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Original Research Article

Determination of antioxidant activity, total phenolic and total flavonoid contents in tamarillo (*Solanum betaceum*) peel's ethanolic extracts

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ABSTRACT

Background: Free radicals are chemical species a molecule, atom with unpaired electrons and reactive as oxidizing agent which cause oxidize stress in tissue. Antioxidants are substances that can protect cells from free radicals by releasing electrons to neutralize free radicals. Tamarillo peel contains phenolic and flavonoid compounds which have antioxidant activity. The aim of this study was to determine the antioxidant activity, total phenolic and flavonoid content of the ethanol extract of tamarillo peel.
Methods: In this research, the maceration method was used to extract phenolic and flavonoid components from tamarillo peel with ethanol 70% solvent. Total phenolic and flavonoid content was determined with UV-vis spectrophotometer. Total phenolic content determined using the Folin-Ciocalteu reagent and expressed in GAE (Garlic acid equivalent) and the total flavonoid content used the AlCl-3 reagent and expressed in QE (Quersetin equivalent). Then, tamarillo peel ethanol extract was tested the antioxidant activity using the DPPH method.
Result: The results showed that the total polyphenol content was 66.6242 mg GAE/g extract or 6.66% w/v extract, total flavonoid content was 0.74246 % w/v or 7.4246 mg QE/g extract. Antioxidant activity in IC_{50} value was 47.9460 ppm.
Conclusions: From the results of the research conducted, it can be seen that the ethanol extract of tamarillo can provide an antioxidant effect in the very strong category.

Keywords: Tamarillo peel extract, Free radical, Antioxidant, Flavonoid and phenolic

INTRODUCTION

The free radicals with unpaired electrons that are reactive and have potent oxidizing functional groups is reactive oxygen species (ROS). It is chemical species as a molecule, atom or with unpaired electrons and produced during cell metabolism. The increasing of ROS can cause oxidative stress and will be able to damage in the tissue.[1] In humans, increased oxidative stress can be disturbed of normal metabolism and trigger various diseases such as cancer, Parkinson's, Alzheimer's, atherosclerosis, heart failure, and myocardial infarction.[2]

Antioxidants are substances that protect cells from transfer the ROS by donating electrons to prevent the production of free radicals, remove damaged molecules, scavenge, suppress, or form chelates with free radicals.[3] The human body produces the endogen antioxidants to fight free radicals. However, the oxidative stress and the aging process can be more produced free radicals, therefore the human body need the exogenous antioxidant to be used.[4]

Antioxidant compounds can be obtained from the natural ingredients, one of them is tamarillo fruit. The reddish purple's a tamarillo peel contains the phenolic, flavonoid quercetin and anthocyanin which have antioxidant activity

for protecting cells from the free radical's damages.[5] Quercetin contained in tamarillo peel is a flavanol compound, which is one of six sub-classes of flavonoids.[6] These flavonoids can be used as antioxidants because free radicals was neutralized through hydrogen donors from hydroxyl groups, which can donate hydrogen atoms to neutralize the free radical's in the body.[7,8] So, the aim of this study was to determine the concentrations of phenolic and total flavonoid in the tamarillo peel ethanol extract and analyzed the antioxidant with DPPH method.

METHODS

Research regarding testing the effectiveness of tamarillo fruit extract was carried out in the period January-February 2023 at the pharmaceutical chemistry laboratory, faculty of pharmacy, universitas Sumatera Utara, Medan, Indonesia.

Preparation of the tamarillo peel's simplicia powder

The samples of tamarillo peel were sorted, completely washed under running water, and weighed. The sample was dried until the tamarillo peel is completely dry in a drying cabinet and the dried tamarillo skin was blended to make the dry simplicia powder.[9]

Preparation of the tamarillo peel's ethanol extract

One part of the simplicia powder and ten parts of the solvent 70% ethanol were mixed to a macerator to obtain the extract. And then, it was soaked for the first six hours while stirring every so often and allowed to stand for eighteen hours. After that, the macerate was filtered. That filtering procedure repeated at least twice with the same kind of solvent and the volume as much as half from the first volume. Then collected the entire macerate and used the rotary evaporator to the evaporate it to get a crude extract.[9]

Phytochemical screening of tamarillo peel's ethanol extract

The chemical compounds of the tamarillo peel's ethanol extract tested for examination of alkaloids, flavonoids, saponins, tannins, glycosides, and triterpenoids/steroids. Three reagents used in the alkaloid test were Buchardate's, Mayer, and Dragendroff's reagents in each test tube. If the white or yellow sediment was formed in Mayer reagents, the Buchardate's reagents was formed the chocolate until black sediment and Dragondroff's reagents was formed orange yellow sediment.[10] These test shown alkaloids are present when two reagents was showed positive result. Flavonoid was analyzed using Mg powder, concentrated HCl and amyl alcohol which was added in the sample. And then, it was shaken and allowed to separate for the presence of flavonoids. If the amyl alcohol was formed the layer with red, yellow, or orange colour. it was showed that flavonoids are present in sample.[11]

The sample was transferred in the hot distilled water. After that, it was cooled and was quickly shaken for 10 seconds, a steady foam forms that lasts for at least 10 minutes and can reach heights of 1 to 10 centimeters. And then was added hydrochloric acid solution, if the foam does not vanish, the presence of saponins is determined. Tannin screening was done added $FeCl_3$ reagents. If the sample showed blue or green black colour, it was shown the presence by of tannin compound. The screening of glycoside was done by addition of Liebermann Burchard reagent. The result showed that blue or green colour to indicate presence of glycoside compound. If the sample showed purple colour ring at the liquid limit with was added the Mollish's reagent, the presence of glucose bonding is determined. The sample added with n-hexane, 2 drops acetic anhydride and 1 drop concentrated sulfate acid. Triterpenoids are present when the result showed red, pink, or purple colors. And then the presence of steroids was showed blue or green colors.[12]

Determination of total phenolic content of tamarillo peel ethanol extract

A total of 10 mg ethanol extract was dissolved in 1 ml of methanol in a 10 ml volumetric flask, and then to which was added with distilled water until 10 ml to get concentration of (1000 ppm). After that, the solution was pipetteted 0.5 ml and added with 2 ml of distilled water and 0.5 ml of 10% Folin-Ciocalteu reagent. After that, it was shaken and allowed to stand for 1 minutes. 1 ml of 20% Na_2CO_3 was added, homogenized, and allowed to incubate for 30 minutes in the dark. The absorbance was measured at a wavelength of 765 nm using UV-vis spectrophotometer. The standard was used gallic acid and the results were reported as (g) of gallic acid equivalent in (mg) of extract (GAE/g).[13,14]

Determination of total flavonoids content of tamarillo peel ethanol extract

A total of 10 mg ethanol extract was dissolved in 1 ml of methanol in a 10 ml volumetric flask, and then it was added with distilled water until 10 ml (1000 ppm). The mixture was pipetted with 0.5 ml of ethanol extract, 1.5 ml of methanol, 0.1 ml of $AlCl_3$ and 0.1 ml of CH_3COONa, and 2.85 ml of distilled water. It was homogenized and incubated for 20 minutes in the dark. The absorbance was measured at a wavelength of 438 nm using a UV-Vis spectrophotometer. The standard was used quercetin and the results were reported as (g) of quercetin equivalent in (mg) of extract (QE/g).[15]

Antioxidant activity test

Preparation of DPPH solution

10 mg of DPPH was transferred into 50 ml of volumetric flaskand dissolved in methanol and calibrated up to the marked line and then added methanol until 50 ml involumetric flasktogeta solution of 200 ppm.[16]

Determination of absorbance curve of DPPH solution

Determination of absorbance curve of DPPH solution was done added 3.8 ml of 200 ppm and 0.2 ml of methanol, and the mixture was incubated for 30 minutes in a dark. After that, the absorbance of the solution was measured at 400-800 nm to obtained absorbance curve and it shown the wavelength of maximum for measurement absorbance.[16]

Antioxidant activity test on vitamin C solution

A 100 ppm solution was prepared by weighing 10 mg of vitamin C powder and dissolving it in 100 ml of methanol. Using 0.05 ml, 0.15 ml, 0.15 ml, 0.2 ml, and 0.25 ml of the 100 ppm vitamin C solution to witch 1 ml of DPPH solution (200 ppm concentration) was added at each concentration, and methanol was added to the mark limit (5 ml volumetric flask), resulting in concentrations of 2, 3, 4, 5, and 6 ppm. After being incubated for 30 minutes, a UV-Vis spectrophotometer with a maximum 516 nm wavelength was used to detect absorbance.[17] Absorbance value is used to obtain the percent free radical scavenging activity. The following of equation can be used to be calculated DPPH radical inhibition (reduction) ability:

$$\% \text{ inhibition} = \left(\frac{A\ control - A\ sample}{\text{A control}} \right) \times 100\%$$

A control=absorbance that does not contain the sample

A sample = absorbance of the sample.[12]

Based on these equations, it can be determined the IC_{50} value was used to indicates the concentration of the solution test for neutralize 50% DPPH free radical activity. It can be calculated from the percentage of inhibition at various concentrations using the equation determined by the linear regression curve.[17]

Antioxidant activity test on tamarillo peel's ethanol extract

10 mg of the tamarillo peel's ethanol crude extract was diluted in 10 ml of methanol, it was obtained to create a 1000 ppm solution. And then, it was pipetteted as much 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, and 0.5 ml in other volumetric flask and was added 1 ml the DPPH solution (200 ppm concentration) at each concentration. After that, the methanol is added until the marking limit was achieved (5 ml volumetric flask) in concentrations of 20, 40, 60, 80, and 100 ppm. it was achieved and incubated for 30 minutes, a UV-Vis spectrophotometer with a maximum 516 nm wavelength was used to analyze the absorbance. The ability to reduce (inhibition) DPPH radicals can be calculated using the same equation with the vitamin C solution antioxidant activity.[18] Based on these equations, it can be determined the IC_{50} value was used to indicates the concentration of the solution test for neutralize 50% DPPH free radical activity. It can be calculated from the percentage of inhibition at various concentrations using the equation determined by the linear regression curve.[17]

Determination of IC_{50} value of tamarillo peel's ethanol extract and vitamin C solution

The half-maximuminhibitory concentration (IC_{50}), which measures the capacity of a substance to inhibit specific biological or metabolic functions, is determined.[19] IC_{50} value was obtained from the calibration curve regression dose response curve obtain by plotting between percentase inhibition anjd concentration. And then, the result was substituted into the calibration curve regression equation y=ax+b, where the y value was substituted by 50, to produce the IC_{50} value of the test sample and vitamin C. The regression equation generated from the calibration curve used to calculate the IC_{50} value is as follows:

y= ax + b

y=Percentage of radical scavenging activity (y=50);

a=Slope/gradien; x=Concentration of Sample /IC_{50} (ppm); b=Intercept/regression coefficient

RESULT

Tamarillo peel ethanol extract

Maceration extract using ethanol 70% solvent. Ethanol will degrade cell walls in samples and can be dissolve polar or non-polar compounds. A diffusion process takes place during maceration process. This process continues until the solution within and outside of cell are in stable state. Once steady state is reached, diffusion process completed.[20] In this research, tamarillo peel ethanol extract was produced a crude extract at 54.63 gm (27.31% yield).

Secondary metabolites tamarillo peel ethanol extract

The phytochemical screening's extract aims to analyze the secondary metabolites from plant. Secondary metabolites are a source of organic compounds synthesized by plants which are used as medicinal compounds in pharmacology sphere.[20] Result of phytochemical screening test is presented in Table 1.

Table 1: Phytochemical screening of tamarillo peel ethanol extract.

Compounds	Reagent	Result
Alkaloids	Dragendorff's	+
	Bouchardat	+
	Meyer	+
Flavonoids	Mg powder + amil alcohol + HCl	+
Glycoside	Molish + H_2SO_4	+
Saponins	Hot distilled water	+
Tannins	FeCl3	+
Triterpenoids/ steroids	Lieberman-Bourchat	+/-

(+): contains a group of compounds, (-): does not contain a group of compound.

Phenol content of tamarillo peel ethanol extract

The results of absorbance and concentration are presented in calibration curve at Figure 1.

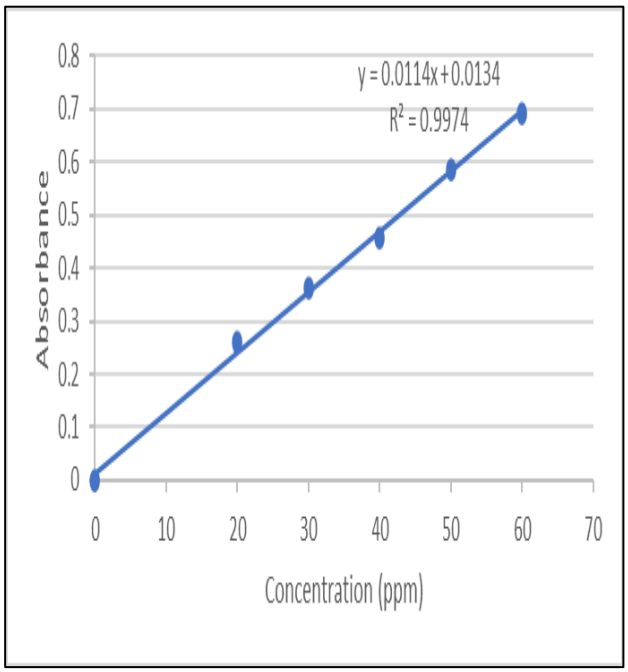

Figure 1: Calibration curve with the correlation between the absorbance and the concentration of the standard for gallic acid.

The maximum absorbance of the standard gallic acid wavelength was at 742 nm. This value was used for measuring absorbance with UV-VIS spectrophotometer to obtained the calibration curve. Based on the Figure 1, the linear regressiin equation is Y=0.0144 X + 0.0134 with the correlation coefficient R^2=0.9974 was obtained and then the total phenol of Tamarillo peel ethanol extract was calculated from the regression equation. The result of calculated the phenolic content in tamarillo peel ethanol extractwas obtained 62.56 mg GAE/g extract or equivalent to 6.256%.

Flavonoids content of tamarillo peel ethanol extract

The results of absorbance and concentration are presented in calibration curve at the Figure 2.

The maximum absorbance results of the standard quercetin was found at 438 nm. And then it was measured using UV-VIS spectrophotometrerto obtained the calibration curve. Based on the Figure 2. the linear regression equation is Y=0.0111X+0.0177 with the correlation coefficient R^2=0.996 is obtained the absorbance measurements and then the total flavonoid of Tamarillo peel ethanol extract was calculated from the regression equation. The result of calculated the total flavonoid content in the tamarillo peel ethanol extract was obtained at 7.42 mg QE/g extractor equivalent to 0.742%.

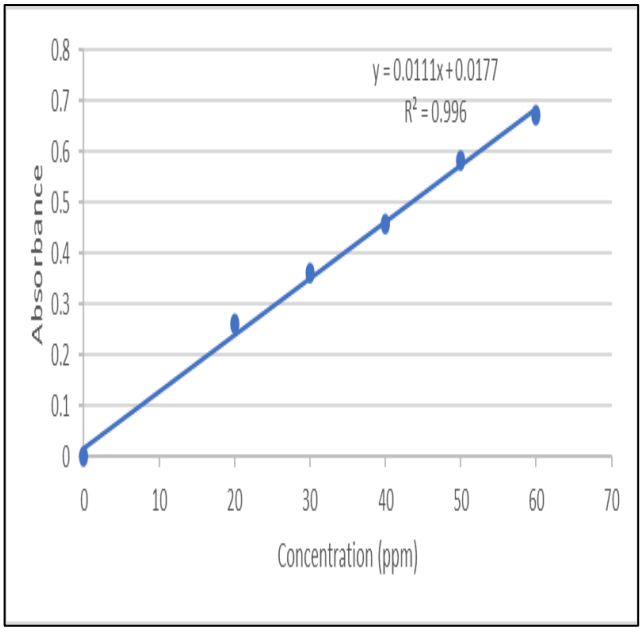

Figure 2: Calibration curve with the correlation between the absorbance and the concentration of the standard for quercetin.

Antioxidant activity of tamarillo peel ethanol extract

The antioxidant activity test was measured in the maximum wavelength at 516 nm. The results of wavelength measurements presented in Figure 3.

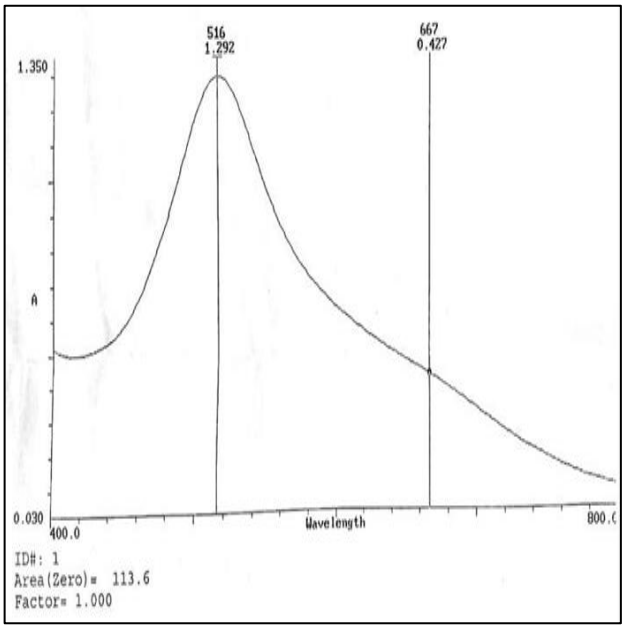

Figure 3: Absorbance curve of 2,2-diphenyl-1-picrylhydrazil solution in methanol (40 µg/ml).

The inhibition concentration (IC50) of ethanol extract of tamarillo peel and vitamin C in scavenging DPPH free radicals can be determined using the regression equation by plotting the concentration of the test sample as the

horizontal axis (X) and the percent attenuation of the test sample as the vertical axis (Y). Where the IC50 value is the inhibition concentration of the extract which causes a 50% loss of DPPH activity.[19] Based on the Figure 3, 4 and Figure 5 the equation Y=0.7187X-15.565 with the correlation coefficient R^2=0.994 for tamarillo peel ethanol extract and Y=13.275 X+2.4795 with the correlation coefficient R^2=0.9934 for vitamin C was calculated the IC50 value. IC_{50} value categories as antioxidant activity can be seen in the Table 2.

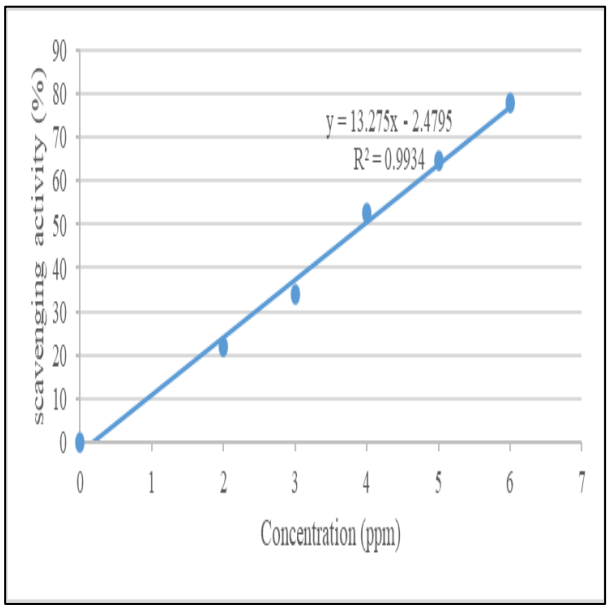

Figure 4: Calibration curve of 1,1-diphenyl-2-picrylhydrazylDPPH free radical scavenging activity (%) of vitamin C.

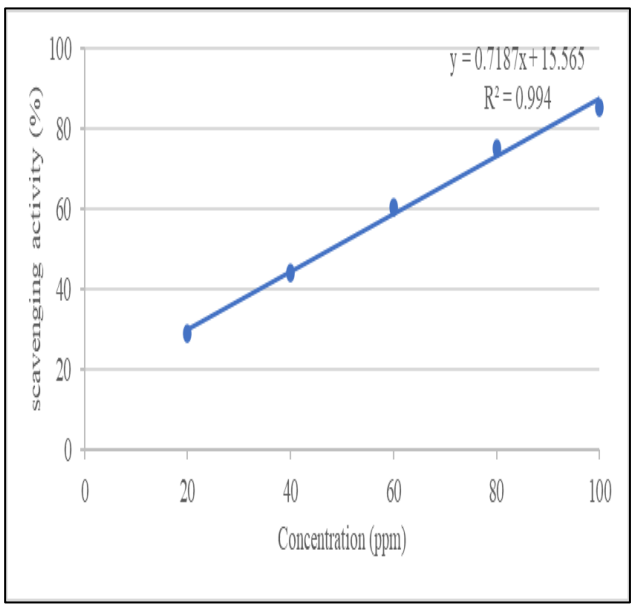

Figure 5: Calibration curve of 1,1-diphenyl-2-picrylhydrazyl DPPH free radical scavenging activity (%) of tamarillo peel extracts ethanol.

Table 2: Antioxidant activity (IC_{50}) value of tamarillo peel ethanol extract and vitamin C.

Sample	IC50 value	Antioxidant category
Tamarillo peel ethanol extract	47.4960 ppm	Very strong (<50 ppm)
Vitamin C	3.9547 ppm	Very strong (<50 ppm)

DISCUSSION

The determination of total phenol was used the standard for gallic acid which is a polyphenol component. It is present in almost all plants, so can be used as a standard for measurement. These organic acids have a pure and stable phenolic content.[20] The determination of total flavonoid levels used quercetin as a standard solution because quersetin is a flavonoid belonging to the flavonol group.[21] In this present study, the result of calculated the phenolic and flavonoid content in tamarillo peel ethanol extract was different from previous research which used different methods and solvents.[22] It can be causes a different the extraction method, a chance that various cultivators, growth environments, nutritional levels, and temperatures which can produce various quantities of flavonoids.[14]

This research was tested with the DPPH method, which is based on the color change of DPPH was caused by the reaction between the DPPH free radical and one electron or hydrogen atom released by a compound contained in the material to form a yellow 1,1-diphenyl-2-picrylhydrazyl compound.[5] The ethanol extract of tamarillo peel was evaluated by DPPH method and vitamin C as a reference. Vitamin C can be utilized as a comparation in this study because it has the potent natural antioxidant.[17] In this research showed that the tamarillo peel ethanol extract is significant antioxidant activity. This study is comparable to the study conducted by Hawa et al, which states that there is significant antioxidant potential from various types. The tamarillo fruit and skin extract using different solvents, namely 80% methanol and aqueous was determined using the DPPH, FRAP, ABTS assay method.[23] Nallakurumban et al also showed that tamarillo has a significant amount of phenolics, flavonoid which contribute to the antioxidant activity of the fruit.[24] In the Diep et al research also demonstrated that tamarillos' antioxidant capacity has comparatively high values, which were highly associated with a high level of total phenolics. The existence of these bioactive substances underscores tamarillo's potential for additional application in the culinary and medicinal sectors.[25]

This study uses the DPPH method, which has a fast, simple and affordable measurement process for measuring antioxidant activity. However, this method can only be dissolved in organic solvents.[26]

CONCLUSION

Based on the result scan be concluded that the ethanol extract of tamarillo peel contains alkaloids, saponins, flavonoids, tannins, and triterpenoids. The total phenolic content using the Folin-Ciocalteu reagent is 62.56 mg GAE/g extract or equivalent to 6.256%, while the total flavonoid content using the $AlCl_3$ reagent is 7.42 mg QE/g extractor equivalent to 0.742%. Based on the antioxidant test using the DPPH method, the antioxidant activity (IC_{50}) the tamarillo peel's ethanol extract value was 47.4960 ppm belong to the activity antioxidant is a very strong activity.

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