Comparative Antioxidant Activity on the *Ficus benjamina* and *Annona reticulata* Leaves

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**ABSTRACT**
Antioxidants can prevent free radical formation. Natural antioxidants found in many plants, such as *Ficus benjamina* and *Annona reticulata*. The study aimed to compare the antioxidant activity of extracts and fractions of *Ficus benjamina* and *Annona reticulata* leaves against 1,1-diphenyl-2-picrilhydrazyl. The steps of this study consist of extraction, fractionation with n-hexane, ethyl acetate and water, phytochemical screening, antioxidant activity determination, and comparing the IC₅₀ values. Percentage scavenging activity of the extracts and fractions against DPPH was calculated to determine the antioxidant activity. The IC₅₀ value of *Ficus benjamina* was 127.86 ppm for ethanolic extract, 94.01 ppm for water fraction, 115.48 ppm for ethyl acetate fraction, and 335.50 ppm for n-hexane fraction. The IC₅₀ value of *Annona reticulata* was 274.31 ppm for ethanolic extract, 211.42 ppm for water fraction, 367.91 ppm for ethyl acetate fraction, and 741.08 ppm for n-hexane fraction. The results showed that the *Ficus benjamina* water fraction was the best antioxidant compared to other extract and fraction.

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**1. INTRODUCTION**
Free radicals defined as chemical species possessing unpaired electrons. These species responsible to many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [1]-[3]. The most effective compound to eliminate free radicals is antioxidants. Antioxidants prevent free radical formation by scavenging them or promoting their decomposition and suppressing such disorders [4]-[6]. There is a growing interest toward natural antioxidants. The idea of natural antioxidant in herbal resources can protect biology systems from oxidative stress [7]-[9]. *Ficus benjamina* (Moraceae) and *Annona reticulata* (Annonaceae) are herbal resources that had antioxidant activity.

*F. benjamina* and *A. reticulate* have been used as traditional medicines. This is due secondary metabolites. *F. benjamina* leaves contain tannins, carbohydrates, phytosterols, flavonoids, phenolics, saponins, oils and fats [10],[11]. The root barks, leaves and stems of *A. reticulata* contain isoquinoline alkaloids and flavonoids [12]. Phenolics, flavonoids, vitamins, and minerals (Cu, Mn, Zn, Se and Fe) are known as natural antioxidants[13]. This study aimed to compare the antioxidant activity of extracts and fractions of *F. benjamina* and *A. reticulata* leaves against 1,1-diphenyl-2-picrilhydrazyl (DPPH).
2. RESEARCH METHOD

2.1. Materials

_F. benjamina_ and _A. reticulate_ leaves obtained from Manoko, West Java, Indonesia. All chemicals with analytical grade are DPPH, vitamin C, ether, hydrochloric acid, sulfuric acid, ethanol, amyl alcohol, n-hexane, ethyl acetate, ammonia, chloroform, magnesium, Dragendorff and Mayer reagent, iron (III) chloride, gelatin, vanillin, and potassium hydroxide.

2.2. Samples Preparation

Simplicia macerated with 70% ethanol for 3 days. Each day, the solvent changed with the fresh one. All macerat collected and vaporated with rotary rotavapor. Dissolve 10 g of ethanolic extract with aquadest to obtain 100 mL solution, then done liquid liquid extraction with n-hexane and ethyl acetate, three times for each solvent. All fraction collected and vaporated. Phytochemical screening was conducted to simplicia, extract, and fraction with Fransworth methods[14].

2.3. Antioxidant Activity Determination

Dissolve 4 mg of DPPH with 96% ethanol in 100 mL volumetric flask (40 μg/mL). Dissolve 5 mg of vitamin C and 50 mg of samples (extract and fraction of _F. benjamina_ and _A. reticulata_) with 96% ethanol, each in 100 mL volumetric flask, then diluted the solutions to prior concentration.

The DPPH radical was used for the determination of free radical-scavenging activity of the extracts and fractions. The modified method of Okada and Okada (1998) was employed[15]. A portion (2 mL) of the different concentrations of extract, fraction or vitamin C, each in tube, was added with 3 mL of 40 μg/mL DPPH. The mixtures were vortexed and incubated in a dark chamber for 30 min, then the absorbancies were measured at 517 nm using spectrophotometer (Ray LEIGH). The blank was 96% ethanol in place of sample. Percentage scavenging activity was calculated using this formula:

\[
\text{% of DPPH inhibition} = \left(\frac{Ab-Aa}{Ab}\right) \times 100
\]

Where Aa and Ab are the absorbance values of the sample and the blank, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value.

3. RESULTS AND DISCUSSION

3.1. Samples Preparation

Maceration is a cold extraction method. These methods was conducted to maximal extraction of the entire secondary metabolites in the sample. _A. reticulata_ leaves (6.94%) has more soluble secondary metabolites in 70% ethanol compared to _F. Benjamina_ leaves (5.68%). Liquid liquid extraction was conducted to separate the secondary metabolites based on its polarity[16]. Water fraction of _F. benjamina_ and _A. reticulata_ bigger than ethyl acetate and n-hexane fraction (Table 1). We concluded that the majority of secondary metabolites in both samples were the polar secondary metabolites.

| Sample       | n-Hexane (%) | Ethyl Acetate (%) | Water (%) |
|--------------|--------------|------------------|-----------|
| _F. benjamina_ | 4.66         | 11.92            | 82.00     |
| _A. reticulata_ | 23.60        | 21.98            | 54.00     |

Table 2. Phytochemical Constituents of _F. benjamina_ and _A. reticulata_
Phytochemicals are synthesized by plants for self-defense from pathogens and environmental stress. These phytochemicals can also be used to cure several diseases. It can be stated that the phytochemicals are the compound that determined the medical potential of any plant. Phytochemical screening with color reaction method was conducted to determine the group of secondary metabolites in the sample. Phytochemical screening showed extract that has the same constituents as simplicia (Table 2). It's mean that maceration with 70% ethanol can extract all groups of secondary metabolites in simplicia.

All ethanolic extract and fraction of *F. benjamina* and *A. reticulata* contain flavonoids (Table 2). Jain *et al* (2013) was found that methanolic extract of *F. bejamina* leaves had high level of phenolic (4.006 mg gallic acid equivalence/g) and flavonoids (16.005 mg quercetin acid equivalence/g) [10]. Flavonoids are the phenolic compounds, which are synthesized by plants due to adaptation in response to biotic and abiotic stresses (infection, water stress, cold stress, and high visible light) [17]. Flavonoids inhibit the oxidation reaction through radical scavenging mechanisms by donating an electron to the unpaired electrons in free radicals. *In vitro*, flavonoids are potent inhibitor to lipid peroxidation, as acatchers of reactive oxygen or nitrogen species, and also able to inhibit the lipoxygenase and cyclooxygenase activity [18]-[20]. The antioxidant activity of phenolic compounds depend on their molecular structure, based on the availability of phenolic hydrogens, which result in the formation of phenoxyl radicals due to hydrogen donation[21].

### 3.2. Antioxidant Activity Determination

| Sample | Concentration (ppm) | % inhibition | Sample | Concentration (ppm) | % inhibition |
|--------|---------------------|--------------|--------|---------------------|--------------|
| *F. benjamina* | Ethanol extract | 40 | 34.82 | *A. reticulata* | Ethanol extract | 100 | 34.40 |
| | 80 | 38.03 | | | 150 | 34.76 |
| | 100 | 44.87 | | | 200 | 42.88 |
| | 120 | 48.71 | | | 250 | 45.14 |
| | 160 | 56.83 | | | 300 | 54.62 |
| Water fraction | 40 | 35.04 | | Water fraction | 50 | 31.60 |
| | 80 | 40.59 | | | 100 | 38.14 |
| | 100 | 46.15 | | | 150 | 38.76 |
| | 120 | 53.84 | | | 200 | 44.01 |
| | 160 | 87.07 | | | 250 | 57.78 |
| *Ethyl acetate* | 40 | 32.26 | | Ethyl acetate | 200 | 29.11 |
| fraction | 80 | 36.96 | | | 250 | 32.50 |
| | 100 | 49.78 | | | 300 | 38.27 |
| | 120 | 55.34 | | | 350 | 48.08 |
| | 160 | 57.47 | | | 400 | 52.14 |
| *n-hexane* | 40 | 17.37 | | n-hexane | 200 | 31.60 |
| fraction | 80 | 21.09 | | | 300 | 32.50 |
| | 100 | 21.58 | | | 400 | 39.50 |
| | 150 | 24.56 | | | 500 | 42.66 |
| | 300 | 51.86 | | | 600 | 44.01 |
Natural antioxidants from medicinal plants are a good choice to control oxidative stress. Because of natural origin, these compounds are usually non-toxic. Antioxidants upon interaction with DPPH radicals transfer a proton to DPPH radicals by direct abstraction of phenol H-atoms and electron transfer process, thus neutralizing its free radical character, which produce DPPH-H (2,2-diphenyl-1-picrylhidrazyn), i.e DPPH with less reactivity. The DPPH radicals solution was purple, because the unpaired nitrogen electrons [22]. The absorbance of 40 ppm DPPH radicals solution was 0.443. These reaction was showed with color alteration from purple to yellow with absorbance reduction at 517 nm. The degree of discoulouration indicates the scavenging potential of the antioxidants [23],[24]. Antioxidant activity were measured from reaction of the sample (extracts and fractions) and vitamin C solutions with DPPH solution, then percentage of DPPH inhibition were counted (Table 3). The IC₅₀ value of extracts and fractions werecounted from the linear regression equation of the curve of concentrations versus% inhibition (Table 4). More smaller the IC₅₀ value, it’s mean more higher antioxidant activity. The antioxidant activity was dose dependent manner.

Jain et al (2013) was determined the IC₅₀ value for the methanolic extract of *F. benjamina* leaves was 59.07 ppm [10]. Jamkhande et al (2014) was determined the IC₅₀ value for the methanolic extract of *A. reticulate* roots was 108.71 ppm [25]. These values lower than our results. These were due to differences in the solvent and the part of the plant which used in extraction, so that the content of secondary metabolites in the extracts was different.

| Sample | Linear Regression Equation | R² | IC₅₀ |
|--------|-----------------------------|----|------|
| *F. benjamina* | | | |
| Ethanolic extract | y=0.1918x+25.475 | 0.9638 | 127.86 |
| Water fraction | y=0.4233x+10.203 | 0.8515 | 94.01 |
| Ethyl acetate fraction | y=0.235x+22.86 | 0.8766 | 115.48 |
| n-hexane fraction | y=0.144x - 1.946 | 0.6720 | 335.50 |
| *A. reticulate* | | | |
| Ethanolic extract | y = 0.1056x + 21.032 | 0.9421 | 274.31 |
| Water fraction | y = 0.1165x + 25.369 | 0.9090 | 211.42 |
| Ethyl acetate fraction | y = 0.1233x + 4.636 | 0.9184 | 367.91 |
| n-hexane fraction | y = 0.0350x + 24.062 | 0.9320 | 741.08 |
| Vitamin C | y=5.3525x+12.497 | 0.9149 | 7.00 |

Table 4 showed that the *F. benjamina* water fraction has the best antioxidant activity (IC₅₀ 94.01 ppm) compared to the other extracts and fractions, both *F. benjamina* and *A. reticulate*. *F. benjamina* water fraction contains polyphenols, quinones, and flavonoids. All these structure having the hydroxyl group which can donate hydrogen to interact with DPPH radical to produce the DPPH-H.A compound is categorized as a very strong antioxidant when the IC₅₀ value is less than 50 ppm, strong antioxidant if the IC₅₀ value is 50-100 ppm, mild antioxidant if the IC₅₀ value is 100-150 ppm, and weak antioxidant if the IC₅₀ values is 150-200 ppm [22]. The *F. benjamina* water fraction categorized as strong antioxidant. The *F. Benjamina* ethanolic extract and ethyl acetate fraction categorized as mild antioxidant. The remnants are considered have no antioxidant activity.

The ratio of antioxidant activity of the IC₅₀ value of *F. benjamina* and *A. reticulate* to vitamin C (7 ppm) was determined to compare the sample reactivity to DPPH radicals. The *F. benjamina* water fraction had the best ratio to vitamin C (Table 5). This means that the *F. benjamina* water fraction was potent antioxidant to be developed.

| Sample | IC₅₀ (ppm) | Ratio |
|--------|------------|-------|
| *F. benjamina* | | |
| Ethanolic extract | 127.86 | 1:18.26 |
| Water fraction | 94.01 | 1:13.43 |
| Ethyl acetate fraction | 115.48 | 1:16.49 |
| n-hexane fraction | 335.50 | 1:46.57 |
| *A. reticulate* | | |
| Ethanolic extract | 274.31 | 1:39.18 |
| Water fraction | 211.42 | 1:30.20 |
| Ethyl acetate fraction | 367.91 | 1:52.55 |
| n-hexane fraction | 741.08 | 1:105.86 |
4. CONCLUSION

The extract and fractions of the *A. reticulate* leaves are considered have no antioxidant activity. In *F. benjamina* leaves, the n-hexane fraction is considered have no antioxidant activity, ethanolic extract and ethyl acetate fraction are categorized as mild antioxidant, and the water fraction is categorized as strong antioxidant.

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