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Physicochemical Parameters, Phytochemical Screening, and Antioxidant Activity of *Capsicum annuum* var. *grossum* Leaves From Indonesia

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ABSTRACT

Dietary plants are believed to contain antioxidants that have positive effects on human health. Free radicals, other reactive oxygen and nitrogen spesies can be eliminated by antioxidants. The presence of flavonoids and phenol in paprika (*Capsicum annuum* var. grossum) fruit contributes to its antioxidant properties. The phytochemistry and antioxidant activity of paprika leaves—especially those cultivated in Indonesia—have not been extensively studied. The aim of this study to investigate psychochemical parameters, phytochemical screening and antioxidant activity of paprika leaves from Indonesia. Several physicochemical parameters and phytochemical screening test were done base on Indonesian Herbs Pharmacopoeia. Antioxidant activity test using DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Dry powder of paprika leaves have some physicochemical parameters which part of its characteristic. Dry powder and extract of paprika leaves contains flavonoid, saponin, phenol and steroid/triterpenoid. The findings of the antioxidant test indicated that the standard vitamin C exhibited an IC50 value of 6.22 µg/ml. In contrast, the extract, n-hexane fraction, ethyl acetate fraction, and water fraction demonstrated IC50 values of 6.41, 9.12, 8.96, and 10.58 µg/ml, respectively. There was potential for paprika leaves to be developed as a natural source of antioxidants

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1. Introduction

Antioxidants have become an important part of our lives because they neutralize or kill "reactive oxygen species" (ROS) or "*free radicals*" before they damage cells (Dontha, 2016). The result of regular cell activity is reactive oxygen species, or ROS. They contribute significantly to signaling pathways and are generated in a variety of cellular compartments. The emergence of numerous human diseases (such as cancer, heart disease, neurological conditions, and metabolic disorders), inflammation, and aging are linked to an excess of reactive oxygen species (ROS) (Snezhkina et al., 2020).

Plants are naturally able to biosynthesize a broad variety of non-enzymatic antioxidants that can reduce oxidative damage caused by reactive oxygen species (ROS)(Kasote et al., 2015). It is widely believed that the consumption of dietary plants, which contain antioxidants, might have positive effects on human health. The capacity of antioxidants to eliminate free radicals and other reactive oxygen and nitrogen species is widely acknowledged as a substantial factor in the development of various chronic diseases (Shetty, 2013).

Paprika, also referred to as bell pepper or sweet pepper, is a solanaceous vegetable (*Capsicum annuum* L. var. grossum)(Sharma et al., 2019). This plant is widely valued all over the world because of its unique flavor, scent, and good color. It is utilized in a variety of culinary contexts, including salad dressings, vegetable preparations, pickling, and processed foods. Paprika is highly valuable in the world of fresh vegetable markets (Roy et al., 2019).\Vitamin C and vitamin A are abundant in fresh red paprika. Furthermore, the substance in question comprises antioxidant components, including β-carotene, lutein, zea-xanthin, and cryptoxanthin (Prabakaran et al., 2017). The presence of flavonoids and phenol in paprika fruit contributes to its antioxidant properties (Oladipo et al., 2023). Research has demonstrated that the hydroxyl groups found inside the aromatic rings of phenolics are primarily responsible for their significant radical-scavenging capabilities (Vuolo et al., 2019).

The phytochemistry and antioxidant activity of paprika leaves—especially those cultivated in Indonesia have not been extensively studied. Furthermore, the objective of this study is to examine the psychochemical parameters, phytochemical screening, and antioxidant activities of paprika leaves sourced from Indonesia.

Studying the physicochemical properties of Capsicum annuum var. grossum leaves can also have advantages for agriculture by offering knowledge about the ideal environmental conditions for the growth of this plant species. Acquiring this knowledge can enhance farming techniques, resulting in higher crop productivity and improved quality.

The results of this study can aid in the advancement of natural antioxidants or medicinal substances obtained from the leaves of Capsicum annuum var. grossum. These compounds can be used in pharmaceutical formulations to treat or prevent certain disorders.

To summarize, studying the physicochemical parameters, phytochemical screening, and antioxidant activity of Capsicum annuum var. grossum leaves from Indonesia has the potential to advance scientific knowledge, support sustainable agriculture, and potentially create new therapeutic agents with diverse health advantages.

2. Materials and Methods

Material

In this study, the main materials employed included DPPH (2,2-diphenyl-1-picrylhidrazyl), vitamin C sourced from Sigma, Dragendorff reagent, Meyer reagent, $AlCl_3$ solution, $FeCl_3$ solution, Liebermann-Burchard reagent for phytochemical screening, and solvents.

Sample preparation

Paprika leaves were gathered in Lembang, west Java, Indonesia. A determination of the plant was made at the Bandung Institute of Technology's Herbarium Bandungense School of Life Science and Technology. In order to make powder, leaves had to be cleaned, dried, and ground.

Physicochemical parameters

Determination of water content

Approximately 25 grams of dry powdered paprika leaves were measured and subsequently transferred into a dry flask. Introduce 200 ml of water-saturated toluene into the flask and proceed to boil the flask with caution for a duration of 15 minutes. Once the toluene has initiated the boiling process, adjust the distillation rate to about 2 drops per second until a significant portion of the water has undergone the dilution process. In addition, it is recommended to enhance the rate of water distillation to 4 drops per second within the receiving tube. The process of distillation should be halted once all the water has been successfully distilled. The interior of the still was cleansed using toluene that was soaked with water. Additionally, a connected tube brush was used to clean the copper wire, which was drenched with toluene. Subsequently, proceed to subject the mixture to a 5-minute distillation process. The receiving tube should be cooled to the ambient temperature. To mitigate the presence of water droplets, apply a layer of rubber affixed to a copper wire and moisten the cooling tube and reception tube with water-saturated toluene until the water droplets diminish. The receiving tube will exhibit a complete separation of water and toluene. Determine the volume of water subsequent to the

complete separation of water and toluene. The water content is determined by expressing it as a percentage of weight/weight (General Director of Pharmaceutical Care and Medical Devices, 2017).

Determination of Total Ash Content

Two to three grams of paprika leaves dry powder were weighed, added to a silica crucible cup, lit, and tared. The crucible with the ash is slowly burned in the furnace (800±25°C) until the charcoal pours out, after which it is cooled in a desiccator and weighed untill constant. The total ash content of dry paprika leaves is determined and expressed as a weight percentage (w/w) (General Director of Pharmaceutical Care and Medical Devices, 2017).

Determination of acid-insoluble ash content

The ash utilized for the determination of the overall ash content underwent a cooking process lasting 5 minutes in 25 mL of diluted hydrochloric acid. Subsequently, the resulting residue was gathered, passed through ash-free filter paper, rinsed with hot water, and subjected to combustion until a uniform weight was achieved. The acid-insoluble ash content of the test sample is determined by expressing it as a weight percentage (General Director of Pharmaceutical Care and Medical Devices, 2017).

Determination of Ethanol Soluble Content

100 mL of 96% ethanol was used to macerate up to 5 grams of paprika leaves dry powder for 24 hours. The mixture was constantly shaken for the first 6 hours and then left for 18 hours. A 20 mL portion of the filtrate was evaporated to dryness in a flat-bottomed shallow cup that had been heated to 105°C. This was done immediately to prevent the ethanol from evaporating. Residue was heated to 105°C and weighed until the weight remained constant. Soluble content ethanol is calculated on dry powdered paprika leaves, stated as a ethanol soluble content percentage (General Director of Pharmaceutical Care and Medical Devices, 2017).

Determination of Water-Soluble Content

A volume of 100 mL of water that has been saturated with kloroform and 5 grams of paprika leaves dry powder were macerated for 24 hours in a plugged flask while being shaken for the first 6 hours and then left for the remaining 18 hours. In a shallow, flat-bottomed dish that has been heated to 105°C, 20 mL of the filtrate was then evaporated to dryness. Residue was heated to 105°C and weighed until the weight remained constant. Paprika leaves dry powder was used to calculate the water-soluble content, which was stated as a water-soluble content percentage (General Director of Pharmaceutical Care and Medical Devices, 2017).

Determination of Drying Shrinkage

Weigh 1 to 2 grams of paprika leaves dry powder and add them to a covered porcelain crucible that has already been heated for 30 minutes at 105°C. Shaking the porcelain crucible causes paprika leaves dry powder to spread out into a layer between 5 mm and 10 mm thick. Place the crutch in the oven, then close it after being opened, weighed, and heated again until the weight is constant. Before each drying, let the closed crucible reach room temperature in a desiccator. The percentage of lost weight to the starting weight is used to calculate drying shrinkage (General Director of Pharmaceutical Care and Medical Devices, 2017).

Phytochemical screening

In order to determine the secondary metabolites present in the dry powder and extract, a qualitative phytochemical screening was performed. The substances that were screened included alkaloids, flavonoids, saponins, tannins, and steroids/triterpenes. The samples were subjected to various detection reagents, including Dragendorf for alkaloids, $AlCl_3$ solution for flavonoids, $FeCl_3$ solution for phenol, and Lieberman-Bourchard reagent for steroids and triterpenes. In order to detect saponins, a strong acid solution was administered following an agitated test (Harbone, 1998)(Farnsworth, 1966). The manufacture of each reagent solution adhered to the guidelines outlined in the Indonesian Herbs Pharmacopoeia (General Director of Pharmaceutical Care and Medical Devices, 2017).

Extraction

200 mg of paprika leaves dry powder was macerated using 96% ethanol. For three consecutive days, bottle macerations are kept at room temperature and shielded from the sun. The soaking results were then filtered. A rotary vacuum evaporator was used to concentrate the liquid extract until a thick extract was produced.

Fractionation

Following the dissolution of 5 grams of extract in 50 mL of hot water, the solution was filtered and subsequently transferred into a separating funnel. Subsequently, a liquid-liquid extraction procedure was conducted, employing a hexane solvent followed by an ethyl acetate solvent. The fractionation results yielded three distinct fractions, namely the n-hexane fraction, the ethyl acetate fraction, and the water fraction. In order to obtain a thick fraction, the water fraction was subjected to freeze drying using freeze dry equipment, while the n-hexane fraction and ethyl acetate fraction were subjected to evaporation using a rotating vacuum evaporator. The n-hexane fraction yielded 0.45 grams, the ethyl acetate fraction yielded 0.92 grams, and water yielded 0.02 grams.

Antioxidant activity test

The determination of antioxidant activity was conducted using DPPH with specific modifications, following the methods outlined by Rohman et al. Each extract and vitamin C were prepared with distinct concentrations. A vial with a maximum volume of 1 ml was used to pipette each sample concentration. Subsequently, 2 ml of DPPH solution was added. The DPPH solution was prepared by dissolving 5 mg of DPPH in pro-analytical methanol in a volumetric flask, resulting in a solution with a concentration of 100 µg/ml. Once the mixture was homogenized, it was then placed in a dark environment for a duration of 30 minutes. The absorbance of a blank ethanol was measured using a UV-VIS spectrophotometer at the maximum wavelength of 516 nm. Furthermore, the control absorbance measurements were conducted by combining 2 ml of DPPH solution with 1 ml of pro-analytical methanol and allowing it to sit in a dark room for 30 minutes. The tests were conducted on three separate occasions. Following the measurement of absorbance, the percentage of inhibition is determined using the following equation:

Inhibition % = [(CA-SA)/(CA)] x 100%

CA : Control Absorbance

SA : Sample Absorbance

The IC50 value, which represents the concentration required to inhibit DPPH by 50%, was determined based on the percentage of inhibition. The IC50 value was calculated by analyzing the linear regression curve between the inhibition percent and different sample concentrations (Rohman et al., 2006).

3. Results and Discussion

Table 1.
Physicochemical Parameters of paprika leaves dry powder

Parameters	Result (%w/w)
Water content	3.3*
Total ash content	7.09
Acid insoluble ash content	3.41
Water-soluble extract content	5.89
Ethanol-soluble extract content	7.28
Drying Shrinkage	5.95

Note: * %v/w

Investigations were conducted on a number of physicochemical parameters of paprika leaves dry powder (table 1). Physicochemical parameters are part of the sample/plant characteristics. The water content is intended to be easily ascertained by checking the water content. Water is a good

medium for microbe growth, the examination's findings that the water content was 3.3 % (less than 10%). It can limit the growth of microorganisms on crude drug. Hence the crude drug that can be preserved for a long period with less than 10% water content. To determine the amount of minerals in the sample, the total ash content is calculated. Organic components will evaporate during the measurement of the ash content, leaving only inorganic compounds. Alkali metal and alkaline earth, such as Ca, K, and Na, are measured by their ability to dissolve in water, whereas heavy metal inorganic compounds, such as Pb and Hg, are identified by their inability to dissolve in acid. To determine how much of a compound's content is dissolved in ethanol and water, respectively, crude drug's water soluble content and ethanol soluble content must be determined. To calculate the number of compounds lost during the drying process at 105°C, drying shrinkage is used.

Table 2.
Phytochemical screening of paprika leaves

Plant constituent	Result	
	Dry powder	Extract
Alkaloid	-	-
Flavonoid	+	+
Saponin	+	+
Tannin	-	-
Quinon	-	-
Phenol	+	+
Steroid/Triterpenoid	+	+

Note: + detected
- not detected

The aim of phytochemical screening is to ascertain the presence of secondary metabolites in the sample (Table 2). It showed that dry powder and extract of paprika leaves contains flavonoid, saponin, phenol and steroid/triterpenoid.

Table 3.
Regretion equation and IC 50 sample

	Vitamin C	Ethanol extract	Etil Acetate Fraction	N-hexane Fraction	Water Fraction
Regretion equation	y = 0.0477x + 0.2038	y = 0.0123x + 0.4208	y = 0.0206x + 0.3358	y = 0.0218x + 0.3078	y = 0.0177x + 0.312
IC50 (µg/mL)	6.21	6.44	8.64	8.82	10.17

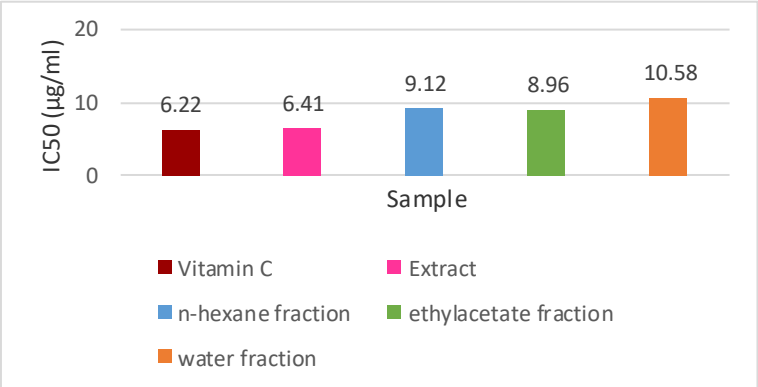

Figure 1. Antioxidant activity test results

The results obtained from the evaluation of antioxidant activity using DPPH indicated that vitamin C exhibited an IC50 value of 6.22 g/ml when used as a reference standard. The ethanol extract of paprika leaves had an IC50 of 6.41 µg/ml, the n-hexane fraction had an IC50 of 9.12 µg/ml, the ethyl acetate fraction had an IC50 of 8.96 µg/ml, and the water fraction had an IC50 of 10.58 µg/ml (Table 3 and Figure 1). Beside vitamin C and carotene content in paprika fruit, the phenol and flavonoid content also contribute to the fruit's antioxidant activity (Chávez-Mendoza et al., 2015). Based on phytochemical screening, it suggested that phenol and flavonoid content also contribute to the paprika leaf's antioxidant activity.

Antioxidants of phenolic compounds achieve these effects primarily through two mechanisms: electron transfer and free radical inactivation. The antioxidant (ArOH) can become radical in the first mechanism when a hydrogen atom is removed by the free radical (R *).

$$R^{\cdot} + ArOH \rightarrow RH + ARO^{\cdot}$$

In the second method, the free radical changes into a cation radical when the antioxidant gives it an electron (Vuolo et al., 2019).

$$R^{\cdot} + ArOH \rightarrow RH^{-} + ARO^{\cdot +}$$

Flavonoids possess the capacity to function as antioxidants by directly capturing free radicals via the provision of hydrogen atoms. Radicals oxidize flavonoids, resulting in the formation of more stable and less reactive radicals. As per the reaction equation depicted in Figure 2 (Arifin & Ibrahim, 2018).

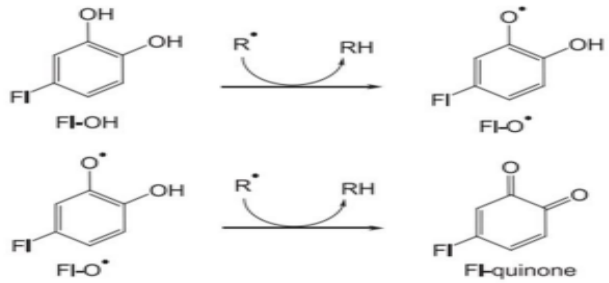

Figure 2. Capture of reactive oxygen species (ROS) by flavonoid
R• is ROS, Fl-OH is flavonoid, Fl-O• is phenoxyl radical reacts with the second radical, producing a stable quinone (Arifin & Ibrahim, 2018)

When comparing this research to earlier studies, it is important to acknowledge that there have been multiple studies conducted on the phytochemical profiles and antioxidant activities of Capsicum annuum, which includes red chili pepper (*Capsicum annuum* L.) (Jang et al., 2024), as well as other types of pepper (Choi et al., 2023)(Kim et al., 2011). These investigations further emphasized the significance of the ripening stage and cooking techniques in determining the presence of bioactive chemicals and antioxidant properties in pepper leaves. Nevertheless, the ongoing research primarily concentrates on *Capsicum annuum var. grossum* and offers a comparative examination of its characteristics.

4. Conclusion

The dry powder derived from paprika leaves possesses certain physicochemical features that contribute to its distinctive characteristics. Flavonoid, saponin, phenol, and steroid/triterpenoid are present in the dry powder and extract of paprika leaves. The findings of the antioxidant test indicated that the standard vitamin C exhibited an IC50 value of 6.22 µg/ml. In contrast, the extract, n-hexane fraction, ethyl acetate fraction, and water fraction demonstrated IC50 values of 6.41, 9.12, 8.96, and 10.58 µg/ml, respectively. Paprika leaves exhibited promising potential as a viable natural reservoir of antioxidants.

The phytochemical screening detected the existence of diverse beneficial substances, but the study did not investigate the identification and measurement of individual components. Utilizing sophisticated techniques like chromatography coupled with mass spectrometry would allow for the

identification and measurement of specific phytochemicals, clarifying their role in the reported antioxidant activity.

Further investigation could prioritize the isolation and characterization of distinct bioactive components from the leaves of *Capsicum annuum var. grossum* through the utilization of sophisticated analytical methods. This analysis would offer valuable information on the range of different chemicals found in the leaves and help us understand how the structure of these chemicals relates to their biological activity.

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