

## SCREENING OF PHYTOCHEMICALS IDENTIFIED AND ANTIOXIDANT ACTIVITY OF *Aegiceras corniculatum* EXTRACT THAT CULTIVATES FROM THE MANGROVE AREA OF TARAKAN CITY

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### ABSTRACT

*Aegiceras corniculatum* is mangrove vegetation that is widely spread in the mangrove area in Karang Rejo, Tarakan City and it has the potential for the development of drugs from natural ingredients from tropical sea coasts. The purpose of this study was to determine the water content, bioactive compounds, and antioxidant activity of *A. corniculatum*. The methods used were the analysis of water content, analysis of bioactive compounds using the phytochemical method, and analysis of antioxidant activity using the DPPH method. This study showed the percentage of moisture or water content in the leaf and fruit extracts of *A. corniculatum* were 1.83% and 1.95%, respectively. The bioactive compounds detected in the leaf and fruit extracts came from flavonoids, alkaloids, tannins, phenol hydroquinone, and saponins. The antioxidant analysis showed that the fruit and leaf crude extracts had an antioxidant activity with IC<sub>50</sub> values were 129.7 ppm and 365.8 ppm, respectively.

**Keywords:** *antioxidants; bioactive compounds; free radical; IC<sub>50</sub>*

### INTRODUCTION

A mangrove forest is a coastal forest ecosystem consisting of groups of vegetation that can live and adapt to an environment of fluctuating salinity. The city of Tarakan is a small island with an area of 657.33 km<sup>2</sup> located in the north of Borneo Island and has an area of mangrove forest resources of 1,224.8 ha (Rachmawani *et al.*, 2010). Mangrove Forest of Karang Rejo is a mangrove conservation forest area in Tarakan City. The extent of the Karang Rejo mangrove area is 22 hectares. This forest provides productive natural resources, including fish resources (fish, shrimp, crabs, gastropods, shellfish), various animals, snakes, monkeys, proboci's monkeys, multiple types of birds, and others, as well as multiple types of mangrove vegetation, including; *Bruguiera* sp., *Avicennia* sp., *Coneratia* sp, *Nypa fructicans*, *Aegiceras* sp., and others. The most common mangrove vegetation found at the edge of the coastal mangrove forest

in Tarakan City is *Aegiceras corniculatum*.

*Aegiceras corniculatum* is a type of mangrove vegetation that grows in the mainland part of the tidal forest, in the form of a shrub or small tree that is always green and grows straight with a tree height of up to 6 meters. The outer bark is gray to reddish-brown, fissured, and has many lenticels. The leaves are light-skinned, shiny green on top and pale green on the bottom, with an inverted oval to the elliptical shape. The crescent-shaped fruit is green or yellowish. *A. corniculatum* has also been investigated regarding the potential of drugs such as the anticoagulant (Tangkery et al., 2013). Trianto et al. (2004) found the antibacterial activity of *A. corniculatum* extract against *Vibrio harveyi* and *Vibrio parahemolyticus*.

Mangrove vegetation is also known to have medicinal potential, such as antioxidant activity. Previous research has established that fruit and bark extract of *Bruguiera gymnorrhiza* had an IC<sub>50</sub> of 13.46 and 56.93 ppm, respectively (Sudirman et al. 2014); Nurjanah et al. 2015). Recent work by Haq et al. (2014) reported that the bark extract of *Sonneratia alba* had an IC<sub>50</sub> of 38 ppm. Imra et al. (2017) showed that methanol extract of *Nypa fruticans* has a very strong antioxidant activity with an IC<sub>50</sub> value of 22.06 ppm. The active compounds that generally play a role in antioxidant activity are tannins, flavonoids, phenolics, saponins, and terpenoids. Margareta et al. (2011) stated that phenolic compounds have a hydroxyl group that captures free radical activity in their molecular structure. Considering all of this evidence, it seems that *A. corniculatum* also contains components that have antioxidant activity. Seeing its benefits, this plant should be able to trace the content of antioxidant activities compounds and be used as a natural source of antioxidants, considering that its utilization is still around the coast and is used traditionally.

Screening of phytochemicals is a crucial way to explore the ability of the natural product. Several studies about phytochemicals in natural products have led to the development of the use of natural products for various purposes. *Saoropus androgynus*, *Apium graveolens* (Awaludin et al., 2019), *Nephrolepis biserrata* (Maulianawati et al. 2018; 2020), *Centella asiatica* (Rukisah et al. 2019) have been testing for phytochemical and used as antibacterial, molting stimulant, sex-reversal, and accelerate gonadal maturation. *Geloina coaxans* from the mangrove area showed antibacterial activity against *Vibrio parahaemolyticus* (Weliyadi et al., 2018). Currently, there are no data on the phytochemicals of *A. corniculatum* from Tarakan. For the first time, the present research explores the active compounds and antioxidant activity of *A. corniculatum*.

## RESEARCH METHOD

### **Materials**

The fruit and leaves of *A. corniculatum*. Other materials used include chemicals for extraction of active compounds (Ethanol 97% Merck), chemical analysis (moisture content and ash content), antioxidant (DPPH SIGMA) and phytochemical testing. The main equipment used in this research is, *shaker*, *vacuum rotary evaporator* RV IKA 05.

### **Corniculatum extract preparation**

The leaves and fruits of *A. corniculatum* were washed with running water, then cut into small pieces, and dried for two days. Drying was continued using an oven at 50°C for 24 hours. Dried fruit and leaves were mashed using a blender.

### **Extraction of *A. corniculatum* (Harbone 1987)**

The powder of leaves and fruit was extracted by the maceration method using 96% ethanol solvent for 48 hours, then filtered (Repeated thrice). The collected filtrate was concentrated using a vacuum rotary evaporator at 40-50°C to obtain a crude extract. The extract was weighed to get the extract yield. The extract was then analyzed for phytochemicals to determine the components of the active compounds. The antioxidant activity test used the 1,1-diphenyl-2-picrylhydrazil (DPPH) method.

### **Moisture Analysis (AOAC 2005)**

Moisture content or water content was determined by using the oven drying method (AOAC 2005). The homogeneous sample was weighed at approximately 1 g ( $W_1$ ) and placed in an empty cup ( $W_0$ ). The cup was then closed and placed in an oven at 105°C for 4 hours or until the weight was constant ( $W_2$ ).

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where  $W_1$  = Sample weighed before dried,  $W_2$  = Sample weighed after dried

### **Determination of Active Compounds (Harbone 1987)**

Active compound testing was carried out to know whether or not bioactive components were contained in the leaf and fruit extract of *A. corniculatum*. The test for alkaloids, steroids/triterpenoids, tannins, saponins, and phenol hydroquinone were described below.

### **Alkaloids**

A 0.01 g of extract was dissolved in a few drops of 2N sulfuric acid. The test used three reagents, namely Dragendorff's, Meyer's, and Wagner's reagent. The test results are declared positive if with Dragendorff's reagent, a red to orange precipitate was formed, a yellowish-white precipitate with Meyer's reagent, and a brown precipitate revealed with Wagner's reagent.

### **Steroids/Triterpenoids**

A total of 0.01 g of extract was dissolved in 2 mL of chloroform in a dry test tube, followed by adding ten drops of acetic anhydride and three drops of concentrated sulfuric acid. A positive reaction is shown with the formation of red color, and then it turns blue and green.

### **Saponins**

Saponins can be detected by foam test in hot water. The foam is stable for 30 minutes and does not disappear after the addition of 1 drop of 2N HCl, indicating the presence of saponins.

### **Phenol Hydroquinone**

A 0.01 g of extract was diluted with 20 mL of 70% ethanol. 1 mL of the solution was taken, and two drops of 5% FeCl<sub>3</sub> solution were added. The formation of the green or bluish-black color showed the presence of phenolic compounds.

### **Determination of Antioxidant Activity**

Antioxidant activity of crude extract of leaves and fruit of *A. corniculatum* was determined using the 1,1-diphenyl-2-picrylhydrazil (DPPH) method based on the Blois method (1958), which concentration has been modified. The DPPH test method is based on the ability of these antioxidant substances to neutralize free radicals. The free radicals used is 1,1-diphenyl -2- picrylhydrazil (DPPH). The free radical DPPH is a synthetic radical which is stable at room temperature and soluble in polar solvents such as methanol and ethanol (Molyneux 2004).

The extract was dissolved in ethanol and prepared with 250, 500, 750, and 1000 ppm diluted concentrations. Ascorbic acid was used as a positive control with a concentration of 2, 4, 6, 8, and 10 ppm. The DPPH reagent solution is made by dissolving DPPH in ethanol with a concentration of 1 mM, kept at a low temperature, and protected from light. 4.5 mL of the extract solution or positive control solution was put in a test tube and then reacted with 0.5 mL of DPPH solution. The test tube was covered with aluminum foil and incubated at a temperature of 37° C for 1 hour. After incubation, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm.

A compound can be said to have antioxidant activity if it can donate its hydrogen atom, which is characterized by a purple to yellow color change (Molyneux 2004). The percentage of free radical inhibition expresses the antioxidant activity. Absorbance from solution blank was also measured to calculate the percent inhibition. The antioxidant activity (%) of the extract was calculated according to the following equation:

$$\% \text{ Scavenging Activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

The concentration and inhibition values of the extract were plotted on the x and y axes, respectively. The equation of the line obtained in the form of  $y = b \ln(x) + a$  is used to find the IC (*Inhibitory Concentration*) value by stating the “y” value as 50 and the “x” value as IC<sub>50</sub>. The IC<sub>50</sub> value represents the concentration of the sample solution required to reduce DPPH by 50%. The smaller the IC<sub>50</sub> value, the higher the antioxidant activity. A compound is said to be a very strong antioxidant if the IC<sub>50</sub> value is less than 50 ppm, strong if the IC<sub>50</sub> value is between 50-100 ppm, mild if the IC<sub>50</sub> value ranges from 100-150 ppm, and weak if the IC<sub>50</sub> value range between 150-200 ppm (Blois 1958).

## RESULTS AND DISCUSSION

### *Extract of A. corniculatum*

*Aegiceras corniculatum* is vegetation that is scattered in the mangrove swamp forest. This vegetation has oval leaves with small white flowers. The fruit size is about 5 cm, thick pencil-shaped curved, and pointed (Figure 3. a), spread in Asia and Australia. The vegetation of *A. corniculatum* used in this study was the leaves and fruit. Leaf and fruit powders that have gone through the preparation process are tested for moisture content. The principle of determining the moisture or water content was to evaporate the water in the material by heating. Heating is carried out at a temperature of 110 °C. The material is then weighed to a constant weight, which means all the water has been evaporated. The results showed that the moisture content of leaf and fruit extracts of *A. corniculatum* were 1.83% and 1.95%, respectively. The moisture content of the fruits is higher than the leaves. The water content of the leaves and, nipah fruit was much lower than that obtained on the *Rhizophora mucronata* fruit, namely 52.38% (Mile et al, 2021).



Figure 3. (a) *A. corniculatum*, (b) maceration processed, (c) fruits preparation, and (d) leaves preparation

Powder of leaves and fruits maceration were carried out using ethanol solvent in a ratio of 1:5 (w/v) for 48 hours. This process was resulting green color for leaves extract and a brownish-yellow for fruit extract. The crude extract was then tested for the content of active compounds and antioxidants.

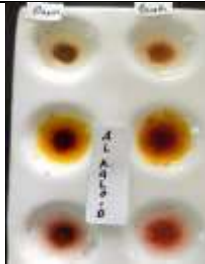

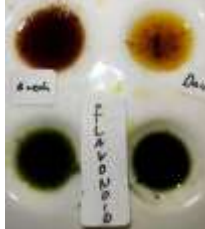
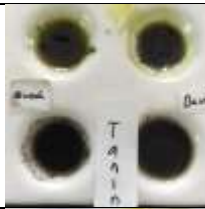


#### **Active Compounds of *A. corniculatum* Extracts**

The active compounds of the leaf and fruit extract of *A. corniculatum* were tested using the phytochemicals method. It aims to preliminary investigate the extract's bioactive compound, including flavonoids, tannins, saponins, phenol hydroquinone, alkaloids, and steroids. The test results for active compounds can be seen in Table 1.

The phytochemical test showed that the leaf extract and fruit contain flavonoids, tannins, phenol hydroquinone, saponins, and alkaloids. Active compounds that generally play a role in antioxidants are tannins, flavonoids, and saponins (Harbone 1987). Margareta *et al.* (2011) reported that antioxidants naturally derived from plants, such as phenolic compounds having a hydroxyl group at the structure of the molecule, which has free radical scavenging activity. If the hydroxyl group is more than one, the antioxidant activity is robust. Supriatna *et al.* (2019) get total phenol content and potential flavonoids antioxidant on skin stem mangroves. Phenolic compounds are soluble compounds in polar and slightly polar compounds.

Phenols include compounds of plant origin and have the same characteristics, namely an aromatic ring containing one or two hydroxyl groups: Monocyclic phenol, phenol propanoic, and phenolic quinones. Quinones are colored compounds and have a basic chromophore, namely the chromophores of benzoquinones (Harborne 1987).

Table 1. The active compound of leaf and fruit extract of *A. comiculatum*

Compound active	Extract		Result	Picture
	Leaf	Fruit		
Alkaloids				
- Wagner	+	+	brown precipitate	
- Meyer	+	+	red-orange precipitate	
- Dragendrof	+	+	White-yellowish precipitate	
Steroids	-	-	Blue and green	
Flavonoids	+	+	Red, yellow, or orange on coating amyl alcohol	
Tannin	+	+	Dark-gree precipitate or dark blue	
Phenol hydroquinone	+	+	Green-blue	
Saponins	+	+	Stable foam after 30 seconds	

Information : (+) detected (-) not detected

Leaf and fruit extracts were identified as positive for flavonoids. Flavonoids are the largest group of phenols. Malik *et al.* (2017) get potential flavonoid compounds antioxidant on mangroves. Compounds classified as tannins are polyphenolic compounds that contain hydroxyl groups and other groups to form hydroxyl groups complex strongly with proteins. Tannins are identified positive on the leaf and the fruit extract of *A. corniculatum*. Tannins spread wide throughout part of the plant and are potentially found in mangroves (Das *et al.*, 2020). Leaf and fruit extracts contain saponins, the main triterpenoids group. Saponins are triterpenoid glycosides which It is also commonly found in plants. Amrati *et al.* (2020) get activity antiproliferative with antioxidants on compound saponins.

### Activity Antioxidant Extract *A. corniculatum*

The antioxidant activity test was carried out using the *1,1-diphenyl -2 picrylhydrazil* (DPPH) method. DPPH is a free radical compound that is the most stable compared to other types of free radicals. If stored in dry storage, this compound will be permanently stable over the years. Ascorbic acid antioxidant was used as a comparison and positive control—the IC<sub>50</sub> value was commonly used to interpret the results. The smaller the IC<sub>50</sub> value, the higher the antioxidant activity (Molyneux 2004). The antioxidant activity of ascorbic acid, leaf, and fruit extracts of *A. corniculatum* can be seen in Table 2.

Table 2. The IC<sub>50</sub> value of leaf and fruit extracts of *A. corniculatum* and ascorbic acid standards using the DPPH method.

Sample	Percent inhibition (%)				IC <sub>50</sub>
	250 ppm	500 ppm	750 ppm	1000 ppm	
Ascorbic acid (0.1 M)	-	-	-	-	78.9
Leaf extract	44.4	60.0	66.7	96.7	365.8
Fruit extract	56.7	68.9	73.3	94.4	129.7

Molyneux (2004) states the IC<sub>50</sub> value is the concentration of the sample solution needed to reduce DPPH by 50%. Blois (1958) stated that a compound is said to be a very strong antioxidant if the IC<sub>50</sub> value is less than from 50 ppm, strong if the IC<sub>50</sub> value is between 50-100 ppm, moderate if the IC<sub>50</sub> value is around 100-150 ppm, and weak if the IC 50 value is between 150-200 ppm. Table 2 shows that the fruit extract has moderate antioxidant activity with an IC<sub>50</sub> value of 129.7 ppm, while the leaf extract has a weak antioxidant activity with an IC50 value of 365.8 ppm.

The fruit extract of *A. corniculatum* has high antioxidant activity. According to the research conducted by Imra *et al.* (2016), it obtained an antioxidant activity in *Nypa* leaf extract using methanol as a solvent with an IC<sub>50</sub> value of 22.5 ppm. Ascorbic acid is vitamin C which can inhibit free radicals and have



robust antioxidant activity. Molyneux (2004) stated that ascorbic acid could reduce free radicals from DPPH compounds. Some mangrove vegetation is also known to have antioxidant activity. Percent inhibition on Free radical reduction is the ability of a material to inhibit free radicals associated with the concentration of the material being tested. The highest percentage of inhibition was produced by a solution with a high concentration of crude extract (1000 ppm). The lowest percentage of inhibition was produced with the smallest crude extract concentration (250 ppm). The result indicates that the higher the concentration of fruit and leaf extracts, the higher the percentage of inhibition.

## CONCLUSION

This study showed the percentage of moisture or water content in the fruit and leaf extracts of *A. corniculatum* were 1.83% and 1.95%, respectively. The bioactive compounds detected in the leaf and fruit extracts came from flavonoids, alkaloids, tannins, phenol hydroquinone, and saponins. The antioxidant analysis showed that the fruit and leaf crude extracts had an antioxidant activity with  $IC_{50}$  values were 129.7 ppm and 365.8 ppm, respectively. Overall, this study strengthens the idea that leaf and fruit extracts of *A. corniculatum* from the mangrove area of Tarakan City have bioactive compounds and antioxidant activity. This research was expected to be used as basic research for future research on *A. corniculatum*.

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