Phytochemical, Antioxidant, and Antibacterial Screening of *Artocarpus integer* from Indonesia

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**Abstract** *Artocarpus integer* is one of the seasonal fruits in Indonesia that is similar to jackfruit. *Artocarpus integer* plant has been widely used as a traditional medicine to cure malaria, tuberculosis, and as antibacterial agents. In this research, the ethanol extracts of leaf and flesh of Indonesian *Artocarpus integer* were screened for their phytochemical characteristics and antioxidant activities using DPPH test. The aim of this research is to investigate and compare the antioxidant activities between *A. integer* flesh fruit and leaves using DPPH method. Furthermore, antibacterial tests against *Streptococcus pyogenes* and *Escherichia coli* were also done for the leaf extract. The phytochemical screening results showed that the ethanol extract from Indonesian *Artocarpus integer* fruit flesh contained alkaloids, flavonoids, saponins, tannins, triterpenoids, and phenolics compounds whereas the leaves did not contain alkaloids and saponins. Meanwhile, the DPPH test showed that both leaf and fruit flesh extracts had good antioxidant activity potential with IC₅₀ of 79.80 ± 5.92 ppm and IC₅₀ of 68.11 ± 6.67 ppm, respectively. The *Artocarpus integer* leaf extract showed antibacterial activity against *Streptococcus pyogenes* with Minimum Bactericidal Concentration (MIC) of 713.76 mg/ml, but showed no antibacterial activity against *Escherichia coli*.

Keywords: *Artocarpus integer*, antioxidant, cempedak, DPPH

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**Introduction**

Exposure to free radicals is increasing and becomes a threat to human life nowadays. Several factors can cause free radicals such as smoke, dust, pollution, and the habit of eating fast food whose nutrition content is not balanced among carbohydrate, protein, and lipid. Free radicals could also damage the biomolecules in human body cells such as protein, lipids, and nucleic acid. Besides, free radicals is one of the factors that contributes in several diseases, such as cancer and heart disease [1]. To neutralize the free radical and inhibit the oxidative stress process, antioxidants can be used [2]. Many plants which contain phenolics and flavonoids are found to be the source of antioxidants. One of mechanisms of the phenolics and flavonoids as antioxidants is to scavenge the free radicals which is influenced by the reduction of potential and dissociation bonding energy between oxygen and hydrogen in phytochemicals [3].

*Artocarpus integer* or “cempedak” plant has fruits with specific odor and belongs to *Moraceae* family [4]. It produces seasonal fruits that look similar to jackfruits but are smaller and have softer flesh [5]. It is distributed widely in Thailand, Malaysia, Sumatra, Java, Sulawesi, Moluccas, and New Guinea [4]. *Artocarpus integer* has some synonyms such as *Artocarpus champeden*, *Artocarpus integrifolia*, *Artocarpus jaca*, *Artocarpus macrocarpon*, *Artocarpus polyphema*, *Polyphema champeden*, *Radermachia integra*, *Saccus arbores*, and *Sitodium macrocarpon* [4]. Traditionally, *Artocarpus integer* seeds have been used as anti-diarrhea medicine, its roots have been used to reduce the fever in the malaria disease, and the young leaves have been used to treat tuberculosis [6]. Many researches have been done to study *A. integer*’s bioactivities, such as antimalarial activity from the aerial parts [7], antibacterial activity against *E. coli* and *S. typhi* from the heartwood [8], antibacterial activity against *E. coli* and *S. aureus* from the leaf [9], and cytotoxic activity from the roots [10]. Based on the literature, several species of *Artocarpus* genus contain a lot of phenolic compounds. Phenolic is a well-known secondary metabolites group in plants, which contains flavonoids and polyphenol compounds. Many flavonoid compounds have been isolated from the *Artocarpus* species such as prenylated flavonoids, flavanone, pyranoflavone, etc [11]. Many studies have shown that lots of polyphenol compounds have better inhibition against free radical compared to ascorbic acid or tocopherol [12]. Based on Abubakar, et al [13], the total phenolics content of all parts...
of the fruits methanol extract is ranging from 3.53 to 42.38 mg GAE/g of dry sample whereas the total flavonoids is ranging from 0.82 to 36.78 mgCE/g of dry sample. The prospect of finding phenolic compounds could investigate the antioxidant activities potential in *Artocarpus integer* plant parts. Therefore, this research was aimed to perform phytochemical screening and to investigate and compare the antioxidant activities between *A. integer* flesh fruit and leaves using DPPH method. Additionally, the antibacterial activity of its leaf against *S. pyogenes* and *E. coli* was also investigated. Antibacterial potentials were deduced from the results of previous research and the traditional therapeutic uses of *A. integer*, such as in therapies against tuberculosis and diarrhea.

**Materials and methods**

**Materials**

For this study, the following materials were used: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (Smart Lab), ethanol (Smart Lab), aquadest, ascorbic acid (Merck), Dragendorff’s reagent, Mayer’s reagent, Lieberman-Burchard’s reagent, chloric acid solution (Smart Lab), magnesium, amyl alcohol, ferric chloride, gelatin solution, Nutrient Agar (NA) (Hi Media), Brain Heart Infusion Agar (BHIA) (Hi Media), Trypticase Soy Broth (TSB) (Oxoid), Nutrient Broth (NB) (Merck), *Escherichia coli* (E. coli) culture, *Streptococcus pyogenes* (S. pyogenes) culture, sheep blood, Dimethyl Sulfoxide (DMSO) (Merck), and Gentamycin sulfate.

**Collection and preparation of A. integer leaves and fruit flesh ethanol crude extract**

Sample of *A. integer* leaves were collected from Lampung and the *A. integer* fruit flesh were collected from Banten in January 2021. Fresh samples (the leaves and fruit flesh) were dried using oven at 60°C and macerated separately in ethanol with ratio of 1:8 (for flesh) and 1:10 (for leaves) for 24 hours at room temperature. The maceration was repeated twice. After maceration, the ethanol extract was then filtered, and ethanol was evaporated using rotary evaporator (Heidolph Hei-Vap Core). The extracts obtained were then frozen and kept at -20°C until further analysis.

**Phytochemical screening**

The phytochemical screening was done according Oladeji, et al [14].

a. Alkaloids

The ethanol extract was added with 1 ml of 2 N HCl and 9 ml of aquadest and then it was heated in water bath for 2 minutes. After cooling, the mixture was divided to two tubes. First tube was added with a few drops of Mayer’s reagent, second tube was added with a few drops of Dragendorff’s reagent. The presence of orange color in the Dragendorff’s reagent tube and yellow precipitate in the second tube indicate the presence of alkaloids.

b. Flavonoids

0.1 g of ethanol extract was added with 10 ml of warm water. The mixture was then boiled and filtered. 5 ml of filtrate was added with 1 ml of concentrated HCl, 0.1 g of magnesium turnings, and 2 ml of amyl alcohol. The presence of flavonoids was detected if pink or magenta-red color was developed.

c. Saponins

0.1 g of ethanol extract was added with 10 ml of hot water in a test tube. After cooling, it was shaken vigorously to froth and was then allowed to stand for 10 minutes. If stable foam was formed, then a few drops of 1% HCl were added. The presence of saponins was observed if stable foam was formed after 1% HCl addition.

d. Tannins

0.1 g of ethanol extract was extracted by 100 ml aquadest, boiled and filtered. 2 ml of filtrate was added with a few drops of 1% FeCl₃. Blue precipitate would indicate the presence of tannins.

e. Steroids and Triterpenoids

0.1 g of ethanol extract was extracted with 25 ml ether for two hours then filtered. 5 ml of filtrate was evaporated until it was dried then it was added with Lieberman-Burchard’s reagent. Blue-green color formation indicates the presence of steroids while the red-magenta color indicates triterpenoids.
Antioxidant activity test
The antioxidant procedure was done based on Molyneux [15]. The antioxidant activities of the crude ethanol extracts from the leaves and fruit flesh were determined using DPPH method [15]. Samples were dissolved in ethanol absolute and then mixed thoroughly using vortex mixer until a homogenous solution was obtained. In the test tube, 2 ml of DPPH 0.23 mM was added to 1 ml sample, and then the mixture was vortexed until homogenous. The resulting samples were incubated in the room temperature in dark condition for 25 minutes. After that, 2 ml of the sample was put in the cuvette. Color changes were observed and measured using UV spectrophotometer at $\lambda = 515$ nm. DPPH and ethanol were used as control. Ascorbic acid was used as positive control. The blank used was ethanol. DPPH Scavenging Effect of the samples was calculated. This assay was done for several sample concentrations to get $IC_{50}$ value. The ethanol extract concentrations used were 70, 80, 90, 100, 110, and 120 ppm for leaves ethanol extract. Meanwhile, the concentrations used for the fruit flesh were 60, 70, 80, 90, 100, 110, and 120 ppm. For A. integer leaf extract, the ascorbic acid control concentrations used were 12, 14, 16, 18, 20, and 22 ppm, whereas for the fruit flesh extract, the ascorbic acid control concentrations were 6, 8, 10, 12, 14, and 16 ppm. The experiments were repeated three times for each sample concentration. The $IC_{50}$ values of leaf and fruit flesh ethanol extract were then compared with the ascorbic acid concentration.

Antibacterial activity
The method was done according to Irulandi et al. [16], with some modifications. Nutrient agar (Merck) inoculated with 1% of E. coli liquid culture was poured on a Petri dish and allowed to solidify. The same was also done to S. pyogenes with Brain Heart Infusion Agar. On the agar plates, the wells were made with diameter of 7.0 mm. After that, serial dilutions of A. integer leaves crude ethanol extract were made with concentration of 400, 800, 1600, and 3200 mg/ml, using DMSO as diluents. Each well contained 20μl extract. The plates were incubated at 37°C for 24 h and the diameter of the clear zone was measured. For each concentration the procedure was done two times. DMSO was used as the negative control while gentamycin sulfate injection 100 mg/ml was used as the positive control.

The data obtained from the antibacterial activity assay was then used to calculate the Minimum Inhibitory (MIC), based on the method of Bloomfield (1991). The ln of the concentration of extract used (in mg/ml) as the x-axis against the square value of the inhibition zone (in mm) as the y axis. Intersection of the linear regression: $Y = aX + b$, has the X being the $Mt$ value, with MIC (in mg/ml) is 0.25 x MBC.

Results and discussion
Phytochemical screening result of the leaf and fruit flesh extract of Artocarpus integer can be seen in Table 1. The data shows that both leaf and fruit flesh ethanol extract contain phenolics and flavonoids, as well as tannins and steroids/triterpenoids.

Table 1. Phytochemical screening results of A. integer leaves and fruit flesh

<table>
<thead>
<tr>
<th>No</th>
<th>Chemical Compounds</th>
<th>Results</th>
<th>Leaves</th>
<th>Flesh</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Steroids / Triterpenoids</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Phenolics</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Notes: (+): detected; (-): not detected

Since phenolic and flavonoid groups of compounds are usually active as antioxidants, there was a high probability that the leaf and fruit flesh ethanol extract could show potential antioxidant activity. This hypothesis could also be verified in the
antioxidant assay using DPPH. Another interesting result was that leaf ethanol extract did not contain alkaloids and saponins, unlike the ethanol fruit flesh extract. Compared to previous result done by Hakim [17] that showed chloroform leaves extract of A. integer only contained alkaloids compounds, it could be assumed that the alkaloids in leaves of A. integer were non polar.

Furthermore, Table 2 shows the result of antioxidant test using DPPH. It can be seen both leaf and fruit flesh ethanol extracts showed no differences in antioxidant activities because the ranges of IC50 between them are overlapping. However, compared to ascorbic acid which has strong antioxidant activity, the IC50 of the extracts were higher. Nevertheless, using the classification adopted in Jadid et al. [17], the strength of antioxidant activity of both leaf and fruit flesh extract could still be considered as intermediate (IC50 between 50 – 100 ppm).

Table 2. IC50 of Antioxidant activity of crude ethanol extract from A. integer leaves and fruit flesh

<table>
<thead>
<tr>
<th>No</th>
<th>Part of A. integer</th>
<th>IC50(ppm)</th>
<th>IC50 of ascorbic acid (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leaves</td>
<td>79.80± 7.26</td>
<td>13.40±0.15</td>
</tr>
<tr>
<td>2</td>
<td>Fruit Flesh</td>
<td>68.11± 6.67</td>
<td>9.06±0.83</td>
</tr>
</tbody>
</table>

Abubakar et al. [13] have also investigated antioxidant activities of the seed, peel, and fruit flesh of Artocarpus integer, but using 80% methanol as extraction solvent. They found that IC50 for antioxidant activity fruit flesh extract was more than 100 ppm, higher than the result of this study (68.11± 6.67 ppm). This difference could indicate that the more potent antioxidant agents in fruit flesh extract was more polar, and therefore could not be fully extracted using 80% methanol.

Figure 1 shows the results of antibacterial activity test on leaf extract against Staphylococcus pyogenes. At extract concentration of 400 mg/ml, the inhibition diameter was 9.81± 1.34 mm, and the highest inhibition was obtained at concentration of 3200 mg/ml, with inhibition diameter of 11.49 ± 0.31 mm. If this data was plotted using ln of concentration as x-axis, and square of clear zone diameter as y-axis, then a regression line with the equation $y = 19.131x - 20.07$ can be fitted. Using this regression line to find the value of extract concentration when the area of clear zone is zero, it could be calculated that the minimum inhibition concentration (MIC) of the A. integer leaf extract on S. pyogenes was 713.76 ppm. This result is lower compared to ginger rhizomes and geranium leaves (both has MIC >1000 ppm) [18]. It suggests that A. integer leaf extract could inhibit S. pyogenes at lower concentration.

![Figure 1. Diameter of clear zone for various leaf extract concentration in antibacterial test against Streptococcus pyogenes](image)

Figure 1. Diameter of clear zone for various leaf extract concentration in antibacterial test against Streptococcus pyogenes

On the other hand, the ethanol leaf extract did not show any antibacterial activity against E. coli. Nevertheless, the leaves of A. integer are used traditionally to treat diarrhea. The non-activity of the ethanol leaf extract suggests that the anti-
Conclusions

A. integer leaf and fruit flesh ethanol extracts contained phenolics, flavonoids, tannins, and steroids/triterpenoids. However, different from fruit flesh ethanol extract, leaf ethanol extract did not contain alkaloids and saponins. Both leaf and fruit flesh ethanol extract had medium antioxidant activity, with IC50 value of 79.80± 7.26 ppm and 68.11± 6.67 ppm, respectively. Leaf ethanol extract of A. integer had inhibitory activity against S. pyogenes with MIC value of 713.76 ppm but did not show inhibitory activity against E. coli. The findings of this research suggest that both leaf and fruit flesh ethanol extract of A. integer are potential antioxidant agents.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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