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Anti-inflammatory and Analgesic Effect of 70% Cinnamon Bark (*Cinnamomum burmannii* Blume.) Ethanolic Extract In Vivo

(Uji Efek Anti-inflamasi dan Analgesik Ekstrak Etanol 70% Kulit Batang Kayu Manis (*Cinnamomum burmannii* Blume.) Secara In Vivo)

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Abstract: Cinnamon bark is known as a medicinal plant to has anti-inflammatory and analgesic effects in vitro. This activity is due to bark of cinnamon containing several compounds including cinnamaldehyde, cinnamic alcohol, cinnamic acid and coumarin. The aim of this study was to observe anti-inflammatory and analgesic effects in vivo. Anti-inflammatory test using Winter method and rats. Rats were divided into five groups negative control (aquadest), positive control (sodium diclofenac), and three extract groups with different doses (300, 400, and 500 mg/kg BW). In an analgesic test using the Siegmund method and mice. Mice were also divided into five groups negative control (aquadest), positive control (sodium diclofenac), and three extract groups with different doses (300, 400, 500 mg/kg BW). Based on a statistical test, 70% ethanol extract of cinnamon bark has anti-inflammatory and analgesic effects, also showed that there was a significant difference between the positive controls and the three extract doses. This shows that the anti-inflammatory and analgesic activity of diclofenac sodium is better than the ethanol extract of cinnamon bark in reducing the edema volume of rats and the amount of mice stretch. In conclusion, cinnamon bark showed no significant increment in high doses of anti-inflammatory and analgesic.

Keywords: Analgesic, anti-inflammatory, *Cinnamomum burmannii* Blume, in vivo.

Abstrak: Kulit batang kayu manis adalah salah satu tanaman mengandung senyawa aktif yang memiliki efek anti-inflamasi dan analgesik secara in vitro. Aktivitas ini disebabkan karena kulit batang kayu manis mengandung beberapa senyawa termasuk sinalmadehid, sinamat alkohol, asam sinamat dan kumarin. Penelitian ini bertujuan untuk mengetahui efek anti-inflamasi dan analgesik in vivo. Pengujian anti-inflamasi menggunakan metode Winter dan hewan uji tikus. Tikus dibagi menjadi 5 kelompok yaitu kontrol negatif (aquadest), kontrol positif (natrium diklofenak), dan 3 kelompok ekstrak dengan dosis 300, 400 dan 500 mg/kg BB. Pada uji analgesik, digunakan metode Siegmund dengan hewan uji mencit. Mencit dibagi ke dalam 5 kelompok yaitu kontrol negatif (aquadest), kontrol positif (natrium diklofenak), dan 3 kelompok ekstrak dengan dosis 300, 400, dan 500 mg/kg BB. Berdasarkan hasil pengujian statistik, diperoleh bahwa ekstrak etanol 70% kulit batang kayu manis memiliki aktivitas anti-inflamasi dan analgesik serta terdapat perbedaan bermakna antara kontrol positif dengan ketiga dosis ekstrak. Hal ini menujukkan bahwa aktivitas anti-inflamasi dan analgesik dari natrium diklofenak masih lebih baik dibanding ekstrak etanol kulit batang kayu manis dalam menurunkan volume udem tikus dan jumlah geliat mencit. Dari hasil pengujian juga didapat bahwa peningkatan dosis tidak memberikan peningkatan efektivitas anti-inflamasi dan analgesik.

Kata kunci: Analgesik, anti-inflamasi, *Cinnamomum burmannii* Blume, in vivo.

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INTRODUCTION

INFLAMMATION was a series of complex changes in tissues due to tissue injury, both caused by bacteria, trauma, chemicals, heat, and pain. Signs of inflammation were redness, heat, swelling, and pain. Pain was a mechanism to protect the body against disturbances and damage in tissues such as inflammation, infection, and pain in the muscles with the release of pain mediators, which include prostaglandins, bradykinin, serotonin, histamine, potassium ions, and acetylcholine[1].

Treatment of inflammation and pain generally uses synthetic drugs but has side effects. Drugs commonly used as anti-inflammatories were NSAIDs (non-steroidal anti-inflammatory drugs) which generally have side effects, so it was necessary to look for alternative treatments to control pain and inflammation with relatively smaller side effects, for example, drugs derived from plants. Therefore, alternative medicine was used to overcome the use of traditional medicine by using plants that have medicinal properties, including cinnamon[2].

Cinnamon was known to have compounds that had anti-inflammatory and analgesic substances. In vitro studies have shown that coumarins and cinnamon extract have anti-inflammatory activity, as indicated by a significant decrease in the inflammatory mediators NO (Nitric Oxide) and PGE2 (Prostaglandin), as well as the cytokine mediators IL-6 (Interleukin-6), IL-1β (Interleukin-1β), and TNF-α (Tumour Necrosis Factor-α) in activated RAW264.7 macrophages[1]. This study aimed to determine the anti-inflammatory and analgesic effects of a 70% ethanol extract of cinnamon bark in vivo. In vivo research was conducted to ensure the efficacy of the anti-inflammatory and analgesic effects of cinnamon bark extract when there was a biological influence (in vivo). In terms of safety, cinnamon bark oil was included in the practically non-toxic category with an LD_{50} of 3679.11 mg/kg BW[2]. In addition, the highest dose in this study was 500 mg/kg BW, so it was safe to use[3]. The anti-inflammatory and analgesic effects of cinnamon bark were also supported by the presence of cinnamaldehyde in cinnamon which has also been shown to inhibit the secretion of pro-inflammatory substances such as NO, cytokines, and IL-1 in RAW264.7[4]. Cinnamon extract also has advantages, its activity in inhibiting the H^+K^+ATP-ase pump in the stomach so that it can prevent peptic ulcers and can overcome the side effects caused by long-term use of NSAIDs[5].

MATERIALS AND METHODS

MATERIALS. Cinnamon bark (*Cinnamomum burmannii Blume.*), DDY male mice, Sprague-Dawley male rats, 3% glacial acetic acid (Merck, Germany) Catalogue Number: 1.00063.2500), 1% carrageenan, Aquadest, diclofenac sodium (Novell, Bogor, Indonesia) feed, 0.5% CMC sodium (Sigma Aldrich, CAS Number 9004-32-4, St Louis, United States)

Equipments. Analytical scales (ADB 200-4, Kern, Germany), plestismometer (Harvard England, Cat. No. 7140).

METHODS. Preparation of Cinnamon Bark Extract. The required amount of cinnamon bark powder was weighed, macerated with 70% ethanol 10 times, and then filtered twice using cotton and filter paper. The filtrate obtained was then taken. The dregs resulting from maceration were then macerated again with ethanol. This process was carried out five times until all the compounds were extracted perfectly. All the filtrates obtained were collected and concentrated with a vacuum rotary evaporator at 45–55°C until a viscous ethanol extract was obtained.

Anti-inflammatory Effect Testing[6]. An experiment to determine the anti-inflammatory effect was carried out using Winter's method with the formation of oedema on the soles of rats' feet. Before the experiment was carried out on mice, the rats were fasted for ± 18 hours while still being given a drink. On the day of the test, the rats were weighed and then marked on the tails of the rats to distinguish one rat from another. 25 rats were taken randomly and divided into five groups, with 5 rats each. will be carried out on the division of groups in Table 1. Before being treated, measure the initial volume of the paws of the rats by dipping the soles of the rats into the pletismometer. In the treatment of the anti-inflammatory test group, rats were given the test substance orally according to the treatment dose for each group. Thirty minutes later, the paws of the rats were induced with 1% carrageenan, as much as 0.2 mL/200 g BW intraplantarly. After administration of carrageenan, measure the oedema volume of the soles of the rats' paws every 1 hour for 5 hours (at 1st, 2nd, 3rd, 4th, and 5th hours).

Analgetic Effect Testing. An experiment to determine analgesic power was carried out using the Siegmund method. Before the experiment was carried out on mice, the mice were fasted first for ± 18 hours while still being given a drink. On the day of the test, the mice were weighed, then marked on the mice's tails to distinguish them from one another. 25 mice were

taken randomly and divided into five groups, with 5 mice each. The treatment that would be carried out in the distribution of groups was shown in Table 2. In the treatment of the analgesic test group, the mice were given the test substance orally according to the treatment dose for each group. Thirty minutes later, the mice were induced intraperitoneally with 3% acetic acid, as much as 0.2 mL/20 g BW. Then the mice were placed in the cage. After administration of acetic acid, the mice gave a writhing response, which was shown by moving a pair of front legs pulled forward and a pair of hind legs pulled back and rubbing their stomachs against the bottom of the cage.

The mice were observed, and then the number of ticks shown by the mice was recorded within 5 minutes for 1 hour (at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 minutes).

Data Analysis. The data obtained from each group for the anti-inflammatory and analgesic test was carried out by calculating the AUC (area under the curve), the percentage of anti-inflammatory and analgesics, and the percentage of effectiveness of anti-inflammatory and analgesics.

Table 1. Anti-inflammatory test group treatment.

Group	Treatment
Negative	Aquadest + 0.2 ml carrageenan 1%
Positive	Natrium diclofenac + 0.2 ml carrageenan 1%,
A	Ethanol extract 70% cinnamon bark 300mg/kgBW + 0.2 ml carrageenan 1%
B	Ethanol extract 70% cinnamon bark 400mg/kgBW + 0.2 ml carrageenan 1%
C	Ethanol extract 70% cinnamon bark 500mg/kgBW + 0.2 ml carrageenan 1%

Note : each group there were five rats.

Table 2. Analgesic test group treatment.

Group	Treatment
Negative	Aquadest + 0.2 ml/20g BW acetic acid 3%
Positive	Diclofenac sodium + 0.2 ml/20g BW acetic acid 3%
A	Ethanol extract 70% cinnamon bark 300mg/kgBW + 0.2 ml/20g BW 3% acetic acid
B	Ethanol extract 70% cinnamon bark 400mg/kgBW + 0.2 ml/20g BW 3% acetic acid
C	Ethanol extract 70% cinnamon bark 500mg/kgBW + 0.2 ml/20g BW 3% acetic acid

Note : each group there were five mice.

RESULTS AND DISCUSSION

Anti-inflammatory Effect Testing. From the results of the average rat paw oedema volume from the 0th hour (before carrageenan induction) to the 5th hour, it can be seen that the positive control and test preparation can reduce the volume of rat paw oedema in the 3rd hour after carrageenan induction. This shows that the positive control and test preparations can inhibit the formation of oedema on the soles of rats.

From the results of the average AUC value in all treatment groups, the negative control AUC value was higher than all other test preparation groups. This shows that all groups of test substances 70% ethanol extract of cinnamon bark and positive control have an anti-inflammatory effect. Of the three extract doses, it was found that the extract at a dose of 500 mg/kg BW was better at inhibiting the formation of oedema on the soles of rats, as indicated by the smallest average AUC value compared to the other two extract groups.

The anti-inflammatory test used the Winter method. This method was chosen because it was the simplest and most commonly used. Udem formation using carrageenan also did not cause injury or tissue damage to the paws of rats and could last for 6 hours before slowly decreasing[9,10]. In addition, another reason for choosing this method was because carrageenan was more sensitive to anti-inflammatory drugs than other anti-irritants[10]. The existence of oedema formation phases also makes it easier to see the work of anti-inflammatory substances that were tested more specifically, especially to see ingredients that were suspected of having a mechanism by inhibiting prostaglandin biosynthesis or COX formation. The parameters seen from testing with the Winter method were the oedema volume of the rat's paws (mL) versus time (hours). Before being given treatment, the rats were fasted for ± 18 hours so that the stomach organs were empty so that the test preparation could be absorbed properly and was not disturbed by the presence of food residue in the stomach. Thus it was hoped that the work of the tested material would be more absorbed.

Analgetic Effect Testing. From the data on the average number of mice wriggling for 1 hour, it can be seen that there was a decrease in the number of mice stretching at 16–20 minutes. This shows that the positive group and the extract were able to reduce the number of mice writhing. From these data, it can also be seen that the peak of the mice's writhing occurred

at 11–15 minutes. This was because the latent time of glacial acetic acid in causing writhing was 15 minutes.

From the results of the average AUC value, it can be concluded that the average AUC of the negative group was the highest compared to the positive group and the extract group. This shows that glacial acetic acid was capable of causing pain, as indicated by the movement of mice. This value also shows that the positive control, namely diclofenac sodium, and the extract group at doses of 300, 400, and 500 mg/kg BW have analgesic activity, as indicated by a reduction or decrease in the amount of writhing in mice. Of the three doses, the extract dose of 500 mg/kg BW showed better analgesic activity than the other extract doses because it had a lower average AUC value than the other extract groups.

From the results of the average number of mice writhing, it was found that the number of negative control mice was the highest compared to the positive control and extract groups. This was because the negative control was only given aquadest. In the positive control and extract groups, there was a decrease in the number of mice writhing because the positive control and extract groups showed that diclofenac sodium and cinnamon bark ethanol extract had analgesic activity. Analgesic activity was also shown by the results of statistical tests, which showed that there were significant differences between the negative control, the positive control, and the extract group. In the results of calculating the percentage of inhibition, it was also found that the positive control and 3 doses of the extract had the ability to reduce the amount of stretching in mice. The decrease in the number of mice writhing showed that diclofenac sodium and extracts had the ability to inhibit prostaglandin synthesis. This was because acetic acid induces pain by stimulating the release of free arachidonic acid from phospholipid tissue, resulting in the formation of COX and prostaglandins. Drugs that can reduce the amount of stretching in mice due to acetic acid induction have the ability to inhibit prostaglandin synthesis.

AUC Values for Anti-Inflammatory Tests and Analgesic Tests. From the average AUC values of the anti-inflammatory and analgesic tests, it was determined that the group with the highest average AUC value was the negative group, namely the group that was only given distilled water and an inducer. The average AUC value in the anti-inflammatory group shows the total volume of oedema formed, while the average AUC in the analgesic group shows the total number of movements of the mice, so the values in Table 3 show that the mice in the negative group have the largest oedema volume compared to the other groups. with mice in the negative group showing the most number of stretches of the other groups.

Percentage of Udema and Stretching Inhibition. The average AUC value obtained can also be used to calculate the percentage of oedema formation inhibition in rats and stretch marks in mice. The percentage of inhibition can be calculated from the average AUC data of the test group and the negative control (Table 4).

From the table, it can be seen that diclofenac sodium and the test preparations at doses of 300, 400, and 500 mg/kg BW have higher analgesic activity than their anti-inflammatory activity. In addition, it was also found that the positive control had better anti-inflammatory and analgesic activity than the extract group.

Percentage of Anti-Inflammatory and Analgesic Effectiveness. In addition to the percentage inhibition of oedema formation and stretching in mice, the AUC value can also be used to calculate the percentage of anti-inflammatory and analgesic effectiveness. The percentage of effectiveness was calculated by

Table 3. The average AUC value of anti-inflammatory and analgesic tests.

Group	Anti-inflamamatory	Analgesic
Negative	8.3450±0.4169	712.5±51.9916
Positive	6.0167±0.0726	372.5±26.0408
Extract 300mg/kgBW	6.8800±0.1913	542.5±45.5864
Extract 400mg/kgBW	6.7783±0.2464	532.5±80.1561
Extract 500mg/kgBW	6.6825±0.3594	481±40.3733

Table 4. Percentage of swelling and stretching inhibition.

Groups	Percentage of inhibition (%)	
	Anti-inflammatory	Analgesic
Negative	0	0
Positive	27.90	47.72
Extract 300mg/kgBW	17.56	23.86
Extract 400mg/kgBW	18.77	25.26
Extract 500mg/kgBW	19.92	32.49

Table 5. Percentage of effectiveness of anti-inflammatory and analgesic.

Group Treatment	Percentage of effectiveness (%)	
	Anti-inflammatory	Analgesic
Extract 300mg/kgBW	62.94	50
Extract 400mg/kgBW	67.28	52.93
Extract 500mg/kgBW	71.40	68.08

comparing the average AUC of the test group with the average AUC of the positive control (Table 5).

From the table, it was found that the anti-inflammatory effectiveness of the extract was better than its effectiveness as an analgesic. In addition, the extract group at a dose of 500 mg/kg BW had better anti-inflammatory and analgesic effectiveness compared to the extract group at a dose of 300 and 400 mg/kg BW.

After the two tests were carried out, it can be seen that the 70% ethanol extract of cinnamon bark has anti-inflammatory and analgesic activity. The existence of these properties was in accordance with previous studies, namely cinnamon bark extract has anti-inflammatory activity with in vitro testing, one of which was by significantly reducing pro-inflammatory agents, one of which was PGE2 [(1).] The anti-inflammatory and analgesic activity of mayu manis extract was thought to be due to the presence of compounds that, according to previous studies, have anti-inflammatory and analgesic roles. These compounds were coumarins, cinnamaldehyde, and flavonoids. However, this needs to be confirmed by the presence of phytochemical screening and if necessary, calculations of the levels of these compounds were also carried out to ensure that the compounds in the cinnamon extract act as anti-inflammatory and analgesic.

CONCLUSION

The 70% ethanol extract of cinnamon bark at a dose of 300 mg/kg BW, 400 mg/kg BW and 500 mg/kg BW has anti-inflammatory and analgesic activity as shown by its ability to inhibit oedema formation on the soles of rats' feet induced by 1% carrageenan solution and its ability to reduce the amount of wriggling of mice induced by 3% acetic acid. The data also showed that the extract has lower anti-inflammatory and analgesic activity than sodium diclofenac sodium. Increasing the dose of 70% ethanol extract of cinnamon bark did not show an increase in anti-inflammatory and analgesic effectiveness as seen from the absence of statistically significant differences between the three extract groups.

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REFERENCES

1. Sandhiutami NMD, Moodiani, M, Laksiamitawati DR, Fauziah N, Maesaroh W. Invitro assessment of antinflammatory activities of coumarin and Indonesian cassia extract in RAW 264.7 murine macrophage cell line. Iran J Basic Med Sci. 2017; 20:99-106
2. Herdwiani W, Soemardji AA, El-Fahmi, Tan M. Gas chromatograph-mass spectrometer analysis and acute oral toxicity of *Cinnamomum burmannii*, Nees Ex Bl. essential oil. Asian Journal of Pharmaceutical and Clinical Research. 2016;9(3):240-5.
3. Rabiatul AA, Hamed SN, Fadzilah AAM, Mohamad RS, Ramlan AA. Assessment of potential toxicological effects of cinnamon bark aqueous extract in rats. International Journal of Bioscience, Biochemistry and Bioinformatics. 2015;5(1):36-44.
4. Chao LK, Hua KF, Hsu HY, Cheng SS, Lin IF, Chen CJ, et al. Cinnamaldehyde inhibits pro-inflammatory cytokines secretion from monocytes/macrophages through suppression of intracellular signaling. Food and Chemical Toxicology 46. 2008;46: 220–23.
5. Tjandrawinata RR, Nailufar F, Arifin PF. Hydrogen potassium adenosine triphosphatase activity inhibition and downregulation of its expression by bioactive fraction DLBS2411 from *Cinnamomum burmannii* in gastric parietal cells. International Journal of General Medicine. 2013;6:807-15.
6. Rahayu L, Dewi SR, Ayu G. Anti-inflammation and analgesic test effect of segani leaves (*Melastoma malabathricum* L.) Infusion. Jurnal Ilmu Kefarmasian Indonesia. 2017;14(1):93-8.
7. John C, Karen C. Swim stress reduces chronic pain in mice through an opioid mechanism. Neuroscience Letters. 1987;74(3):358-63.

8. Bogdan T, Raadu I, George F, Magda L. Experimental models for the evaluation of somatic pain in rodents.
9. J Necas, N Bartosikova. Carrageenan: a review. Veterinarni Medicina. 2013;58(4):187–205
10. Sukmawati S, Yuliet Y, Hardani R. Uji aktivitas antiinflamasi ekstrak etanol daun pisang ambon (*Musa paradisiaca* L.) terhadap tikus putih (*Rattus norvegicus* L.) yang diinduksi karagenan. Galenika Journal of Pharmacy. 2015;1(2):126-32.
11. Suwandi DW, Puspita T, Nuari DA, Hamdani S. Analgetic and anti-inflammatory activity of ethanol extract and fraction of guava leaf (*Syzygium jambos* L.) in vivo. Jurnal Sains dan Kesehatan. 2021;3(2):218-26.
12. Hendra P, Susana W, Permatasari I, Florensia J, Siregar MN. Analgesic and anti-inflammatory activities of *Eurycoma longifolia* root in Mice. Jurnal Ilmu Kefarmasian Indonesia. 2018;16(2):182-7.
13. Khairani S, Rahayu L, Sandhiutami NM, Dewi RS, Rahmawati I. Test anti-inflammatory and analgesic effect from decoction of bamboo kuning leaves (*Bambusa vulgaris* Schard). Jurnal Ilmu Kefarmasian Indonesia. 2021;19(2):266-71.
14. Sandhiutami NM, Atayoglu AT, Sumiyati Y, Desmiaty Y, Hidayat RA. The combination of *Colocasia esculenta* L. and *Zingiber officinale Potentially* inhibits inflammation and pain. Jurnal Ilmu Kefarmasian Indonesia. 2023;2(1):81-9.
15. Nurhidayati LG, Listina O, Cahyanta AN, Purwati E. Combination of moringa leaf extract (*Moringa Pterygosperma* Gaertn.) and lamtoro leaf (*Laucaena Leucocephalab* Lmk.) as analgesic in male white mice with writhing method. Jurnal Sains dan Kesehatan. 2022;4(6):627-34.