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Analysis of quercetin content as an anti-inflammatory agent in *Akasia* leaf extract (*Acacia auriculiformis*) using the HPLC method

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Abstract

Background: Inflammation is a natural defense response, characterized by five classic symptoms, namely swelling, heat, redness, pain, and impaired organ function. However, uncontrolled inflammation can trigger various chronic diseases, so effective and safe anti-inflammatory agents are needed. One natural compound that has this potential is quercetin, a flavonoid with antioxidant and anti-inflammatory activities through inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, as well as modulation of the NF-κB transcription factor. Acacia auriculiformis leaves are known to contain quercetin and other bioactive compounds, so they have the potential to be developed as a source of natural anti-inflammatory agents. **Objective**: This study aims to analyze the quercetin content in ethanol extract of Acacia leaves using high-performance liquid chromatography (HPLC). **Methods:** Extraction was performed by maceration using 96% ethanol. Acacia leaf simplicia weighing 300 grams was successfully extracted and produced a thick extract of 30 ml with a characteristic dark green-brown color. Then, it was analyzed using HPLC based on validation parameters of LOD (0.1172 mg/L) and LOQ (0.3906 mg/L). **Results:** The quercetin content was found to be below the detection limit (<0.1172 mg/L), making quantitative determination unfeasible. **Conclusion:** The results suggest that quercetin is present in very low concentrations or possibly in a conjugated form. Further phytochemical and GC-MS analyses are required to confirm the presence and activity of other flavonoids contributing to the anti-inflammatory potential of Acacia leaf extract.

Keywords: Quercetin; *Acacia auriculiformis*; HPLC; Flavonoid; Anti-inflammatory

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INTRODUCTION

Inflammation is a complex biological response of vascular tissue to harmful stimuli, such as irritation, infection, or cell damage. This process involves the activation of immune cells and the release of inflammatory mediators aimed at localizing and eliminating the triggers of damage. Although essential for healing, prolonged or chronic inflammation is often associated with the development of various serious diseases [1,2].

The need for anti-inflammatory agents continues to grow, but commonly used synthetic drugs often cause undesirable side effects with long-term use. Therefore, the exploration of natural resources, particularly medicinal plants, is crucial to discover safer and more effective active compounds [3,4]. One plant with great potential is Acacia (Acacia auriculiformis). This plant has been used traditionally, and modern research shows that its leaves contain various secondary metabolites such as flavonoids, tannins, and alkaloids [5]. These compounds are known to have high antioxidant activity, which is an important precursor in anti-inflammatory mechanisms. Flavonoids in particular have the ability to modulate inflammatory signaling pathways [6]. One of the most widely studied flavonoids is quercetin, which is known to suppress inflammation through inhibition of COX and LOX enzymes and modulation of the expression of the transcription factor NF-κB [7,8].

Based on this potential, this study aims to analyze the content of quercetin as a flavonoid compound that plays a key role in anti-inflammatory activity in ethanol extracts of Acacia (Acacia auriculiformis) leaves. The analysis was conducted using the High-Performance Liquid Chromatography (HPLC) method, known for its high accuracy and sensitivity in detecting bioactive compounds in natural products [9,10]. The results of this study are expected to provide scientific data that will serve as a basis for further understanding the potential of Acacia leaf extract as a natural source of anti-inflammatory agents.

METHODS

Materials and equipment

The primary material used was Acacia (Acacia auriculiformis) leaves collected from the Mendalo area, Jambi, Indonesia. Solvents included 96% ethanol, methanol, aquadest, and phosphoric acid (H_3PO_4). The main instruments were an oven, blender, maceration container, water bath, and HPLC system with a C18 column and UV-Vis detector.

Preparation of simplicia

Fresh, disease-free Acacia leaves were collected using a purposive sampling method, washed thoroughly, and air-dried. Drying continued in an oven at 40–60°C until the moisture content met simplicia standards. The dried leaves were ground into fine powder and stored in airtight containers [11,12].

Extraction process

A total of 300 g of powdered Acacia leaf simplicia was macerated in 3 L of 96% ethanol (1:10 ratio) for 48 hours at room temperature with occasional stirring. The filtrate was separated, and the solvent was evaporated using a water bath to obtain approximately 30 mL of concentrated extract [13,14].

Determination of quercetin content by HPLC

Quantification of quercetin was carried out using an HPLC system equipped with a C18 column and UV-Vis detector at 365–375 nm. The mobile phase consisted of a gradient of methanol and 0.1% phosphoric acid in distilled water, with a flow rate of 0.8–1.0mL/min. Standard quercetin was used to construct the calibration curve. The extract sample was dissolved in methanol, centrifuged, and filtered through a 0.45 µm membrane filter prior to injection. Quercetin concentration was determined by comparing the peak area of the sample with that of the standard curve [15,16].

*Ethical concideration*s

This research was approved by the Health Research Ethics Commission, Faculty of Medicine and Health Sciences, Universitas Jambi, under approval number 2355/UN21.8/PT.01.04/2025.

RESULTS

Organoleptic observations

The maceration process successfully produced a thick ethanol extract with a yield of approximately 30 mL from 300 g of powdered leaves. Acacia extract produced a total of 30 ml of thick extract with a yield of 120/300x100% = 40% of the total weight of the simplicia powder. This shows that the maceration method with 96% ethanol solvent is quite efficient in extracting secondary metabolites from Acacia Auriculiformis leaves.

Table 1. Organoleptic observations

Characteristic	Description	Interpretation
Texture	Thick paste	Thick consistency indicates a high level of dissolved solids (secondary metabolite compounds after going through the solvent evaporation process)
Color	Dark greenish brown	This color reflects a combination of compounds extracted from 96% ethanol, Phenolic Compounds (such as tannins and flavonoids), which generally give a dark greenish brown or yellowish color; and Chlorophyll Pigment, a green pigment from leaves that is soluble in ethanol.
Aroma	The strong, distinctive aroma of acacia leaves	The presence of a distinctive aroma indicates that volatile compounds specific to the A. auriculiformis plant are also extracted by ethanol and are not completely lost during the concentration process at a low temperature of 40-50°C.

The extract exhibited a dark greenish-brown color and viscous consistency. This appearance indicates the successful extraction of polar compounds such as chlorophyll and polyphenols, which are known to impart dark coloration to ethanol extracts [17].

Quercetin level analysis testing with HPLC method

The HPLC method used for quercetin quantification was validated based on sensitivity parameters. The obtained LOD and LOQ values are shown in Table 2. These results demonstrate that the HPLC method possesses sufficient sensitivity to detect quercetin at very low concentrations, ensuring analytical reliability for natural product analysis.

Table 2. Quercetin level analysis.

Parameters	Units	Results	Method
Quercetin LOD: 0,1172 LOQ: 0,3906	mg/L	<LOD	HPLC

Quercetin content analysis

The HPLC analysis revealed that the quercetin concentration in the ethanol extract of Acacia leaves was below the detection limit (<0.1172 mg/L). This suggests that the extract contains very low levels of free quercetin (aglycone form). In plants, quercetin predominantly exists in conjugated forms (glycosides) rather than as free aglycone [18]. Because acid hydrolysis was not performed in this study, the glycosidic forms of quercetin were not detected using aglycone standards. Therefore, the undetectable level likely indicates the dominance of bound quercetin in the extract [19]. Although free quercetin was not detected, previous studies have demonstrated that Acacia leaf extracts exhibit anti-inflammatory and antioxidant activities [20,21]. These effects may be attributed to other phenolic compounds, flavonoid derivatives, or glycosylated quercetin, which will be identified in future phytochemical and GC-MS analyses [22].

DISCUSSION

The absence of detectable free quercetin in Acacia leaf ethanol extract is consistent with the common occurrence of flavonoid glycosides in plants, which requires hydrolysis for quantification as aglycones. Despite the undetectable free quercetin, Acacia leaf extracts have shown anti-inflammatory and antioxidant activity in previous studies, suggesting that other compounds, including flavonoid glycosides, tannins, and phenolic acids, contribute to its bioactivity [23,24].

The extraction method may also influence quercetin detection. Maceration with ethanol effectively extracts polar compounds but may not release glycosylated flavonoids without acid or enzymatic hydrolysis [25,26]. Optimization of extraction methods in future studies, along with complementary analytical techniques such as GC-MS or LC-MS/MS, could provide a more comprehensive profile of bioactive compounds and clarify their role in anti-inflammatory mechanisms.

Pharmacologically, anti-inflammatory activity may result from the synergistic effects of multiple compounds rather than a single constituent. Acacia extracts have been reported to inhibit COX and LOX enzymes and downregulate NF-κB activity, supporting the potential of Acacia leaves as a natural anti-inflammatory agent even in the absence of detectable free quercetin [27,28].

CONCLUSIONS

The analysis of Acacia (*Acacia auriculiformis*) leaf ethanol extract using HPLC revealed that the quercetin content was below the detection limit (<0.1172 mg/L). This indicates that quercetin is likely present in conjugated (glycoside) forms that are not

measurable by the current analytical method. Future research will focus on phytochemical and GC-MS analyses to confirm the presence and activity of other flavonoid compounds contributing to the anti-inflammatory potential of the extract.

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 used artificial intelligence (AI) tools and methodologies in the following capacities AI-based language models, such as [for example, ChatGPT, Quillbot], were/was employed to: Language refinement (improving the grammar, sentence structure, and readability of the manuscript).

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