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Analysis of Phytochemical Compounds, Total Phenolic Content, and Antioxidant Activity Test of Ethanol Extract of Okra (*Abelmoschus Esculentus* L.) from the Traditional Market of Kendari

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Abstract: Okra (*Abelmoschus esculentus L.*) is often used as a vegetable and has many benefits, as it contains bioactive compounds beneficial to health and acts as a source of nutrition. This research aimed to determine the phytochemical content, total phenolic content, and antioxidant activity in the ethanolic extract of green okra (Abelmoschus esculentus) fruit obtained from a traditional market in Kendari, Southeast Sulawesi Province, Indonesia. The research included four stages: sampling; sample preparation (the making of simplicia); extraction using the maceration method with 96% ethanol solvent; concentration of the extract using a rotary evaporator, followed by qualitative tests on the content of phenolics, alkaloids, flavonoids, saponins, terpenoids, and steroids using reagents as per the test parameters. Determination of the total phenolic content was carried out through the Folin-Ciocalteu solution method using standard UV-visible spectrophotometry for gallic acid at a wavelength of 765 nm. Antioxidant activity was tested by employing the DPPH (1,1-diphenyl-2-picrylhydrazyl) trapping method, which was measured using a UV-visible spectrophotometer with a wavelength of 515 nm. The results of the phytochemical screening of the okra fruit showed that the ethanolic extract of the fruit contained phenolic compounds, alkaloids, flavonoids, saponins, steroids, and terpenoids and had a total phenolic value of 13,456 ppm. The ethanol extract of okra fruit has weak antioxidant activity, with an IC50 value of 157.071 mg/mL. This showed that green okra fruit can be used as a basic ingredient for biodiversity-based medicines.

Keywords: *Abelmoschus esculentus L.*, total phenolic content, antioxidant, IC50, 1,1-diphenyl-2-picrylhydrazyl.

肯达里传统市场秋葵乙醇提取物的植物化学成分分析、总酚含量及抗氧化活性测试

摘要：秋葵(冬虫夏草.)通常用作蔬菜并具有许多好处，因为它含有对健康有益的生物活性化合物并作为营养来源。本研究旨在确定从印度尼西亚东南苏拉威西省肯达里的一个传统市场获得的绿色秋葵(南芥)果实的乙醇提取物中的植物化学成分、总酚含量和抗氧化活性。研究包括四个阶段：抽样；样品制备（简单的制作）；采用 96%乙醇溶剂浸渍法提取；使用旋转蒸发仪对提取物进行浓缩，然后根据测试参数使用试剂对酚类、生物碱、黄酮类、皂苷、萜类和类固醇的含量进行定性测试。总酚含量的测定通过福林-乔卡尔特乌溶液法使用标准紫外-可见分光光度法在 765 纳米波长处测定没食子酸。通过采用 DPPH (1，1-二苯基-2-苦基肼) 捕获方法测试抗氧化活性，该方法使用波长为 515 纳米的紫外可见分光光度计进行测量。秋葵果实的植物化学筛选结果表明，果实的乙醇提取物中含有酚类化合物、生物碱、黄酮类、皂苷、甾体和萜类化合物，总酚值为 13，456 ppm。秋葵果实的乙醇提取物抗氧化活性较弱，我知道了 50 值为 157.071 毫克/毫升。这表明绿色秋葵果实可以用作生物多样性药物的基本成分。

关键词：冬虫夏草 ，总酚含量，抗氧化剂，我知道了 50，1，1-二苯基-2-苦基肼。

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1. Introduction

Indonesia's biodiversity, including the diversity of foodstuffs and vegetables, represents a source of food and feed with high nutritional content. It contains bioactive compounds that are potential sources of valuable food and medicine, are beneficial for health, and have economic potential. This biodiversity must be managed effectively and responsibly. To determine the content of chemical compounds in plants, a phytochemical analysis was carried out [1].

One of the benefits of these compounds is as an antioxidant. Antioxidants are compounds that can bind free radicals and highly reactive molecules by inhibiting oxidation reactions, so that cell damage caused by free radicals can be inhibited [2].

Free radicals contain one or more unpaired electrons in their outer orbitals, so these compounds are very reactive when they bind to the electrons in the surrounding molecules. This will result in impaired cell function, damage to cell structure, modified molecules that cannot be recognized by the immune system, and even mutations. These disorders can trigger various diseases, such as degenerative diseases and cancer. Therefore, our bodies need antioxidants that can reduce free radicals and their negative effects [3].

Green okra (*Abelmoschus esculentus*) fruit is often used as a vegetable ingredient and has many benefits. It contains a source of nutrition and secondary metabolite compounds, such as flavonoids, phenolics, alkaloids, tannins, terpenoids, steroids, and saponins, all of which are beneficial to health. Okra (*Abelmoschus esculentus*) is also a type of functional vegetable that has many health benefits [4]. The benefits of okra for health include preventing diabetes, lowering cholesterol, use as an antioxidant, use as an antibacterial, preventing the development of cancer, and it is good for the digestive system [5, 25]. Okra contains protein, carbohydrates, and fat [6, 7].

However, the chemical content of okra fruit is not widely known. Therefore, research is needed to analyze its phytochemical content and antioxidant activity, which may have health benefits for the community.

2. Research Method

2.1. Materials

Okra fruit (*Abelmoschus esculentus*) test samples, ethanol, methanol, Wagner's reagent, Mayer's reagent, magnesium (Mg) powder, concentrated hydrochloric acid (HCl), Dragendorff's reagent, chloroform (CHCl 3), acetic acid anhydride (C4H6O3), concentrated sulfuric acid (H2SO4), iron chloride (FeCl3) 3%, distilled water (H2O), sodium hydroxide 1 N (NaOH), natrium carbonate (Na2CO3) 7.5%, iron (III) chloride (FeCl3), spiritus, aluminum foil, DPPH, ethanol 96%, methanol, vitamin C, quercetin, gallic acid, Folin-Ciocalteu reagent, and aluminium chloride (AlCl3).

2.2. Tools

A set of glassware and extraction tools, UV-Vis spectrophotometer, Erlenmeyer, cuvette, test tube, beaker, measuring cup, spatula, analytical balance, tube rack, tongs, cool box, blender, 60-mesh sieve, aluminum tray, rotary vacuum evaporator, incubator, vacuum filter, flask, dropper, funnel, Petri dish, spoon substance, volume pipette, and reagent bottle.

2.3. Preparation and Extraction of the Raw Ingredients

The sampling of the okra (*Abelmoschus esculentus*) was conducted in the morning at the Kendari City traditional market. The okra fruit was labeled and stored in a closed container during the journey to the laboratory. The preparation of the okra (*Abelmoschus esculentus*) began with the washing process. Fresh okra fruit was washed using running water. The sample was then drained. Then, the sample was cut, or chopped, into pieces, placed in a container, and dried. After drying, the sample was put into an oven at a temperature of 40–50^{0}C until it was completely dry. It was then blended and sieved through a 60-mesh sieve to become powder. The refined sample was then weighed with an analytical balance for further extraction [8].

Maceration was carried out for 24 hours using 96% ethanol as a solvent. The ratio of simplicia powder and solvent used was 1:10. During maceration, the sample was stirred every hour for the first six hours. After 24 hours, filtration was carried out to obtain the maserate, and the maceration process was repeated twice. The result of maceration in the form of a solution is then filtered with filter paper to obtain the filtrate and residue. The filtrate was evaporated until the solvent separated from the extract using a Rotary Vacuum Evaporator at a temperature of less than 50°C. This extract is formulated by adding diluent or aquadest as a solvent [8, 9]. The extraction results were evaporated to obtain a thick extract of okra fruit (Fig. 1). The extract was stored in a tightly closed glass container and protected from exposure to sunlight.

Fig. 1 The thick extract of the fruit of *Abelmoschus esculentus* L. (okra)

2.4. Phytochemical Screening

2.4.1. Flavonoid

Several extracts were added with methanol and heated over a water bath; 0.1 mg of powder and five drops of concentrated HCl were added. A positive reaction of flavonoids will be indicated by the formation of a red, yellow, or orange color [2].

2.4.2. Alkaloids

A total of 0.5 g of extract was added with 1 ml of HCl 2N and 9 ml of distilled water, heated in a water bath for 2 minutes, cooled, and filtered. The filtrate obtained was used for the alkaloids test. 2 test tubes were taken, and then 0.5 ml of filtrate was added to each test tube; 2 drops of Mayer's reagent and Dragendorff's reagent were added to each test tube. If there is a precipitate or turbidity, positive results for alkaloids will be shown [10, 14].

2.4.3. Terpenoids and Steroids

Examination of triterpenoids and steroids was carried out by the Liebermann-Burchard reaction. 2 mL of the test solution was evaporated in a porcelain cup. The residue was dissolved with 0.5 mL of chloroform, and then 0.5 mL of anhydrous acetic acid was added. 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 the presence of triterpenoids, whereas a greenish blue ring indicates the presence of steroids [11].

2.4.4. Saponins

A 2.5 mL sample was added with a few drops of distilled water and then shaken vigorously (a positive test result was indicated by the appearance of foam that lasted more than 30 seconds and did not disappear when one drop of HCl 2N was added [10, 11].

2.4.5. Phenolics

Phenolic compounds were screened by dissolving 0.1 gram of okra fruit ethanol extract with 10 mL of distilled and filtrated water. Take 1 ml of the filtrate and add two drops of 5% $FeCl_3$ solution. A positive reaction of phenol indicates the formation of a green or blue-green color [12, 13].

2.4.6. Total Phenolic Test

The total phenolic content test was carried out with a solution of the Folin-Ciocalteu method using UV-Vis spectrophotometry, referring to gallic acid as a comparison standard [17]. Ten milligrams of the extract were added with 0.4 mL of Folin-Ciocalteau reagent and incubated for 4-8 minutes. Next, the solution was added with 4.0 mL of 7% Na_2CO_3 and distilled water. After 2 hours of incubation, the absorbance of the solution was measured at a wavelength of 765 nm. The total phenol content was expressed as gallic acid equivalent (GAE) in mgGAE/g extract [16-18].

2.5. Antioxidant Activity Test (IC50) Using the DPPH Method

The antioxidant activity test using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method refers to [15]. The absorbance of each sample was determined using a UV-visible spectrophotometer at a wavelength of 515 nm. Test solutions with concentrations of 20, 40, 80, 160, and 320 mg/L were used to determine the antioxidant activity in the present research. Each test solution (0.5 mL) was added to 1 mL of DPPH solution in ethanol. The resulting solution was incubated for 30 min, and the absorbance was measured as described above. According to Fitriana et al. [19], free radical scavenging activity can be calculated using the following formula:

$$\% \, Inhibition = (Acontrol - Asample)/Acontrol \times 100$$

The inhibition of free radical activity was evaluated from the absorbance value of the sample. The regression equation was obtained from the relationship between the sample concentration and the percentage of free radical activity inhibition. The concentration of the test solution that inhibited free radical activity by 50% (IC50) was calculated using a linear regression equation [19, 20].

2.6. Data Analysis

Statistical analysis was carried out on the data relating to the content of phytochemical compounds, the total content of phenolic compounds, and the antioxidant activity of each sample. The data are presented as tables and graphs, which were created using Microsoft Excel.

3. Results and Discussion

3.1. Phytochemical Screening

Phytochemical screening was carried out to determine the levels of secondary metabolites or bioactive compounds in the ethanol extracts obtained from the okra fruits.

The results of phytochemical screening (Table 1) show that the ethanolic extracts of okra fruit contained phenolic compounds, alkaloids, flavonoids, saponins, terpenoids, and steroids.

Table 1 Phytochemical screening results of okra fruit ethanol extract

Number	Phytochemical Test	Explanation
1	Phenolic	+
2	Alkaloids	+
3	Flavonoids	+
4	Saponins	+
5	Steroids/Terpenoids	+

The secondary metabolites contained in natural ingredients can be identified through qualitative

analysis with phytochemical screening. Extracts from natural ingredients also contain various kinds of secondary metabolites with biological activity. These secondary metabolites can be identified using certain reagents that recognise the specific chemical groups they contain [21].

3.2. Total Phenolic Content

The total phenolic content of each sample was determined by UV-vis spectrometry using Folin–Ciocâlteu reagent. The principle of this test is the formation of a blue complex compound due to the reaction between phenolic compounds and Folin–Ciocâlteu reagent; the complex can be detected at a wavelength of 765 nm [16].

The standard curve equation for gallic acid was used to determine the total phenolic content of the okra fruit extract. Gallic acid was used as a standard because it effectively forms complex compounds with Folin–Ciocâlteu reagent. Therefore, the resulting reaction is more sensitive and intensive [16]. The maximum wavelength absorption measurement was carried out according to the maximum wavelength absorbed by the gallic acid standard (i.e., 765 nm). That maximum absorbance wavelength was used in the investigation of the okra fruit ethanol extract sample. The calculations show that the total phenolic content of the okra fruit ethanol extract was 13,456 mg gallic acid equivalent (GAE)/g extract.

3.3. Antioxidant Activity Test

In the present research, antioxidant activity was determined using the DPPH method. There was a decrease in absorbance attributable to DPPH in the ethanol extract of the okra fruits, reflecting a decrease in antioxidant activity (Table 2). This was due to the scavenging activity of the extract.

Table 2 Antioxidant activity test results of the ethanol extract of okra fruits

No	Okra fruit extract concentration (μg/mL)	Absorbance (A) λ = 515 nm	Antioxidant activity of okra fruit extract (%)	IC-50 Value (μg/mL)
1	20	0,406	21,92	157,071
2	40	0,380	26,92	
3	80	0,338	35,00	
4	160	0,253	51,35	
5	320	0,094	81,92	
6	Control	0,520		

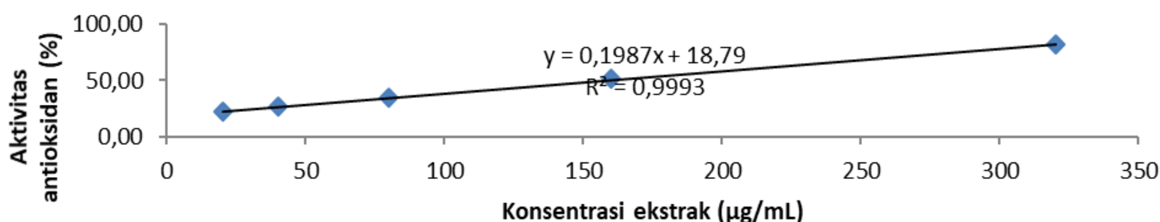

Fig. 2 The curve of the relationship between the antioxidant activity and the extract concentration of okra fruits

The calculated IC50 value for the okra fruit ethanol extract, obtained from the linear regression equation shown in Fig. 2, indicates that the regression equation for the extract is y = 0.1987x + 18.79, with $R^2 = 0.9993$ and r = 0.9996. The y-coefficient in this equation is IC50, whereas the x-coefficient indicates the optimum concentration of the extract. The obtained value of x is the extract concentration required to reduce the activity of DPPH radicals by 50%. The value of r = 0.9996 is close to +1 (a positive value), illustrating that the greater the extract concentration, the greater the antioxidant activity. This can be seen from the correlation curve of the okra fruit ethanol extract concentration versus the percent inhibition (Fig. 2).

According to the system of categorizing antioxidants proposed by [22], an IC50 of < 50 indicates very strong antioxidant activity, an IC50 of 50–100 indicates strong activity, an IC50 of 101–150 indicates moderate activity, and an IC50 of 151–200 indicates weak activity. The calculated IC50 of the okra fruit ethanol extract shown was 157.071 μg/mL (Table 2). This indicates that an okra fruit ethanol extract has weak antioxidant activity (weak category: IC50 151–200), and is capable of neutralizing 50% of free radicals because it contains phenolic compounds, alkaloids, flavonoids, saponins, terpenoids, and steroids.

The working principle of this experiment depends on the presence of stable free radicals, namely DPPH radicals, mixed with antioxidants that have the ability to donate hydrogen, so that the free radicals are reduced [23]. The DPPH method comprises quantitatively determining antioxidant activity by using UV-vis spectrophotometry to evaluate the ability of an antioxidant to scavenge DPPH radicals. The free radical scavenging activity is then expressed as the IC50 (inhibitory concentration) value. The IC50 value is the concentration of the test compound that can reduce the level of free radicals by 50%. The lower the IC50 value, the higher the free radical scavenging activity [22].

An antioxidant is a compound that can inhibit or

prevent the oxidation of a substrate. Antioxidants are needed to protect the body from free radical attack. Free radicals can cause degenerative diseases such as cancer, diabetes mellitus and its complications, stroke, and atherosclerosis due to oxidative stress [24].

4. Conclusion

The present research indicates that an ethanolic extract of okra fruit: contains bioactive secondary metabolites, including alkaloids, flavonoids, phenolic compounds, steroids, and terpenoids; has a total phenolic content of 13,456 mg GAE/g; and has weak antioxidant activity (i.e., an IC50 value of 157.071 μg/mL). These results prove that okra fruit is a functional food because it contains the secondary metabolites mentioned above. Such metabolites are bioactive compounds and have been scientifically proven to improve health. This study's novelty is that the ethanolic extract of green okra fruit contains phytochemical compounds and phenols and has antioxidant activity. This is evidence that green okra fruit can be a basic ingredient for biodiversity-based medicines.

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