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Determination of total phenolic content and antioxidant activity of fruit mix from Malino, Gowa Regency, South Sulawesi

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Abstract. This study aimed to determine the total phenolic content and antioxidant activity of single fruit extracts and mixtures of tomatoes (*Solanum lycopersicum*), purple passion fruit (*Passiflora edulis* var. Sims), and strawberries (*Fragaria sp.*). Tomatoes, purple passion fruit and strawberries were extracted using 96% ethanol as solvent using the maceration method. Determination of total phenolic content using the Folin-ciocalteu method, measurement of antioxidant activity using the DPPH method (1,1-diphenyl-2-picrylhydrazil) spectrophotometrically and measuring the degree of acidity (pH) using a pH meter. Data were analyzed using ANOVA with Tukey's further test. The results showed that the total phenolic content of tomatoes, purple passion fruit and strawberries before and after mixing were tomato extract (1,731 mg GAE/g), purple passion fruit extract (1,577 mg GAE/g), strawberry extract (1,917 mg GAE/g), tomato and purple passion fruit extract (1,758 mg GAE/g), tomato and strawberry extract (2,020 mg GAE/g), strawberry and purple passion fruit extract (1,924 mg GAE/g) and tomato, purple passion fruit extract and strawberries (2.107 mg GAE/g). The antioxidant activity showed that there was a significant difference between the purple passion fruit treatments (78.695%), tomatoes and strawberries (86.160%) and tomatoes, purple passion fruit and strawberries (88.328%), but not significantly different from the tomatoes (80.683%), tomatoes and purple passion fruit (82.059%) as well as the treatment of strawberries (83.690%), strawberries and purple passion fruit (84.097%), but significantly different from the BHA control (93.526%). It can be concluded that the total phenolic content and antioxidant activity of the mixed extracts of the three fruits, namely tomatoes, purple passion fruit and strawberries, were higher than those of the single fruit extracts without mixing.

1. Introduction

A healthy lifestyle is a lifestyle that pays attention to all aspects of health conditions ranging from food, drink, nutrition consumed and daily behavior. For some people living a healthy life is not easy, especially for those who have busy work so they prefer to consume fast food and drinks. However, consuming fast food and drinks in excess can cause various diseases caused by an increase in free radicals. Free radicals are molecular fragments that contain one or more unpaired electrons in their orbitals. Free radicals are considered dangerous because they can be very reactive in gaining their electron pair. Due to its highly reactive nature and irregular movements, free radicals can cause damage to various parts of living cells and can cause various degenerative diseases [14].

The human body can neutralize free radicals if the amount is not excessive, with an antioxidant



defense mechanism [18]. Antioxidants are compounds that can prevent and slow down the damage caused by free radicals through the inhibition of oxidative mechanisms. Increased production of free radicals formed due to stress factors, excessive consumption of fast food and drinks, ultraviolet radiation, air and environmental pollution resulted in inadequate defense systems, so additional antioxidants from outside the body were needed [1].

Antioxidants outside the body can be obtained in synthetic and natural forms. Synthetic antioxidants such as *buthylated hydroxytoluene* (BHT), *buthylated hydroxyanisol* (BHA), and *tert-butylhydroquinone* (TBHQ) can effectively inhibit oxidation. However, the use of synthetic antioxidants is limited by government regulations because if their use exceeds the limit, they can cause toxins in the body and are carcinogenic, so safe natural antioxidants are needed [17]. Antioxidant compounds that are proven to have high antioxidant potential and activity are found in fruits or vegetables that contain vitamin C, vitamin E, beta-carotene and plants that contain phenolic compounds [12].

Phenolic compounds are compounds that have one or more hydroxyl groups attached to an aromatic ring, so they are easily oxidized by donating hydrogen atoms to free radicals [4]. Epidemiological studies show that the natural antioxidant content found in fruits is beneficial in protecting the human body against damage caused by reactive substances. One of the fruits that contain natural antioxidants are tomatoes, purple passion fruit and strawberries [5]. Malino, Gowa Regency, South Sulawesi is one of the areas that has a diversity of vegetables and fruits.

Tomato plants are developed in several places in Indonesia, one of which is Malino, South Sulawesi. Tomatoes contain nutrients that are very beneficial for the health of the body, especially vitamin A, vitamin C and the antioxidant lycopene. Lycopene, a red pigment found in fruits, is a powerful antioxidant and free radical scavenger. Tomato fruit contains many chemical compounds such as alkaloids, saponins, malic acid, citric acid, amino acids, bioflavonoids including lycopene, fat, protein, fiber, natural sugars in the form of glucose and fructose, minerals and histamine [7].

Passion fruit that is widely cultivated in Indonesia is purple passion fruit or commonly known as sour passion fruit. Purple passion fruit has been developed in several places in Indonesia, one of which is Malino, South Sulawesi [13]. Purple passion fruit is a fruit that has high nutritional value, one of which is the content of antioxidant compounds. Purple passion fruit juice contains lots of vitamin A, vitamin C, β -carotene and flavonoid compounds [8]. β -carotene in purple passion fruit has a strong antioxidant effect that can prevent LDL oxidation so as to reduce the risk of atherosclerosis [9].

Strawberries are one of the subtropical plants that have long been cultivated in Indonesia [11]. One of them is strawberries that have been cultivated by several farmers in Malino [10]. Strawberries contain phenolic compounds such as flavonoids, anthocyanins and tannins. Anthocyanins can act as antioxidants and can also be a source of natural dyes [15]. Strawberry fruit (*Fragaria sp.*) acts as protection against cancer cells, prevention of ischemic heart disease, antitumorogenic, anti-inflammatory, antiallergic, antimutagenic and antimicrobial [16].

The high demand for fruit and vegetable consumption means that many people use very varied techniques in consuming fruit. One technique used is to mix several types of fruit and process them into juices and smoothies. This technique is widely used by the community because it can streamline the time to consume fruit, so it is often an option for people who are very busy [6].

The problem that can arise by mixing these fruits is what about the total phenolic content and antioxidant activity of mixed fruit between tomatoes, purple passion fruit and strawberries, are they the same, higher or lower than the total phenolic content and antioxidant activity of single fruit without being mixed. Determination of the total phenolic content and antioxidant activity of processed fruit is important to determine the health potential of these foods. Therefore, a study was conducted to determine the total phenolic content and antioxidant activity of a mixture of tomatoes (*Solanum lycopersicum*), purple passion fruit (*Passiflora edulis* var. Sims) and strawberries (*Fragaria sp.*) from Malino, Gowa district, South Sulawesi.

2. Materials and Methods

2.1. Fruit sample preparation

The three tomatoes (*Solanum lycopersicum*), purple passion fruit (*Passiflora edulis* var. Sims) and strawberries (*Fragaria sp.*) were washed and then drained and each fruit was weighed as much as 500 grams. The weighed fruit is mashed using a blender.

2.2. Sample extraction

Sample extraction was carried out using the maceration method. Fruit samples that have been mashed in the form of juice are put into glass jars and soaked in 500 mL 96% ethanol. The glass jar containing the fruit sample was covered with aluminum foil. The solution mixture was filtered and the dregs were re-extracted 3 times with the same solvent. The results of the immersion were concentrated using an oven to obtain a thick ethanol extract. The extract was calculated as the percentage yield using the formula:

$$\% \text{ Ekstrak yield} = \frac{\text{Total weight of extract}}{\text{Total simplicia weight}} \times 100$$

2.3. Phytochemical analysis

Phytochemical analysis was carried out to determine the secondary metabolites contained in the ethanol extract of fruits. The types of secondary metabolites tested qualitatively include [3]:

a. Alkaloid Test

• Wagner Test

0.1 gram sample was dissolved in 10 ml of methanol. The solution mixture was taken 2 ml of the filtrate and added 1 ml of Wagner's reagent. If the solution has a reddish-brown precipitate, it indicates the presence of an alkaloid group of compounds.

• Meyer Test

0.1 gram sample was dissolved in 10 ml of methanol. The solution mixture was taken 2 ml of the filtrate and added 1 ml of Meyer's reagent. If the solution has a yellowish white color, it indicates the presence of alkaloids.

b. Flavonoid Test

The test of Mg and HCl reagents was 0.1 gram of sample dissolved in 10 ml of methanol. 2 ml of the solution mixture was taken then added 0.05 mg of Mg powder and 1 ml of concentrated HCl, then shaken. A positive test is indicated by the formation of a red, yellow or orange color.

c. Triterpenoid and Steroid Test

0.1 gram sample was dissolved in 2 ml of chloroform and 0.5 ml of acetic anhydride was added and then 2 ml of concentrated sulfuric acid was added through the tube wall. The formation of a brownish or violet ring on the boundary of the solution indicates a positive terpenoid, if a blue-green ring is formed, it indicates a positive steroid.

d. Tannin Test

0.1 gram sample was dissolved in 10 ml of methanol. The solution mixture was taken 2 ml and added 3 drops of 3% FeCl₃. If the solution contains a blackish green precipitate, it indicates the presence of tannins.

e. Saponin Test

The 0.5 gram sample was added to 5 ml of distilled water in a test tube. The solution was shaken slowly and heated for 2-3 minutes. If the solution forms a stable foam for 15-30 minutes, it indicates the presence of saponins.

2.4. Determination of total phenolic content

a. Determination of phenolic content for control

0.5 grams of gallic acid was diluted by adding 10 ml of ethanol and made up with sterile distilled water until the solution reached 100 ml. 6 pieces of 100 ml erlenmeyer each were added with distilled

water and given treatment. In erlenmeyer 1 no stock solution was added, while in erlenmeyer 2-6, 1 ml, 2 ml, 3 ml, 5 ml and 10 ml of stock solution were added, respectively. The concentrations used were 10 mg/ml, 50 mg/ml, 150 mg/ml, 250 mg/ml and 500 mg/ml. 6 test tubes, where in the tube (blank) 20 µl of distilled water was added. Tubes 2-6 were added with 20 µl of stock solution. Then 1.58 ml H₂O, 100 µl *Folin-Ciocalteu* reagent were added to all test tubes and left for 7 minutes. After that, 300 µl of sodium carbonate (Na₂CO₃) was added. Stored for 30 minutes in a dark room and calculate the absorbance using a spectrophotometer with a wavelength of 765 nm.

b. Determination of the phenolic content of fruit extracts

Each fruit extract was diluted by weighing a sample of 0.05 grams and adding 5 ml of distilled water (H₂O). The solution mixture was taken as much as 20 µl was put into a test tube and added 1.58 ml of H₂O and 100 µl of *Folin-Ciocalteu* reagent in a test tube, then left for 7 minutes. After that, 300 µl of sodium carbonate (Na₂CO₃) was added. Stored for 30 minutes in a dark room and calculate the absorbance using a spectrophotometer with a wavelength of 765 nm. The treatment was repeated 3 times.

The total content of phenolic compounds in the extract was expressed as milligrams of gallic acid equivalent per gram dry weight (mg GAE/g) of the extract [2]. The content of phenolic compounds in fruit extracts can be calculated by the formula:

$$T = C.V/M$$

Information : T : Total phenolic content (mg/g)

C : Concentration calculated from absorbance obtained

V : Volume of test compound (ml)

M : Mass of test compound (mg)

2.5. Antioxidant activity test (DPPH method)

a. DPPH Solution Preparation

DPPH was weighed as much as 0.0011 grams and dissolved in 50 ml of methanol in an erlenmeyer.

b. Preparation of Test Solution

Each fruit extract was weighed as much as 0.05 grams. Each fruit extract was dissolved with 10 ml of methanol in a sample bottle and then homogenized.

c. Determination of Antioxidant Activity of Fruit Extracts

77 µl of each extract solution was taken and put into a test tube, after which 3 ml of DPPH solution was added. The solution mixture was homogenized and incubated for 30 minutes in the dark. Measurement of antioxidant activity was measured using a spectrophotometer at a wavelength of 517 nm. The treatment was repeated 3 times.

d. Determination of Antioxidant Activity for Standard Solutions

BHA (*Butylated Hydroxyanisole*) was weighed as much as 0.025 grams and dissolved in 10 ml of methanol. 3 ml of DPPH solution was taken and 77 µl of BHA solution was added to the test tube. The solution mixture was homogenized and incubated for 30 minutes in the dark. Measurement of antioxidant activity was measured using a spectrophotometer at a wavelength of 517 nm. The treatment was repeated 3 times.

e. Determination of Antioxidant Activity for Blank

Determination of antioxidant activity for blanks only used 77 µl of methanol and added 3 ml of DPPH solution. The solution mixture was homogenized and incubated for 30 minutes in the dark. Measurement of antioxidant activity was measured using a spectrophotometer at a wavelength of 517 nm. The free radical scavenging activity of the sample is calculated using the formula:

$$\text{Free radical scavenging activity (\%)} = \frac{\text{Abs.Control} - \text{Abs.Sample}}{\text{Abs.Control}} \times 100 \%$$

2.6. pH measurement

Samples of tomatoes, purple passion fruit and strawberries before and after mixing were measured using a pH meter to determine the degree of acidity (pH) of each fruit.

2.7. Data analysis

Data analysis was carried out using ANOVA while to find out the difference between treatments, *Tukey's* further test was carried out. Data were analyzed using SPSS 24.00 for Windows.

3. Results and Discussion

3.1. Yield of tomato, purple passion fruit and strawberry extracts

Tomato, purple passion fruit and strawberry samples were extracted using maceration method using 96% ethanol as solvent. The yield value showed that the extraction of tomatoes, purple passion fruit and strawberries before and after mixing obtained tomato extract as much as 38.351 grams with an extract yield value of 7.670%. Purple passion fruit extract was 61.210 grams with a yield value of 12.242%. Strawberry extract as much as 40.260 grams with a yield value of 8.052%. Tomato and purple passion fruit extracts were 41,683 grams with a yield value of 8.337%. Tomato and strawberry extract as much as 38.972 grams with a yield value of 7.794%. Strawberry and purple passion fruit extracts were 45,420 grams with a yield value of 9.084% and tomato, purple passion fruit and strawberry extracts were 48.944 grams with a yield value of 9.789%. Comparison of the percentage yield of fruit extracts can be seen in Figure 1.

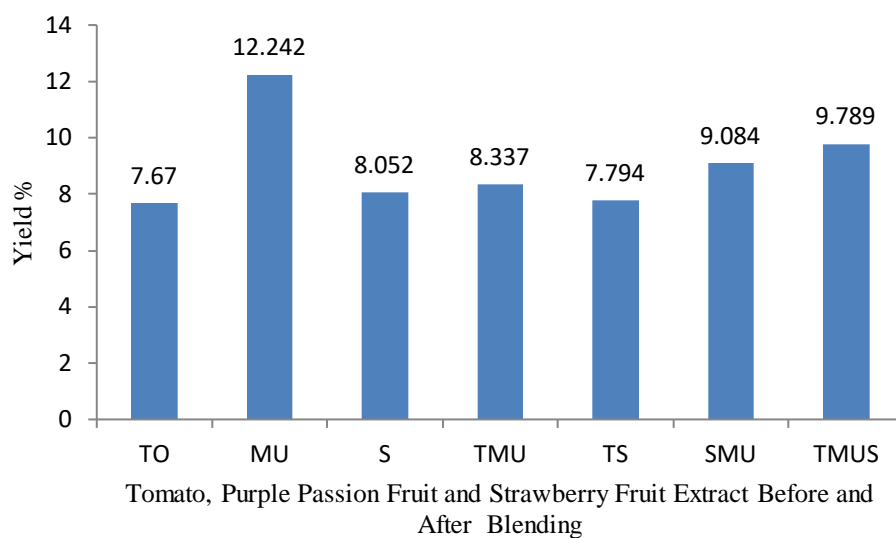


Figure 1. Comparison of yield percentage of tomato, purple passion fruit and strawberry extracts before and after mixing

3.2. Phytochemical screening

The results of phytochemical screening for several compounds can be determined by a color test using several reagents for groups of alkaloids, flavonoids, triterpenoids and steroids, tannins and saponins. The results of phytochemical screening of tomato, purple passion fruit and strawberry extracts can be seen in Table 1.

Table 1. Phytochemical screening results of tomato, purple passion fruit and strawberry extracts

Compound Identification	Reactor	Sample		
		Tomato	Purple Passion Fruit	Strawberry
Alkaloid	Mayer	+	+	+
	Wagner	+	+	+
Flavonoid	Mg +HCL Powder	+	+	+
Triterpenoid and steroid	Acetic Acid	+	+	+
	Anhydride+Sulfuric Acid	-	-	-
Tannin	FeCl ₃	+	+	+
Saponin	Hot Aquadest	+	+	+

Information :

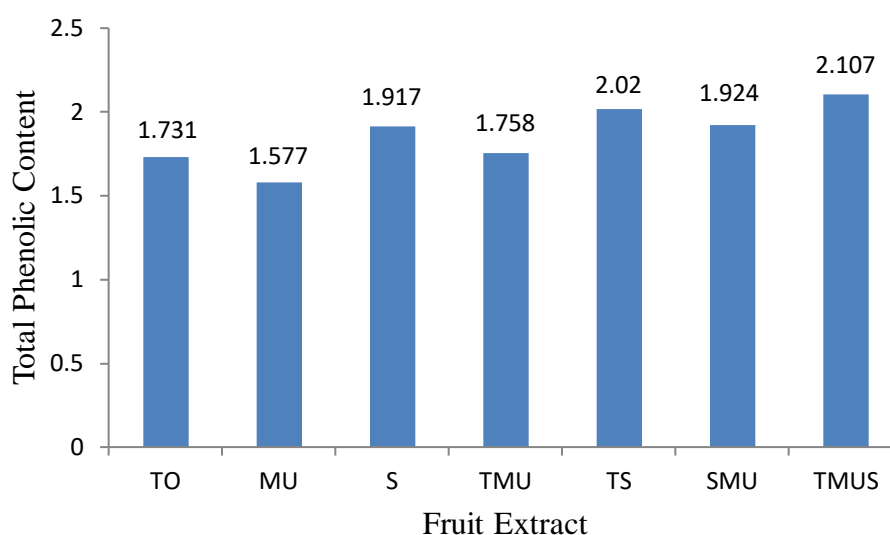
(+) = Gives a positive reaction

(-) = Gives a negative reaction

The results of the phytochemical screening test for tomato, purple passion fruit and strawberry extracts showed that there was a positive reaction to the secondary metabolite compounds, namely alkaloids, flavonoids, triterpenoids, tannins and saponins.

3.3. Total phenolic content of fruit extract

The total phenolic content of tomato, purple passion fruit and strawberry extracts before and after mixing was measured using the Folin-Ciocalteu reagent method which is equivalent to gallic acid. The results of the determination of total phenolic showed that the total phenolic content of tomato extract was (1,731 mg GAE/g), purple passion fruit extract (1,577 mg GAE/g), strawberry extract (1,917 mg GAE/g), tomato and purple passion fruit extract was (1,758 mg GAE/g), tomato and strawberry extract (2,020 mg GAE/g), strawberry and purple passion fruit extract (1,924 mg GAE/g) tomato, purple passion fruit and strawberry extract (2.107 mg GAE/g). Comparison of total phenolic from tomato, purple passion fruit and strawberry extracts before and after mixing can be seen in Figure 2.

**Figure 2.** Total phenolic extract of tomato, purple passion fruit and strawberry before and after mixing

Where : TO = Tomato
M = Purple passion fruit
S = Strawberry

- TMU = Tomato and purple passion fruit
 TS = Tomato and strawberry
 SMU = Strawberries and purple passion fruit
 TMUS = Tomato, purple passion fruit and strawberry

3.4. Antioxidant activity

Measurement of antioxidant activity of tomato, purple passion fruit and strawberry extracts before and after mixing was carried out using the DPPH method. The absorbance of DPPH was measured using a spectrophotometer at a wavelength of 517 nm in order for maximum absorption to occur. The percentage value of free radical scavenging activity was analyzed using the Tukey test to see that the average of each treatment was significantly different or not significantly different. The data table of the results of the Tukey test analysis on antioxidant activity can be seen in Table 2.

Table 2. Antioxidant activity of tomato, purple passion fruit and strawberry extracts before and after mixing

Treatment	Absorbance Value			Average (% DPPH)
	Replay			
	1	2	3	
BHA (control)	92,660	93,730	94,190	93,527 ^f
Tomato	80,275	80,734	81,040	80,683 ^b
Purple passion fruit	79,052	78,593	78,440	78,695 ^a
Strawberry	84,098	83,792	83,180	83,690 ^c
Tomato and purple passion fruit	81,651	82,569	81,958	82,059 ^b
Tomato and strawberry	86,697	86,391	85,392	86,160 ^d
Strawberry and purple passion fruit	84,862	83,945	83,486	84,098 ^c
Tomato, purple passion fruit and strawberry	88,685	88,532	87,786	88,328 ^e

Explanation: The same letter shows the results that are "not significantly different" based on Tukey's results with a confidence level of = 0.05

Based on the Tukey test in table 2 it shows that the combination extracts of the three fruits, namely tomatoes, purple passion fruit and strawberries, had the highest antioxidant activity compared to other treatments. The high antioxidant activity of the combination of the three fruits was caused by the compounds contained in each fruit mixer which had high antioxidant activity. The antioxidants found in these three fruits are vitamin C. The content of vitamin C in 100 grams of fruit is 40 mg of tomatoes, 30 mg of purple passion fruit and 56-60 mg of strawberries. This is what causes when combined the three fruits show high antioxidant activity compared to single fruit without being mixed. The antioxidant content in vitamin C can not only fight free radicals in the body but can also improve the immune system and reduce the risk of chronic diseases [9]. This is in line with the results of Hala and Ali (2020) study which stated that the antioxidant capacity of fruits before being mixed was around 0.2727 – 0.2977%, after being mixed the antioxidant capacity was 0.3223%. This shows that mixing fruit in the form of fruit juice does not decrease the antioxidant capacity of the fruit and even tends to increase the antioxidant capacity of the fruit.

The high percentage of treatment of tomato, purple passion fruit and strawberry extracts before and after mixing compared to the negative control (blank) treatment may occur due to the metabolite compounds contained in the three fruit extracts. Based on phytochemical screening, the three fruit extracts, namely tomatoes, purple passion fruit and strawberries, contained alkaloids, flavonoids, saponins, tannins and also a class of terpene compounds such as triterpenoids. Where these compounds have potential as antioxidants. Firdiyani *et al* (2015) stated that compounds that have potential as antioxidants are generally flavonoids, phenolics, alkaloids, saponins, steroids and triterpenoids.

3.5. Degree of acidity (pH) tomatoes, purple passion fruit and strawberries

The degree of acidity (pH) of tomatoes, purple passion fruit and strawberries before and after mixing was measured using a pH meter. The results of measuring the degree of acidity (pH) can be seen in Table 3.

Table 3. The degree of acidity (pH) of tomatoes, purple passion fruit and strawberries before and after mixing

Treatment	Degree	Of Acidity	(pH)	Average
	I	II	III	
Tomato	4,613	4,597	4,561	4,590
Purple passion fruit	3,093	3,088	3,092	3,091
Strawberry	3,717	3,692	3,708	3,706
Tomato and purple passion fruit	3,353	3,350	3,346	3,350
Tomato and strawberry	3,802	3,796	3,805	3,801
Strawberry and purple passion fruit	3,340	3,337	3,349	3,342
Tomato, purple passion fruit and strawberry	3,573	3,582	3,578	3,578

Fruits have a sour taste that varies greatly, from very sour (low acidity) to very sweet [6]. Based on Table 3 shows that mixing fruit can increase the acidity (pH) of low fruit, namely purple passion fruit (3.091) and can reduce the degree of acidity (pH) of high fruit, namely tomato (4.590) to 3.801. Hala and Ali (2020) in their research stated that the degree of acidity of fruits before mixing ranged from 3.76 for grapes to 4.44 for pears. After mixing the degree of acidity of the fruit to 4.14. This shows that mixing fruit in the form of fruit juice can increase the pH of low fruit (3.76) and lower the pH of high fruit (4.44) to pH 4.14. The thing that causes mixing fruit in the form of fruit juice can increase the acidity (pH) of low fruit and can reduce the pH of high fruit because this is related to the pH value of tomatoes which is higher than the pH value of purple passion fruit and strawberries. This shows that the pH value of the mixed fruit used can affect the resulting pH value. Agree with the results of research by Yusmarini *et al* (2015) which states that the results of the analysis show that the addition of watermelon juice will increase the pH value of pineapple juice. This is because the pH value of watermelon juice is higher than the pH value of pineapple juice.

4. Conclusion

Based on the results of research that has been done, it can be said that the total phenolic content of tomato (*Solanum lycopersicum*), purple passion fruit (*Passiflora edulis* var. Sims) and strawberry (*Fragaria sp.*) extracts after being equivalent to gallic acid showed that the highest total phenolic content was found in strawberry extract, which was 1.917 mg GAE/g, then tomato fruit extract was 1,731 mg GAE/g and purple passion fruit extract was 1,577 mg GAE/g. The antioxidant activity of the extracts of tomatoes (*Solanum lycopersicum*), purple passion fruit (*Passiflora edulis* var. Sims), and strawberries (*Fragaria sp.*) showed that the highest antioxidant activity was found in the extracts of strawberries, namely 83.690%, then tomato fruit extracts, namely 80.683% and purple passion fruit extract is 78.695% and the total phenolic content and antioxidant activity of a mixture of tomatoes (*Solanum lycopersicum*), purple passion fruit (*Passiflora edulis* var. Sims), and strawberries (*Fragaria sp.*) showed that the highest total phenolic content of mixed fruit was in the extracts of tomatoes, purple passion fruit and strawberries, namely 2.170, while the highest antioxidant activity of mixed fruit was in the extracts of tomatoes, purple passion fruit and strawberries, which was 88.328%.

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