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Phytochemical and Antioxidant Assay of *Tabebuia aurea* Stem Extract: A Potential Source of Bioactive Compounds

Fadhlur Rohman Al Akbar, Rahmat Kurniawan *

Department of Chemistry, Sumatra Institute of Technology, South Lampung-35365 (Indonesia)

** Corresponding E-mail: rahmat.kurniawan@ki.itera.ac.id*

Abstract: *Tabebuia aurea* has been recognized for its traditional medicinal applications and promising antioxidant properties. The research addresses a gap in understanding the phytochemical and antioxidant characteristics of *T. aurea* in Indonesia. This study aims to conduct phytochemical screening and evaluate the antioxidant activity of *T. aurea* stem extracts. The stems of *T. aurea* were collected from the Botanical Garden of the Sumatra Institute of Technology and authenticated at the Bogoriense-BRIN Herbarium in Bogor to ensure the accuracy of the plant species. The samples were cleaned and dried indoors to prevent the degradation of active compounds before being ground into fine simplicia powder. The extraction process was carried out through maceration utilizing different solvents, including methanol, aquadest, ethyl acetate, and n-hexane. Phytochemical analysis revealed the presence of tannins in the methanol extract. The aquadest extract contains tannins, terpenoids, and flavonoids. Phytochemical screening indicated that the ethyl acetate extract comprises alkaloids and flavonoids, while the n-hexane extract contains alkaloids and saponins. An antioxidant assay using the DPPH method shows that *T. aurea* stem extracts exhibit a significant ability to scavenge free radicals. Methanol extract has IC_{50} value of 51.24 µg/mL (strong), aquadest extract has IC_{50} of 65.34 µg/mL (strong), ethyl acetate extract has IC_{50} of 79.88 µg/mL (strong), and n-hexane extract has IC_{50} of 256.73 µg/mL (very weak). For comparison, the vitamin C control has an IC_{50} of 43.95 µg/mL (very strong). The findings of this study demonstrate that the extract from the stem of *Tabebuia aurea* contains bioactive compounds with potential as a natural antioxidant source. This antioxidant property highlights the potential of *T. aurea* stem as a valuable raw material for developing health and cosmetic products that leverage its antioxidant capabilities.

Keywords: A*ntioxidant assay, phytochemical assay, Tabebuia aurea*

Introduction

Tabebuia aurea (Silva Manso) Benth. & Hook.f. ex S. Moore, commonly referred to as the golden trumpet tree, is a species within the Bignoniaceae family, native to South America. Its distribution spans from Venezuela to Argentina, including tropical regions. In Brazil's Northeastern area, this species is locally known as "Craibeira", "Paratudo", and "Ipê-amarelo" [1]. This species has attracted scientific interest due to its traditional medicinal applications: it is used as infusions or alcohol-based macerations, and its stem bark is chewed to counteract the effects of snakebite. Additionally, it is employed for its anti-inflammatory properties and in the treatment of influenza [2]. Further, *T. aurea* shows antioxidant activity, as well as its potential to produce bioactive compounds [3].

Earlier studies on the phytochemical composition of *Tabebuia* species have identified the presence of various secondary metabolites, including tannins, flavonoids, alkaloids, and saponins [4]. These compounds are known to have antioxidant properties. Antioxidants contribute to neutralizing free radicals and preventing cell damage due to oxidative stress [5].

Previous studies have demonstrated the antioxidant properties of various *Tabebuia* species, including *T. aurea*. Methanol extracts of *T. aurea* leaves and bark have shown strong antioxidant activity with IC_{50} values ranging between 53.03 and 87.2 µg/mL, classifying them as potent natural antioxidants [4].

Although Tabebuia is promising in Indonesia, research on its phytochemical content and antioxidant capacity remains limited [4]. In addition, studies comparing the effectiveness of various solvent extracts of *T. aurea* in

scavenging free radicals remain limited. This research seeks to address this gap by performing an in-depth phytochemical analysis and assessing the antioxidant activity of *T. aurea* stem extracts obtained with a range of solvents, including methanol, aquadest, ethyl acetate, and n-hexane.

This study has two main objectives: first, to identify the phytochemical constituents present in the stem extracts of *T. aurea*; and second, to evaluate the antioxidant potential of these extracts using the DPPH assay. The significance of this research lies in establishing *T. aurea* as a potential natural antioxidant source for the formulation of health and cosmetic products. Furthermore, the results of this study will contribute to the growing body of knowledge in natural product research, particularly in the discovery of new bioactive compounds with therapeutic potential.

Method

Materials

The materials utilized in this research consist of *T. aurea* stems, methanol, ethyl acetate, aquadest, n-hexane, 1% $FeCl_3$ reagent, $MgCl_2$ reagent, Lieberman-Burchard reagent, Mayer reagent, Dragendorff reagent, and a solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH), which were sourced from Sigma-Aldrich. The experimental apparatus consisted of standard laboratory equipment, including volumetric flasks, beakers, stirring rods, analytical balances, glassware, aluminum foil, graduated cylinders, spatulas, test tubes, tube racks, micropipettes, filter paper, chromatography chambers, rotary evaporators, cuvettes, and UV-Vis spectrophotometers.

Sampling and Determination

The stem *of T. aurea* used in this study was obtained from the Botanical Garden, Sumatra Institute of Technology. This specimen was then sent to the Bogoriense-BRIN Herbarium in Bogor for species identification, and it was confirmed as *Tabebuia aurea*. The determination of *plant species of T. aurea* aims to ensure the authenticity of the materials used in this study.

Drying Method

The stems of *T. aurea* are cleaned and dried indoors to prevent degradation of the active compounds from direct sunlight. Once dried, the stems are ground into a fine simplicia powder [6].

Maceration Process

The extraction of *T. aurea* stems, which have been processed into fine simplicia powder, is conducted using four different solvents: methanol, ethyl acetate, n-hexane, and aquadest. For each solvent, 50 grams of powder is weighed. The extraction utilizes the maceration method, where 50 grams of the powdered sample is soaked in 500 mL (1:10 w/v) of solvent for 72 hours at room temperature, shielded from light, with stirring for at least 30 minutes each day. After maceration, the mixture is filtered through filter paper to obtain the filtrate. The filtrate is then concentrated using a rotary vacuum evaporator at 50°C and 110 rpm, yielding a concentrated extract. This extract is subsequently ready for phytochemical screening and antioxidant testing [7].

Phytochemical Assay

A total of 1 gram of extract is dissolved in 10 mL of solvent, using methanol, ethyl acetate, n-hexane, and aquadest. From this extract solution, 1 mL (approximately 20 drops) is drawn with a pipette and placed into seven test tubes for each extract type. The initial tube serves as a control or blank, while the subsequent six tubes are prepared for additional testing by incorporating the appropriate reagents [8].

• Alkaloid Examination

For the alkaloid screening, two methods were employed: the Mayer test and the Dragendorff test. The Mayer test was conducted by combining 1 mL of extract with 1 mL of Mayer reagent in test tubes containing methanol, ethyl acetate, n-hexane, and aqueous extracts. The formation of a white precipitate in the Mayer test indicates a positive presence of alkaloids. Similarly, in the Dragendorff test, the appearance of a reddish precipitate when 1 mL of extract is combined with 1 mL of Dragendorff reagent in the same test tubes confirms the presence of alkaloids [9].

• Flavonoid Examination

The flavonoid test involves combining 1 mL of extract with 1 mL of $MgCl_2$ reagent. A positive result is indicated by the formation of red, yellow, or orange flavillium salts, confirming the presence of flavonoids [10], [11].

• Steroid and Terpenoid Examination

The steroid and terpenoid tests involve combining 1 mL of extract with 1 mL of Liebermann-Burchard reagent. A green-blue color indicates a positive result for steroids, while a red-purple color signifies the presence of terpenoids [12].

• Saponin Examination

The saponin test involved combining 1 mL of extract with 1 mL of aquadest and shaking vigorously for 30 seconds. A positive result was indicated by the formation of foam at least 1 cm high within 10 minutes, which persisted after the addition of a drop of 2N HCl [13].

• Tannin Examination

The tannin test involved combining 1 mL of extract with 1 mL of 1% $FeCl_3$ reagent. A positive result was indicated by the formation of a dark blue, blue-black, or greenish-black solution, confirming the presence of tannins [12].

Antioxidant Assay

The antioxidant activity of *the T. aurea stem extract was quantified* using the DPPH method. This method measures the extract's ability to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals, simulating the antioxidant mechanism in inhibiting DPPH as depicted in Figure 1. The sample's absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm [14]. The IC_{50} value (the concentration of the sample required to inhibit 50% of DPPH activity) was calculated using a linear regression equation derived from the relationship between sample concentration and the percentage of inhibition based on the antioxidant assay data.

The experimental procedure involves preparing standard and sample solutions, preparing a 0.1 mM DPPH solution, and conducting an antioxidant activity assay. A 100 µg/mL vitamin C standard solution was prepared by dissolving 1 mg of vitamin C in 10 mL of methanol and diluting to a series of concentrations (6.25 µg/mL; 12.5 µg/mL; 25 µg/mL; 50 µg/mL; 100 µg/mL). Similarly, a 100 µg/mL sample solution was prepared by dissolving 1 mg of extract in 10 mL of methanol and diluting it to the same concentration series. A 0.1 mM DPPH solution was prepared by dissolving 0.9858 mg of DPPH in 25 mL of methanol. In the antioxidant assay, 2 mL of 0.1 mM DPPH solution was combined with 2 mL of the standard or sample solution at varying concentrations, and the mixture was incubated for 30 minutes in a dark room. Absorbance was then measured at 517 nm using a UV-Vis spectrophotometer [14].

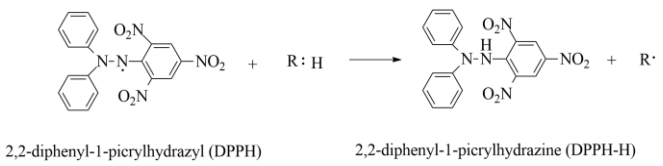

Figure 1. Reaction Mechanism of Antioxidant Compounds in Inhibiting DPPH [15]

Results And Discussion

Result of Phytochemical Assay

Phytochemical assay is an initial screening step in research to identify the classes of secondary metabolites present in a plant. This method involves adding specific reagents to the plant extract to identify the chemical compounds it contains. The addition of specific reagents in phytochemical assay enables the identification of certain compounds based on color changes or precipitate formation. For instance, color changes upon the addition of Mayer's or Dragendorff's reagents can be used to detect alkaloids, while the Liebermann-Burchard test can identify terpenoids and steroids. Tannin testing involves adding the FeCl3 reagent, while flavonoid detection relies on a reaction with $MgCl_2$. Saponins are identified by adding aquadest.

Phytochemical data from *T. aurea* stems revealed varied results. In the methanol extract, tannins were detected with the appearance of a greenish-black color. The aquadest extract showed positive results for tannins (blackish color), terpenoids (reddish color), and flavonoids (reddish-orange color). The ethyl acetate extract was found to contain alkaloids, as evidenced by a reddish precipitate in the Dragendorff test and a white precipitate in the Mayer test, as well as flavonoids, indicated by a reddish-orange color. Meanwhile, the n-hexane extract showed the presence of alkaloids with results similar to those of the ethyl acetate extract, and saponins, indicated by the formation of foam.

The positive detection of alkaloids, terpenoids, tannins, flavonoids, and saponins in the methanol, aquadest, ethyl acetate, and n-hexane extracts suggests that the stems of *T. aurea* are abundant in bioactive compounds with potential pharmacological properties. This information is essential for comprehending the plant's chemical composition and its possible applications in the

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health and pharmaceutical sectors. The phytochemical assay results for the methanol, aquadest, ethyl acetate, and n-hexane extracts of the *T. aurea* stem samples are presented in Figure 2.

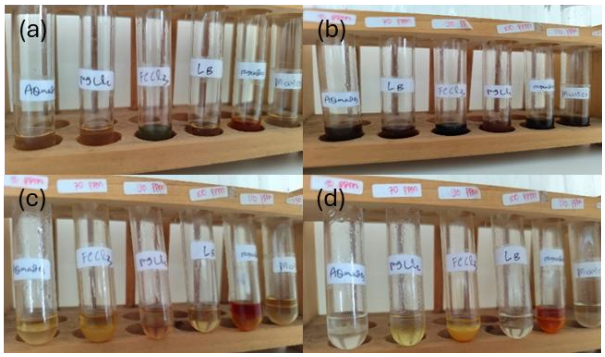

Figure 2. Phytochemical Assay of Methanol (a), Aquadest (b), Ethyl Acetate (c), n-hexane (d) Extract from Stems of *T. aurea*

Result of Antioxidant Assay

The antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, with absorbance measured at 517 nm using a UV-Vis spectrophotometer [14]. DPPH is a stable free radical compound characterized by its delocalized unpaired electrons, which impart a distinct purple color [14]. The principle of this method lies in the ability of antioxidant compounds to donate hydrogen atoms to free radicals, converting DPPH into its non-radical form, DPPH-H, as indicated by a color change from purple to yellow (Figure 3). This discoloration indicates the reduction of free radicals by antioxidants [16].

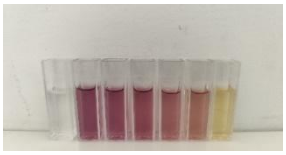

Figure 3. The Results of Antioxidant Assay

In this research, vitamin C served as the positive control, and the antioxidant activity of *T. aurea* stem extracts in methanol, aqueous, ethyl acetate, and n-hexane solvents was evaluated. The absorbance of each extract at varying concentrations (6.25, 12.5, 25, 50, and 100 µg/mL) was measured to assess the extract's capacity to inhibit DPPH radicals, expressed as percentage inhibition (% inhibition). The IC_{50} value, which represents the concentration required to inhibit 50% of DPPH radicals, was used as a measure of antioxidant potency. A lower IC_{50} value indicates a stronger antioxidant activity of the sample [17]. The results demonstrated that DPPH radical inhibition increased progressively with rising concentrations of both vitamin C and the tested extracts. Methanol extracts exhibited the highest antioxidant activity, followed by aquadest extracts and ethyl acetate extracts, whereas n-hexane extracts demonstrated significantly lower activity. The IC_{50} values obtained were 43.95 µg/mL for vitamin C, 51.24 µg/mL for methanol extract, 65.34 µg/mL for aquadest extract, 79.88 µg/mL for ethyl acetate extract, and 256.73 µg/mL for extract n-hexane. This shows that methanol, aquadest, and ethyl acetate extracts have strong antioxidant activity, while extracts in n-hexane are relatively weak in their antioxidant activity [17].

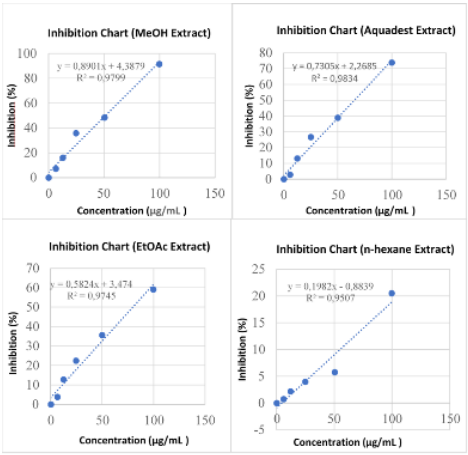

Figure 4. Inhibition Chart of Methanol, Aquadest, Ethyl Acetate, n-hexane Extract from Stems of *T. aurea*

The significant antioxidant activity observed in the methanol and aqueous extracts is likely due to the solvents' ability to extract polar secondary metabolites, such as flavonoids and tannins, which are renowned for their potent antioxidant properties. These compounds can neutralize free radicals by donating a hydrogen atom. Instead, extract n-Hexane, which is non-polar and tends to contain non-polar compounds such as lipids with low antioxidant potential. Thus, the variation in antioxidant activity among the tested extracts can be explained by the differences in solvent polarity and the types of metabolites extracted [18].

Table 1. Phytochemical and Antioxidant Profile

Extract Sample	Activity Antioxidant	Phytochemical Profile					
	IC_{50}	Alkaloids	Flavonoids	Steroids	Terpenoids	Saponins	Tannins
Methanol	51,24	-	-	-	-	-	+
Aquadest	65,34	-	+	-	+	-	+
Ethyl Acetate	79,88	+	+	-	-	-	-
n-hexane	256,73	+	-	-	-	+	-

The data on antioxidant activity and phytochemical composition presented in Table 1 demonstrate that the *T. aurea* stem extract contains bioactive compounds with potential as a natural antioxidant source. The observed antioxidant activity in the methanol, aqueous, and ethyl acetate extracts, each containing secondary metabolites such as tannins, flavonoids, and alkaloids, supports the viability of *T. aurea* stems as a raw material for the development of health and cosmetic products that leverage their antioxidant properties. These findings confirm the potential of *T. aurea* as a natural source of antioxidant compounds, advancing natural therapies and antioxidant-based medicinal and cosmetic formulations.

Conclusions

The phytochemical analysis confirmed the presence of tannins, alkaloids, flavonoids, terpenoids, and saponins in *T. aurea* stem extract, with variations dependent on the solvent type. Antioxidant activity testing using the DPPH method revealed that methanol, aqueous, and ethyl acetate extracts exhibited strong antioxidant activity, with IC_{50} values of 51.24 µg/mL, 65.34 µg/mL, and 79.88 µg/mL, respectively, while n-hexane extract demonstrated weak antioxidant activity, with an IC_{50} value of 256.73 µg/mL. Vitamin C, used as a positive control, displayed very strong antioxidant activity with an IC_{50} value of 43.95 µg/mL. These findings suggest that *T. aurea* stem extract contains bioactive compounds with potential as a natural antioxidant source, supporting its application as a raw material in the development of health and cosmetic products.

Conflicts of interest

All authors declare that there are no conflicts of interest related to this study.

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