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Antidiabetic potential of *Acacia auriculiformis* leaf extract based on antioxidant activity (DPPH assay)

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Abstract
Background: Diabetes mellitus is a chronic metabolic disorder associated with oxidative stress, which leads to complications in various organs. Natural antioxidants from plants have gained attention as potential antidiabetic agents due to their ability to neutralize reactive oxygen species (ROS). **Objective**: This study aimed to evaluate the antioxidant potential of Acacia auriculiformis leaf extract as a preliminary indicator of its antidiabetic activity using the DPPH radical scavenging method. **Methods:** The antioxidant activity was determined by the in vitro DPPH (2,2-diphenyl-1-picrylhydrazyl) assay using methanolic extracts of A. auriculiformis leaves at concentrations of 6–100 ppm. The percentage of inhibition was measured spectrophotometrically at 492 nm, and IC_{50} values were calculated using nonlinear regression analysis with GraphPad Prism 10.3.1. **Results:** The extract showed a concentration-dependent increase in DPPH radical inhibition, with an IC_{50} value of 70.09 ppm, classifying it as a strong antioxidant (IC_{50} < 100 ppm). The correlation analysis ($r = 0.9972$, $p < 0.001$) confirmed a strong linear relationship between concentration and antioxidant activity. **Conclusion:** These findings suggest that A. auriculiformis leaf extract exhibits strong antioxidant activity, likely attributed to its phenolic, flavonoid, and tannin constituents, supporting its potential as a natural candidate for antidiabetic therapy development.

Keywords: *Acacia auriculiformis*; DPPH assay; antioxidant; IC_{50}; natural antidiabetic agent; ROS (Reactive Oxygen Species).

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INTRODUCTION

Diabetes mellitus (DM) is one of the most prevalent metabolic disorders worldwide, characterized by chronic hyperglycemia resulting from defect in insulin secretion, insulin action or both [1]. According to the International Diabetes Federation (IDF, 2022), an estimated 537 million adults were living with diabetes globally, and this number is projected to increase to 783 million by 2045, highlighting the growing global burden of this disease [2]. Chronic hyperglycemia causes oxidative stress, leading to complications various organs such as the heart, kidneys, and nerves [3,4]. Oxidative stress production Reactive Oxygen Species (ROS) through several metabolic pathways, such as the polyol pathway, the hexosamine biosynthetic pathway (HBP), and the activation of protein kinase C [5]. This imbalance between ROS production and antioxidant defenses leads to cellular damage, inflammation, ad contributes to diabetic complications like neuropathy, retinopathy, and cardiovascular disease [6].

Antioxidants play a vital role in scavenging free radicals. Thereby maintaining the integrity of pancreatic β-cell and improving insulin sensitivity [7,8]. Although synthetic antidiabetic drugs such as metformin, sulfonylureas, and acarbose are widely used, their long-term use is often associated with adverse side effects [9,10]. Consequently, natural products have gained increasing attention as promising alternatives for diabetes management due to their diverse mechanisms of action, lower toxicity, and minimal side effects [11].

Plants containing phenolic and flavonoid compounds have been reported to exhibit significant antidiabetic effects through both antioxidant activity and inhibition of carbohydrates-hydrolyzing enzymes such as a-glucosidase and a-amylase [12]. Acacia auriculiformis commonly known as earleaf acacia, is leguminous tree widely distributed in Southeast Asia including Jambi, Indonesia. Its leaves are known to contain a variety of secondary metabolites, including flavonoid, tannin, and phenolic compounds. Previous studies have demonstrated that the leaf extract of A. auriculiformis exhibit string antioxidant activity as evidenced by DPPH (2,2-diphenyl-1-picrylhyrazyl) radical scavenging assays, suggesting its potential as a natural antidibateic agents using the DPPH assay as a preliminary indicator of its potential as a natural antidiabetic agent [13]. Overall, this research focuses on evaluating the antioxidant activity of Acacia auriculuformis leaf extract using the classical DPPH radical scavenging method to assess its biological potential. Through this approach, the study aims to confirm the ability of A. auriculiformis antioxidants to neutralize free radicals and inhibit the formation of reactive oxygen species (ROS), ehich play a central role in the pathogenesis of diabetes mellitus. The findings of this study are expected to provide a scientific foundation for developing A. auriculiformis as a promising natural antidiabetic candidate based on its antioxidant properties.

METHODS

Study design

This study was an experimental laboratory research conducted to evaluate the antioxidant activity of Acacia auriculiformis leaf extract using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The experiment was performed in vitro to determine the extract's ability to scavenge free radicals as an indicator of its potential antidiabetic properties.

Materials and instruments

The materials used included DPPH (Sigma-Aldrich), ascorbic acid (as the standard antioxidant), methanol (p.a.), dimethyl sulfoxide (DMSO), and distilled water. The equipmen used consisted of analytical balance, micropipettes (100 µL and 1000 µL), microtubes (1.5 mL), centrifuge tubes, multichannel pipettor, 96-well microplate, vortex mixer, and microplate reader (set at 492 nm). All glassware and instruments were cleaned and calibrated prior to use.

Sample preparation

Fresh leaves of Acacia auriculiformis were collected from the Muaro Jambi Regency, Jambi, washed, and air-dried at room temperature. The dried leaves were powdered and subjected to maceration with ethanol (or methanol) at a ratio of 1:10 (w/v) for 48 hours with occasional stirring. The extract was filtered, concentrated using a rotary evaporator, and stored at −20°C until further analysis. A stock solution of 100,000 ppm was prepared by dissolving 100 mg of the extract in 1 mL of DMSO. Serial dilutions were made using DMSO to obtain five concentrations of 6,13,25,50,100 ppm, which were used for the DPPH assay.

Preparation of reagents and standard solutions

The DPPH stock solution (1 mM) was prepared by dissolving 39.4 mg of DPPH in 100 mL of methanol. This solution was stored in a tightly closed amber bottle and kept away from light. The working solution (0.1 mM DPPH) was prepared freshly before use by diluting the stock solution with methanol. Ascorbic acid was used as a positive control. The stock solution (1,000 ppm) was prepared by dissolving 10 mg of ascorbic acid in 10 mL of distilled water, followed by serial dilutions to obtain concentrations ranging from 25 ppm to 0.20 ppm. All reagent preparations were carried out in minimal light conditions to prevent degradation.

DPPH radical scavenging assay

The antioxidant activity was determined following the standard operating procedure (POB No. 009/POB-LK/XII/2023). Briefly, 80 µL of each extract or standard solution was pipetted into a 96-well microplate, followed by the addition of 80 µL of 0.1 mM DPPH solution. Each concentration was tested in triplicate. Three wells containing only solvent and DPPH served as blanks. The microplate was covered with aluminum foil and incubated at 25°C for 30 minutes in the dark. After incubation, the absorbance was measured at 492 nm using a microplate reader [14]. The percentage of DPPH inhibition was calculated using the equation (1).

$$\%\text{inhibition} = \frac{A\ Blank - A\ Sample}{A\ Blank} \times 100 \quad (1)$$

Where Ablank is the absorbance of the control (DPPH+solvent) and Asample is the absorbance of the test sample or standard. The IC50 value the concentration required to inhibit 50% of the DPPH radicals) was determined from the four-parameters logistic regression model equation of the dose-response curve between the percentage of inhibition and the extract concentration.

Statistical analysis

Data analysis was performed using GraphPad Prism version 10.3.1. IC_{50} values were determined using nonlinear regression with the model "log(inhibitor) vs. normalized response – variable slope" based on a four-parameter logistic equation fitted by the least-squares method. All experiments were conducted in triplicate, and the results were reported as mean ± standard deviation (SD) [15].

RESULTS

The antioxidant activity of Acacia auriculiformis leaf extract was tested using the DPPH (2,2-diphenyl-1-picryhydrazyl) method at various concentrations of 6, 13, 25, 50, and 100 ppm. The results showed an increase in DPPH free radical inhibition as the concentration of the extract increased. This indicates a dose-dependent relationship with antioxidant activity.

Table 1. DPPH radical inhibitory activity of Acacia auriculformis leaf extract.

Conc. (%)	Conc. (ppm)	%inhibition			Avarage	SD
		1	2	3		
0.01	100	65.62	64.61	65.62	65.28	0.58
0.005	50	35.87	35.39	34.23	35.16	0.84
0.0025	25	14.06	15.45	14.97	14.83	0.71
0.0013	13	4.96	3.66	4.96	4.53	0.75
0.0006	6	0.91	2.46	2.6	1.99	0.93

Conc. = Concentration; Avg. = Average; SD = Standard Deviation (n = 3)

Based on these results, the average inhibition value increased from 1.99% at a concentration of 6 ppm to 65.28% at the highest concentration of 100 ppm.

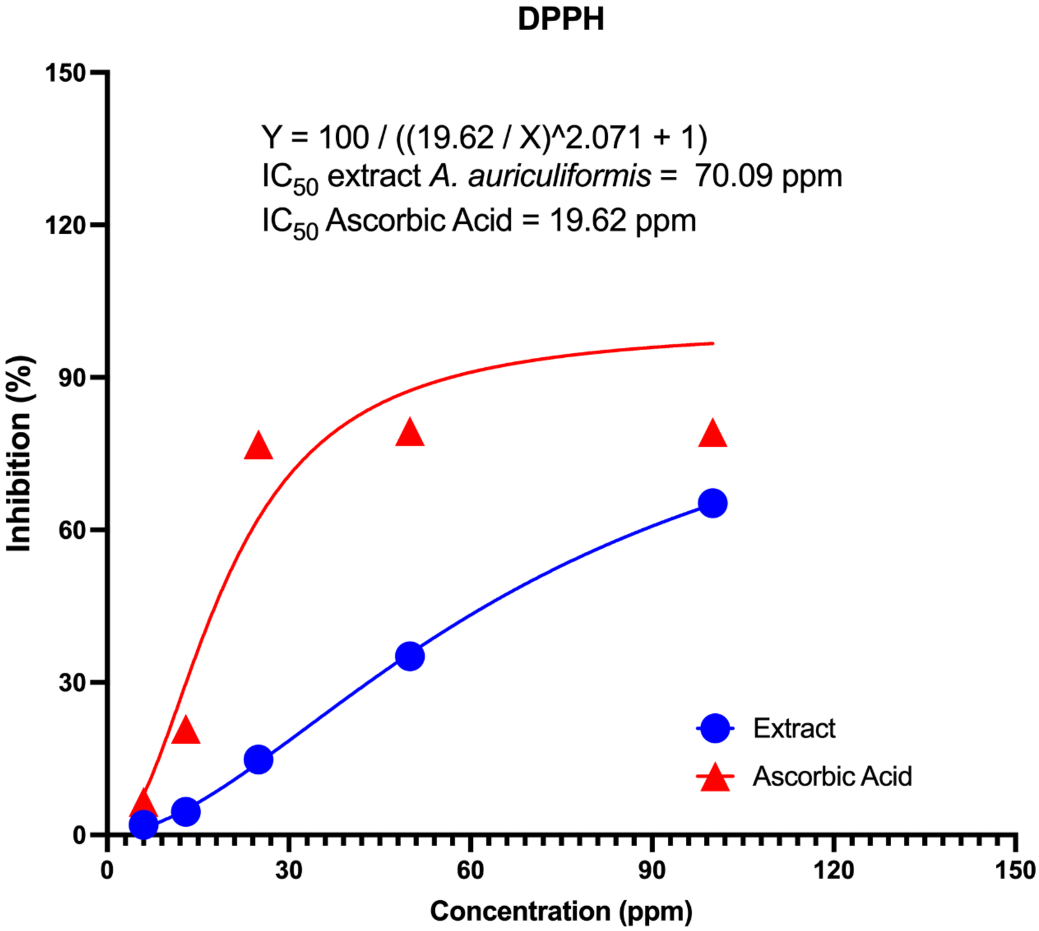

Figure 1. Antioxidant activity curve of A. auriculiformis extract and ascorbic acid using DPPH method. Remarks: IC50 value was calculated based on four-parameter logistic regression model with least squares method. Model [inhibitor] vs normalized response-variable slope with Graphad Prism 10.3.1.

A. auriculiformis extract showed antioxidant activity with an IC_{50} value of 70.09 ppm, significantly different from ascorbic acid as a positive control which had an IC50 of 19.62 ppm. The inhibition curve showed a typical dose-dependent pattern, where increasing extract concentration was positively correlated with the percentage of DPPH radical inhibition. The nonlinear regression model used produced a high coefficient of determination (R^2) for the extract (0.9995/99.95%) and ascorbic acid (0.8713/87.13%) indicating the model's suitability to the experimental data. Statistical analysis proved that the Pearson correlation (r) between concentration and inhibitory activity reached 0.9972 ($p<0.001$) for the extract indicating a strong linear relationship between the administration of *A. auriculiformis* extract and antioxidant activity. In contrast, ascorbic acid showed a lower correlation (r=0.7184, p=0.17) which was not statistically significant. This difference indicates a distinct free radical inhibition mechanism between the pure synthetic compound and the direct extract containing a diverse mixture of secondary metabolites.

The Hill Slope value of the extract (1.745) was lower than that of ascorbic acid (2.071), indicating a lower response gradient. This parameter suggests that the extract requires a higher concentration range to achieve maximum inhibition, likely due to synergistic or antagonistic interactions between the bioactive components. Comparison of antioxidant activity revealed that the extract had a 3.57-fold lower potency than ascorbic acid based on the IC50 ratio. However, the IC50 value below 100 ppm classifies the extract as a potent antioxidant according to the Blois standard. These findings validate the potential of A. auriculiformis extract as a natural antioxidant source that can be further developed for pharmaceutical or nutraceutical applications.

DISCUSSION

The findings of this study demonstrate that Acacia auriculiformis leaf extract proccess strong antioxidant activity as evaluated by the DPPH assay. The percentage of free radical inhibition increased progressively with extract concentrations ranging from 6 to 100 ppm, indicating a clear dose-dependent relationship in its free radical scavenging capacity. This observation aligns with the fundamental principle of the DPPH assay, in which higher concentrations of antioxidant compounds enhance the capacity to donate electrons, thereby more effectively neutralizing free radicals [16].

The IC_{50} value of 70.09 ppm obtained in this study shows that A. auriculiformis leaf extract is included in the strong antioxidant category (IC_{50}<100ppm). These results are consistent with the findings of Sundari & Wijayanti, (2021), who reported that the ethanol extract of A. Auriculiformis leaves showed very high antioxidant activity with an IC_{50} value of 9 ppm using the DPPH method [17]. The ethanol fraction of A. auriculiformis leaves contains high levels of phenolic and flavonoid compounds, which play a crucial role in free radical scavenging mechanisms [18,19]. Phenolic and flavonoid compounds are known to act as proton or electron donors, enabling them to stabilize free radicals and prevent oxidative chain reactions in the body [13,20,21].

The flavonoids, tannins, and phenolic compounds present in A. auriculiformis leaf extract exhibit various biological activities, including anti-inflammatory, antimicrobial, and antidiabetic effects [13,19,22]. They work by counteracting free radicals and synergizing in inhibiting the enzymes a-glucosidase and a-amylase which play a role in the process of hydrolyzing carbohydrates into glucose [13,23]. This finding is consistent with the study by Hossain et al. which reported that interactions between phenolic hydroxyl groups and enzyme active sites can form hydrogen bonds and hydrophobic interactions, thereby reducing the affinity of α-glucosidase and α-amylase for their substrates. As a result, the breakdown of polysaccharides into monosaccharides is slowed, leading to reduced glucose absorption in the intestine and attenuation of postprandial glucose spikes [24].

The relationship between antioxidant activity and glycemic control plays a significant role in the pathogenesis of diabetes mellitus [25]. Elevated production of reactive oxygen species (ROS) damages pancreatic β-cells, leading to impaired insulin secretion and reduced insulin sensitivity in peripheral tissues. Additionally, ROS accumulation contributes to the chronic complications of diabetes, including neuropathy, nephropathy, and cardiovascular disease [26,27]. Therefore, the presence of natural antioxidants, such as those found in A. auriculiformis leaves, may help inhibit ROS formation, protect pancreatic β-cells from oxidative damage, and improve glucose metabolism. Overall, the findings of this study suggest that Acacia auriculiformis is a promising source of natural bioactive compounds with strong antioxidant activity. Further research is warranted, including phytochemical analyses to identify the primary active constituents, as well as additional studies to confirm the extract's efficacy and safety as a natural antioxidant source.

CONCLUSIONS

The results of this study demonstrated that Acacia auriculiformis leaf extract possesses strong antioxidant activity as determined by the DPPH free radical scavenging method. The inhibition of DPPH radicals increased proportionally with the extract concentration, showing a clear dose-dependent relationship. The IC_{50} value of 70.09 ppm indicates that the extract has potent antioxidant activity (IC_{50} < 100 ppm) and supports its classification as a natural antioxidant source. These findings suggest that A. auriculiformis contains bioactive compounds, such as phenolics and flavonoids, that contribute to its antioxidant potential. Further research is required to conduct phytochemical screening and advanced biological evaluations to identify the major active compounds and to confirm the extract’s safety and efficacy for potential use in pharmaceutical or nutraceutical applications.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

This study employed artificial intelligence (AI) tools, including ChatGPT and SciteAI, for language refinement, specifically to improve the grammar, sentence structure, and overall readability of the manuscript. We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. All final decisions, analyses, and interpretations presented in this article were made solely by the authors.

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