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Evaluation of the Analgesic Potential of *A. cocculus* Stem Bark Using In Vivo Assays

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ABSTRACT

A. cocculus, an endemic plant of Papua, Indonesia, has been traditionally utilized by indigenous Papuan communities for the empirical treatment of various health conditions. This study aimed to evaluate the analgesic potential of the stem bark extract of A. cocculus in mice using the Tail Flick method. A total of 25 mice were randomly divided into five groups (n = 5 per group). Group I served as the negative control (5% CMC-Na, 5 mL/kg, oral.); Group II received mefenamic acid (1.3 mg/30 g BB, oral.) as the positive control; Group III received stem bark extract of A. cocculus (AC) at 17.5 mg/kg, oral.; Group IV received AC at 35 mg/kg, oral.; and Group V received AC at 70 mg/kg, oral. Data were analyzed using paired-sample t-tests and independent-sample t-tests. Treatment with the stem bark extract of A. cocculus significantly increased pain tolerance in mice ($p < 0.05$) compared with the negative control group receiving CMC-Na suspension. Moreover, no statistically significant difference was observed ($p > 0.05$) when compared with the positive control group treated with mefenamic acid. Oral administration of the stem bark extract at doses of 17.5, 35, and 70 mg/kg demonstrated significant analgesic activity ($p < 0.05$) in mice (Mus musculus). These findings suggest that A. cocculus stem bark extract possesses promising analgesic properties and may have potential applications in pain management.

Keywords: Analgesic; A. Cocculus; Endemic

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INTRODUCTION

Pain is a complex biological phenomenon involving interactions between the peripheral and central nervous systems in response to potentially tissue-damaging stimuli. In addition to its protective function, pain is also a leading cause of reduced quality of life and disability globally, in both acute and chronic conditions. The Global Burden of Disease Report indicates that chronic pain contributes significantly to the global burden of disease, particularly in the productive age group. Therefore, effective and safe pain management remains a major challenge in modern clinical practice (Raja et al., 2021)(Meguellati et al., 2026)(Vi et al., 2026).

To date, the management of pain has predominantly relied on synthetic analgesic agents, particularly nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids. Although these medications are clinically effective, prolonged use of NSAIDs is associated with significant adverse effects, including gastrointestinal irritation and bleeding, renal impairment, and an increased risk of cardiovascular events. Opioids, on the other hand, despite their strong analgesic efficacy, are limited by the risks of tolerance, physical dependence, and respiratory depression. These limitations highlight the urgent need for the development of alternative analgesic agents that are safer, effective, and suitable for long-term use (Harirforoosh et al., 2013)(Oliveira et al., 2025),(Kedir & Ayele, 2024).

In this context, medicinal plants represent one of the most promising sources for the discovery of new analgesic candidates. Historically, various traditional medical systems around the world have utilized plants for the treatment of pain and inflammation. Modern scientific investigations have subsequently confirmed that the analgesic activity of medicinal plants is closely associated with the presence of bioactive phytochemical compounds, such as alkaloids, flavonoids, terpenoids, phenolics, and tannins. These compounds have been reported to modulate pain pathways through multiple mechanisms, including the inhibition of prostaglandin synthesis, suppression of cyclooxygenase (COX) enzymes, modulation of inflammatory mediators, and interactions with nociceptive receptors in both the peripheral and central nervous systems (Denlinana & Abram, 2020)(Patel et al., 2025).

Validation of the analgesic activity of medicinal plants is generally conducted through preclinical approaches using extensively validated in vivo test models. Several studies in the past five years have reported that medicinal plant extracts exhibit significant, dose-dependent analgesic activity, even comparable to standard analgesics, thus strengthening the potential of medicinal plants as a source for the development of new analgesics (Binte et al., 2025).

Anamirta cocculus is an endemic medicinal plant that has been traditionally used by communities in Papua for the management of various health conditions. Phytochemical investigations have reported that *A. cocculus* contains diverse classes of bioactive compounds, particularly alkaloids, flavonoids, terpenoids, steroids, tannins and phenolic compounds, which are known to exhibit significant pharmacological activities (Masitoh et al., 2024)(Hardiansyah et al., 2024)(Nur Fadhilah et al., 2025).

Several recent studies have evaluated the biological activities of *A. cocculus*, particularly its antibacterial and antioxidant properties. Extracts from the stem bark and other plant parts have been reported to inhibit the growth of certain pathogenic bacteria and to exhibit considerable free radical–scavenging activity (Tumpu et al., 2025)(Nurwahida et al., 2025)(Erawati et al., 2024)(Masitoh et al., 2024). In addition, *A. cocculus* has also been reported to exhibit antianemic and antihyperglycemic activities (Muslihin et al., 2025)(Budiyanto et al., 2026). Phytochemical analyses have also confirmed the presence of alkaloids and flavonoids in the stem bark of *A. cocculus*, compounds that are pharmacologically associated with analgesic and anti-inflammatory activities in many other medicinal plants (Nur Fadhilah et al., 2025)(Hardiansyah et al., 2024). However, to date, scientific studies that specifically evaluate the analgesic activity of *A. cocculus* stem bark using in vivo experimental models remain very limited or have not yet been systematically reported.

Therefore, scientific evaluation of the stem bark of *A. cocculus* is essential to address the existing knowledge gap and to provide scientific evidence supporting its traditional use as an analgesic. Furthermore, this research is aligned with global efforts to develop safer and more sustainable drug candidates derived from natural products.

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METHODS

Experimental Section

This study is a laboratory experimental research using mice as the test subjects. The research will be conducted from July to August 2025 at the Pharmacy Laboratory of Universitas Pendidikan Muhammadiyah Sorong.

Plant Collection and Identification

A. cocculus bark was collected from the forest area of Misool Island, Raja Ampat Regency, Southwest Papua Province, Indonesia during the period May 2025 – June 2025.

Experimental Animals

A total of 25 healthy adult mice, weighing 25–30 g, were acclimatized for seven days under natural light–dark cycle conditions to allow adaptation to the laboratory environment. During this period, the animals were provided with standard laboratory chow and had ad libitum access to clean drinking water, which was replaced regularly to maintain hygiene and quality. All experimental procedures involving animal subjects were conducted in accordance with established ethical guidelines and were approved by the Research Ethics Committee of the Makassar College of Pharmacy (Approval No. 07.153/KOMETIK/STIFA/VII/2025).

Preparation of Extracts

The *A. cocculus* bark that had been collected were dried using an oven at a temperature of 50°C. The dried sample was then powdered and extracted using methanol as the solvent with a ratio of 1:4. The mixture was allowed to stand for 3×24 hours in a sealed container, protected from direct light, then occasionally stirred and filtered. After the extraction process was complete, the extract was evaporated using a rotary evaporator at 50^0C until a thick extract was obtained. The percentage yield was calculated and recorded (Muslihin et al., 2025).

Analgesic testing

A total of 25 mice were used in this study. The 25 mice were divided into 5 groups, each consisting of 5 mice. Group I: Negative Control (5% CMC Na 5 mL/kg, oral), Group II: Mefenamic Acid (1,3 mg/30 g BB, oral), Group III: *A. cocculus* (AC) (17,5 mg/kg, oral), Group IV: *A. cocculus* (AC) (35 mg/kg, oral), Group V: *A. cocculus* (AC) (70 mg/kg, oral). The analgesic activity was evaluated using the Tail Flick Test, a standard method for assessing centrally mediated analgesic effects based on the reflex response to thermal stimulation. The principle of this assay is based on measuring the latency time required for the experimental animals to respond to a thermal stimulus applied to the tail, which is characterized by a rapid withdrawal or flicking movement of the tail (Meegada et al., 2021)(Aburas et al., 2023). The test was performed using a Tail Flick Analgesy-Meter, with the temperature of the thermal stimulus maintained at a constant 50 °C. Prior to treatment, the baseline response latency of each mouse to the thermal stimulus was measured. The time elapsed between the onset of thermal exposure and the appearance of the reflex response was recorded as T_0 (Rezaee-asl et al., 2014)(Aburas et al., 2023). Subsequently, the animals were administered treatments orally according to their respective groups, consisting of a negative control, a positive control, and treatment groups receiving the stem bark extract of *A. cocculus* at predetermined doses. Thirty minutes after administration, the Tail Flick test was repeated using the same apparatus (Maulana et al., 2024)(Ariasti & Muhsin, 2024). The latency time of the nociceptive response was recorded at 30, 60, 90, and 120 minutes following treatment. The reaction time was noted when the mice exhibited a reflex response in the form of tail flicking or withdrawal from the heat source (Rohmania et al., 2024)(Karrat et al., 2022).

Measures and Data Analysis

All research data were analyzed using IBM SPSS version 25 for Windows programs. The data analysis used was One-way ANOVA followed by LSD test. The results were then presented as an average of SD ±. The value of $p < 0.05$ was inferred to be statistically significant.

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RESULTS

Table 1. Analgesic Activity of *A. cocculus* Stem Bark Extract

Variable	Control - (Na. CMC)	Control + (Mefenamic Acid dose 1.3 mg/kgBW, po)	Group-I (AC 17,5 mg/KgBB)	Group-II (AC 35 mg/KgBB)	Group III (AC 70 mg/KgBB)	p-value
Pret-test	12.44±2.23	8.10±0.97	9.02±2.86	8.72±1.73	7.78±0.89	0.007*
Post-test (Δ%)						
30th minute	-11.83±6.27	49.92±33.11	35.39±59.57	18.89±34.39	58.55±34.45	0.056
60th minute	-9.58±28.70	65.12±38.08	57.65±72.59	32.86±34.07	73.17±36.54	0.054
90th minute	-11.85±47.17	77.31±37.63	72.91±87.85	42.29±35.32	91.60±42.47	0.046*
120th minute	-13.12±49.42	100.11±44.06	85.59±94.05	58.24±39.00	116.62±52.70	0.021*

Notes: Values are expressed as Mean ± SD, n = 5, **Δ%** = % Difference between pre-treatment and post-treatment, *p < 0.05 One-way ANOVA, AC = *A. cocculus*

The results showed that the administration of *A. cocculus* stem extract resulted in an increase in analgesic activity that tended to depend on the time of observation. The analgesic effect began to be seen from the 30th minute and increased until the 120th minute in the treatment group. Group III that obtained a dose of 70 mg/kg BW, showed the strongest and most consistent analgesic response compared to the other extract groups; even at the final observation showed higher effectiveness than positive controls. Meanwhile, group I and group II showed a lower analgesic response, with group II providing the least relative effect among the extract groups. Statistically, differences in responses between groups have not shown significance in the 30th and 60th minute observations. However, at the 90th and 120th minutes, the difference became significant ($p < 0.05$), suggesting that the analgesic effect between groups was more pronounced at longer observation times. Overall, these findings indicate that *A. cocculus* stem extract, especially at a dose of 70 mg/kg BW, has the potential to produce increased analgesic activity over time and exhibit a comparable or stronger response compared to positive controls at the end of the observation period.

Table 2. Comparison of analgesic efficacy between the control group and the treatment group

Measurement Time	Groups		p-value	95% CI (Lower±Upper)
90th minute	**Control + (Mefenamic Acid dose 1.3 mg/kgBW, po)**	Group-I (AC 17,5 mg/KgBB)	0.898	-66.42±75.23
		Group-II (AC 35 mg/KgBB)	0.315	-35.80±105.85
		Group III (AC 70 mg/KgBB)	0.678	-85.11±56.54
	Control - (Na. CMC)	Group-I (AC 17,5 mg/KgBB)	0.021*	-155.59±-13.93
		Group-II (AC 35 mg/KgBB)	0.126	-124.97±16.68
		Group III (AC 70 mg/KgBB)	0.006*	-174.28±-32.62

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Measurement Time	Groups		p-value	95% CI (Lower±Upper)
120th minute	Control + (Mefenamic Acid dose 1.3 mg/kgBW, po)	Group-I (AC 17,5 mg/KgBB)	0.702	-63.59±92.64
		Group-II (AC 35 mg/KgBB)	0.277	-36.24±119.98
		Group III (AC 70 mg/KgBB)	0.664	-94.62±61.61
	Control - (Na. CMC)	Group-I (AC 17,5 mg/KgBB)	0.016*	-176.82±-20.60
		Group-II (AC 35 mg/KgBB)	0.071	-149.48±6.75
		Group III (AC 70 mg/KgBB)	0.002*	-207.85±-51.63

Notes: LSD = *Least Significant Difference*, which is *a post hoc* test to find out significantly different pairs of groups after ANOVA analysis shows differences between groups. **CI** = *Confidence Interval* (95% confidence interval). **Control (+)** = positive control and **Control (−)** = negative control. Comparison data are presented with **a 95% CI.** The difference is considered significant if $p < 0.05$ and is denoted by (*).

Post *hoc* analysis of LSD showed that the analgesic effectiveness of the treatment groups at the 90th and 120th minutes was not significantly different compared to the positive controls (mefenamic acid dose 1.3 mg/kg BW, po), indicating that the analgesic response of the extract at different doses was statistically comparable to the mefenamic acid dose of 1.3 mg/kg BW. In contrast, compared to negative controls, the administration of extracts at doses of 17.5 and 70 mg/kgBB produced significant differences at both observation times, while doses of 35 mg/kgBB did not show a significant difference. These findings suggest that the extract's analgesic activity is primarily apparent at doses of 17.5 and 70 mg/kg BB, with effectiveness that can be statistically comparable to positive controls. Overall, the results of the analysis support the more pronounced analgesic effect of *A. cocculus* stem extract at certain doses and is increasingly visible in the follow-up observation period.

DISCUSSION

The analgesic activity of medicinal plants is closely associated with the presence of bioactive secondary metabolites produced through plant metabolic processes. These compounds represent an important chemical defense system in plants and may exert pharmacological effects when administered to experimental animals or humans. In the context of pain modulation, plant-derived secondary metabolites can influence multiple biological targets involved in nociception, including inflammatory mediators, peripheral nociceptors, ion channels, neurotransmitter systems, and central pain pathways. Therefore, the analgesic potential of a plant extract is generally considered the result of the combined or synergistic activity of several phytochemical constituents rather than the action of a single compound alone. Previous studies have demonstrated that flavonoids, alkaloids, tannins, and steroidal compounds possess pharmacological activities that may contribute to the attenuation of pain through both peripheral and central mechanisms (Bouyahya et al., 2022; Alhassen et al., 2021; Iek et al., 2026).

Flavonoids are among the most extensively studied plant secondary metabolites due to their diverse pharmacological properties, including antioxidant, anti-inflammatory, and analgesic activities. Their potential analgesic effects are associated with the modulation of the arachidonic acid pathway and the suppression of inflammatory mediator synthesis. Certain flavonoids have been reported to inhibit cyclooxygenase (COX)

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enzymes, thereby reducing the conversion of arachidonic acid into prostaglandins (Novisma et al., 2024; R. Ferraz et al., 2020; Panche et al., 2016; Baker, 2022).

Alkaloids may also contribute to the analgesic potential of medicinal plants. Certain alkaloids have been reported to exert peripheral analgesic effects by inhibiting cyclooxygenase activity and subsequently reducing prostaglandin production. This mechanism may attenuate peripheral nociceptor sensitization and decrease the intensity of pain signals generated in response to noxious stimuli (Vadhel et al., 2023; Salwa et al., 2025; Souza et al., 2020; Jiang et al., 2022; Letchuman et al., 2025; Jiang et al., 2022).

Tannins may also contribute to analgesic activity through their potential anti-inflammatory and neuroprotective effects. Their potential analgesic properties have been associated with the inhibition of inflammatory mediators and the modulation of signaling pathways involved in nociception. Previous studies have suggested that tannins may exhibit effects partly comparable to those of nonsteroidal anti-inflammatory drugs (NSAIDs), particularly through the suppression of inflammatory pathways and the attenuation of peripheral sensitization (Kovac et al., 2023; Zinia et al., 2012; Jing et al., 2022; Tong et al., 2022; Omar et al., 2022; Manville et al., 2024; Kim et al., 2022).

Steroidal compounds detected in medicinal plants may also be relevant to their potential analgesic effects. Corticosteroids can inhibit phospholipase A_2 activity, thereby reducing the release of arachidonic acid from membrane phospholipids. Because arachidonic acid is a precursor for the synthesis of prostaglandins and leukotrienes, its reduced availability may decrease the production of multiple inflammatory mediators. This upstream mechanism distinguishes the anti-inflammatory action of corticosteroids from that of conventional NSAIDs, which primarily inhibit cyclooxygenase enzymes downstream of arachidonic acid release (Couch et al., 2024; Vyvey, 2010; Passos et al., 2022).

Taken together, the presence of flavonoids, alkaloids, tannins, and steroidal compounds suggests that the analgesic activity of the plant extract may involve multiple complementary mechanisms. Flavonoids may predominantly reduce prostaglandin synthesis and oxidative stress, whereas alkaloids may modulate both peripheral inflammatory mediators and central neuronal signaling. Tannins may contribute through the suppression of nitric oxide, cytokines, and inflammatory mediators, while steroidal compounds may inhibit the release of arachidonic acid and suppress upstream inflammatory pathways. The simultaneous presence of these secondary metabolites may therefore produce additive or synergistic effects that result in a stronger overall analgesic response (Junior et al., 2022).

The potential synergistic interaction among these compounds is an important consideration in the evaluation of plant-derived analgesics. In a crude extract, bioactive constituents are not present as isolated compounds but coexist within a complex chemical matrix. Consequently, the observed pharmacological activity may result from interactions among several metabolites acting on different molecular targets. A compound that primarily suppresses inflammatory mediator production may complement another compound that directly modulates neuronal excitability or neurotransmitter release. This multi-target activity is one of the major characteristics of medicinal plant extracts and may explain their ability to produce analgesic effects through more than one biological pathway (Wink, 2022) (Rajabian et al., 2022).

Based on these considerations, the observed analgesic activity of the plant extract may be attributed to its phytochemical composition and the collective pharmacological action of its secondary metabolites. Nevertheless, the specific contribution of each metabolite cannot be conclusively determined based solely on preliminary phytochemical screening and in vivo analgesic testing. The presence of a particular metabolite class indicates a potential pharmacological contribution but does not confirm that all compounds within that class are responsible for the observed activity. Further investigations involving bioassay-guided fractionation, compound isolation, structural elucidation, and mechanistic studies are therefore necessary to identify the specific constituents responsible for the analgesic effect. Such approaches would provide stronger evidence

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regarding the molecular basis of the observed pharmacological activity and may support the future development of the plant as a potential source of novel analgesic agents (Mani et al., 2022).

CONCLUSION AND IMPLICATIONS

Oral administration of *A. cocculus* bark extract at doses of 17.5, 35 and 70 mg/kg showed significant analgesic activity in mice using the tail flick method. The increase in pain tolerance indicates the presence of substances with analgesic activity in *A. cocculus* bark extract, which are beneficial for managing pain. Consequently, *A. cocculus* bark extract provides beneficial effects on pain. Further research, involving the isolation of phytochemical compounds combined with in vivo evaluation, is needed to elucidate the exact mechanism of the analgesic effect of these specific compounds. In addition, toxicity testing of *A. cocculus* plant extract should be conducted.

LIMITATIONS AND FUTURE RESEARCH

This study has several limitations that should be considered when interpreting the findings. First, the analgesic activity of the *A. cocculus* stem bark extract was evaluated using an animal model (*Mus musculus*); therefore, the findings cannot be directly extrapolated to humans. Second, the assessment of analgesic activity was primarily based on the use of an analgesy meter, which may limit the breadth of information obtained regarding the different mechanisms underlying the observed antinociceptive effects. In addition, the study did not include a comprehensive evaluation of the safety profile, subacute or chronic toxicity, or the stability of the extract suspension. Furthermore, the extract was not standardized based on its bioactive constituents, making it difficult to determine the specific compounds responsible for the observed analgesic activity.

Future research should focus on the phytochemical standardization and formulation of *A. cocculus* stem bark extracts, including the identification and quantification of major bioactive compounds potentially responsible for its analgesic effects. More robust dose–response studies should also be conducted to establish the optimal effective dose and therapeutic window. In addition, the analgesic potential should be evaluated using complementary in vivo models that represent different types and mechanisms of nociception, including both peripheral and central pain pathways. Further investigations into the underlying mechanisms of action, together with acute and repeated-dose toxicity studies, are also needed to provide a more comprehensive assessment of the therapeutic potential and safety of *A. cocculus* stem bark as a prospective source of analgesic agents.

DECLARATIONS

Ethics approval: All experimental procedures involving animal subjects were conducted in accordance with established ethical guidelines and were approved by the Research Ethics Committee of the Makassar College of Pharmacy (Approval No. 07.153/KOMETIK/STIFA/VII/2025).

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