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RESEARCH ARTICLE

Antioxidant activities of aqueous and 70% ethanol extracts of akar kuning (*Arcangelisia flava* (L.) Merr) stem using the DPPH method

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Abstract

Background: Numerous herbs can be used as natural exogenous antioxidants and clinically proven effective as antioxidants. **Objective:** The primary objective of this study is to assess the antioxidant activity of the stems of akar kuning (*Arcangelisia flava* (L.) Merr). **Method:** This plant was extracted with aqueous solution using the decoction method and 70 % ethanol using the maceration method. Antioxidant activity was evaluated using the TLC-Bioautography sprayed with DPPH reagent for the qualitative method and microplate assay with DPPH for the quantitative method. This research also identified the total phenol from each extract which was measured using the Folin-Ciocalteu method. **Result:** Based on phytochemical screening, each extract contained flavonoids, polyphenols, anthraquinones, terpenoids, and saponins. TLC-bioautography showed flavonoids, polyphenols, and anthraquinones were responsible for antioxidant activity. Quantification of antioxidant activity results of aqueous and 70% ethanol extracts of akar kuning (*Arcangelisia flava* (L.) Merr) showed IC_{50} value at 78.8 ± 1.2 µg/mL and 142.8± 2.9 µg/mL respectively. The content of total phenolic compounds was 80.544 ± 2.6 mg GAE/ g and 52.522 ± 1.9 mg GAE/g. **Conclusion:** As observed in the study, *Arcangelisia flava* (L.) Merr extracts from both extraction methods possess an antioxidant.

Introduction

From 2009 to 2019, the prevalence of deaths in Indonesia caused by degenerative diseases has increased (Vos *et al.*, 2020). These diseases are generally caused by cell damage due to exposure to oxidative stress and free radicals (Pham-Huy *et al.*, 2008). Various studies have shown that antioxidant compounds play a major role in preventing and reducing the risk of degenerative diseases and other diseases caused by free radicals (Bharti *et al.*, 2013). Plants are still a source of natural antioxidants often utilised by the community and are clinically proven effective as antioxidants (Ariani *et al.,* 2023).

Arcangelisia flava (L.) Merr or Yellow Root is one of the plants that have the potential as a traditional medicine to treat various diseases, one of which is jaundice in the Dayak tribe in Kalimantan (Herianto *et al.*, 2018). Several plant extracts have been reported to have antioxidant activity, such as methanol extract (Keawpradub *et al.*, 2005) and 96% ethanol extract (Suratno *et al.*, 2019).

This research was conducted to explore potential aqueous and ethanolic extracts from *Arcangelisia flava* (L.) Merr stems as an antioxidant. It also identified the total phenolic content from each extract. Phenol compounds are key to antioxidant activity (Dimitrios, 2006). This activity was seen from its ability to scavenge free radicals with the DPPH method. Then, the total phenolic content was measured using the Follin-Ciocalteu method.

Methods

Chemicals and reagents

The reagents used in this study include 70% ethanol, distilled water, Follin-Ciocalteu reagent, sodium carbonate (Merck); gallic acid (Sigma-Aldrich); 1,1-diphenyl-2- picryl-hydrazil (Sigma-Aldrich) microplate reader (Biochrom EZ READ 2000).

Plant materials

Stems of *Arcangelisia flava* (L.) Merr were obtained from Soren Forest, Kalimantan, Indonesia. The plant was identified in Materia Medika Malang.

Extract preparations

Extraction was done using decoction and maceration methods. The 200 g powdered stems were extracted with aqueous using the decoction method and 70% ethanol using the maceration method (1: 10 b/v). The decoction took 30 minutes, and the filtrate was concentrated using freeze-dried. The maceration was done in three days, and this process was repeated thrice. The filtrate from maceration was concentrated in a rotary evaporator at 40ºC.

Phytochemical screening

Phytochemical screening was conducted using the TLC qualitative method derivatised by some reagents. The presence of flavonoids was tested with borax citrate, polyphenols with $FeCl_3$ reagent, alkaloids with Dragendorf reagent, anthraquinones with KOH 10 %, terpenoids with anisaldehyde-H_2SO_4, and saponins using the foam test. All extracts were evaluated by mobile phase Chloroform—Aceton—Formic acid (8:4:1).

TLC bioautography sprayed with DPPH

The stationary phase employed in this study was silica gel 60 F254. The mobile phase consisted of a mixture of Chloroform, Acetone, and Formic acid in a ratio of 8:4:1. Samples (25,000 ppm) were spotted on the plate. After evaluation, the plate was sprayed with 0.02% DPPH to identify radical scavenging activity. The TLC-Bioautography test was carried out to observe the colour change of the spot to the yellow spot, which means that the sample has radical scavenging activity (Gu *et al.*, 2009).

Quantification of antioxidant activities using the DPPH method

The antioxidant activities of all extracts were quantified using the DPPH method, which was accomplished by utilising a 96-well microplate reader. 100 µL of blanko extracts (15.6 – 500 µg/mL) and positive control (0.5-4 µg/mL) were transferred into a 96-well plate. Then, 100 µL of 0.25 mM DPPH reagent was added to the 96-well plate. The well plate was incubated for 30 min in the dark and covered. After that, the DPPH reduction reaction was measured at an absorbance of 517 nm. Gallic acid was used as a positive control. The percentage inhibition was calculated using the formula:

$$\% \text{ inhibition} = 1- \{\frac{(\bar{x}A\ sampel - \bar{x}\ A\ blanko)}{\bar{x}\ A\ kontrol}\} \times 100\%$$

Quantification of total phenolic content (TPC) using the Follin-Ciocalteu method

The total phenolic content of all extracts was quantified by the Follin-Ciocalteu method performed with a 96-well microplate reader (Ahmad *et al.*, **2017**). In summary, the aliquot of the extract or standard solution (25 µL) was introduced into a 96-well plate, followed by 100 µL of a 25% Folin-Ciocalteu reagent. The mixture was thoroughly mixed and left undisturbed for six minutes. Next, a solution of sodium carbonate (75 µL) was introduced, thoroughly mixed, and allowed to incubate for 90 minutes at ambient temperature while shielded from light.

The absorbance measurement was conducted with a 96-well microplate reader spectrophotometer UV-VIS, specifically at a wavelength of 750 nm. A range of concentrations of Gallic acid, ranging from 12.5 to 400 µg/mL, was employed as a standard in the experiment. The resulting regression formula, Y = 0.005x - 0.0795, was derived with a R-value of 0.9962. In this formula, Y represents the concentration of Gallic acid, while X represents the corresponding absorbance value. This equation was employed to calculate each extract's total phenolic content (TPC).

$$\text{TPC (mg GAE/g sample)} = \{\frac{(C\ x\ V)}{M}\}$$

Statistical analysis

The 50% inhibitory concentration (IC_{50}) and total phenolic compound values were determined using Microsoft Excell software by plotting the concentration of extracts as X-axis and % scavenging DPPH for the antioxidant assays, and concentration of extracts as Y-axis and absorbance as ordinate for the total phenolic compounds assays. Results were expressed as mean ± standard deviation (SD) of three experiments.

Results

Phytochemical screening

Phytochemical screening identified various phytochemical constituents using qualitative methods. The results are shown in Table I.

Based on phytochemical screening, all extracts contained flavonoids, polyphenols, and anthraquinones. However, the results showed differences in the phytochemical constituents of each extract. Terpenoids only existed in 70% ethanol extract, and saponins only in the aqueous extract.

Table I: Phytochemical screening results of both extracts

Extract	Flavonoids	Polyphenols	Anthraquinones	Alkaloids	Terpenoids	Saponins
Aqueous extract	++	++	+	+	-	+
70% Ethanol extract	++	+	+	+	+	-

*++: two spots observed; *+: one spot observed; *-: spot not observed

TLC Bioautography sprayed with DPPH

DPPH is a free radical that is dark purple. This colour fades to pale yellow as the free electrons on the molecule pair with antioxidant molecules. Its presence of free radical scavenging activity (Gupta, 2015).

The TLC Bioautography results indicated that all extracts exhibited free radical scavenging activity, as evidenced by the colour change of the stain to a pale-yellow hue on the TLC plate, as shown in Figure 1.

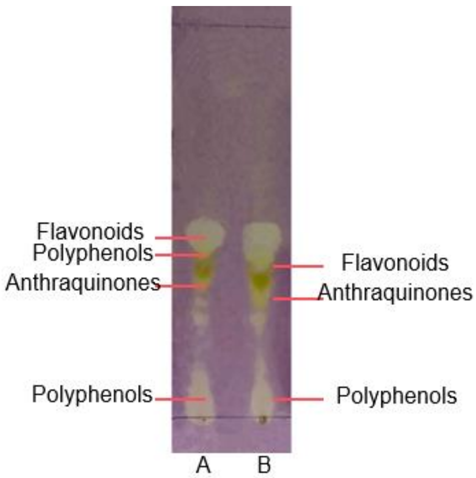

Figure 1: TLC Bioautography using DPPH results of aqueous extract (A) and 70% ethanol extract (B) *Arcangelisia flava* (L.) Merr stems

Quantitative antioxidant activities using DPPH

The results of IC_{50} value measurements on aqueous extracts, 70% ethanol extracts, and gallic acid were 78.8 ± 1.2 µg/mL, 142.8 ± 2.9 µg/mL, and 1.5 ± 0.1 µg/mL, respectively. The results are shown in Figure 2.

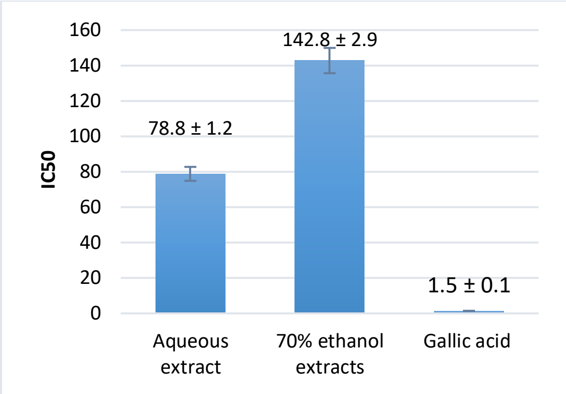

Figure 2: IC_{50} results of both extracts *Arcangelisia flava* (L.) Merr stems and control

Total phenolic content (TPC) using Follin-Ciocalteu

Phenolic compounds have the most role in causing antioxidant activity both *in vivo* and *in vitro* (Zeb, 2020). The results showed phenol in the aqueous extract and 70% ethanol extract of the *Arcangelisia flava* (L.) Merr stems were 80.544 ± 2.6 mg GAE/g and 52.522 ± 1.9 mg GAE/g, respectively. The results are shown in Figure 3.

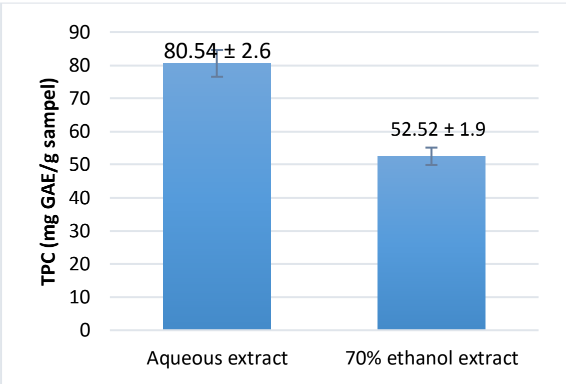

Figure 3: Total phenolic content results of both extracts *Arcangelisia flava* (L.) Merr stems

Discussion

Oxidative stress deregulates a series of cellular functions, leading to various pathophysiological conditions such as osteoarthritis, cardiovascular dysfunction, diabetes, and degenerative diseases (Yeshi *et al.*, 2022). In the human body, several mechanisms have been developed to protect the body from oxidative or nitrosative stress induced by free radicals. The body naturally produces antioxidant compounds to overcome the presence of free radicals (Shi *et al.*, 1999). However, besides natural antioxidant compounds obtained from the body, antioxidant intake can be obtained from outside the body (Flieger *et al.*, 2021). Plants are a source of natural antioxidants often utilised by the community (Amarowicz *et al.*, 2019).

Based on phytochemical screening, all extracts contained flavonoids, polyphenols, and anthraquinones. A comparison between TLC plates from phytochemical screening and TLC Bioautography showed flavonoids, polyphenols, and anthraquinones were the compounds responsible for antioxidant activity in the extracts based on their radical scavenging ability.

This research also identified quantitative antioxidant activity from each extract. A microplate reader quantified all extracts using the DPPH method to get their IC_{50} value. The results of IC_{50} value measurements on aqueous extracts, 70% ethanol extracts, and gallic acid were 78.8 ± 1.2 µg/mL, 142.8 ± 2.9 µg/mL, and 1.5 µg/mL respectively. The results of the IC_{50} values of the two extracts had significant differences in their antioxidant strength. Based on the antioxidant strength category, water extract has strong antioxidant activity with an IC_{50} value in the range of 50-100 µg/mL, while 70% ethanol extract has moderate antioxidant strength with an IC_{50} value in the range of 100-150 µg/mL (Jun *et al*., 2003).

Furthermore, all extracts were measured for total phenol content using the Follin-Ciocalteu method. This measurement showed that the aqueous extract had a higher total phenol content than the 70% ethanol extract. This finding is consistent with the observed antioxidant activity exhibited by the aqueous extract, which surpassed that of the ethanol extract. The study results showed that phenolic compounds were among the compounds that could be responsible for the antioxidant capacity of the extract. The main mechanism was donating hydroxyl groups to free radical substrates, resulting in non-radical substrate species (RH, ROH, or ROOH) and antioxidant free radicals (Zeb, 2020).

Conclusion

Both the aqueous extract and the 70% ethanol extract exhibit antioxidant properties and possess the potential to be formulated into pharmaceutical agents that can confer health benefits to humans. These extracts can safeguard the body against diseases induced by free radicals and prevent ailments like cancer and hepatitis triggered by the presence of these reactive species.

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