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RESEARCH ARTICLE

POTENTIAL NATURAL ANTIOXIDANT CONTENT IN SONGAN KINTAMANI TOMATO EXTRACT BANGLI

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Abstract
Tomato is one of the plants that have the potential as a source of antioxidant compounds. Tomatoes contain lycopene which is a powerful antioxidant. Lycopene has been shown to reduce the risk of heart disease, cancer, and disease in old age. Songan Village, Bangli is a tomato-producing area in Bali. This study aimed to determine the content of antioxidant compounds in tomatoes from Songan-Kintamani Bangli village. The research method used is a descriptive experiment. Tomato fruit was extracted by the maceration method using hexane and methanol. The results of the phytochemical screening test of tomato extract showed that the tomato extract contained bioactive compounds of alkaloids, phenolics, saponins, and flavonoids. The extracts were then tested for lycopene, polyphenols, flavonoids, and antioxidant activity levels. Tomato fruit extract had a lycopene content of 0.878 mg/100g, polyphenol content of 7.55 mg/L GAE, and flavonoid content of 1,278 mg/100 g QE. Tomato extract has strong antioxidant activity with an AAI value of 1.387
Keywords: phytochemical, lycopene, antioxidants, tomatoes, Songan, Bangli

1 | INTRODUCTION

Nowadays, attention to natural antioxidants and their relationship to health is increasing. One source of natural antioxidants with great potential is antioxidants derived from plants. Plants produce several secondary metabolites, which are components of antioxidants to fight free radical molecules. One of the plants that have the potential as antioxidants are the tomato.

Tomato is a vegetable fruit that has a Latin name (*Lycopersicum esculentum* Mill). Tomatoes from horticultural plants originating from Mexico are known as

tomatoes. Tomato fruit is a multipurpose commodity that can be used as vegetables, cooking spices, drinks with various processed forms[1][2]. Tomatoes contain bioactive compounds found in tomatoes: (:(-carotene, lycopene, vitamins, and phenolic compounds have physiological properties, including antioxidant, anti-inflammatory, anti-allergic, and antimicrobial [2].

Tomatoes are considered one of the best sources of lycopene production and contain vitamins A and C, which are quite high. Tomatoes contain lycopene 30-200 mg/kg fresh[3], 3 - 5 mg/L[4] [5]. During the ripening process, the lycopene content increases sharply[6]. Lycopene often referred to as carotene, is a bright red pigment carotenoid found in tomatoes and other red fruits. Lycopene can be absorbed directly from tomato juice, ketchup, and supplements. Lycopene levels in serum were shown to increase significantly after consuming tomato products and supplements, accompanied by decreased oxidation biomarkers, including oxidation of serum lipids, LDL cholesterol, serum proteins, and DNA.[7]. Lycopene is a powerful antioxidant compound. Lycopene has been shown to reduce the risk of heart disease, cancer, and disease in old age[8].

Tomato is one of the most common plants in Indonesia. One of the tomato-producing areas in Bali is in the village of Songan-Kintamani Bangli. Songan village, Kintamani Bangli, is located at the foot of Mount Batur and is located on the outskirts of Lake Batur. Since the Covid-19 pandemic season has resulted in a decline in horticultural products, one that is very pronounced is the tomato commodity. Quoted from NusaBali September 15, 2020, the price of tomatoes at the farmer level is currently between Rp. 1500-Rp. 2000 per kilogram. The fall in tomato prices was caused by the low purchasing power of the people and the abundant production of tomatoes. To overcome the fluctuating price of tomatoes, the absorption of tomato yields is processed into valuable things such as making tomatoes valuable extract for the body and health.

Supplementary information The online version of this article (https://doi.org/10.15520/mcrr.v4i07.174) contains supplementary material, which is available to authorized users.

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2 | MATERIALS AND METHODS

Raw material preparation

Tomato samples of 10 kg of tomatoes of uniform size were washed in running water, and the seeds were removed. It was dried in an oven at 500 C for three days. Then mashed with a blender and sieved 20 mesh.

Extraction

Tomato powder was weighed 500 g and extracted using hexane and methanol (CH3OH). The sample was soaked in hexane solvent for 48 hours, occasionally stirring, filtering, and maceration for 24 hours. The extract was washed with distilled water and put into a separatory funnel with a shaker to separate the extract from the impurities. After forming two layers, take all the nonpolar layers and then tamping them into a glass beaker. Then the top layer (nonpolar), red like a tomato, was evaporated using a rotary evaporator at a temperature of 500, the pressure of 254 mbar with a rotating speed of 80 rpm until a concentrated extract was obtained. Then the analysis was carried out.

Phytochemical Screening

1. Alkaloid screening (Test Mayer)

A total of 1 ml of the sample solution was dissolved with HCl, then added with Mayer's reagent. The presence of white taste indicates the presence of flavonoid compounds.

2. Flavonoid Screening (Shindo Test)

A total of 1.3 ml of the sample was mixed with 0.5 grams of magnesium, then boiled for 5 minutes.

A color change from orange to red indicates the presence of flavonoids.

3. Tannin Screening (Wohler's Test)

A total of 1.6 ml of the sample was dripped with lead acetate solution. The presence of a white precipitate indicates the presence of tannins.

4. Phenol Screening

A total of 2 ml of the sample was pipetted, and a few drops of FeCl3 were added. The presence of a greenish color indicates the presence of high or low content.

5. Saponin Screening (Frothing Test)

Take 10 ml of the filtrate, add 5 mL of distilled water, and then shake vigorously until foam is formed. Then add three drops of olive oil to the foam, then shake it again and observe the formation of an emulsion.

Lycopene Level

Conventional lycopene test

Total lycopene in 2 g of a sample using the method of Sadler et al. (1990) modified by Perkins-Veazie et al. (2001). The assay required 25 ml of 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, 25v ml of 95% ethanol, and 50 ml of hexane per sample tested.

Measurement of lycopene levels

Each vial added 5 ml of 0.055 (w/v) BHT in acetone, 5 ml of 95% ethanol, and 10 ml of hexane. Hexane was taken with a volume pipette calibrated to produce 10 ml at 200C. The next test uses a volume dispenser to take the organic solution. Both techniques give comparable results in terms of precision and accuracy.

The non-polar layer, red like a tomato, is stirred and pipetted into a test bottle according to the specified weight. Depending on the balance used, the weight of the sample is determined to an accuracy of 0.01 g or 0.001 g. The number of samples used in the educated volume test can range from 0.4 to 0.6 g. The weight of the sample added to the test vial is at least close to 0.01 g to maintain an acceptable level of weighing precision. The bottle is placed sideways in a rectangular container, closed with a second container containing ice, placed on an orbital shaker, and then shaken at 180 rpm for 15 minutes. After 15 minutes of shaking, 3 ml of deionized water was added to each vial, and the sample was shaken for 5 minutes. Shaking was stopped, and the vial was left at room temperature for 5 min for phase separation. The absorbance of the hexane layer was measured in a quartz cuvette with a wavelength of 503 nm compared to the empty hexane solvent. The formula then estimates the lycopene content:

C = A/(E1%1 cm x B)

Where : C : concentration (g/100 ml),

A : Absorbance

B : thickness of cuvette

E1%1cm refers to the absorbance of 1 cm of the solution layer that is concentrated at the specified wavelength and

Polyphenol content

A total of 0.05 grams of crude extract sample, extracted with 5 ml of 99.9% methanol, homogenized and centrifuged at 3000 rpm for 15 minutes to obtain the supernatant. The supernatant was filtered to obtain a filtrate. The 0.4 ml pipette filtrate was placed in a test tube, added 0.4 ml of Folin–Ciocalteu reagent, vortexed until homogeneous, and allowed to stand for 5 minutes before adding 4.2 ml of 5% sodium carbonate solution. The sample was allowed to stand for 30 minutes at room temperature before reading the color absorption at a wavelength of 760 nm. Standard curves were made by dissolving gallic acid in distilled water with various concentrations of 10-100 mgL-1. Calculation of total phenol using the regression equation y = ax + b 13.

Flavonoid Level

A total of 0.05 grams of crude extract sample, extracted with 5 ml of 99.9% ethanol, homogenized and centrifuged at 3000 rpm for 15 minutes to obtain a supernatant. The supernatant was filtered to obtain a filtrate. The 0.5 ml pipette filtrate was placed in a test tube, added 0.5 ml of ethanol and 1.0 ml of 2% AlCl3 reagent, vortexed until homogeneous, and left for 30 minutes at room temperature before reading the color absorption at a wavelength of 415 nm. Standard curves were prepared by dissolving quercetin in 99.9% ethanol with various concentrations of 0-30 mgL-1. Calculation of flavonoids using the regression equation y = ax + b 14.

Antioxidant Activity

Antioxidant activity was measured by determining the inhibitory value (IC) by making the concentration of the extracted sample, then reacted with 0.1 mM DPPH radical and read its color absorption at a wavelength of 517 nm. The formula determines IC 50 value:

Inhibition Index = Control OD- Sample OD x 100%

OD control

Note: OD = optical density (absorbance value)

3 | RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening was carried out to provide an overview of the class of compounds contained in the extract[9]. In this case, it is a tomato fruit extract. The phytochemical screening of tomato fruit extracts obtained in this study showed that positive alkaloids, phenolic compounds, saponins, and flavonoids were contained in the tomato extract. This shows that the tomato extract contains an alkaloid, phenolic, saponin, and flavonoid bioactive compounds qualitatively. The results of the phytochemical screening test can be seen in Table 1.

TABLE 1: Phytochemical screening test results

No	Variabels	Check up result	Information
1	Alkaloids	positive	White precipitate
2	Phenolic	positive	Greenish color
3	Saponins	positive	There is foam
4	Flavonoids	positive	Reddish color
5	Tannin	negative	No sediment

Lycopene Level

The results of absorbance and lycopene content of tomato fruit extract can be seen in Table 2. Based on the table above, tomato extract has an average lycopene content of 0.878 mg/100g.

Lycopene is a carotenoid pigment that causes the red color of tomatoes. The lycopene content of tomatoes

TABLE 2: Lycopene content of tomato fruit extract

No	Sample	replay	Sample weight	Absorbance	Lycopene Level (mg/100g)	Average lycopene levels (mg/100g)
1	Tomato Extract	1	0.0005	0.460	0.878	0.878
		2	0.0005	0.461	0.879	
		3	0.0005	0.460	0.877	

is strongly influenced by the ripening process and differences in varieties (for example, red varieties contain more lycopene than yellow ones).[10]. The level of lycopene in tomatoes is determined by the genetic potential of the species and environmental conditions, especially temperature and light. During the ripening period, the lycopene composition of the fruit increases markedly[11]. The lycopene content of tomatoes in 100 grams is 3.041 mg/100g[12]. In the compound extraction process, the solvent used dramatically affects the amount of lycopene obtained. This is because the interaction of lycopene compounds with the solvent used is the dispersal of solvent molecules. Lycopene compounds tend to be perfect if the solvent used is non-polar. This happens because of the intermolecular forces between similar compounds with the same strength[13].

Polyphenol content

The results of the total phenol examination in tomato extracts examined using the UV-Vis spectrophotometric method with Folin-Ciocalteu phenol reagent can be seen in Table 1. The total phenol calculation using the regression equation y = ax + b, namely y = 0.0111 x 0.0138 with a linear line R2 = 0.9994. Table 3. shows the results of the calculation of total phenol that has been carried out. The average value of total phenol is 7.55 mg/L GAE. Polyphenol content in fruit is influenced by fruit variety, planting technique, and environmental conditions. The polyphenol content of tomatoes with organic cultivation was 10.06 mg/100g GAE, and conventional cultivation was 5.58 mg/100g GAE.[14].

Flavonoid Level

The total flavonoid examination results using UV-Vis spectrophotometry were carried out to deter-

TABLE 3: Polyphenol content of tomato fruit extract

Tube	Sample weight (g)	Total phenol (mg/100g GAE)	Average
1	0.05	7.640	7.55
2	0.05	7.640	
3	0.05	7.369	

mine how much flavonoid content was in the tomato extract. As a standard solution, the quercetin standard solution was shown in Table 4. Calculation of total flavonoids using the regression equation y = ax + b, namely y = 0.0316 x 0.0089 with a linear line R2 = 0.9944. Table 3. shows the results of the calculation of total phenol that has been carried out. The average value of total phenol is 1,278 mg/100 g QE. Tomato fruit flavonoid content (1.42-1.43 mgQE/100 g fruit)[14]. The flavonoid content of tomatoes in Songan was lower due to different types of environmental conditions. In addition, the extraction solvent is very influential on the levels of flavonoids. Most of the flavonoid compounds in tomatoes are polar and non-polar depending on the number of hydroxyl groups they have[14].

TABLE 4: Flavonoid content of tomato fruit extract

Tube	Sample weight (g)	Total flavonoids (mg/L QE)	Average
1	0.05	1.2747	1,278
2	0.05	1.2837	
3	0.05	1.2747	

Antioxidant Activity (IC 50)

Measurement of antioxidant activity spectrophotometrically with the DPPH method. DPPH antioxidant activity test is based on the DPPH radical scavenging reaction by antioxidant compounds through a hydrogen atom donation mechanism. DPPH-H (non-radical form) is produced and causes a decrease in the intensity of the purple color of DPPH. The level of antioxidant power with the DPPH method can be seen in Table 5.

Free radical scavenging activity is usually expressed as the percent inhibition of DPPH, but it can also be expressed as the concentration that causes a 50% loss of DPPH activity (IC50). The IC50 value is

TABLE 5: The level of antioxidant power by the DPPH method [15]

Intensity	IC50 value Nilai
Very strong	AAI>2 ppm
Strong	AAI>1-2 ppm
Medium	AAI>0.5-1 ppm
Weak	AAI<0.5 ppm

considered a good measure of the antioxidant efficiency of pure compounds or extracts[16]. The smaller the IC50 value of a test compound, the more active the compound is as an antioxidant[17]. The results of the antioxidant activity test can be seen in Table 6. Based on the table above, tomato extract has an AAI value of 1.387, indicating strong antioxidant activity. This is following research[18]which states that tomatoes (*Solanum lycopersicum* L.) have very strong antioxidant activity. The antioxidant contained in tomatoes is lycopene. In addition, tomatoes contain phenolic and flavonoid compounds. Compounds that have potential as antioxidants are generally flavonoids, phenolics, and alkaloids. Flavonoid compounds and polyphenols are antioxidants, antidiabetic, anticancer, antiseptic, and anti-inflammatory, while alkaloid compounds inhibit the growth of cancer cells.[19].

TABLE 6: Results of Measurement of Antioxidant Activity of Tomato Fruit Extract

Cons	Abs	Inhibition	AAI
mg/mL		%	
0.00	0.984	0.000	
0.24	0.436	55,700	
0.47	0.338	65,657	1.387
0.71	0.265	73.075	
0.94	0.189	80,797	
1.18	0.126	87,198	

4 | CONCLUSIONS

Based on the research results above, the hexane and methanol extracts of tomatoes had a lycopene content of 87.835%, polyphenol content of 7.55 mg/L GAE, and flavonoid content 12.78 mg/L. Tomato

extract has strong antioxidant activity with an AAI value of 1.387.

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