Phytochemicals Screening and Antioxidant Activity of Rambai (*Sonneratia x urama*) Leaves

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**Abstract.** Sonneratia is a mangrove plant used in traditional medicine. This work investigated the phytochemicals and antioxidant activity of *Sonneratia x urama* from South Kalimantan. Phytochemical screening was carried out on *S.x urama* leaves. *S.x urama* leaves were extracted using different solvents such as methanol, acetone, ethyl acetate, and hexane. Each extract was assayed for antioxidant activity using the DPPH (2,2-diphenyl-1-picryl-hydrazyl) method. This work showed that *S. x urama* leaves gave positive results for flavonoids, tannins, alkaloids, saponins, terpenoids, and reducing compounds in the phytochemical screening test. The acetone and methanol extracts showed the highest antioxidant activity. Therefore, acetone and methanol extracts of *S.x urama* leaves have the potential to be a source of antioxidants.

# INTRODUCTION

Antioxidants are health-promoting compounds that counteract excess oxidants, including reactive oxygen species and free radicals, which can occur in the human body due to 'oxidative stress' conditions. This condition can eventually lead to various diseases such as cellular ageing, mutagenic changes, cancer, cardiovascular, and neurodegenerative diseases [1]. The human body can fight oxidative stress by producing antioxidants in cells or supplied from outside through food or supplements [2]. Plants can be a source of natural antioxidants [3].

*Sonneratia* is a genus of true mangrove plants and mainly grows on the banks of tidal creeks and rivers or the coast [4, 5]. Banjar people in South Kalimantan commonly call *Sonneratia* plants as rambai. *Sonneratia x urama* is a mangrove hybrid of *S. alba* vs *S. lanceolata*, distributed in Australia, Indonesia, and Papua New Guinea [6]. People used plants of the *Sonneratia* for food and medicinal materials such as stopping bleeding and treating hepatitis, fever, sprains, asthma, and diabetes [7, 8].

Recently, *Sonneratia* plants have received more attention for their bioactivity and phytochemical compounds. Methanol fraction of *S. apetala* fruit contains carbohydrates, tannins, flavonoids, steroids, alkaloids, glycosides, and terpenoids, and it has antiproliferative and antioxidant activities [9]. Ethanol extract of *S. caseolaris* leaves has antioxidant and antidiabetic activities in vitro and in vivo [8]. *S. caseolaris* leaf ethanol extract is a potential super-antioxidant and has methicillin-resistant *Staphylococcus aureus* (MRSA) activity. In addition ethanol extract of *S. caseolaris* contains tannins, flavonoids, and steroids [10]. Methanol extract of different parts of *S. ovata,* including stem barks, roots, leaves, and fruits, have antioxidant activity, which leaves have very strong antioxidant activity [11]. *S. alba* fruit vinegar can reduce blood glucose levels in rats [12]. Methanol and ethanol extract of *S. alba* roots and leaves have antimicrobial and antioxidant activities [13]. However, there is no publication about the phytochemical constituents and antioxidant activity of *S. x urama* leaves. Therefore, we will report a preliminary test of *S. x urama* leaves on phytochemical constituents and antioxidant activity.



1. (b) (c) (d)

**FIGURE 1**. *Sonneratia x urama* (a) tree, (b) flower, (c) fruit, (d) leaves

# MATERIALS AND METHODS

## Plant Materials

Leaves of *S. x urama* were collected from Barito Kuala District, South Kalimantan, Indonesia. The samples were identified by Dr. Gunawan, a botanist from the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat, Indonesia. After collection, the leaves were cleaned and air-dried. The dried leaves were ground to obtain a powder.

## Chemicals

Technical grades of methanol, acetone, ethyl acetate, and hexane were purchased from the Jong Java Store. Pro-analytical grades of methanol and chloroform were obtained from Merck. Other chemicals were 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich), ascorbic acid (Merck), gallic acid (Sigma Aldrich), hydrochloric acid (Merck), sulfuric acid (Merck), ferric chloride (Merck), sodium hydroxide (Merck), and glacial acetic acid (Merck). Benedict's solution, Mayer's reagent, Wagner's reagent, and Dragendorf's reagent were purchased from Nitra Kimia.

## Phytochemical Screening

Phytochemical screening was carried out on *S.x urama* leaves. The test was screened for the presence of tannins, phlobatannins, saponins, terpenoids, alkaloids, cardiac glycosides, combined anthraquinones, free-anthraquinones, carotenoids, and reducing compounds [14].

### Tannins Test

One gram of leaf powder was added to 30 mL of distilled water, then boiled in a water bath and filtered while hot. The cooled filtrate was taken up to 1 mL in a test tube and diluted with distilled water to 5 mL, then 2-3 drops of 10% FeCl3 solution were added. The formation of a bluish-green or brownish-green precipitate indicated the presence of tannins.

### Saponins Test

One gram of leaf powder was added to 30 mL of distilled water, boiled in a water bath. Then, it was filtered while hot and allowed to cool. The 2.5 mL filtrate was transferred to a test tube, diluted with distilled water to 10 mL, and shaken vigorously for 2 minutes. The formation of a stable foam indicated the presence of saponins.

### Phlobatanins Test

One gram of leaf powder was added to 30 mL of 1% HCl and boiled in a water bath. If a red precipitate forms, it indicates the presence of phlobatanin.

### Terpenoids Test

One gram of leaf powder was extracted with chloroform and then filtered. The filtrate was added with concentrated H2SO4 so that the solution formed a layer. If a reddish-brown precipitate forms, it indicates the presence of terpenoids.

### Flavonoids Test

One g of leaf powder was boiled with 10 mL of distilled water for 5 minutes and then filtered. A few drops of 20 % NaOH were added to this cooled filtrate. The colour change from deep yellow to colourless when dilute HCl was added indicated the presence of flavonoids.

### Cardiac Glycosides Test

One gram of leaf powder was extracted with methanol. Five ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This mixture was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxysugar characteristics of cardenolides. A violet ring may appear below the ring, while a greenish ring may be formed in the acetic acid layer.

### Combined Anthraquinones Test

One gram of leaf powder was boiled with 15 ml of 10 % hydrochloric acid. The mixture was filtered while hot. The cooled filtrate was partitioned against an equal volume of chloroform. The chloroform layer was transferred to a test tube, and an equal volume of 10 % ammonia solution was added. Then, the solution was shaken and allowed to separate. The aqueous layer was observed for any colour change; a pale pink colour indicated the presence of anthraquinone.

### Free Anthraquinones Test.

One gram of leaf powder sample was added to 10 mL of chloroform, then stirred and filtered to obtain the filtrate. The 1 mL filtrate was transferred to a test tube, and the same volume of 10% ammonia was added and shaken. If a bright pink solution forms in an aqueous layer, it indicates the presence of free anthraquinones.

### Carotenoids Test

One gram of leaf powder sample was added to 10 mL of chloroform, then stirred and filtered to obtain the filtrate. The 2 mL filtrate was transferred to a test tube, and three drops of concentrated H2SO4 were added slowly through the tube wall. The formation of a blue-coloured solution on the surface indicated the presence of carotenoids.

### Reducing Compounds Test

One gram of leaf powder was boiled with 10 mL of distillate water and filtered while hot. The cooled filtrate (2 ml) was placed in a test tube, and three drops of 20%NaOH were added to make the solution alkaline. Then, the same volume of qualitative Benedict's solution was added and boiled in a water bath. The formation of a brick-red precipitate indicated the presence of reducing compounds.

### Alkaloid Test

One gram of leaf powder was boiled with 30 mL of distilled water and 10 mL of HCl in a water bath and then filtered. The filtrate (1 mL) was transferred to three test tubes, and ammonia solution was added until the pH reached 6-7. Three drops of each reagent, i.e., Mayer's, Wagner's, and Dragendrof's reagents, were added to the solution. If a white precipitate formed on the Mayer reagent, a brown residue on the Wagner reagent, and a light orange residue on the Dragendrof reagent, it indicates the presence of alkaloids.

## Preparation of Extracts

The maceration method was employed for the extraction. An amount of 20 g leaves powder of *S.x urama* was macerated with 200 mL methanol for 24 hours. The extract was filtrated through Whatman paper No.1 and concentrated by drying at room temperature in a fume hood. The same procedure was done for other solvents such as acetone, ethyl acetate, and hexane. The following equation determined the yield of the extract:

Finally, 10 mg of each extract was dissolved in 1 mL DMSO (10 mg/mL) and ready to use for the experiment.

## Antioxidant Activity Assay

DPPH (2,2-diphenyl picrylhydrazyl) radical scavenging activity method was used to evaluate antioxidant activity according to [15]. Two millilitres of extract solutions of different concentrations were mixed with 2 mL of 0.15 mM DPPH in a test tube. The mixed solution was incubated at room temperature in the dark for 30 min. The absorbance of solutions was measured using a Uv-Vis spectrophotometer (Genesys 10) at 515 nm. The inhibition or free radical scavenging activity was calculated according to the following equation:

The antioxidant activity of the extracts was expressed as IC50.

# RESULTS AND DISCUSSION

## Phytochemicals Screening

The phytochemical constituents are summarised in Table 1.

**Table 1**. Phytochemical constituents of *S. x urama* leaves.

|  |  |  |
| --- | --- | --- |
| **Phytochemicals** | **Test** | **Results** |
| Tannins | Ferric chloride | + |
| Saponins | Foaming | + |
| Phlobatanins |  | - |
| Terpenoids | Salkowski | + |
| Flavonoids | Alkali | + |
| Cardiac glycosides | Killer Killiani | - |
| Combine anthraquinones | Borntragor | - |
| Free anthraquinones | Borntragor | - |
| Carotenoids |  | - |
| Reducing compounds | Benedict | + |
| Alkaloids | Mayer | - |
| Alkaloids | Wagner | + |
| Alkaloids | Dragendorf | + |

Note: (+) = presence

(-) = absence

Preliminary phytochemical screening of *S. x urama* leaves revealed the presence of tannins, saponins, flavonoids, terpenoids, reducing compounds, and alkaloids. Meanwhile, phlobatanin, cardiac glycoside, carotenoid, combined anthraquinone, and free anthraquinone were not detected in *S. x urama* leaves.

The phenolic compounds detected in the leaves of *S. x urama* were flavonoids and tannins. Other phenolics were absent, i.e., phlobatanin, combined anthraquinones, and free anthraquinones. The reducing compound test gave positive results, indicating the presence of sugary compounds (glycosides) in the leaves of *S. x urama*. These reducing compounds may be due to phenolics-glycosides such as flavonoid glycosides and tannins, which gave positive results in phytochemical tests. Moreover, it is in line with the results of the other phytochemical test, which showed the presence of saponins. Saponins are triterpenoid glycosides or steroids [16].

Phytochemical constituents are responsible for various bioactivities. Phenolic compounds have pharmacological effects such as antioxidant and antimicrobial [17], anti-inflammatory, antihypertensive [18] and antidiabetic [19]. Alkaloids have various pharmacological effects, such as anti-inflammatory, anticancer, and antiviral effects, on the cardiovascular system [20]. Saponins have antiphotoaging and anti-melanogenesis activity [21], while terpenoids have α-glucosidase inhibitory activities [22] and anti-inflammatory activity [23].

## Extraction

**Table 2**. Yield of extract *S.x urama*

|  |  |
| --- | --- |
| **Extract** | **Yield (%)** |
| Methanol | 7.86 |
| Acetone | 7.73 |
| Ethylacetate | 3.09 |
| Hexane | 0.73 |

*S. x urama* leaves powder was extracted with different polarity solvents such as methanol, acetone, ethyl acetate, and hexane. The order of solvent polarity is methanol > acetone > ethyl acetate > hexane. Table 2 summarises the yield of *S.* *x urama* extracts. The yield of methanol extract was the highest of the others. The yield of the acetone extract was close to that of the methanol extract. Hexane extract had the lowest yield. These results suggest that methanol and acetone are suitable solvents for extracting phytochemicals compared to ethyl acetate and hexane. It was probably because most of the compounds in the extracted plants are polar and semi-polar, therefore soluble in polar and semi-polar solvents, such as methanol and acetone, compared to solvents that tend to be more non-polar, namely ethyl acetate and hexane.

## Antioxidant Activity

The antioxidant activity of *S. x urama* leaf extract was measured based on its ability to scavenge DPPH free radicals and expressed as IC50 values. DPPH is a stable free radical because of delocalization electron. When DPPH radicals react with a substance that can donate a hydrogen atom (an antioxidant compound), it causes violet DPPH loss[15]. Table 3 showed that methanol, acetone, and ethyl acetate extracts were active antioxidants, categorized as strongly active, while hexane extract was inactive, based on criteria by Phongpaicit [24].

**Table 3**. Antioxidant Activity (IC50) of *S.x urama* leaves extracts

|  |  |
| --- | --- |
| **Sample** | **IC50 (µg/mL)** |
| Methanol | 21.25±0.03 |
| Acetone | 19.30±0.02 |
| Ethylacetate | 45.12±0.11 |
| Hexane | 920.64±5.94 |
| Gallic acid | 1.91±0.13 |
| Ascorbic acid | 7.52±0.03 |

The order of antioxidant strength starts with the most active, acetone > methanol > ethyl acetate. Although acetone was the most active extract, its antioxidant activity was still weaker than gallic and ascorbic acid. Ascorbic acid and gallic acid are well-known as natural antioxidant compounds.

The extracts from *S. x urama* leaves had antioxidant activity because they contain phytochemical compounds, especially phenolic compounds such as flavonoids and tannins (Table 1). Methanol extract had slightly weaker antioxidant activity than acetone extract. It might be because the methanol extract extracted more polar flavonoid compounds, such as flavonoid-glycosides, while the acetone extract extracted more semi-polar flavonoids (might aglycone flavonoids/free flavonoids). Glycosylation in flavonoid compounds can suppress antioxidant ability by reducing the number of free OH groups on flavonoids used to scavenge free radicals [25].

The antioxidant activity of acetone extract (IC50 19.30±0.02 µg/mL) and methanol extract (IC50 21.25±0.03 µg/mL) of *S. x urama* leaves was stronger than 95% ethanol extract of *S. caseolaris* leaves (IC50 24.22 ppm) [26], but weaker than the methanol extract of *S. ovata* leaves from Kotabaru, South Kalimantan (IC50 4.07 ppm) [11] and stem bark of *S. ovata* from Aru islands, Maluku (IC50 4.73 µg/mL) [27]. The acetone and methanol extracts of the leaves of *S. x urama* had a stronger antioxidant activity than the methanol (IC50 0.038±0.003 mg/mL) and chloroform (IC50 0.27±0.017 mg/mL) extracts of *S. alba* [13]. The antioxidant activity of methanol extract of *S. x urama* leaves was higher than the methanol extract of *Dipterocarpos liitoralis* stem bark (IC50 57,59 µg/mL)[28].

# conclusion

Leaves of *S. x urama* contain flavonoids, tannins, terpenoids, saponins, alkaloids, and reducing compounds. The acetone and methanol extracts showed the highest antioxidant activity. Furthermore, acetone and methanol extracts of *S. x urama* leaves have the potential to be sources of antioxidant agents.

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