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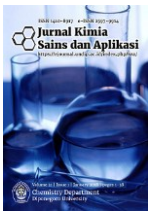

# Potential Antioxidant Activity of the Unused Part of Tamarillo (*Solanum betaceum* Cav.)

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Abstract

Tamarillo (*Solanum betaceum* Cav.) is a member of the Solanaceae family that contains phenolic and flavonoid compounds. The fruit of the tamarillo is commonly consumed. However, other parts that are rarely used also have many potential benefits, including antioxidant activity. This study aims to evaluate antioxidant activity of used part (flesh) and unused part (leaves and peel) extracts of tamarillo through ascorbic acid equivalent antioxidant capacity (AEAC), phenolic and flavonoids content, correlation the phenolic and flavonoid content on antioxidant activity, correlation the DPPH and CUPRAC methods and the levels of flavonoid compound in selected extract. Three organs (flesh, leaves, peel) were extracted using polarity solvents (n-hexane, ethyl acetate, ethanol). Antioxidant activity was conducted using DPPH and CUPRAC assays, and total phenolic acid and flavonoid acid were also investigated. The result showed that the highest antioxidant activity of tamarillo extracts using DPPH and CUPRAC methods was given by ethanol leaves, 94.917 ± 3.920 and 110.182 ± 7.987 mg AEAC/g sample. The highest TPC in the ethanol leaves extract 10.416 g GAE/100 g, while the highest TFC in the n-hexane leaves extract 8.367 g QE/100 g. Phenolic and flavonoid compounds were identified as significant contributors to the antioxidant activity, with both assessment methods showing a strong linear correlation. In conclusion, Tamarillo leaves had the potential to be a natural antioxidant source, with a rutin content of 0.088%, according to HPLC analysis.

## 1. Introduction

Free radicals are atoms or molecules possessing one or more unpaired electrons; as a result, they are very reactive and unstable. Free radicals are naturally produced through chemical processes occurring in the body, such as oxidation, cellular metabolism, and inflammation. In addition, free radicals are also generated due to external factors such as exposure to cigarette smoke, ultraviolet radiation, chemicals in food, and other pollutants [1, 2]. Free radicals cause damage to other molecules by extracting electrons from them to achieve stability [3]. This damage led to various diseases, such as degenerative diseases and cancer [4].

One way to counteract the entry of free radicals into the body is by optimizing the body's defense through antioxidants. Antioxidants are substances that could counteract free radicals by providing the necessary electron to stabilize them, thus impeding the formation of free radicals. Naturally, the human body could produce antioxidants, such as superoxide dismutase and glutathione peroxidase, which play a role in the immune system. However, with the high exposure to radicals from ultraviolet, smoke, and pollution from the environment, our body needs additional antioxidants from outside [4]. Several studies suggest that the presence of chemical compounds such as phenol and flavonoid is correlated with the antioxidant activity. Those compounds are components easily found in fruits and vegetables, such as tamarillo (*Solanum betaceum* Cav.).

Tamarillo (*Solanum betaceum* Cav.) is a perennial shrub from South America. This 2-4 m high plant belongs to the Solanaceae family, the same group as potato

(*Solanum tuberosum*), tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), and eggplant (*Solanum melongena*). The tamarillo fruit is egg-shaped, like a tomato. The fruit has diverse colors, ranging from yellow, orange, red, and purple, depending on the chemical composition of the carotenoids, chlorophyll, and anthocyanin in the fruit pericarp. Tamarillo fruit has been used by Indonesians to enhance the immune system, to maintain eye and skin health, to prevent diabetes, cardiovascular disease, cancer, and anemia [5].

Tamarillo fruit is the main part consumed in Indonesia. It is commonly consumed as a healthy drink by grinding the fruit. Some people choose to peel the tamarillo's skin because they do not like the texture of the skin, while others decide to use the whole fruit. To this end, most studies about tamarillo's antioxidant properties use the fruit as the object. However, they did not specify which part of the fruit they used. Therefore, which part of the fruit has the antioxidant activity remains unknown, whether it is the tamarillo's peel or the tamarillo's flesh.

In tamarillo plantations, only the fruit is harvested, as the other parts lack commercial value and are typically discarded. Previous research has focused solely on the fruit, leaving the chemical composition and pharmacological potential of other parts, particularly the leaves, largely unexplored. Identifying pharmacological benefits in these underutilized parts could enhance the overall market value of the plant while reducing agricultural waste.

A previous study about tamarillo was done by Rito *et al.* [6]. They compared the antioxidant capacity of four genotypes of tamarillo fruit: Red tamarillo from Mealhada, Portugal, red and orange tamarillo from the Botanical Garden in Portugal, and imported tamarillo from Portugal. The result showed that red tamarillo from Mealhada had the highest antioxidant activity using DPPH, FRAP, ABTS, and Beta carotene bleaching methods. Another study by Silva *et al.* [7] showed that the ethanolic extract of tamarillo fruit had high antioxidant activity using four antioxidant methods: DPPH, ABTS, FRAP, and ORAC. However, to date, no studies have compared the antioxidant activities of the leaves, peel, and flesh parts of tamarillo using DPPH (2,2-Diphenyl-1-picryl-hydrazyl) and the CUPRAC (Cupric Reducing Antioxidant Capacity) method.

According to that background, this study aims to determine the antioxidant activities of several parts of tamarillo: peel, flesh, and leaves, using two different antioxidant methods, which are DPPH (2,2-Diphenyl-1-picryl-hydrazyl) and CUPRAC (Cupric Reducing Antioxidant Capacity). Two methods were chosen to confirm the validity of the antioxidant measurement. These methods have different mechanisms. The DPPH method has a radical scavenging mechanism, while the CUPRAC method measures antioxidant compounds that could reduce $Cu^{2+}$ to $Cu^{+}$ [8]. Moreover, this study also determines the phenol and flavonoid content of the extracts because those components might correlate with the antioxidant properties, and determines the major compound content in the extracts. In the end, the statistical correlation was conducted to understand which component plays a significant role in the antioxidant properties of the extract.

## 2. Experimental

### 2.1. Materials

Quercetin, gallic acid, neocuproine, and DPPH (2,2-diphenyl-1-picrylhydrazyl) were obtained from Sigma-Aldrich; ferric chloride and cupric chloride were obtained from Aldrich. Other chemicals used were of analytical grade.

### 2.2. Preparation of the Sample

Three organs from tamarillo (*Solanum betaceum* Cav.) that were leaves named as LV, flesh as FS, and peel as PL were obtained from Ciwidey (7°4'0" S 107 26'5" E), West Java, Indonesia, and identified in Herbarium Bandungense, School of Life Science and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. Samples were cleaned of contaminants, cut, dried in a cabinet dryer at 45–50°C, and milled into powder.

### 2.3. Extraction of Leaves, Flesh, and Peel of Tamarillo

Extraction was carried out by reflux using three solvents of increasing polarity: n-hexane (nonpolar), ethyl acetate (semipolar), and ethanol (polar). A total of 300 g of powdered sample was extracted three times with each solvent at a sample-to-solvent ratio of 1:5 (w/v). Each extraction was conducted for 2–3 hours after the solvent reached its boiling point. The resulting liquid extracts were concentrated using a rotary evaporator, yielding the following samples: n-hexane leaf extract (LV1), n-hexane flesh extract (FS1), n-hexane peel extract (PL1), ethyl acetate leaf extract (LV2), ethyl acetate flesh extract (FS2), ethyl acetate peel extract (PL2), ethanol leaf extract (LV3), ethanol flesh extract (FS3), and ethanol peel extract (PL3).

### 2.4. Determination of the Total Phenolic Content (TPC)

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method with gallic acid as the standard. Gallic acid solutions (60–130 µg/mL) were prepared, and 50 µL of each standard was mixed with 500 µL of 10% Folin–Ciocalteu reagent and 400 µL of 1 M sodium carbonate in an Eppendorf tube. After incubation for 30 minutes, absorbance was measured at 765 nm using a Thermo Scientific Orion Aquamate 8100 UV–Vis spectrophotometer to construct the calibration curve. Methanol was used as the solvent, while Folin–Ciocalteu reagent and sodium carbonate served as the blank [9]. The same procedure was applied to the extracts, with each measurement performed in sextuplicate. TPC was calculated from the linear regression equation of the gallic acid calibration curve and expressed as g gallic acid equivalent per 100 g extract (g GAE/100 g).

### 2.5. Determination of the Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined using the method of Chang *et al.* [10], with quercetin as the

standard (40–110 µg/mL). Quercetin solution (100 µL) was mixed with 300 µL methanol, 560 µL distilled water, 20 µL of 10% $AlCl_3$, and 20 µL of 1 M sodium acetate in an Eppendorf tube. The mixture was incubated for 30 minutes, and absorbance was measured at 415 nm using a UV-Vis spectrophotometer. The same procedure was applied to the extracts, with each measurement performed in sextuplicate. TFC was calculated from the linear regression equation of the quercetin calibration curve and expressed as g quercetin equivalent per 100 g extract (g QE/100 g).

### 2.6. Determination of the Antioxidant Activities by DPPH Assay

Antioxidant activity was evaluated using the DPPH method, with ascorbic acid as the standard. A 50 µg/mL DPPH solution was prepared as the control, and methanol was used as the blank. Ascorbic acid solutions of various concentrations were prepared by mixing 125 µL of the sample with 750 µL of DPPH solution, followed by incubation for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The same procedure was applied to the extracts, with each measurement performed in sextuplicate. The percentage of DPPH inhibition was calculated and substituted into the regression equation of the ascorbic acid standard curve. Results were expressed as mg ascorbic acid equivalent antioxidant capacity per gram of extract (mg AEAC/g extract) [11].

### 2.7. Determination of the Antioxidant Activities by CUPRAC Assay

Antioxidant activity was also determined using the CUPRAC method. The CUPRAC reagent was prepared by dissolving 8.5 mg $CuCl_2$ in 5 mL of distilled water and 7.8 mg neocuproin in 5 mL of ethanol, then mixing the solutions in a 1:1 ratio. The mixture was adjusted to a final concentration of 100 µg/mL with 1 M ammonium acetate buffer (pH 7). Ascorbic acid was used as the standard, CUPRAC reagent (100 µg/mL) as the control, and ammonium acetate buffer as the blank. Ascorbic acid solutions of various concentrations were prepared by mixing 250 µL of standard solution with 750 µL of CUPRAC reagent, followed by incubation for 30 minutes. Absorbance was measured at 450 nm using a UV–Vis spectrophotometer. The same procedure was applied to the extracts, with each measurement performed in sextuplicate. The percentage increase in reducing capacity was calculated and expressed as mg ascorbic acid equivalent antioxidant capacity per gram of extract (mg AEAC/g extract) [12].

### 2.8. Statistical Analysis

One-way ANOVA followed by Tukey's post hoc test was performed using Minitab 20 to assess significant differences ($p < 0.05$). Pearson's correlation analysis was also conducted to evaluate relationships between variables.

### 2.9. Identification and Determination of Flavonoid Compounds in Selected Extracts

Several flavonoid standards suspected to be present in the ethanol leaf extract of tamarillo (300,000 µg/mL) were prepared, including luteolin-7-O-glucoside, rutin, quercetin, kaempferol, and apigenin (500 µg/mL each). Identification and quantification were carried out using a Shimadzu LC-20AD liquid chromatography system equipped with a UV-Vis SPD-20A detector at 360 nm. Separation was achieved on a LiChrospher 100 RP-18 column (5 µm) with a mobile phase of methanol (eluent B) and water (eluent A) under a linear gradient of 40–60% B for 5 minutes, 70% B for 10 minutes, and 40% B for 15 minutes. The flow rate was 1 mL/min, injection volume 20 µL, and column temperature 30°C (Oven CTO-2A, Shimadzu). Quantification was performed using the one-point method by comparing the area under the curve (AUC) with that of the standards.

## 3. Results and Discussion

Tamarillo is one of the plants commonly consumed as a healthy drink in Indonesia. This study explored the antioxidant potency of rarely used parts of tamarillo, such as peel and leaves, and compared it with the fruit, which is the most consumed part of tamarillo. Extraction was done using the reflux method because the compounds that might contribute to antioxidant activities, such as phenolic and flavonoid compounds, are relatively thermostable. Moreover, three polar solvents (n-hexane, ethyl acetate, and ethanol) were used to get a higher compound yield for each polarity.

In this study, two antioxidant methods, DPPH and CUPRAC, were chosen. Antioxidant activity assay based on chemical reactions classified into single electron transfer (SET) and hydrogen atom transfer (HAT) based assays. DPPH (2,2-diphenyl-1-picrylhydrazyl) is an example of an HAT-based assay, while CUPRAC is an example of a SET-based assay [13].

### 3.1. Antioxidant Activities of Leaves, Flesh, and Peel Extracts from Tamarillo

DPPH is a stable free radical at room temperature, detectable by UV-Vis spectrophotometry at 517 nm. When mixed with antioxidant molecules, DPPH is reduced, resulting in a color change from deep violet to light yellow and a corresponding decrease in absorbance. This decrease is linearly proportional to the antioxidant concentration [13].

The CUPRAC method is based on the ability of antioxidants to reduce $Cu^{2+}$ to $Cu^{+}$ in the presence of neocuproine (2,9-dimethyl-1,10-phenanthroline), an organic heterocyclic compound and chelating agent. The $Cu^{+}$ ions selectively react with neocuproine to form a colored complex, with absorbance measured at 450 nm [14]. In this method, the percentage increase in reducing capacity is calculated. Samples exhibiting antioxidant activity contain compounds with a reduction potential lower than that of the $Cu^{2+}/Cu^{+}$ complex (0.159 V) [14].

**Table 1**. Antioxidant activities of tamarillo extracts using the DPPH method

| Sample | Antioxidant activity (mg AEAC/g) | | |
|---|---|---|---|
| | n-Hexane extract | Ethyl acetate extract | Ethanol extract |
| Leaves | 1.551 ± 0.041$^{ax}$ | 22.739 ± 0.756$^{ay}$ | 94.917 ± 3.920$^{az}$ |
| Flesh | 1.109 ± 0.040$^{bx}$ | 8.337 ± 0.525$^{by}$ | 8.583 ± 0.746$^{by}$ |
| Peel | 2.299 ± 0.069$^{cx}$ | 56.250 ± 5.612$^{cy}$ | 53.008 ± 4.326$^{cy}$ |

a–c = different letters in one column, indicating the significant difference ($p< 0.05$)
x–z = different letters in one row, indicating the significant difference ($p<0.05$)
AEAC = ascorbic acid equivalent antioxidant capacity, n = 6

Ascorbic acid was used as a reference standard for preparing the calibration curve. The equation represented the linear regression of the ascorbic acid calibration curve with the DPPH method was $y = 11.289x + 15.05$; $R^2 = 0.992$, and the CUPRAC method was $y = 3.879x + 48.253$; $R^2 = 0.9904$. The antioxidant activities of both methods were reported as sample mg ascorbic acid equivalent antioxidant capacity per gram of extract (mg AEAC/g extract).

In this research, antioxidant activity using DPPH from leaves, flesh, and peel extracts of tamarillo varied in the range of 1.109–94.917 mg AEAC/g, as shown in Table 1. The higher AEAC value reflects the stronger antioxidant activity of the extracts. The highest antioxidant activity was shown by LV3 94.917 ± 3.920 mg AEAC/g sample, followed by PL2 56.250 ± 5.612 mg AEAC/g sample and PL3 53.008 ± 4.326 mg AEAC/g sample.

Mutalib *et al.* [15] showed that the antioxidant activity ($IC_{50}$) of ethanol fruit extract (0.80 mg/mL) was higher than that of ethyl acetate fraction (1.40 mg/mL) and water fraction (1.75 mg/mL) of tamarillo. A lower $IC_{50}$ value expresses higher antioxidant activity. These results were consistent with this study, where the ethanol extract of tamarillo fruit showed higher antioxidant activity than n-hexane and ethyl acetate extracts.

Research by Noor Atiqah *et al.* [16], antioxidant activity was shown by $EC_{50}$ values. Smaller $EC_{50}$ values indicated higher antioxidant activity. The study investigated the antioxidant capacity of tamarillo, red cherry tomato, yellow cherry tomato, and tomato in water extracts and 70% ethanol. The results expressed that the ethanol extract of tamarillo had the lowest $EC_{50}$ value, while red cherry tomato, yellow cherry tomato, and tomato had similar $EC_{50}$ values. Therefore, the ethanol fruit tamarillo extract had the highest antioxidant activity. According to Azeez *et al.* [17], antioxidant activity decreased during the ripening process because of polyphenol oxidase oxidation of phenolic and flavonoid compounds. In addition, low antiradical efficiency may be caused by phenolic compounds bound to other molecules, such as carbohydrates, which greatly reduce radical scavenging activity.

**Table 2**. Antioxidant activities of tamarillo extracts using the CUPRAC method

| Sample | Antioxidant activity (mg AEAC/g) | | |
|---|---|---|---|
| | n-Hexane extract | Ethyl acetate extract | Ethanol extract |
| Leaves | 5.657 ± 0.335$^{ax}$ | 35.232 ± 2.442$^{ay}$ | 110.182 ± 7.987$^{az}$ |
| Flesh | 5.460 ± 0.322$^{abx}$ | 12.715 ± 0.943$^{by}$ | 3.944 ± 0.316$^{bx}$ |
| Peel | 5.087 ± 0.399$^{bcx}$ | 95.007 ± 6.686$^{cy}$ | 75.473 ± 6.349$^{cz}$ |

a–c = different letters in one column, indicating the significant difference ($p< 0.05$)
x–z = different letters in one row, indicating the significant difference ($p<0.05$)
AEAC = ascorbic acid equivalent antioxidant capacity, n = 6

Rahmawati *et al.* [18] reported that the peel of tamarillo had very strong antioxidant activity with an $IC_{50}$ of 45.15 µg/mL. The research proved that the unused part of the tamarillo had a high potential for antioxidant activity. These results were consistent with this study, which showed that the ethyl acetate and ethanol peel extracts of tamarillo had antioxidant activities of 56.250 ± 5.612 mg AEAC/g and 53.008 ± 4.326 mg AEAC/g, as determined by the DPPH method, indicating that the peel of tamarillo has the potential as a source of antioxidants. The higher AEAC value reflects the stronger antioxidant activity of the extracts.

Antioxidant activity using CUPRAC from leaves, flesh, and peel extracts of tamarillo was found to vary between 3.944 and 110.182 mg AEAC/g, as shown in Table 2. The highest antioxidant activity was demonstrated in the LV3, 110.182 ± 7.987 mg AEAC/g sample, followed by PL2, 95.007 ± 6.686 mg AEAC/g, and PL3, 75.473 ± 6.349 mg AEAC/g. The highest antioxidant activity using CUPRAC is ethanol leaf extract, the same result as DPPH. However, after looking thoroughly, the antioxidant activity results of the CUPRAC and DPPH methods showed a different order. This is because the CUPRAC method has a different mechanism from DPPH; therefore, they gave different results.

Diep *et al.* [19] investigated the antioxidant activity of three tamarillo cultivars—Amber, Laird's Large, and Mulligan—grown in Whangarei, Northland, New Zealand, using the CUPRAC method. The samples used in this study were the peel and flesh of tamarillo. In this study, antioxidant activity was measured by Trolox equivalent antioxidant capacity (TEAC) per dry weight. The results showed that the antioxidant activity of Mulligan peel (265.29 µmol TEAC/g DW) was higher than that of Laird's Large (136.68 µmol TEAC/g DW) and Amber (117.59 µmol TEAC/g DW). Meanwhile, the antioxidant activity of Mulligan, Laird's Large, and Amber flesh was 71.57, 52.42, and 42.92 µmol TEAC/g DW, respectively. Overall, the peel showed the highest antioxidant activity, approximately 3.7, 2.8, and 2.6 times higher than the flesh of Mulligan, Amber, and Laird's Large, respectively [19]. Consistent with these findings, a previous study reported that ethyl acetate and ethanol extracts of tamarillo peel (95.007 ± 6.686 mg AEAC/g and

75.473 ± 6.349 mg AEAC/g, respectively) exhibited higher antioxidant activity than the flesh (12.715 ± 0.943 mg AEAC/g and 3.944 ± 0.316 mg AEAC/g) when measured using the CUPRAC method.

### 3.2. TPC and TFC in Leaves, Flesh, and Peel Extracts from Tamarillo

Tamarillo was known to contain various flavonoid compounds such as rutin, quercetin, kaempferol, and luteolin [20]. Flavonoid and phenolic compounds were known to contribute to antioxidant activity. Total flavonoid content was determined using the method developed by Pourmorad *et al.* [9]. In this method, gallic acid is used as a standard. Total phenolic content of each extract was calculated using the linear regression equation of the gallic acid calibration curve and expressed as gallic acid equivalent per 100 g of extract (g GAE/100 g). The linear regression equation obtained from the gallic acid calibration curve was y = 0.0046x + 0.0025; $R^2$ = 0.9927. Based on Table 3, the phenol content from leaves, flesh, and peel extracts of tamarillo ranged from 0.263 to 10.416 g GAE/100 g. The highest value was observed in LV3 (10.416 ± 0.506 g GAE/100 g), followed by PL2 (7.727 ± 0.751 g GAE/100 g) and PL3 (6.698 ± 0.191 g GAE/100 g).

Mutalib *et al.* [15] stated that the TPC in tamarillo was influenced by the type of extract and fraction used. The detected phenol content in ethanol extract was 2.53 mg GAE/g DW, followed by ethyl acetate fraction (1.77 mg GAE/g DW), n-butanol fraction (2.10 mg GAE/g DW), and water fraction (1.49 mg GAE/g DW). The study found that the total phenol content in ethanol extract was higher compared to ethyl acetate, n-butanol, and water fractions, which were consistent with the results of this study.

Previous research by Noor Atiqah *et al.* [16] denoted the total phenol of three plants from the Solanaceae family, tamarillo, tomato, and cherry tomato, using ethanol and water as solvents. The TPC in the water extract of tamarillo was higher than that of yellow cherry tomato, tomato, and red cherry tomato. Statistical analysis showed no significant difference among the samples. When comparing the solvents, the ethanol extract had higher phenol content than the water extract. This indicates that different solvents, extraction methods, and environmental conditions can give different results.

**Table 3**. Total phenolic content in tamarillo extracts

| Sample | Total phenolic content (g GAE/100 g) | | |
|---|---|---|---|
| | n-Hexane extract | Ethyl acetate extract | Ethanol extract |
| Leaves | $2.364 \pm 0.054^{ax}$ | $2.819 \pm 0.170^{ay}$ | $10.416 \pm 0.506^{ay}$ |
| Flesh | $0.263 \pm 0.020^{bx}$ | $1.983 \pm 0.069^{by}$ | $1.744 \pm 0.234^{bz}$ |
| Peel | $0.549 \pm 0.053^{cx}$ | $7.727 \pm 0.751^{cy}$ | $6.698 \pm 0.191^{cz}$ |

a–c = different letters in one column, indicating the significant difference ($p < 0.05$)

x–z = different letters in one row, indicating the significant difference ($p<0.05$)

**Table 4**. Total flavonoid content in tamarillo extracts

| Sample | Total flavonoid content (g GAE/100 g) | | |
|---|---|---|---|
| | n-Hexane extract | Ethyl acetate extract | Ethanol extract |
| Leaves | $8.367 \pm 0.662^{ax}$ | $4.332 \pm 0.413^{ay}$ | $4.379 \pm 0.399^{ay}$ |
| Flesh | $0.712 \pm 0.036^{bx}$ | $1.894 \pm 0.114^{by}$ | $0.399 \pm 0.031^{bz}$ |
| Peel | $3.454 \pm 0.283^{cx}$ | $3.039 \pm 0.086^{cy}$ | $1.089 \pm 0.103^{cz}$ |

a–c = different letters in one column, indicating the significant difference ($p < 0.05$)

x–z = different letters in one row, indicating the significant difference ($p<0.05$)

Diep *et al.* [19] found that the peel of tamarillo had significantly higher total phenol and antioxidant content compared to the flesh part of the fruit. Among the three regions studied, the Mulligan region had relatively higher TPC, with 2225.06 and 874.09 mg GAE/100 g DW for peel and flesh, respectively. The TPCs in the peel and flesh of tamarillo varied among cultivars, with the peel showing almost twice the phenol content compared to the flesh. The highest phenol content was found in the peel of Mulligan tamarillo (2225.06 mg GAE/100 g DW) and the lowest in the flesh of Amber tamarillo (678.98 mg GAE/100 g DW).

Determination of total flavonoid was based on the method of Chang *et al.* [10]. The principle of complex formation is the formation of stable acid complexes between $AlCl_3$ and flavonoids that have a keto at C-4 and hydroxyl groups at C-3 or C-5 on ring A. $AlCl_3$ also forms labile complexes under acidic conditions with ortho-dihydroxyl groups on ring A or ring B of flavonoids [10]. The standard used in TFC is quercetin, and the results were reported as quercetin equivalent per 100 g of extract (g GAE/100 g). The linear regression equation obtained from the quercetin calibration curve was y = 0.0055x + 0.0004; $R^2$ = 0.9997. As shown in Table 4, the flavonoid content of tamarillo leaf, flesh, and peel extracts ranged from 0.399 to 8.367 g QE/100 g. The highest value was recorded in LV1 (8.367 ± 0.662 g QE/100 g), followed by LV3 (4.379 ± 0.399 g QE/100 g) and LV2 (4.332 ± 0.413 g QE/100 g).

Mutalib *et al.* [15] showed that the water extract produced very low TFC. From these results, it was clear that the ethanol extract showed a much higher total flavonoid value compared to the water extract. Ethanol has a better ability to identify metabolite compounds compared to water. Ethanol extract had a similar polarity level to flavonoid compounds, making it more soluble in polar solvents such as ethanol than in water. In parallel, total flavonoid content decreased in tamarillo samples with different fractions: n-butanol > ethyl acetate > water fraction.

The previous research revealed that the TFC of ethanol extract from tamarillo was higher than that of cherry tomato and tomato. Tamarillo water extract had the highest flavonoid content, followed by cherry tomato

and tomato. Cherry tomato and tomato had similar average TFC and were not significantly different. All ethanol extracts gave a higher average TFC compared to water extracts [16].

The other study reported the TFC in ethanol extract from different parts of tamarillo, including peel, pulp, seed, and fruit. Tamarillo peel showed the highest TFC, which was 265.70 mg QE/100 g of powdered herbal material, and the lowest was found in the fruit of tamarillo, which was 157.69 mg QE/100 g of powdered herbal material [21].

### 3.3. Correlation of TPC and TFC with DPPH and CUPRAC Antioxidant Activities in Tamarillo Extracts

Quantitative correlation analysis between TPC and TFC against antioxidant activity using DPPH and CUPRAC methods in leaves, flesh, and peel extracts of tamarillo was measured using Minitab with the Pearson correlation method. A positive and significant correlation in Table 5 indicated a correlation between total phenol and total flavonoid and antioxidative activity, meaning that phenol and flavonoid substances contributed to its antioxidant activity.

According to Schober *et al.* [22], Pearson's correlation can be categorized as follows: 0.00–0.10 indicates no correlation, 0.10–0.39 weak correlation, 0.40–0.69 moderate correlation, 0.70–0.89 strong correlation, and 0.90–1.00 very strong correlation. As shown in Table 5, the total phenolic and total flavonoid contents of tamarillo leaf, flesh, and peel extracts exhibited strong to very strong correlations with antioxidant activity in both DPPH and CUPRAC assays. These results indicate that phenols and flavonoids significantly contribute to the antioxidant activity of tamarillo extracts.

Variations in antioxidant activity are partly due to differences in phenolic and flavonoid structures. Antioxidant activity increases with higher amounts of phenolic compounds, hydroxyl groups, and conjugated double bonds. Specifically, ortho-dihydroxy groups at positions 3' and 4' in ring B enhance stability of radical forms and facilitate electron delocalization, contributing to strong antioxidant activity [23]. Additionally, a 3-OH group in combination with a double bond between C-2 and C-3 further improves antioxidant activity [24]. Flavonoids with double bonds at C-2 and C-3 conjugated with a keto group at C-4 in ring C, or with hydroxyl groups at C-3 and C-5 and a keto group at C-4 in rings A and C, also exhibit high antioxidant activity [23].

The correlation between the antioxidant activity of the test extract using the DPPH and CUPRAC methods was statistically tested using Pearson's method in Minitab. In Table 4, both the DPPH and CUPRAC methods showed a strong to very strong correlation. Therefore, it can be concluded that both test methods yield linear results for the antioxidant activity of the leaves, flesh, and peel extracts of tamarillo.

**Table 5.** Correlation of the TPC and TFC of tamarillo extracts with antioxidant activity

| Antioxidant parameter | Pearson's correlation coefficient (r) | |
|---|---|---|
| | TPC | TFC |
| DPPH LV1 | 0.962**** | 0.929**** |
| DPPH FS1 | 0.925**** | 0.970**** |
| DPPH PL1 | 0.976**** | 0.997**** |
| CUPRAC LV1 | 0.880*** | 0.919**** |
| CUPRAC FS1 | 0.789*** | 0.909**** |
| CUPRAC PL1 | 0.902**** | 0.941**** |
| DPPH LV2 | 0.862*** | 0.993**** |
| DPPH FS2 | 0.779*** | 0.941**** |
| DPPH PL2 | 0.920**** | 0.826*** |
| CUPRAC LV2 | 0.952**** | 0.909**** |
| CUPRAC FS2 | 0.995**** | 0.836*** |
| CUPRAC PL2 | 0.925**** | 0.982**** |
| DPPH LV3 | 0.985**** | 0.984**** |
| DPPH FS3 | 0.976**** | 0.932**** |
| DPPH PL3 | 0.844*** | 0.946**** |
| CUPRAC LV3 | 0.961**** | 0.793*** |
| CUPRAC FS3 | 0.943*** | 0.920**** |
| CUPRAC PL3 | 0.860*** | 0.972**** |

*** strong correlation, **** very strong correlation, LV = leaves, FS = flesh, PL = peel, 1 = n-hexane extract, 2 = ethyl acetate extract, 3 = ethanol extract

### 3.4. Identification and Determination of Flavonoid Compounds in Selected Extracts

Statistical correlation indicated that extracts with the highest antioxidant activity were closely associated with their phenolic and flavonoid contents. Phenols constitute a large group of compounds, including flavonoids, phenolic acids, coumarins, lignans, and lignins. Therefore, this study focused on identifying the flavonoid compounds in the extracts. Flavonoid identification and quantification were performed on LV3, the tamarillo leaf extract exhibiting the highest antioxidant activity in both DPPH and CUPRAC assays, using HPLC (LC-20AD) equipped with a UV-Vis SPD-20A Shimadzu detector at 360 nm. Tamarillo is known to contain flavonoids such as rutin, quercetin, and kaempferol [20]. Similarly, leaves of other Solanaceae plants, such as tomato, contain rutin and quercetin [25]; thus, rutin, quercetin, and kaempferol were used as standards in this study. The results are presented in Figure 1.

Based on Figure 1, the peak at a retention time of 5.333 minutes in the extract closely matched the rutin peak at 5.301 minutes, indicating the presence of rutin in the tamarillo ethanol leaf extract. The retention time and AUC of the extract compared to the rutin standard are presented in Table 7. Using the one-point calibration method to compare the sample AUC with that of the standard, the rutin content in the tamarillo ethanol leaf extract was calculated to be 0.088%.

**Table 6**. Correlation of the TPC and TFC of tamarillo extracts with antioxidant activity

| Antioxidant parameter | Pearson's correlation coefficient (r) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | CUPRAC LV1 | CUPRAC FS1 | CUPRAC PL1 | CUPRAC LV2 | CUPRAC FS2 | CUPRAC PL2 | CUPRAC LV3 | CUPRAC FS3 | CUPRAC PL3 |
| DPPH LV1 | 0.979**** | - | - | - | - | - | - | - | - |
| DPPH FS1 | - | 0.924**** | - | - | - | - | - | - | - |
| DPPH PL1 | - | - | 0.983**** | - | - | - | - | - | - |
| DPPH LV2 | - | - | - | 0.908**** | - | - | - | - | - |
| DPPH FS2 | - | - | - | - | 0.871*** | - | - | - | - |
| DPPH PL2 | - | - | - | - | - | 0.921**** | - | - | - |
| DPPH LV3 | - | - | - | - | - | - | 0.800*** | - | - |
| DPPH FS3 | - | - | - | - | - | - | - | 0.913**** | - |
| DPPH PL3 | - | - | - | - | - | - | - | - | 0.908**** |

*** strong correlation, **** very strong correlation, LV = leaves, FS = flesh, PL = peel, 1 = n-hexane extract, 2 = ethyl acetate extract, 3 = ethanol extract

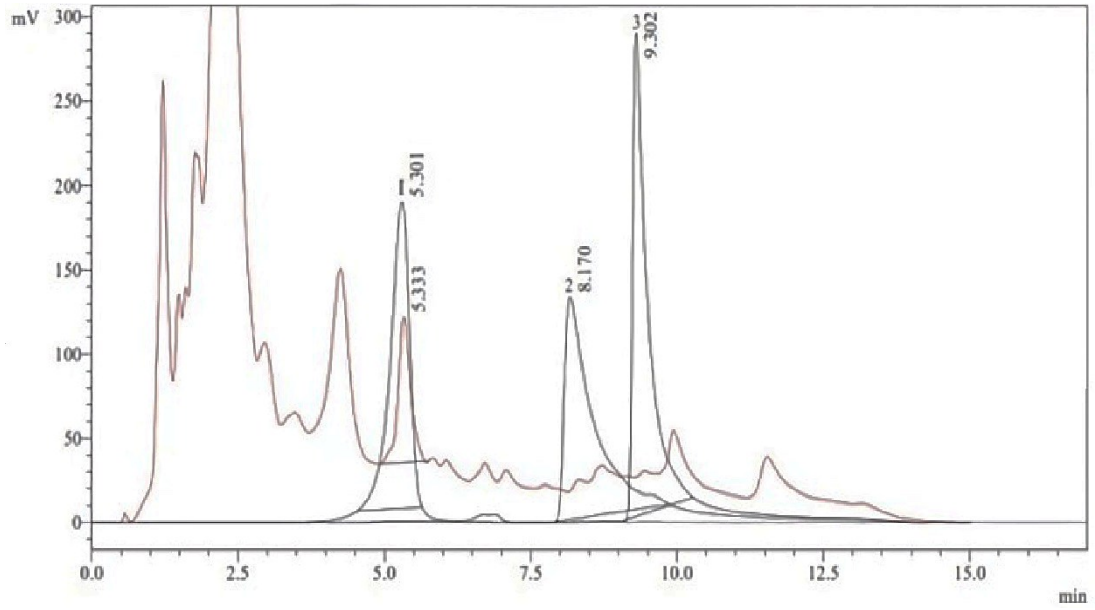


**Figure 1**. HPLC chromatogram of tamarillo LV3 and three standard flavonoids: (1) rutin, (2) quercetin, and (3) kaempferol. The black line represents the standards; the red line represents the sample

**Table 7**. Retention time and AUC of the sample and rutin

| Standard | Retention time (minutes) | | AUC | |
|---|---|---|---|---|
| | Sample | Standard | Sample | Standard |
| Rutin | 5.333 | 5.301 | 2184076 | 4132432 |

## 4. Conclusion

The antioxidant activity of extracts from leaves, flesh, and peel of tamarillo by using the DPPH and CUPRAC methods ranged from 1.109 to 94.917 mg AEAC/g and 3.944 to 110.182 mg AEAC/g. Ethanol leaves showed the highest total phenolic content extract at 10.416 g GAE/100 g, and the highest total flavonoid content was found in n-hexane leaves extract at 8.367 g QE/100 g. Phenolic and flavonoid compounds significantly contributed to the antioxidant activity of leaves, flesh, and peel extracts of tamarillo using DPPH and CUPRAC methods. The DPPH and CUPRAC methods showed linear correlation for the antioxidant activity of tamarillo leaves, flesh, and peel extracts. The ethanol extract of tamarillo showed the highest antioxidant activity using the DPPH and CUPRAC methods. The ethanol leaves extract of tamarillo contained rutin, with a content of 0.088%. Tamarillo leaves that are unused have potential as a natural antioxidant source.

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