INTRODUCTION
Antioxidants protect the human body against free radicals, especially reactive oxygen species (ROS) [1]. ROS, which are products of the metabolism process, can be found in all living cells, such as the respiratory chain in the mitochondria. UV lights, pollutants, radiation, xenobiotic, and bugs are the most common sources of extracellular ROS [1]. Several types of ROS exist, including hydrogen peroxide, superoxide anion radicals, reactive hydroxyl radicals, singlet oxygen, hypochlorite radicals, nitric oxide radicals, and lipid oxide radicals [1, 2]. ROS are not harmful in controlled conditions [4]. The antioxidative action mechanism interferes with ROS in many ways, such as scavenging free radicals, protecting cells from lipid peroxidation-induced damage, inhibiting malondialdehyde formation, protecting the cell from HBP-induced damage, inhibiting thioribarbituric acid formation, and reducing glutathione depletion [4]. The flavonoid action mechanism interferes with ROS and quenches free radicals by chelating metals, stimulating antioxidant enzymes, and releasing a hydrogen atom that suppresses glutathione depletion [4]. The flavonoid action mechanism interferes with ROS and quenches free radicals by chelating metals, stimulating antioxidant enzymes, and releasing a hydrogen atom that suppresses glutathione depletion [4].

The antioxidative activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. These extracts were then analyzed using thin layer chromatography (TLC) and phytochemical tests to identify the extracts' secondary metabolites.

RESULTS
The phytochemistry tests were positive for glycosides, alkaloids, steroids, triterpenoids, tannins, and flavonoids. The TLC analysis revealed that the extracts contained two to three organic compounds. The ethanol Hibiscus sabdariffa extracts with an IC50 value 103.63 ppm showed stronger antioxidant activity than the ethyl acetate extract.

CONCLUSION
Ethanol Hibiscus sabdariffa extracts may be a potential source of natural antioxidant.

KEYWORDS
Antioxidant, DPPH, Hibiscus sabdariffa, IC50, Phytochemical

INTRODUCTION
Antioxidants protect the human body against free radicals, especially reactive oxygen species (ROS) [1]. ROS, which are products of the metabolism process, can be found in all living cells, such as the respiratory chain in the mitochondria. UV lights, pollutants, radiation, xenobiotic, and bugs are the most common sources of extracellular ROS [1]. Several types of ROS exist, including hydrogen peroxide, superoxide anion radicals, reactive hydroxyl radicals, singlet oxygen, hypochlorite radicals, nitric oxide radicals, and lipid oxide radicals [1, 2]. ROS are not harmful in controlled amounts, but if the production of ROS exceeds endogenous antioxidants which produces within the body, oxidative stress will occur and may lead to DNA mutation and protein denaturation and affect carbohydrate and lipid metabolism [1]. Oxidative stress is also associated with the etiology of many diseases, such as cancer, arthritis, cardiovascular disease, and diabetes [1]. Therefore, maintaining a balance between antioxidant levels and ROS is vital [3]. Antioxidants may interfere with ROS in many ways, such as scavenging free radicals, protecting cells from lipid peroxidation-induced damage, inhibiting malondialdehyde formation, protecting the cell from HBP-induced damage, inhibiting thioribarbituric acid formation, and reducing glutathione depletion [4]. The flavonoid action mechanism interferes with ROS and quenches free radicals by chelating metals, stimulating antioxidant enzymes, and releasing a hydrogen atom that suppresses the enzymes that lead to free radical formation [1].

There are two classes of antioxidants, natural and synthetic antioxidants. Natural antioxidants are preferred because synthetic antioxidants produce substances that have a carcinogenic effect [5]. Moreover, natural antioxidants can be easily found in all plant parts [4]. Therefore, research concerning natural antioxidants from plants has intensified. For example, Hibiscus sabdariffa, commonly known as Roselle and part of Malvaceae family, and is widely cultivated in tropical and subtropical countries (i.e., Indonesia) [4]. Roselle calyces are consumed in herbal drinks, jam, pudding, teas, gelatin, and cake [4]. Roselle has been consumed as an herbal drink due to its medicinal properties; it has anti-inflammatory, antibacterial, antipyretic, antifungal, antinociceptive, antiparasitic, hepatoprotective, nephroprotective, and diuretic effects due to its potent antioxidants, including anthocyanins, flavonoids, organic acids, and polysaccharides [4]. The amount of antioxidants in Hibiscus sabdariffa plants varies based on environment, ecology, variety, genetics, and harvest conditions [4]. The Hibiscus sabdariffa antioxidants may be used as anticancer therapies due to their cytotoxicity [4]. For example, the protocatechuic acid, polyphenol, and anthocyanin in Roselle extracts has an apoptotic effect on cancer cell lines, reduces carcinogenic action, and inhibits tumour metastasis in tissue [4]. Therefore, the purpose of this research is to evaluate the phytochemical profile and antioxidant activity of Hibiscus sabdariffa. The Hibiscus sabdariffa sample was extracted and macerated in three different solvents: ethyl acetate, ethanol, and n-hexane. Then, the antioxidant activity was evaluated using the DPPH method. Finally, the extract was analyzed using a phytochemistry test and thin layer chromatography to identify its organic compounds.

MATERIALS AND METHODS
Extraction of Hibiscus sabdariffa samples
The Hibiscus sabdariffa flowers were purchased in a local market, the AEON Mall, in Serpong. The flowers were dried, ground, and immersed in a container with three different solvents: ethyl acetate, ethanol, and n-hexane. The maceration process was done in stages. First, the flowers were macerated into 500 ml of the first solvent (n-hexane) for 24 h. Then, they were filtered and the solid filtrate was macerated into 500 ml of the second solvent (ethyl acetate). After filtration, the solid filtrate was macerated into 500 ml of the third solvent (ethanol). Subsequently, liquid filtrate of n-hexane, ethyl acetate and ethanol respectively, were concentrated using a vacuum-drying process in a rotary evaporator for 12–48 h.

Antioxidant analysis
The DPPH (2,2-diphenyl-1-picrylhydrazyl) method was used to determine the Hibiscus sabdariffa extract’s antioxidant activity. The ethanol extracts in five different concentration (3.75; 6.25; 12.5; 25; 50) and 100 ml ethyl acetate were placed into a microcentrifuge tube. Then, 2.9 ml of the DPPH molecule at a 0.004% concentration was added to the sample tube, positive control

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Hibiscus sabdariffa

The glycosides test was conducted by evaporating 0.1 ml of the extract without the added sample and the absorbance of the sample refers to the absorbance of the DPPH with the added sample.

**Thin layer chromatography**

Thin layer chromatography (TLC) was used to determine the number of organic compounds in the extract. This method separated the organic compound in the sample based on polarity. A thin plate was coated with an inert material, such as silica gel used as a stationary phase. The mobile phase is a mixture of chloroform (CHCl3) and methanol (CH3OH) in ratio 4:1, which was placed inside the chamber. Chemical compounds containing in the sample migrated and eluted at different rates based on its polarity. After completion, spots of chemical compound in the TLC plate was visualized by UV lamp at the wavelength of 254 nm. The compound was identified based on the retention factor (Rf) of its spot. The retention factor is a parameter for interpretation of the migrated sample, which represent the position of the spot in the stationary phase after elution. The Rf formula is as follows:

\[ Rf = \frac{\text{Distance moved by sample}}{\text{Distance moved by solvent}} \]

**Phytochemical test**

Phytochemical tests were used to determine secondary metabolites, such as glycoside, alkaloid, steroid, triterpenoid, saponin, tannin, and flavonoid, containing in the Hibiscus sabdariffa extracts. The test procedures for each metabolite are the following:

**Glycoside test**

The glycosides test was conducted by evaporating 0.1 ml of the Hibiscus sabdariffa ethanol, ethyl acetate, and n-Hexane extracts separately, over a water bath. The remaining solution was diluted in 5 ml of acetic acid anhydrate. Then, 10 drops of concentrated sulfuric acid were added. The formation of a blue or green product indicated a positive result for glycosides.

**Alkaloid test**

The alkaloid test was conducted by evaporating the solution in a porcelain cup. The residue was diluted in 5 ml of 2 N HCl Then, it was divided into three tubes. The first tube was the blank, and it was mixed with 2 N HCl. Three drops of a Dragendorff reagent were added to the second tube. Then, three drops of a Mayer reagent were added to the third tube. The formation of a yellow precipitate in the third tube and orange precipitate in the second tube indicated that the solution was positive for alkaloids.

**Steroid and triterpenoid test**

The Liebermann-Burchard reaction was used in the steroid and triterpenoid test. This test was conducted by evaporating 2 ml of the solution in a porcelain cup. The residue was then diluted in 0.5 ml of chloroform (CHCl3) and 0.5 ml of anhydrate acetic acid. Then, 2 ml of concentrated sulfuric acid was added through the tube wall. The presence of steroids was indicated by the formation of a blue-green ring, whereas the presence of triterpenoids was indicated by the formation of a violet or brownish ring.

**Saponin test**

The saponin test was conducted by adding 10 ml of the solution to a tube. The tube was then shaken vertically for approximately 10 seconds. For the next 10 seconds, the tube was kept in a standing position. If the solution was positive for saponins, the presence of stable foam formation around 1–10 cm in height was visible for less than 10 min. The addition of 1 drop of 2 N HCl was used in the confirmation test as it showed that the foam still existed.

**Tannin test**

The tannin test was conducted by dissolving 1 ml of the Hibiscus sabdariffa extracts in a solution of 10% (v/v) of FeCl3. A greenish-black or dark blue color indicated the presence of tannin.

**Flavonoid test**

Flavonoid screening was conducted by evaporating 1 ml of the solution in a porcelain cup until it was dry. The remaining solution was moistened with acetone and mixed with smooth oxalic acid and boric acid powder. The porcelain cup was heated at boiling temperature (80 °C - 100 °C) in a water bath. Then, the residue was mixed with 10 ml of ether. The porcelain cup was read under a 365 nm UV light. The presence of a yellow fluorescence indicated a positive result.

**RESULTS AND DISCUSSION**

**Antioxidant activity OF Hibiscus sabdariffa**

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method was used to analyze the antioxidant activity of the Hibiscus sabdariffa extracts. This method has been widely used to evaluate antioxidant activity due to its simplicity, low cost, and efficiency [6]. The presence of antioxidants may reduce the DPPH by binding a hydrogen atom from the antioxidant and a nitrogen atom from the DPPH [4]. The effect of the antioxidant is evident when the original violet color of the DPPH changes to yellow [6]. The absorption of the DPPH is monitored using a 517 nm UV light [6]. In this work, non-polar n-hexane extract of Hibiscus sabdariffa was not evaluated its antioxidant activity by DPPH because it cannot dissolved well in DPPH reagent, therefore polar and semipolar extracts such as ethanol and ethylketate extract are more suitable to be analyzed by DPPH method [7].

The DPPH method uses the half maximal inhibitory concentration (IC50) value as a parameter for interpretation of antioxidant activity. IC50 represents the concentration of substance (extract) that reduces the DPPH activity by 50% [6]. IC50 value is inversely proportional to the antioxidant activity, so the lower IC50 value of the substrate or extract, the higher its antioxidant activity [6]. The results of antioxidant activity evaluation of Hibiscus sabdariffa extracts are summarized in table 1.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>% Inhibition</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.75</td>
<td>2.8</td>
<td>0.0326</td>
</tr>
<tr>
<td>6.25</td>
<td>3.7</td>
<td>0.0173</td>
</tr>
<tr>
<td>12.5</td>
<td>7.9</td>
<td>0.0011</td>
</tr>
<tr>
<td>25</td>
<td>15.1</td>
<td>0.0142</td>
</tr>
<tr>
<td>50</td>
<td>24.1</td>
<td>0.0085</td>
</tr>
</tbody>
</table>

The linear equation that represented the relationship between the IC50 value and the concentration of the Hibiscus sabdariffa ethanol extract is \( y = 0.4668x + 1.6261 \) and \( R^2 = 0.984 \). Fig. 1 illustrates the relationship between the percentage of DPPH activity inhibition and the concentration of the Hibiscus sabdariffa ethanol extract. The x-axis in the graph represents the concentration of the ethanol extract required to reduce the DPPH activity by 50%, while the y-axis represents the IC50 value.
Fig. 1: The relationship between the percentage of inhibition and concentration of the *Hibiscus sabdariffa* ethanol extracts

Based on the results, none of the concentrations that were analyzed achieved a 50% inhibition of the DPPH activity. However, the IC\textsubscript{50} value of the ethanol extract can be calculated using the linear equation $y = 0.4668x + 1.6261$, where the $y$-axis represents the percentage of DPPH activity inhibition and the $x$-axis represents the concentration of the ethanol extract. Therefore, the IC\textsubscript{50} value required to inhibit 50% of the DPPH activity in the ethanol extract was 103.63, which suggests moderate antioxidant activity. The percentage of inhibition in the highest extract concentration, 50 µg/ml, was 24.1%.

The ethyl acetate extract test results are summarized in table 2.

### Table 2: The antioxidant activity of *Hibiscus sabdariffa* ethyl acetate extract

<table>
<thead>
<tr>
<th>Concentrations (ppm)</th>
<th>% Inhibition</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.75</td>
<td>1.1</td>
<td>0.0109</td>
</tr>
<tr>
<td>6.25</td>
<td>3.6</td>
<td>0.0106</td>
</tr>
<tr>
<td>12.5</td>
<td>3.7</td>
<td>0.0065</td>
</tr>
<tr>
<td>25</td>
<td>7.5</td>
<td>0.0457</td>
</tr>
<tr>
<td>50</td>
<td>11.8</td>
<td>0.0018</td>
</tr>
</tbody>
</table>

The linear equation that represents the relationship between the IC\textsubscript{50} value and the concentration of the *Hibiscus sabdariffa* ethyl acetate extract is $y = 0.2163x + 1.3277$, where $R^2 = 0.9594$. The $x$-axis represents the concentration of the ethyl acetate extract required to inhibit 50% of the DPPH activity, while the $y$-axis represents the IC\textsubscript{50} value. Fig. 2 illustrates the relationship between the percentage of DPPH activity inhibition and the *Hibiscus sabdariffa* ethyl acetate extract concentration.

The IC\textsubscript{50} value of the ethyl acetate extract can be calculated using the linear equation $y = 0.2163x + 1.3277$, where the $y$-axis represents the percentage of DPPH activity inhibition and the $x$-axis represents the concentration of the ethyl acetate extract. Therefore, the IC\textsubscript{50} value of ethyl acetate extract that is required to inhibit 50% of the DPPH activity is 225.02, which indicates that ethyl acetate extract of *Hibiscus sabdariffa* has a weak antioxidant activity. The percentage of inhibition at the highest extract concentration (50 µg/ml) was 11.8%.

### Table 3: A comparison of the IC\textsubscript{50} values of the *Hibiscus sabdariffa* ethyl acetate and ethanol extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>IC\textsubscript{50} value (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>225.02</td>
</tr>
<tr>
<td>Ethanol</td>
<td>103.63</td>
</tr>
</tbody>
</table>

The IC\textsubscript{50} values are separated into four levels: very strong, strong, moderate, and weak. An IC\textsubscript{50} value below 50 ppm indicates very strong antioxidant activity, an IC\textsubscript{50} value of 50–100 ppm indicates strong antioxidant activity, an IC\textsubscript{50} value of 101–150 ppm indicates moderate antioxidant activity, and an IC\textsubscript{50} value above 150 ppm indicates weak antioxidant activity. Therefore, the *Hibiscus sabdariffa* ethanol extract had stronger antioxidant activity than the ethyl acetate extract as the ethanol extract had moderate antioxidant activity, while the ethyl acetate extract had weak antioxidant activity.

The results from the current study are different from previous research, in which the ethanol extract of *Hibiscus sabdariffa* had a low IC\textsubscript{50} value of 46.13±3.37 ppm, indicated a strong antioxidant activity [9]. The differences may be the result of many factors, including the different varieties of the flowers, genetics, the environment of the plant; the choice of solvent; and the solubility of the product [1]. Ethyl acetate is typically used to extract terpenoids, flavonoids, and alkaloids, while ethanol is typically used to extract polyphenols, saponins, flavanols, and alkaloids [10].

### Thin layer chromatography

The retention factor (RF) value and TLC results of the *Hibiscus sabdariffa* extracts are displayed in table 4 and fig. 3, respectively.

### Table 4: The retention factor (RF) of n-hexane, ethanol of the *Hibiscus sabdariffa*

<table>
<thead>
<tr>
<th>Extract</th>
<th>RF value</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td></td>
<td>0.143</td>
<td>0.671</td>
<td>0.742</td>
<td>0.914</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol</td>
<td></td>
<td>0.114</td>
<td>0.671</td>
<td>0.842</td>
<td>0.857</td>
<td>0.942</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td></td>
<td>0.228</td>
<td>0.643</td>
<td>0.721</td>
<td>0.886</td>
<td>-</td>
</tr>
</tbody>
</table>
The phytochemical test for the *Hibiscus sabdariffa* extracts showed that the sample was positive for glycosides, alkaloids, steroid, triterpenoid, tannin, and flavonoids. Only the ethyl acetate and n-Hexane extracts were positive for glycosides, alkaloids, and steroids. Triterpenoid is only present in the ethanol extracts, whereas tannin is present in the ethanol and ethyl acetate extracts. Flavonoids are present in all the extracts. The results agree with previous several studies that found that *Hibiscus sabdariffa* mostly contains flavonoids, anthocyanins, and polyphenolic acids [12].

Flavonoids are compounds extracted from the plant that form the aroma and color of the flowers; they can be found in several parts of a plant [13]. The subclasses of the flavonoids contained in the *Hibiscus sabdariffa* are as follows: quercetin, hibiscitrin, chlorogenic acid, isorhamnetin, gossypetin, protocatechic acid, gossypyrin, eugenol, pelargonic acid, and sterols [1]. Flavonoids have many benefits in human health due to several mechanisms, including scavenging free radicals. Flavonoids react with and stabilize radical compounds, making them less radical, through direct and indirect pathways. For example, rutin and epicatechin have inhibitory properties that affect the XO enzyme [12, 13]. This study is limited to the qualitative measurement of secondary metabolites.

**CONCLUSION**

The *Hibiscus sabdariffa* ethanol extracts showed potent antioxidant activity and had many organic compounds. Therefore, *Hibiscus sabdariffa* may be used as a natural antioxidant and developed as a therapeutic alternative for cancer and inflammatory disease. Further study should evaluate the specific amount and type of antioxidants and their therapeutic applications.

**ACKNOWLEDGMENT**

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**AUTHORS CONTRIBUTIONS**

All the authors have contributed equally.

The TLC analysis was used to qualitatively identify the number of organic compounds in the *Hibiscus sabdariffa* extracts. The analysis revealed that n-hexane and ethyl acetate extracts contain four spots of chemical compounds, while the ethanol extract contains five spots of chemical compounds. The n-hexane and ethanol extracts had two similar spots with the same Rf values, 0.571 and 0.742. However, further study can be conducted to evaluate the specific organic compounds. For example, to evaluate the presence of flavonoids, ammonia may be sprayed on the plate, and the test results may be read under a 366 nm UV light. A blue colored notch at Rf 0.54 and 0.92 indicates a positive result [11].

**PHYTOCHEMICAL PROFILE**

The phytochemical profile of *Hibiscus sabdariffa* is summarized in table 5.

**Table 5: The phytochemical composition of *Hibiscus sabdariffa***

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Ethanol</th>
<th>Ethyl Acetate</th>
<th>N-Hexane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoside</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannin</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**CONFLICT OF INTERESTS**

The authors declare no conflict of interest in this study.

**REFERENCES**