free radicals formed during melanin synthesis in the skin. According to Alhabsyi et al. (2014), there was a positive correlation between antioxidant activity and sun protective factor. The stronger the antioxidant activity, the higher the Sun Protective Factor (SPF) value produced. The activity of the extract is strongly influenced by the level of its active substances. It is important to determine the most optimal solvent for extracting the active substances in pepino fruit. The selection of solvent in the extraction process plays a crucial role in the extraction of phenolic and flavonoid compounds used as antioxidants, tyrosinase enzyme inhibitors, and sunscreens. A study on the extraction of phenolic and flavonoid compounds from pepino fruit was conducted by Sudha et al. (2012b) using 95% ethanol solvent, which obtained a total phenolic content of 14.44 mg GAE/g, represented as gallic acid equivalents (GAE), and total flavonoid content of 23.62 mg RE/g, represented as rutin equivalents (RE). Another study by Sudha et al. (2012a) extracted pepino fruit using ethyl acetate solvent and obtained a total phenolic content of 20.43 mg GAE/g and total flavonoid content of 53.85 mg RE/g. The research results of Sriarumtias (2016) showed that the β-carotene content of pepino fruit extract using 95% ethanol solvent was 12 x 10^{-3}%.

Based on the previous studies above, ethanol or ethyl acetate solvents can be used to extract phenolic and flavonoid compounds in pepino fruit. However, a study to determine the optimal concentration of these ethanol or ethyl acetate solvents has not been conducted. It is important to determine the optimal concentration of solvent because it affects the solvent polarity, which in turn affects the concentration of active compounds extracted from the plant (Riwanti et al., 2020).

The solubility of a compound in a solvent is highly determined by the compatibility of the chemical properties or structure of the solute with the solvent, according to the principle "*like dissolves like*" (Hismath et al., 2011). Activity assay of extracts obtained from variations of these solvents will provide information on the optimal solvent types and concentrations. The most active extract can be developed further into active ingredients in cosmetic formulations.

Ethyl acetate solvent is preferred due to its semi-polar nature, capable of extracting compounds with a wide range of polarity from polar to nonpolar (Putri et al., 2013). Meanwhile, ethanol is chosen as a solvent due to its universal nature, being able to dissolve both polar and nonpolar compounds (Voight, 1995). Pepino fruit has been known to contain phenolic compounds, flavonoids, and beta-carotene.

The extraction process of phenolic and flavonoid compounds from pepino fruit is commonly carried out using the maceration method. The selection of the maceration method considers the binding of phenolic and flavonoid compounds according to the polarity level of the solvent (Handoyo, 2020). The maceration method can also prevent the risk of damaging plant compounds that are heat-sensitive, such as phenolic and flavonoid compounds (Susanty and Bachmid, 2016).

METHODS

Material and Instrument

The pepino fruits were obtained from Wonolelo Village, Sawangan, Magelang, Central Java, aged 3 months and harvested in March 2022. The materials used were 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Aldrich), Vitamin C (Sigma), p.a. ethanol (Merck), p.a. methanol (Merck), mushroom tyrosinase (Sigma), L-dihydroxyphenylalanine (L-DOPA) (Sigma), phosphate buffer, kojic acid (Sigma), octyl-p-methoxycinnamate (Sigma), dimethyl sulfoxide (DMSO) (Merck), cuvettes, glassware (iwaki). The tools used were UV-Vis spectrophotometer (Shimadzu CROP UV-1900), volumetric pipettes, vortex (Maxi Mix), analytical balance (Scout Pro), and pH meter (Omega).

Preparation of Pepino Fruit Extract

Simplex powder of pepino fruit, 150 grams each, was extracted using the maceration method. The solvents used were ethanol or ethyl acetate, each at concentrations of 50%, 70%, and 96%, respectively. The ratio of simplex and solvent was 1:10 for 72 hours at room temperature (37°C) and protected from light. The solution was filtered, then evaporated using a rotary evaporator at 50°C, and a water bath at 50°C until a thick extract was obtained (Sudha et al., 2012a with modifications).

Antioxidant Activity Assay of Pepino Fruit Extract

The antioxidant activity assay was conducted using the DPPH method. Positive control (vitamin C) was prepared in series at concentration of 0.5, 1, 2, 3, and 4 µg/mL. The ethyl acetate extract of pepino fruit was prepared at concentrations of 100, 150, 300, and 450 µg/mL, while the ethanol extract was prepared at concentrations of 200, 400, 600, and 800 µg/mL. Next, each extract was reacted with 2 mL of 0.01% DPPH solution in methanol and incubated for 25 minutes.

$$\%\ Inhibition\ = \frac{Absorbance\ of\ negative\ control - Absorbance\ of\ sample}{Absorbance\ of\ negative\ control} x\ 100\% \quad (1)$$

Table 1. Preparation of tyrosinase inhibition test materials

Material	Sample Treatment (µL)	Positive Control (µL)	Negative Control (µL)
Phosphate Buffer Solution pH 6.8	100	100	100
Sample Solution (100, 200, 300, 400, 500 µg/mL)	200	-	-
Kojic Acid Solution (5, 10, 15, 20, dan 25 µg/mL)	-	200	-
L-DOPA 0.85 mM	100	100	100
Tyrosinase Enzyme 340.12 U/ml	200	200	200

The antioxidant activity was then measured using a UV-Vis spectrophotometer at a maximum wavelength of 517 nm (Rohmah et al., 2020, with modifications). The determination of the antioxidant activity of the extracts was calculated based on equation (1).

From the percentage inhibition results, a linear regression equation between concentration (x) and percentage inhibition (y) of each treatment was determined. From the equation y = bx + a, EC_{50} calculation was then conducted.

Tyrosinase Inhibition Assay

The tyrosinase inhibition assay was conducted according to the method used by Chang (2009) with three testing groups consisting of samples, positive controls, and negative controls. The preparation can be seen in Table 1. Upon the addition of phosphate buffer (pH 6.8) and 0.85 mM L-DOPA solution, incubation was carried out for 10 minutes, then the mixture was transferred to Eppendorf tubes and further incubated at 37°C for 30 minutes after the addition of tyrosinase solution (340.12 U/mL). Absorbance was measured at the maximum absorption using a UV-Vis spectrophotometer (Wigati & Rahardian, 2018; Chang, 2009).

$$\%\ Inhibition = 100 - \frac{A \times 100}{B} \quad (2)$$

Absorbance measurement using UV-Vis at a maximum wavelength of 483 nm was carried out to determine the absorbance of dopachrome formation. The absorbance measurement results were used to calculate the percentage of anti-tyrosinase activity using the formula as in equation (2).

A = Absorption value in sample with inhibitor (kojic acid)
B = Absorption value in sample without inhibitor

From the percentage inhibition results, a linear regression equation between the inhibitor concentration (x) and the percentage inhibition (y) of each treatment was determined. From the equation y = bx + a, the calculation of IC_{50} was then performed.

***In Vitro* SPF Test**

SPF testing *in vitro* was conducted using the method by Mansur et al. (1986), while referring to the methods by Santos et al. (1999) and Sari (2018). A total of 2 mL of standard octyl-p-methoxycinnamate solution at a concentration of 5% in ethanol was measured for absorbance at wavelengths of 290-320 nm in triplicate. Ethanol extract samples (50%, 70%, 96%), and ethyl acetate extract samples (50%, 70%, 96%) were also tested for SPF using the same procedure. Absorbance was measured using a UV-Vis spectrophotometer, and the SPF value was calculated based on equation (3). The values of EE x I at a wavelength of 290-320 nm are shown in Table 2.

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad (3)$$

Remarks:
CF = Correction factor
EE = Erythemal effect spectrum
I = Solar intensity spectrum
Abs = Absorbance of sunscreen product

Table 2. Values of EE x I at a wavelength of 290-320 nm (Adawiyah, 2019)

Wavelength (λ nm)	EE × I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Data Analysis

The data obtained was analyzed by using the One-Way ANOVA test with the processing program IBM SPSS (Statistical Product and Service Solution) v.24.

RESULTS AND DISCUSSION

The ethanol extract yield of pepino fruit at concentrations of 50%, 70%, and 96% were 51.8%; 87.4%; and 54.6%, respectively (Firsty et al., 2023), and for ethyl acetate extract at 50%, 70%, and 96% were 23.979%; 20.431%; 14.34%, respectively. Based on the obtained yields, the 70% ethanol solvent provided the highest yield, whereas the highest yield for ethyl acetate was obtained at a concentration of 50%. The use of 70% ethanol solvent resulted in the highest extract yield due to its polarity, which is similar to most components in pepino fruit, such as polar compounds like flavonoids and phenolics (Abdillah et al., 2015). The increase in yield with decreasing ethyl acetate concentration aligns with the research findings of Rahardhian et al. (2019). Furthermore, each extract was qualitatively and quantitatively identified for phenolic and flavonoid content.

The screening results of the ethanol extract of pepino fruit were reported in the study by Firsty et al. (2023), indicating that the ethanol extract of pepino fruit contained the flavonoid compound quercetin (QE) and the phenolic compound gallic acid.

The research showed that the optimal concentration of ethanol for extracting active substances was 70%. At this concentration, the calculated amount of flavonoids was 0.56 ± 0.03 mg QE/g, represented as quercetin equivalents (QE), and the amount of phenolics represented as gallic acid was 3.63 ± 0.12 mg GAE/g. In this study, it was found that the phenolic content in ethyl acetate extract at 50%, 70%, and 96% concentrations were 3.81 ± 0.02 mg GAE/g, 7.50 ± 0.13 mg GAE/g, and 11.63 ± 0.17 mg GAE/g respectively, and the total flavonoid content was 2.12 ± 0.04 mg QE/g, 11.11 ± 0.22 mg QE/g, and 20.54 ± 0.03 mg QE/g. Based on this data, it was evident that the 96% ethyl acetate solvent concentration was the most effective solvent for extracting flavonoids and phenolics from pepino fruit.

The high level of flavonoids and phenolics in the 96% ethyl acetate extract indicate that the extracted flavonoids are those with low polarity such as quercetin, naringenin, kaempferol, and luteolin.

This aligns with the separation profile of pepino fruit flavonoids using HPLC in the study by Hsu et al. (2011) which detected several flavonoids, such as myricetin, naringenin, quercetin, and rutin. The increased phenolic content with the rise in concentration level indicates that with a higher ethyl acetate composition, more nonpolar phenolic compounds are extracted from the extract (Harborne, 1987). The separation profile of phenolates in pepino fruit, respectively starting from high polarity, includes: chlorogenic acid, caffeoylquinic acid isomers, caffeoylsinapoyl-quinic acid, and 3-caffeoyl-qunic acid (Hsu et al., 2011).

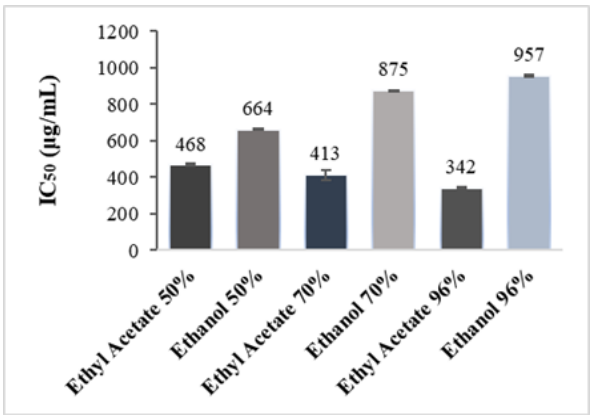

Figure 1. Antioxidant activity of ethanol and ethyl acetate extracts of pepino fruit
Data are presented in mean and standard deviation provided as error bar (n=3)

Studies on the ethanol and ethyl acetate extracts of pepino fruit were also determined based on their activities. The determination results of the IC_{50} antioxidant activity and tyrosinase enzyme inhibition, as well as the SPF value of pepino fruit extracts, with ethanol and ethyl acetate solvents at various concentrations are presented in Figures 1, 2, and 3.

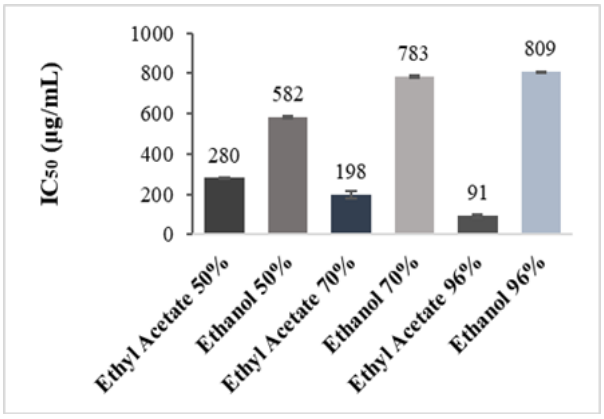

Figure 2. Tyrosinase enzyme inhibition activity of ethanol and ethyl acetate extracts of pepino fruit
Data are presented in mean and standard deviation provided as error bar (n=3)

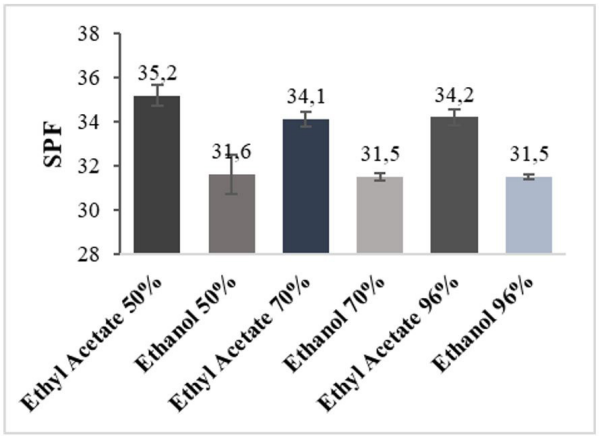

Figure 3. SPF value of ethanol and ethyl acetate extracts of pepino fruit
Data are presented in mean and standard deviation provided as error bar (n=3)

Based on the data in Figure 1, 2, and 3, it was found that the ethyl acetate extract exhibited better antioxidant activity, tyrosinase enzyme inhibition, and SPF value compared to the ethanol extract. This is likely due to the polarity of the phenolic and flavonoid compounds present in pepino fruit, which tend to be semi-polar, thus being more readily extracted in ethyl acetate. The polarity index of ethyl acetate is 4.4, while that of ethanol is 5.2. This indicates that ethyl acetate is more semi-polar compared to ethanol. According to previous research by Suhendra and Arnata (2009), ethyl acetate solvent was shown to be more optimal than ethanol to be used in the extraction

of fennel (*Foeniculum vulgare* Mill) seed, based on the antioxidant activity. This was also related to the higher yield of active phenolic compounds obtained from ethyl acetate extract compared to ethanol extract.

Phenolic compounds contain hydroxyl groups bound to the aromatic carbon ring that can catch free radicals (Saxena et al., 2013). Phenolic compounds react with free radicals by providing electrons (reduction), thereby producing more stable products and inhibiting free radical chain reactions (Plaza et al., 2014).

Phenolic compounds serve as photoprotective agents with conjugation systems similar to sunscreen compounds. The conjugated phenolic bonds are present in the benzene nucleus, which undergo resonance upon exposure to ultraviolet light through electron transfer (Prasiddha et al., 2016). Ebrahimzadeh et al. (2014) reported that antioxidants can enhance the SPF value as antioxidant compounds like phenolics can prevent the formation of free radicals and lipid peroxidation. This was observed in studies using *Dracocephalum moldavica* and *Vanda tricolor*. Research by Santhanam et al. (2013) also indicates that the increase in SPF value is influenced by the phenolic compound content of the extract. The higher the phenolic content, the higher the SPF value of an extract. A study by Latif et al. (2022) shows that increasing the concentration of coffee extract with antioxidant activity can enhance the SPF value. Similarly, research by Sari et al. (2022) demonstrates that increasing the concentration of *Daemonorop*s *acehensis* leads to improved antioxidants and SPF value.

Flavonoid compounds interact with free radicals produced at the enzyme's active site or on the copper ion (Cu) that serves as the tyrosinase enzyme's active site. The copper ion (Cu) acts as a cofactor in the tyrosinase enzyme's activity. The catalytic ability of the tyrosinase enzyme decreases with the removal of Cu from the enzyme's active site, preventing dopachrome formation (Kim et al., 2006). Both phenolic and flavonoid compounds have hydroxyl (-OH) and carboxylic acid (COOH) functional groups that resemble the tyrosinase substrate L-tyrosine or L-DOPA, leading to competitive inhibition mechanisms (Park et al., 2013).

The chromophore group in flavonoid compounds strongly absorbs ultraviolet radiation in both UVA and UVB ranges due to the presence of a conjugated aromatic system (Prasiddha et al., 2016). High concentrations of rutin, as a flavonoid compound, have also been utilized to prevent UV radiation-induced free radical formation, thereby increasing skin protection effects (Ebrahimzadeh et al., 2014). Research by Ibrahim et al. (2022) also demonstrates that the addition of *Moringa oleifera* extract with high phenolic and flavonoid contents, exhibiting strong antioxidant activity, has led to sunscreen formulations with photostability and enhanced protection against UVA/UVB.

The result of study showed that 96% ethyl acetate exhibited the strongest antioxidant activity and tyrosinase enzyme inhibition activity. This supports previous findings by Rachmawati et al. (2020), suggesting that active antioxidant and tyrosinase inhibition agents tend to be semi-polar. These antioxidant contents will enhance the SPF value. However, the highest SPF value was achieved with the 50% ethyl acetate extract. Based on statistical tests, it was revealed that the solvent concentration difference did not significantly affect the SPF value. Therefore, it could be concluded that the SPF value across various concentrations of ethyl acetate had nearly equal sunscreen potential. This potential may be due to the semi-polar nature of the antioxidant contents.

Based on the statistical calculation using Two-Way ANOVA, regarding the antioxidant activity and tyrosinase enzyme inhibition assay of the extracts, a significance value of $p<0.05$ was obtained, indicating a significant difference in the results based on the use of different types of solvents, which were ethyl acetate and ethanol, as well as variations in each solvent concentrations of 50%, 70%, and 96%, for extraction. In the sunscreen testing using SPF values, based on the use of different types of solvents for extraction, the statistical result showed a significance of $p<0.05$, indicating a significant difference in the SPF values produced by ethyl acetate and ethanol extracts. However, based on variations in solvent concentrations of 50%, 70%, and 96%, a p-value of $p>0.05$ was obtained, indicating no significant difference in the SPF values produced by the extracts from difference solvent concentrations within each solvent used for extraction.

CONCLUSION

It can be concluded that the most optimal solvent for extracting pepino fruit as promising compound for sunscreen formulation is 96% ethyl acetate.

ACKNOWLEDGMENT

This research was conducted with the support of Research Grant MU Batch VI No: 1687.390/PD/I.3/D/2022.

CONFLICT OF INTEREST

There is no conflict of interest

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