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# ANTIOXIDANT ACTIVITY OF THE COMBINATION OF AMBARELLA LEAVES (*Spondias dulcis* Parkinson) AND SOURSOP LEAVES (*Annona muricata* Linn) EXTRACT

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**ABSTRACT**
Degenerative diseases can be caused by free radicals, whereas antioxidants can overcome free radicals. Ambarella leaves (*Spondias dulcis* Parkinson) and soursop leaves (*Annona muricata* Linn.) are potential sources of suitable antioxidant compounds. This study aims to determine the antioxidant activity and the relationship between antioxidants and total phenolics from the combination of *S. dulcis* and *A. muricata* leaf extracts. *S. dulcis* leaves, and *A. muricata* leaves were macerated with 96% ethanol and then formulated with a combination of extracts (1:1, 2:1, and 1:2). Each was tested for its antioxidant activity by the DPPH method. The ethanol extract of *S. dulcis* leaves had an antioxidant activity value of 36.72±4.01 ppm with a total phenol of 76.84±0.50 mgGAE/g extract, an ethanol extract of *A. muricata* leaves of 81.45±8.19 ppm with a total phenol of 104.48±7.55 mg GAE/g extract and a combination of extracts with a ratio of ethanol extract of *S. dulcis* leaves and ethanol extract of *A. muricata* leaves (2:1) of 29.98 ± 0.37 ppm with a total phenol of 36.99 ± 0.98 mgGAE/g extract. The results showed that the phenolic content of the ethanol extract of *A. muricata* leaves was more significant than the combination (2:1), which had the most excellent antioxidant activity among the other comparisons. Total phenolic is inversely related to antioxidant activity. Antioxidant activity is not only played by phenolic compounds.

**Keywords:** Antioxidant; Extract; Fenolic; Free radicals

## 1. INTRODUCTION

Indonesia has a very abundant wealth of natural resources that can benefit the health sector; one example is an antioxidant. Antioxidants are used as free radical scavenging compounds (Fatimah & Sundu, 2020). Free radicals are unstable atoms or molecules with high reactivity so that they can cause damage to the body. Various attempts have been made to explore sources of antioxidants, one of which is sourced from natural ingredients such as plants which tend not to produce reactions or side effects (Rahmi, 2017; Ipandi et al., 2016).

Traditional plants that can be used as a source of natural antioxidants are ambarella (*Spondias dulcis* Parkinson) (Pitojo, 2005) and soursop (*Annona muricata* Linn.) (Haryati, 2005). Species of the genus *Spondias* contain secondary metabolites such as saponins, sterols, triterpenes, tannins, flavonoids, and phenolics, which have antioxidant, antimicrobial, cytotoxic, potential thrombolytic, anti-inflammatory, and antihypertensive activities (Sameh et al., 2018). The ethyl acetate fraction of *S. dulcis* leaves had an antioxidant activity with an $IC_{50}$ value of 48.32 ppm with the highest total phenol value of 0.13 mgGAE/mg sample. The *n*-butanol and methanol-water fractions with $IC_{50}$ were 53.95 ppm and 83.27 ppm, *n*-hexane fraction with an $IC_{50}$ value of 370.76 ppm (Aulia, 2017).

*A. muricata* leaves contain acetogenins compounds which have anti-inflammatory and antioxidant properties (Wahyuningsih, 2010). Naspiah et al, (2013) antioxidant activity test on *A. muricata* leaves with *n*-hexane, ethyl acetate, methanol, and butanol solvents with $IC_{50}$ values obtained successive results of 30.10, 18.05, 23.60; and 14.80 ppm (Hakim *et al*., 2020) and total phenol of 276.90 mg GAE/g extract (Aulia & Widjanarko, 2018).

Combining the two plant extracts is thought to increase the antioxidant potential (Lingga, 2012). The combination of ethanol extracts of *Syzygium polyanthum* leaves and *Moringa oleifera* leaves 2:1 has the best activity with total phenolic of 61.48 mgGAE/g and antioxidant activity of 28.55 ppm and the combination of *S. dulcis* and *A. muricata* leaf extracts have the potential to be developed to obtain activity best antioxidants. This study used a combination of *S. dulcis* and *A. muricata* leaf extracts to explore their antioxidant activity and total phenolic content (Rudiana et al, 2020).

## 2. METHOD

### 2.1. Tools and materials

Laboratory glassware, a set of UV-Vis spectrophotometer (Optima), vacuum rotary evaporator (IKA), micro pipette and filter paper, *S. dulcis* and *A. muricata* leaves, methanol (technical grade), 96% ethanol, gallic acid (Sigma Aldrich), natrium carbonate (Merck), Ascorbic acid (Sigma Aldrich), Folin Ciocalteu reagent (Merck), distilled water, and 0.002% DPPH (Merck).

### 2.2. Preparation and Extraction

*S. dulcis* leaves, and *A. muricata* leaves were obtained from Pandeglang Regency, Banten. The two samples were sorted, washed, drained, chopped, and dried to obtain Simplicia. The simplicial was crushed by grinding using a blender; then, each simplicia was macerated using 96% ethanol solvent. Each macerate was concentrated using a rotary evaporator.

### 2.3. Antioxidant Activity Test

The antioxidant activity test followed the procedure of Rudiana et al. (2018), which has been modified. Prepare a positive control (vitamin C), negative control (methanol), and ethanol extract of *S. dulcis* and *A. muricata* leaves with varying concentrations of 50-250 ppm, and a combination of ethanol extracts of *S. dulcis* and *A. muricata* leaves with a ratio of 1:1, 2:1, and 1:2 (w/w) with a concentration of 62.5 – 1000 ppm. Sample solution of 5 mL was put into a test tube and then added 5 mL of 0.002% DPPH. The absorbance of each sample was measured using a UV-Vis spectrophotometer at λmax 515 nm (Rudiana et al, 2020).

### 2.4. Total Phenol Analysis

The sample was added with distilled water and Folin-Ciocelteau reagent. The test solution was incubated, and 20% natrium carbonate was added. The solution was incubated again in the water bath. A gallic acid solution is used as standard. Absorbance was measured at a wavelength of 745 nm (Rudiana et al, 2020).

## 3. RESULT AND DISCUSSION

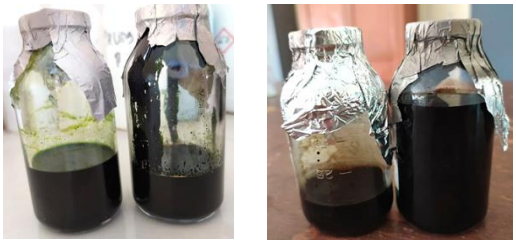
### 3.1. *S. dulcis* and *A. muricata* leaf extracts

Each simplicia of *S. dulcis* and *A. muricata* leaf powder was macerated using 96% ethanol for 1x24 hours (Rudiana et al., 2020). The yield of each extract is presented in **Table 1**.

**Table 1.** Yield of *S. dulcis* and *A. muricata* leaf extracts

| Ethanol extract | Extract mass (g) | % Yield |
|---|---|---|
| *S. dulcis* leaves | 22.25 | 7.41 |
| *A. muricata* leaves | 25.61 | 5.20 |

The ethanol extract of *S. dulcis* leaves had a higher % yield value compared to the ethanol extract of *A. muricata* leaves (**Table 1**). *S. dulcis* leaf extract has a high % yield because *S. dulcis* leaves contain secondary metabolites, which are abundant compared to *A. muricata* leaves. Ethanol solvent in dissolving all components of secondary metabolites both nonpolar, semipolar, and polar (Rudiana et al., 2020).



(a) (b)

**Figure 1**. Concentrated extracts of: (a) S. dulcis leaves; (b) A. muricata leaves

The ethanol extracts of *S. dulcis* and *A. muricata* leaves had different colors of each extract showed that there were differences in the active substance contained in the extract (**Figure 1**). The ethanol extract of *S. dulcis* leaves has a red color due to the presence of anthocyanins. Anthosionins belong to a class of flavonoid compounds which function as a source of antioxidants (Jusuf et al., 2008). The ethanol leaf extract of *A. muricata* has a green color caused by the chlorophyll pigment found in the chloroplasts (Sumenda, 2011).

### 3.2. Antioxidant Activity and Total Phenolic Analysis

Extracts and combinations of ethanol extracts of *S. dulcis* and *A. muricata* leaves were analyzed for their free radical scavenging activity with DPPH as the radical source. Antioxidant activity data are presented in **Table 2**.

**Table 2.** Antioxidant activity and total phenolic extracts and combinations of ethanol extracts of *S. dulcis* and *A. muricata* leaves

| Ethanol Extract Sample | $IC_{50}$ value (ppm) | Total Phenolic (mg GAE/g) |
|---|---|---|
| *S. dulcis* leaves | 36.72 ± 4.01 | 76.4 ± 0.50 |
| *A. muricata* leaves | 81.45 ± 8.19 | 104.48 ± 7.55 |
| Combination *S. dulcis*: *A. muricata* (1:1) | 52.53 ± 3.83 | - |
| Combination *S. dulcis*: *A. muricata* (2:1) | 29.8 ± 0.37 | 36.99 ± 0.98 |
| Combination *S. dulcis*: *A. muricata* (1:2) | 69.25 ± 14.28 | - |

The ethanol extract of *S. dulcis* leaves has solid antioxidant activity (Molyneux, 2004) with an $IC_{50}$ value of 36.72 ± 4.01 ppm, while the antioxidant activity value of *A. muricata* leaves is 81.45 ± 8.19 ppm included in the strong category (**Table 2**). *S. dulcis* leaves have the best free radical scavenging activity; this aligns with Aulia (2017) research, which tested *n*-hexane, ethyl acetate, butanol, and methanol-water extracts with an $IC_{50}$ value of 370.76; 48.32; 53.95 and 83.27 ppm. Methanol has more polar properties than ethanol because the ethyl group in ethanol makes ethanol have a more nonpolar polarity when compared to methanol which only contains one methyl group. It is suspected that semipolar compounds act as active compounds in *S. dulcis* leaves; this can be seen in the ethyl acetate extract, which has an outstanding activity value. Correspondingly, ethanol solvent has a low polarity when compared to methanol (Martono et al., 2012). The ethanol extract of *A. muricata* leaves an antioxidant activity value of 81.45 ± 8.19 ppm. Hakim et al. (2020) stated that the ethanol extract of *A. muricata* leaves has antioxidant activity with an $IC_{50}$ value of 14.48 ppm. The difference in antioxidant activity is thought to be due to different sources of simplicia, which results in different content of secondary metabolites. The content of secondary metabolites is influenced by the environment around which the plant grows (Ap et al., 2022)

The results of the analysis of the effectiveness of the extract combinations showed that the combination (2:1) had the highest $IC_{50}$ value. This is an effect of increasing antioxidant activity. The antagonistic effect of the extract was given in combinations (1:1) and (1:2). This was indicated by the decrease in DPPH free radical scavenging activity by the combination extract (**Table 2**). The difference in $IC_{50}$ values in single and combination extracts is due to interactions between the chemical compounds in each extract (Hidayat, 2011). The stability of flavonoids is also thought to reduce or even eliminate antioxidant activity in dried ingredients, one of which is the change in the form of glycosides to aglycones (Yordi et al., 2012).

Each extract and combination of extracts (2:1) were tested quantitatively for total phenolic content (**Table 2**). The leaves of *A. muricata* had the highest total phenolic, 104.48 ± 7.55 mgGAE/g. The high total phenolic did not affect the strength of the antioxidant. An increase did not follow the high antioxidant activity in the combination extract (2:1) in total phenolics; the total phenol results obtained were inversely proportional to the antioxidant activity. This is presumably because the antioxidant activity is not only played by phenolic compounds but also by the influence of other compounds such as tannins, alkaloids, and other compounds. It was stated that tannins, flavonoids, and phenolic content correlated with antioxidant activity. Tannins contributed 97.63% to reducing DPPH radicals and 90.91% to reducing ABTS radicals in the *Miristica fragrance* Houtt fruit extract (Antasionasti et al., 2021). The limitations of this study were only analyzing the antioxidant activity of each ethanol extract of *S. dulcis* and *A. muricata* leaves and the combination of extracts with a ratio of 2:1, 1:1, and 1:2. The combination of extracts with the best activity, each extract of *S. dulcis* and *A. muricata* leaves were analyzed for total phenolic content.

## 4. CONCLUSION

Combining ethanol from *S. dulcis* leaves, and *A. muricata* leaves (2:1) can synergistically increase antioxidant activity. The ethanol extract of *S. dulcis* leaves of *A. muricata* leaves had an antioxidant activity with $IC_{50}$ values of 36.72 ± 4.01 and 81.45 ± 8.19 ppm. The combination of the ethanol extract of *S. dulcis* and *A. muricata* leaves (2:1) had the best antioxidant activity with an $IC_{50}$ value of 29.98 ± 0.37 ppm with a total phenol of 36.99 ± 0.98 mg GAE/g extract. The total phenol yield obtained is inversely proportional to the antioxidant activity; this is presumably because the antioxidant activity is not only caused by phenolics/flavonoids but can be caused by alkaloid compounds, tannins, and other compounds that can donate protons. Further research is needed to determine the antioxidant activity of other combination formulas to obtain the best antioxidant activity and to predict the effect of the combination on increasing antioxidant activity, total phenolics and flavonoids

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## 6. CONFLICT OF INTEREST

In this study the authors declare free from conflicts of interest.

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