

BUKTI KORESPONDENSI

Lampiran	:	Peer review proses korespondensi submit Publikasi Jurnal Ilmiah Internasional Bereputasi
Nama Jurnal	:	Acta Pharmaceutica Scientia Vol 61 No 1 2023 Hal: 37-48
Index	:	Terindeks pada database internasional bereputasi dan berfaktor dampak (SCOPUS Q3)
Judul Jurnal	:	Antioxidant and Antibacterial Activities of Ethanolic Extract of Sintok Lancang (<i>Cinnamomum javanicum</i> Blume) from Central Kalimantan

No	Item	Tanggal	Halaman
1	Submission article and acknowledgement of submission (PJ_19_277)	30 Mei 2022	1
2	Review Process (Manuscript Revision)	11 Agustus 2022	2
3	Acknowledgement of Manuscript Revision	07 September 2022	14
4	Acceptance of Manuscript	26 Oktober 2022	15
5	Article Published	Februari 2023	15
6	Ethical approval	21 Juli 2020	28

1. Submission article

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Sen, 30 Mei 2022 jam 15.18

ACTA PHARMACEUTICA SCIENCIA

Dear Susi Novaryatiin,

Thank you for submitting your manuscript entitled "Antioxidant and Antibacterial Activities of Ethanolic Extract of Sintok Lancang (*Cinnamomum javanicum* Blume) from Central Kalimantan" to ACTA Pharmaceutica Scientia. Your manuscript will first be evaluated by the editors and if it meets the Journal's standards, will be forwarded to referees for scientific review. You will be able to follow the stage of your manuscript in the review process through the author center to which you will have access with your user name and password. You can use the author center for revisions and new submissions.

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2. Review Process (Manuscript Revision)

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Kam, 11 Agu 2022 jam 17:59

ACTA PHARMACEUTICA SCIENCIA

Dear Other Susi Novaryatiin,

The Editors have now assessed the reviewer response and have concluded that, in its present form, the manuscript is not yet ready for publication in the Journal. Below you will find the relevant review comments and editorial notes. Acceptance of the paper is contingent upon effectively revising the work by taking these comments into serious consideration, and by responding or rebutting them in detail. We ask you to submit your revision through the online system within 30 days. The web site is located at <http://actapharmsci.com>.

Please upload the file containing your revised manuscript. The rebuttal letter should be placed in "cover letter" section. Please note that you should submit your revised letter by clicking on "Submit Revision" link, not as a new manuscript. If you have any problem please send an e-mail to editor@actapharmsci.com.

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Sincerely,

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Suggestions

1. Reviewer Comments

1. Very general expressions are used in the Introduction section. There are even some speculative claims. Attention has been drawn to the antioxidant effects of Indonesian herbs, but this information is more or less valid for every plant extract. These unnecessary parts need to be removed.
2. If the antioxidant and antimicrobial effects of this plant have been studied many times before, what is the difference to be revealed in this study? More emphasis should be placed on this in the introduction.
3. The botanical author name can only be used where the name of the plant is first mentioned. It should be deleted where it is used repeatedly.
4. The herbarium number of the plant and the herbarium in which it was stored should be written.
5. It is not necessary to include information other than the method in the material method section. If the difference between percolation and maceration is to be emphasized, it should be included in the conclusion.
6. How the solvent is removed from the plant extract is not mentioned.
7. In the antimicrobial effect, the effect was observed less for all 3 bacteria at 10% concentration. I think there is an error here. The experiment needs to be repeated.
8. In the images in Figure 1, it is seen that the disc diameters expand as the extract concentration increases. The reason for this is not understood. (not zone diameter, disc diameter)
9. The number of 40 references is too many. I suggest simplifying the references.

2. Reviewer Comments

Reviewer Comments for manuscript number APS-1976: Antioxidant and Antibacterial Activities of Ethanolic Extract of Sintok Lancang (*Cinnamomum javanicum* Blume) from Central Kalimantan

It is an original study. In general, the manuscript was written scientifically. It contains useful results. On the other hand, it contains some mistakes and deficiency. So, major revisions are required.

Corrections were pointed out by line number.

My opinions and comments about this manuscript are as follows:

A. GENERAL REMARKS:

1. No negative control is written in the study. It must be written if negative control is used. Negative control should be used in such studies.
2. Incubation of *Cutibacterium* acnes can perform in an anaerobic conditions. This situation is not included in the manuscript.
3. *Cutibacterium acnes* is the new name of *Propionibacterium acnes*. Please correct it in the whole manuscript.
4. ml should be written as mL. Please correct it in the whole manuscript.

B. SPECIFIC REMARKS:

Line 19: I think the word "ini" was written wrong. Please correct it.

Line 22-24: I think the sentence must end with the word "respectively".

Total alkaloids, flavonoids and tannins content were 32.81 ± 0.77 µg caffeine equivalent/mg, 126.96 ± 3.17 µg quercetin equivalent/mg, and 42.89 ± 0.77 µg catechin equivalent/mg.

Line 54: There must be a space between C. and loureiriol. Please correct it.

Line 55-58: The sentence is unclear. It should be revised.

Line 76: I think the word "dan" was written wrong in the "Research Center for Plant Conservation dan Botanic Gardens"

Line 99: Please replace "0,5 mL" with "0.5 mL".

Line 100: What is the name of the solution? You wrote only solution.

Add to the 0.5 mL solution, 3.0 mL vanillin 4% and 1.5 mL concentrated HCl.

Line 104: Please replace "0,4 mM" with "0.4 mM".

Line 153-155: I did not understand the words "This compound". Maybe the words "These compounds" is suitable.

Phenolics and flavonoids are commonly found in all parts of the plant. This compound is a group of secondary metabolites made up of a large group of polyphenols that can scavenge free radicals and inhibit lipid oxidation³⁰.

Line 331: In reference 36: Please replace 'coffe' with 'coffee'.

Reviewer 2

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Manuscript Information

Manuscript ID:	APS-1976
Title in English:	Antioxidant and Antibacterial Activities of Ethanolic Extract of Sintok Lancang (<i>Cinnamomum javanicum</i> Blume) from Central Kalimantan
Small Title in English:	No information entered
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Keywords in English:	Cinnamomum javanicum Blume, DPPH, FRAP, Disc-diffusion, Medicinal plant
Manuscript Type:	Original investigation
Processing Status:	Major Revision

Antioxidant and Antibacterial Activities of Ethanolic Extract of Sintok Lancang (*Cinnamomum javanicum* Blume) from Central Kalimantan

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ABSTRACT

C. javanicum Blume is a typical plant that can be founded in Central Kalimantan. This research aimed to find out the total flavonoids, alkaloids, tannins, and compounds and to evaluate the antioxidant and antibacterial activity of ethanol extract of the stem of *C. javanicum*. The antioxidant activity was tested by two different assays: DPPH and FRAP. Total alkaloids, flavonoids and tannins content were 32.81±0.77 µg caffeine equivalent/mg, 126.96±3.17 µg quercetin equivalent/mg, and 42.89±0.77 µg catechin equivalent/mg, respectively. The IC₅₀ value of DPPH was 20.63±0.82 ppm quercetin equivalent while the FRAP method was 968.38±22.25 µmol trolox/g. *C. javanicum* extract had antibacterial potential against the three bacteria tested, with the inhibition zones in the range of 3.17±0.90 - 8.90±1.50 mm. It can be stated that the ethanol extract of the stem of *C. javanicum* has high potential antioxidant activity, however, the antibacterial activity is classified as weak activity.

Keywords: *Cinnamomum javanicum* Blume, DPPH, FRAP, Disc-diffusion, Medicinal plant

INTRODUCTION

Free radicals are known for their central role in various physiological conditions as well as their involvement in a variety of diseases. The accumulation of free radicals in the body produces oxidative stress, which has been reported to be associated with various diseases such as neurodegenerative disorders, diabetes mellitus, respiratory diseases, cardiovascular diseases, coronary heart diseases,

rheumatoid arthritis, the development of cataracts, inflammatory diseases, autoimmune disorders, aging, and various cancers¹⁻³. Antioxidants play an important role in neutralizing free radicals³.

In addition, the resistance of bacterial species to many antibiotics is another major problem in antibiotic therapy that continuously encourages researchers to develop new antibiotics. Recently, most of the newly approved antibacterial agents are derived from natural products or their derivatives. Herbal remedies are one of the most important sources of natural antibacterial agents¹. The antimicrobial activity was mainly related to the existence of secondary metabolites of phenols, terpenes, and alkaloids present in the plant extracts². Therefore, there is a need to explore new, safe and inexpensive sources of natural antioxidant and antibacterial compounds.

Cinnamomum (family Lauraceae) is a genus known for its fragrant leaves and bark. This genus has more than 300 species that are widespread in North America, Asia, Australasia, and Southeast Asia. Commercial species sold as spices are *C. verum*, *C. cassia*, *C. burmannii*, *C. zeylanicum*, and *C. loureiroi*⁴.

Sintok lancang (*Cinnamomum javanicum* Blume) is a typical plant of Central Kalimantan, in this study found at Mungku Baru Forest (KHDTK), an educational forest managed by the collaboration of Muhammadiyah University of Palangkaraya with the Borneo Nature Foundation (BNF). Empirically, Sintok lancang was used for various diseases such as treating abdominal pain, wounds, and diabetes mellitus.

There has not been much research on *C. javanicum* from Indonesia regarding its potential use as an antioxidant and antibacterial agent. One of the studies done on *C. javanicum* from Malaysia showed that the leaf and bark extracts had antioxidant activity⁵. The antibacterial activity of the essential oil of *C. javanicum* found in Borneo was investigated using the broth dilution method, which showed that the inhibition against *S. aureus* with MIC and MBC values was 250 µg/mL and 500 µg/mL, respectively⁴. Previous studies reported that the ethanolic extract of *C. javanicum* leaves from Central Kalimantan had a high potential for antioxidant activity as tested by DPPH and FRAP methods⁶. However, no studies on the antioxidant activity of these ethanolic extracts of *C. javanicum* stem from Central Kalimantan have been conducted. This study was advanced from the previous study, with the aim being to determine the total number of flavonoid, alkaloid, and tannin compounds and to evaluate the antioxidant and antibacterial activity of the ethanolic extract of stems of *C. javanicum*. This study will help to explore the potential of Indonesian medicinal plants as natural antioxidants and antibacterial agents.

METHODOLOGY

Plant material

C. javanicum was collected from Mungku Baru Forest (KHDTK), an educational forest managed by the cooperation of the Muhammadiyah University of Palangkaraya with the Borneo Nature Foundation (BNF). The collected plant material was identified by Dr. R. Hendrian, MSc (Indonesian Institute of

Sciences, Research Center for Plant Conservation and Botanic Gardens, Bogor, Indonesia) with the document number B-833/IPH.3./KS/VII/2020.

Preparation of *Cinnamomum javanicum* extract

The stems of *C. javanicum* were dried in an oven at 45 °C. The dried stems were powdered by the grinder and then percolated using 96% ethanol at room temperature. Percolation is a continuous process in which the saturated solvent is continuously replaced by a new solvent⁷. The solvent is removed from the extract by using a rotary evaporator. Percentage yield (w/w) of the extracts is calculated by formula⁸: (Weight of the extract ÷ Weight of the starting plant material) x 100%

Phytochemical qualitative screening

The ethanolic extract of *C. javanicum* was preliminarily screened for phytochemicals such as alkaloids, flavonoids, saponins, tannins, and steroids⁹⁻¹².

Total alkaloids content

Ten mg of *C. javanicum* extract were weighed and then dissolved in 10 mL of ethanol. The absorbance of one mL of the extract was measured with a spectrophotometer at 272 nm. The standard used for the calibration curve was caffeine¹³. Total alkaloid content is expressed as µg alkaloids per mg of extract.

Total flavonoids content

Five mg of *C. javanicum* extract were weighed and then dissolved in 10 mL of ethanol. One mL of the extract was placed in the volumetric flask, and one mL of AlCl₃ (2% b/v) and 8 mL of acetic acid (5% v/v) were added. After mixing, the solution was incubated for 20 min¹⁴. The solution absorbance was measured with a spectrophotometer at 412 nm. The standard used for the calibration curve was quercetin¹⁵. Total flavonoid content is expressed as µg of flavonoids per mg of extract.

Total tannins content

A total of 30 mg of sample was weighed and placed in a 10 mL volumetric flask. Add to the 0.5 mL catechin standard solution, 3.0 mL vanillin 4% and 1.5 mL concentrated HCl. The mixture was incubated for 10 min¹⁵⁻¹⁶. Absorbance was measured with a UV Vis spectrophotometer at 498 nm. The standard used for the calibration curve was catechin¹⁷. Total tannin content is expressed as µg tannins per mg extract.

Antioxidant activity by DPPH assay

A 0.4 mM DPPH solution was prepared and the absorbance was measured at 512 nm. The resulting absorbance of the DPPH solution is the absorbance control. The stem extract of *C. javanicum* (sample) was first dissolved in methanol with five variant concentrations of 10, 20, 30, 40, 50 ppm. One mL of 0.4 mM DPPH solution was added in a 5 mL volumetric flask, and then 4 mL of sample solutions of different concentrations were added. The prepared mixture was placed at 25 °C for 30 min, the absorbance was measured at 512 nm¹⁸. The calculation of the percent inhibition or DPPH scavenging effect was used according to the following formula:

$$\text{DPPH Scavenging effect \%} = \frac{(A - B)}{A} \times 100\%$$

Where A was the absorbance of DPPH solution and B was the absorbance of sample solution¹⁹⁻²⁰.

Antioxidant activity by FRAP assay

Sample (0.2 g) dissolved with ethanol in a 10 mL volumetric flask. Two mL of sample solution were added to 3 mL of FRAP reagent in a test tube, followed by incubation for 16 minutes. Absorbance was measured with a UV Vis spectrophotometer at 595 nm. Antioxidant activity expressed in $\mu\text{mol trolox/g}$ ¹⁹⁻²¹.

Antibacterial activity test

The antibacterial activity was determined by disc diffusion method with four variant concentrations 1%, 5%, 10%, and 15% against three bacterial strains: *Cutibacterium acnes/C. acnes* (ATCC 11827), *Staphylococcus epidermidis/S. epidermidis* (ATCC 12228) and *Staphylococcus aureus/S. aureus* (ATCC 25923). Ten mL of McFarland 0.5 standard were prepared in sterilized tubes. The suspension of bacteria was prepared by diluting the colonies of bacteria in a normally sterile saline solution and the turbidity was adjusted to $1-2 \times 10^8$ CFU/mL (based on McFarland 0.5 standard). A sterile cotton swab was dipped in a standardized suspension of bacteria and used to inoculate on Mueller-Hinton agar plates²². All discs were immersed in the ethanolic extract of *C. javanicum* and then placed on the plates. A disc immersed in 1% clindamycin gel (positive control) was also placed on the plate. **These plates were incubated in the aerobic incubator for 24 h at 37 °C.** A caliper was used to measure the diameter of the inhibition zone of each extract and positive controls.

RESULTS AND DISCUSSION

Extraction yield

One thousand two hundred grams of *C. javanicum* were extracted into 60.29 g of extract. Based on yield calculation, *C. javanicum* extraction yielded 5.03%. The yield value relates to the number of secondary metabolites that were captured during extraction⁸.

Qualitative phytochemical screening

Phytochemical screening of *C. javanicum* stem by using the following standard methods⁹⁻¹⁰. The results of the phytochemical qualitative test of *C. javanicum* stem showed the presence of alkaloids using Mayer's reagents¹¹, flavonoids using the Shinoda test¹², tannins, saponins, and steroids (Table 1.)

Table 1. The qualitative phytochemical of ethanolic extract of *C. javanicum* stem

Phytochemical compound	Result
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Steroids	+

Total alkaloids, flavonoids, and tannins content

C. javanicum stem was extracted by percolation method. Total alkaloids, flavonoids, and tannins content were calculated by standard protocols, with the results of 32.81 ± 0.77 μg caffeine equivalent/mg, 126.96 ± 3.17 μg quercetin equivalent/mg, and 42.89 ± 0.77 μg catechin equivalent/mg, respectively (Table 2.). The total flavonoid content is the largest compared to the total alkaloids and tannins content. In addition, the total alkaloids, flavonoids, and tannins of *C. javanicum* stem ethanol extract in this study were greater than the total content of the ethanolic extract of the leaves of *C. javanicum* obtained in the previous studies⁶.

Phenolics and flavonoids are commonly found in all parts of the plant. These compound is a group of secondary metabolites made up of a large group of polyphenols that can scavenge free radicals and inhibit lipid oxidation²³. Most antioxidant activities from plant sources correlate with phenolic and flavonoid contents²⁴. Therefore, the measurement of phenol and flavonoid content can be used as a basis for rapid screening of antioxidant activities¹.

Table 2. Total alkaloids, flavonoids, and tannins content of ethanolic extract of *C. javanicum* stem

Sample	Ethanolic extract of <i>C. javanicum</i>
Assay	
Total Alkaloid (μg caffeine equivalent/mg)	32.81 ± 0.77
Total Flavonoid (μg quercetin equivalent/mg)	126.96 ± 3.17
Total Tannin (μg catechin equivalent/mg)	42.89 ± 0.77

Antioxidant activity

DPPH and FRAP methods were used to evaluate the antioxidant activity of *C. javanicum* extract. The antioxidant activity results showed that the IC_{50} value of DPPH was 20.63 ± 0.82 ppm quercetin equivalent while the FRAP method was 968.38 ± 22.25 μmol trolox/g (Table 3).

Table 3. Antioxidant activity of ethanolic extract of *C. javanicum* stem

Sample	Ethanolic extract of <i>C. javanicum</i>	Quercetin
Assay		
DPPH (IC_{50} ppm)	20.63 ± 0.82	6.98
FRAP (μmol trolox/g)	968.38 ± 22.25	-

According to some publications, the antioxidant activity with the DPPH method was classified by IC_{50} as very strong (< 50 ppm), strong (50-100 ppm), moderate (101-150 ppm), and low (> 150 ppm)^{19,25}, while the antioxidant activity with the FRAP method was classified as very low FRAP (< 10 $\mu\text{mol/g}$), low FRAP (10-50 $\mu\text{mol/g}$), good FRAP (50-100 $\mu\text{mol/g}$), high FRAP (100-400 $\mu\text{mol/g}$) and very high FRAP (> 400 $\mu\text{mol/g}$)²⁶. A low IC_{50} value (the extract concentration, required to scavenge 50% of DPPH free radicals) means strong antioxidant activity²⁷. The ethanolic extract of *C. javanicum* stem is included in the very strong antioxidant activity (20.63 ± 0.82 ppm) and very high FRAP (968.38 ± 22.25 $\mu\text{mol trolox/g}$). The antioxidant activity in this study was better than in the previous study, where the ethanolic extract of *C. javanicum* leaves included very strong antioxidant activity (26.99 ± 0.27 ppm) and very high FRAP (779.73 ± 19.66 $\mu\text{mol trolox/g}$)⁶. When compared with the antioxidant activity of *C. javanicum* leaves and bark study conducted in Malaysia with the DPPH method (223.5 ppm and 197.4 ppm)⁵, *C. javanicum* in Indonesia, especially Central Kalimantan gives better antioxidant activity. DPPH is a stable free radical with unpaired electrons distributed throughout the molecule and is widely used to test the free radical scavenging capacity of a variety of samples²⁸. The DPPH assay is based on both electron transfer and hydrogen atom transfer reactions. The reduction in absorbance of DPPH caused by antioxidants is due to a reaction between the antioxidant molecules and the radical, which results in radical scavenging by hydrogen donation. This is visualized as purple-to-yellow discoloration. The advantage of the DPPH test is that it is simple, fast, and economical. Although the DPPH test is simple, its sensitivity can be affected by several factors, such as solvent type, reaction time, temperature, and freshness of DPPH reagent^{1,29}, while the FRAP test is a nonspecific, redox-linked, colorimetric assay related to the molar concentration of the antioxidant present. The FRAP assay is a typical method based on electron transfer, which measures the reduction of ferric ion (Fe^{3+})-ligand complex to the ferrous (Fe^{2+}); complexed by antioxidants in acidic media. One limitation of the FRAP assay is its tendency to precipitate, form suspensions, and color the cuvette. Therefore, the timing of FeCl_3 addition is essential to prevent error interpretation. However, the FRAP or DPPH assay is simple, economical, fast, and requires no specialized equipment²⁹.

Antibacterial activity

In this study, an antibacterial activity test was performed for *C. javanicum* extract and clindamycin gel. Clindamycin is used as a positive control because it is a lincosamide antibiotic used to treat anaerobic, streptococcal, and staphylococcal infections, with in vitro bactericidal activity against a wide variety of anaerobic bacteria, including *Bacteroides fragilis* as well as some *Staphylococcus* sp.³⁰. It is also known to be one of the antibiotics used to treat acne³¹. The diameters of the zones of inhibition produced by the clindamycin gel against *C. acnes*, *S. epidermidis*, and *S. aureus* were 34.17 ± 2.48 mm, 28.87 ± 0.75 mm, and 29.53 ± 1.06 mm, respectively (Table 4).

Table 4. Antibacterial activity of ethanolic extract of *C. javanicum* stem

Materials	Concentration (%)	Inhibition zone diameter (mm) (mean±SD; n=3)		
		<i>C. acnes</i>	<i>S. epidermidis</i>	<i>S. aureus</i>
Clindamycin gel	1	34.17 ± 2.48	28.87 ± 0.75	29.53 ± 1.06
Ethanolic extract of <i>C. javanicum</i> stem	1	4.20 ± 1.58	7.47 ± 3.87	4.33 ± 0.38
	5	5.10 ± 1.02	7.53 ± 1.80	4.80 ± 0.28
	10	4.00 ± 1.80	7.33 ± 0.38	4.03 ± 1.03
	15	3.17 ± 0.90	8.90 ± 1.50	5.83 ± 0.76

This study showed that *C. javanicum* extract was effective against the three bacteria tested and its inhibition zone was in the range of 3.17 ± 0.90 - 8.90 ± 1.50 mm (Figure 1). The highest antibacterial activity was found for *C. javanicum* extract against *S. epidermidis*, with inhibition zone diameters of 7.47 ± 3.87 mm, 7.53 ± 1.80 mm, 7.33 ± 0.38 mm and 8.90 ± 1.50 mm at concentrations of 1%, 5%, 10% and 15% (Table 4). The antibacterial activity of the extract can be divided into three levels: weak activity (inhibition zone less than 12 mm), moderate activity (inhibition zone 12-20 mm) and strong activity (inhibition zone greater than 20 mm)³².

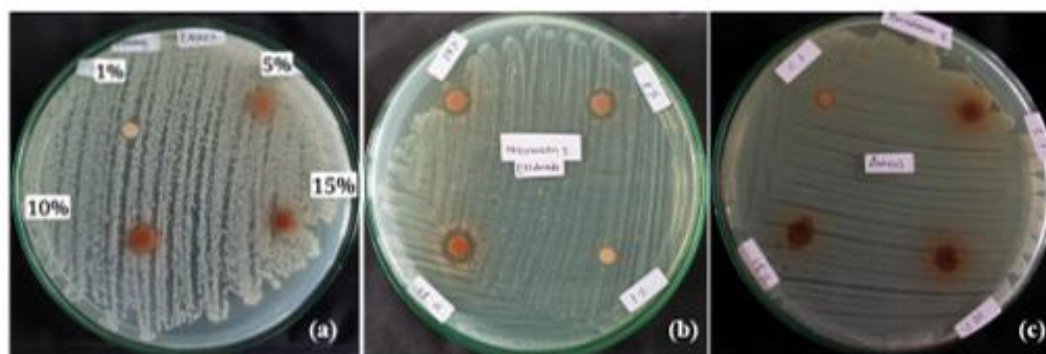


Figure 1. Antibacterial activity of ethanolic extract of *C. javanicum* stem against *C. acnes* (a), *S. epidermidis* (b), and *S. aureus* (c)

It can be concluded that the ethanol extract of the stem of *C. javanicum* has high potential antioxidant activity, but the antibacterial activity is classified as weak activity. The inhibition zones produced in this study fall on the concentration of extract of 10%. Further studies are needed to identify the factor that affects the rise and fall of the inhibition zone. In addition, negative control must be used in further study.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This research was funded by an internal grant from Muhammadiyah University of Palangkaraya and supported by a RisetMu grant batch 5 from Majelis Diktilitbang PP Muhammadiyah for publication.

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3. Acknowledgement of revision

Response to Reviewer:

Reviewer 1

1. The unnecessary parts were removed from the manuscript.
2. More emphasis is placed on the introduction in the manuscript. "However, no studies on the antioxidant activity of these ethanolic extracts of *C. javanicum* stem from Central Kalimantan have been conducted. This study was advanced from the previous study, with the aim being to determine the total number of flavonoid, alkaloid, and tannin compounds and to evaluate the antioxidant and antibacterial activity of the ethanolic extract of stems of *C. javanicum*."
3. The botanical author's name is deleted in the manuscript where it is used repeatedly.
4. The herbarium number was written in the manuscript.
5. The information about maceration was deleted from the manuscript.
6. The technique for solvent removal was mentioned in the manuscript.
7. There are many factors that can affect the rise and fall of the inhibition zone. Further research needs to be done, not by repeating the experiment. **We include the research suggestions in the manuscript, to identify the factor that affects the rise and fall of the inhibition zone.**
8. In the images in Figure 1, actually the disc diameters do not expand. It looks like that is due to the color of the plant extract which gets more concentrated as the extract concentration increases.
9. The number of references decreased, to less than 40 (32 references).

Reviewer 2

A. General Remarks:

1. We didn't use negative control in this study. **We include the research suggestion in the manuscript that negative control must be used in further study.**
2. We incubated *C. acnes* in aerobic conditions because of the lack of lab equipment. **The incubation using the aerobic incubator is mentioned in the manuscript.**
3. The name of *P. acnes* was replaced with *C. acnes* in the whole manuscript.
4. ml was corrected to mL in the whole manuscript.

B. Specific Remarks: All of the specific remarks were corrected in the manuscript.



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Dear Susi Novaryatiin,

This is to acknowledge receipt of your revised manuscript entitled Antioxidant and Antibacterial Activities of Ethanolic Extract of Sintok Lancang (*Cinnamomum javanicum* Blume) from Central Kalimantan. You can follow the stage of your manuscript in the review process through the author center. Thank you.

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Dear Other Susi Novaryatiin,

I am pleased to inform you that your manuscript entitled as "Antioxidant and Antibacterial Activities of Ethanolic Extract of Sintok Lancang (*Cinnamomum javanicum* Blume) from Central Kalimantan" has been accepted for publication in an upcoming issue of ACTA Pharmaceutica Scientia. We will send the proof of your article for your approval prior to publication. You can reach the final information about your manuscript at online manuscript center (<http://actapharmsci.com>).

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5. Article Published

ISSN: 2636-8552 - e-ISSN: 1307-2080

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Antioxidant and antibacterial activities of ethanolic extract of sintok lancang (*Cinnamomum javanicum* Blume) from Central Kalimantan

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DOI: 10.23893/1307-2080.APS6103

The *C. javanicum* Blume is a typical plant that can be founded in Central Kalimantan. This research aimed to find out the total flavonoids, alkaloids, tannins, and compounds and to evaluate the antioxidant and antibacterial activity of ethanolic extract of the stem of *C. javanicum*. The antioxidant activity was tested by two different assays: DPPH and FRAP. Total alkaloids, flavonoids and tannins content were 32.81±0.77 µg caffeine equivalent/mg, 126.96±3.17 µg quercetin equivalent/ mg, and 42.89±0.77 µg catechin equivalent/mg, respectively. The IC50 value of DPPH was 20.63±0.82 ppm quercetin equivalent while the FRAP method was 968.38±22.25 µmol trolox/g. *C. javanicum* extract had antibacterial potential against the three bacteria tested, with the inhibition zones in the range of 3.17±0.90 - 8.90±1.50 mm. It can be stated that the ethanol extract of the stem of *C. javanicum* has high potential antioxidant activity, however, the antibacterial activity is classified as weak activity.

Keywords : *Cinnamomum javanicum* Blume, DPPH, FRAP, disc-diffusion, medicinal plant

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Antioxidant and antibacterial activities of ethanolic extract of sintok lancang (*Cinnamomum javanicum* Blume) from Central Kalimantan

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ABSTRACT

The *C. javanicum* Blume is a typical plant that can be founded in Central Kalimantan. This research aimed to find out the total flavonoids, alkaloids, tannins, and compounds and to evaluate the antioxidant and antibacterial activity of ethanol extract of the stem of *C. javanicum*. The antioxidant activity was tested by two different assays: DPPH and FRAP. Total alkaloids, flavonoids and tannins content were 32.81 ± 0.77 μg caffeine equivalent/mg, 126.96 ± 3.17 μg quercetin equivalent/mg, and 42.89 ± 0.77 μg catechin equivalent/mg, respectively. The IC_{50} value of DPPH was 20.63 ± 0.82 ppm quercetin equivalent while the FRAP method was 968.38 ± 22.25 μmol trolox/g. *C. javanicum* extract had antibacterial potential against the three bacteria tested, with the inhibition zones in the range of 3.17 ± 0.90 - 8.90 ± 1.50 mm. It can be stated that the ethanol extract of the stem of *C. javanicum* has high potential antioxidant activity, however, the antibacterial activity is classified as weak activity.

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(Received 30 May 2022, Accepted 26 Oct 2022)

Keywords: *Cinnamomum javanicum* Blume, DPPH, FRAP, disc-diffusion, medicinal plant

INTRODUCTION

Free radicals are known for their central role in various physiological conditions as well as their involvement in a variety of diseases. The accumulation of free radicals in the body produces oxidative stress, which has been reported to be associated with various diseases such as neurodegenerative disorders, diabetes mellitus, respiratory diseases, cardiovascular diseases, coronary heart diseases, rheumatoid arthritis, the development of cataracts, inflammatory diseases, autoimmune disorders, aging, and various cancers^{1,3}. Antioxidants play an important role in neutralizing free radicals³.

In addition, the resistance of bacterial species to many antibiotics is another major problem in antibiotic therapy that continuously encourages researchers to develop new antibiotics. Recently, most of the newly approved antibacterial agents are derived from natural products or their derivatives. Herbal remedies are one of the most important sources of natural antibacterial agents¹. The antimicrobial activity was mainly related to the existence of secondary metabolites of phenols, terpenes, and alkaloids present in the plant extracts². Therefore, there is a need to explore new, safe and inexpensive sources of natural antioxidant and antibacterial compounds.

Cinnamomum (Family Lauraceae) is a genus known for its fragrant leaves and bark. This genus has more than 300 species that are widespread in North America, Asia, Australasia, and Southeast Asia. Commercial species sold as spices are *C. verum*, *C. cassia*, *C. burmannii*, *C. zeylanicum*, and *C. loureiroi*⁴.

Sintok lancang (*Cinnamomum javanicum* Blume) is a typical plant of Central Kalimantan, in this study found at Mungku Baru Forest (KHDTK), an educational forest managed by the collaboration of Muhammadiyah University of Palangkaraya with the Borneo Nature Foundation (BNF). Empirically, Sintok lancang was used for various diseases such as treating abdominal pain, wounds, and diabetes mellitus.

There has not been much research on *C. javanicum* from Indonesia regarding its potential use as an antioxidant and antibacterial agent. One of the studies done on *C. javanicum* from Malaysia showed that the leaf and bark extracts had antioxidant activity⁵. The antibacterial activity of the essential oil of *C. javanicum* found in Borneo was investigated using the broth dilution method, which showed that the inhibition against *S. aureus* with MIC and MBC values

was 250 µg/mL and 500 µg/mL, respectively⁴. Previous studies reported that the ethanolic extract of *C. javanicum* leaves from Central Kalimantan had a high potential for antioxidant activity as tested by DPPH and FRAP methods⁶. However, no studies on the antioxidant activity of these ethanolic extracts of *C. javanicum* stem from Central Kalimantan have been conducted. This study was advanced from the previous study, with the aim being to determine the total number of flavonoid, alkaloid, and tannin compounds and to evaluate the antioxidant and antibacterial activity of the ethanolic extract of stems of *C. javanicum*. This study will help to explore the potential of Indonesian medicinal plants as natural antioxidants and antibacterial agents.

METHODOLOGY

Plant material

C. javanicum was collected from Mungku Baru Forest (KHDTK), an educational forest managed by the cooperation of the Muhammadiyah University of Palangkaraya with the Borneo Nature Foundation (BNF). The collected plant material was identified by Dr. R. Hendrian, MSc (Indonesian Institute of Sciences, Research Center for Plant Conservation and Botanic Gardens, Bogor, Indonesia) with the document number B-833/IPH.3./KS/VII/2020.

Preparation of *Cinnamomum javanicum* extract

The stems of *C. javanicum* were dried in an oven at 45 °C. The dried stems were powdered by the grinder and then percolated using 96% ethanol at room temperature. Percolation is a continuous process in which the saturated solvent is continuously replaced by a new solvent⁷. The solvent is removed from the extract by using a rotary evaporator. Percentage yield (w/w) of the extracts is calculated by formula⁸: $\text{Weight of the extract} \div \text{Weight of the starting plant material}) \times 100\%$

Phytochemical qualitative screening

The ethanolic extract of *C. javanicum* was preliminarily screened for phytochemicals such as alkaloids, flavonoids, saponins, tannins, and steroids⁹⁻¹².

Total alkaloids content

Ten mg of *C. javanicum* extract were weighed and then dissolved in 10 mL of ethanol. The absorbance of one mL of the extract was measured with a spectrophotometer at 272 nm. The standard used for the calibration curve was caffeine¹³. Total alkaloid content is expressed as µg alkaloids per mg of extract.

Total flavonoids content

Five mg of *C. javanicum* extract were weighed and then dissolved in 10 mL of ethanol. One mL of the extract was placed in the volumetric flask, and one mL of AlCl_3 (2% b/v) and 8 mL of acetic acid (5% v/v) were added. After mixing, the solution was incubated for 20 min¹⁴. The solution absorbance was measured with a spectrophotometer at 412 nm. The standard used for the calibration curve was quercetin¹⁵. Total flavonoid content is expressed as μg of flavonoids per mg of extract.

Total tannins content

A total of 30 mg of sample was weighed and placed in a 10 mL volumetric flask. Add to the 0.5 mL catechin standard solution, 3.0 mL vanillin 4% and 1.5 mL concentrated HCl. The mixture was incubated for 10 min¹⁵⁻¹⁶. Absorbance was measured with a UV Vis spectrophotometer at 498 nm. The standard used for the calibration curve was catechin¹⁷. Total tannin content is expressed as μg tannins per mg extract.

Antioxidant activity by DPPH assay

A 0.4 mM DPPH solution was prepared and the absorbance was measured at 512 nm. The resulting absorbance of the DPPH solution is the absorbance control. The stem extract of *C. javanicum* (sample) was first dissolved in methanol with five variant concentrations of 10, 20, 30, 40, 50 ppm. One mL of 0.4 mM DPPH solution was added in a 5 mL volumetric flask, and then 4 mL of sample solutions of different concentrations were added. The prepared mixture was placed at 25 °C for 30 min, the absorbance was measured at 512 nm¹⁸. The calculation of the percent inhibition or DPPH scavenging effect was used according to the following formula:

$$\text{DPPH Scavenging effect \%} = \frac{(A - B)}{A} \times 100\%$$

Where A was the absorbance of DPPH solution and B was the absorbance of sample solution¹⁹⁻²⁰.

Antioxidant activity by FRAP assay

Sample (0.2 g) dissolved with ethanol in a 10 mL volumetric flask. Two mL of sample solution were added to 3 mL of FRAP reagent in a test tube, followed by incubation for 16 minutes. Absorbance was measured with a UV Vis spectrophotometer at 595 nm. Antioxidant activity expressed in μmol trolox/g¹⁹⁻²¹.

Antibacterial activity test

The antibacterial activity was determined by disc diffusion method with four variant concentrations 1%, 5%, 10%, and 15% against three bacterial strains: *Cutibacterium acnes/C. acnes* (ATCC 11827), *Staphylococcus epidermidis/S. epidermidis* (ATCC 12228) and *Staphylococcus aureus/S. aureus* (ATCC 25923). Ten mL of McFarland 0.5 standard were prepared in sterilized tubes. The suspension of bacteria was prepared by diluting the colonies of bacteria in a normally sterile saline solution and the turbidity was adjusted to $1-2 \times 10^8$ CFU/mL (based on McFarland 0.5 standard). A sterile cotton swab was dipped in a standardized suspension of bacteria and used to inoculate on Mueller-Hinton agar plates²². All discs were immersed in the ethanolic extract of *C. javanicum* and then placed on the plates. A disc immersed in 1% clindamycin gel (positive control) was also placed on the plate. These plates were incubated in the aerobic incubator for 24 h at 37 °C. A caliper was used to measure the diameter of the inhibition zone of each extract and positive controls.

RESULTS AND DISCUSSION

Extraction yield

One thousand two hundred grams of *C. javanicum* were extracted into 60.29 g of extract. Based on yield calculation, *C. javanicum* extraction yielded 5.03%. The yield value relates to the number of secondary metabolites that were captured during extraction⁸.

Qualitative phytochemical screening

Phytochemical screening of *C. javanicum* stem by using the following standard methods⁹⁻¹⁰. The results of the phytochemical qualitative test of *C. javanicum* stem showed the presence of alkaloids using Mayer's reagents¹¹, flavonoids using the Shinoda test¹², tannins, saponins, and steroids (Table 1).

Table 1. The qualitative phytochemical of ethanolic extract of *C. javanicum* stem

Phytochemical compound	Result
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Steroids	+

Total alkaloids, flavonoids, and tannins content

C. javanicum stem was extracted by percolation method. Total alkaloids, flavonoids, and tannins content were calculated by standard protocols, with the results of 32.81 ± 0.77 μg caffeine equivalent/mg, 126.96 ± 3.17 μg quercetin equivalent/mg, and 42.89 ± 0.77 μg catechin equivalent/mg, respectively (Table 2). The total flavonoid content is the largest compared to the total alkaloids and tannins content. In addition, the total alkaloids, flavonoids, and tannins of *C. javanicum* stem ethanol extract in this study were greater than the total content of the ethanolic extract of the leaves of *C. javanicum* obtained in the previous studies⁶.

Phenolics and flavonoids are commonly found in all parts of the plant. This compound is a group of secondary metabolites made up of a large group of polyphenols that can scavenge free radicals and inhibit lipid oxidation²³. Most antioxidant activities from plant sources correlate with phenolic and flavonoid contents²⁴. Therefore, the measurement of phenol and flavonoid content can be used as a basis for rapid screening of antioxidant activities¹.

Table 2. Total alkaloids, flavonoids, and tannins content of ethanolic extract of *C. javanicum* stem

Assay	Sample	Ethanolic extract of <i>C. javanicum</i>
Total Alkaloid (μg caffeine equivalent/mg)		32.81 ± 0.77
Total Flavonoid (μg quercetin equivalent/mg)		126.96 ± 3.17
Total Tannin (μg catechin equivalent/mg)		42.89 ± 0.77

Antioxidant activity

DPPH and FRAP methods were used to evaluate the antioxidant activity of *C. javanicum* extract. The antioxidant activity results showed that the IC_{50} value of DPPH was 20.63 ± 0.82 ppm quercetin equivalent while the FRAP method was 968.38 ± 22.25 μmol trolox/g (Table 3).

Table 3. Antioxidant activity of ethanolic extract of *C. javanicum* stem

Assay \ Sample	Ethanolic extract of <i>C. javanicum</i>	Quercetin
DPPH (IC ₅₀ ppm)	20.63 ± 0.82	6.98
FRAP (µmol trolox/g)	968.38 ± 22.25	-

According to some publications, the antioxidant activity with the DPPH method was classified by IC₅₀ as very strong (< 50 ppm), strong (50-100 ppm), moderate (101-150 ppm), and low (> 150 ppm)^{19,25}, while the antioxidant activity with the FRAP method was classified as very low FRAP (< 10 µmol/g), low FRAP (10-50 µmol/g), good FRAP (50-100 µmol/g), high FRAP (100-400 µmol/g) and very high FRAP (> 400 µmol/g)²⁶. A low IC₅₀ value (the extract concentration, required to scavenge 50% of DPPH free radicals) means strong antioxidant activity²⁷. The ethanolic extract of *C. javanicum* stem is included in the very strong antioxidant activity (20.63 ± 0.82 ppm) and very high FRAP (968.38 ± 22.25 µmol trolox/g). The antioxidant activity in this study was better than in the previous study, where the ethanolic extract of *C. javanicum* leaves included very strong antioxidant activity (26.99 ± 0.27 ppm) and very high FRAP (779.73 ± 19.66 µmol trolox/g)⁶. When compared with the antioxidant activity of *C. javanicum* leaves and bark study conducted in Malaysia with the DPPH method (223.5 ppm and 197.4 ppm)⁵, *C. javanicum* in Indonesia, especially Central Kalimantan gives better antioxidant activity.

DPPH is a stable free radical with unpaired electrons distributed throughout the molecule and is widely used to test the free radical scavenging capacity of a variety of samples²⁸. The DPPH assay is based on both electron transfer and hydrogen atom transfer reactions. The reduction in absorbance of DPPH caused by antioxidants is due to a reaction between the antioxidant molecules and the radical, which results in radical scavenging by hydrogen donation. This is visualized as purple-to-yellow discoloration. The advantage of the DPPH test is that it is simple, fast, and economical. Although the DPPH test is simple, its sensitivity can be affected by several factors, such as solvent type, reaction time, temperature, and freshness of DPPH reagent^{1,29}, while the FRAP test is a non-specific, redox-linked, colorimetric assay related to the molar concentration of the antioxidant present. The FRAP assay is a typical method based on electron transfer, which measures the reduction of ferric ion (Fe³⁺)-ligand complex to the ferrous (Fe²⁺); complexed by antioxidants in acidic media. One limitation of the FRAP assay is its tendency to precipitate, form suspensions, and color

the cuvette. Therefore, the timing of FeCl_3 addition is essential to prevent error interpretation. However, the FRAP or DPPH assay is simple, economical, fast, and requires no specialized equipment²⁹.

Antibacterial activity

In this study, an antibacterial activity test was performed for *C. javanicum* extract and clindamycin gel. Clindamycin is used as a positive control because it is a lincosamide antibiotic used to treat anaerobic, streptococcal, and staphylococcal infections, with *in vitro* bactericidal activity against a wide variety of anaerobic bacteria, including *Bacteroides fragilis* as well as some *Staphylococcus* sp.³⁰. It is also known to be one of the antibiotics used to treat acne³¹. The diameters of the zones of inhibition produced by the clindamycin gel against *C. acnes*, *S. epidermidis*, and *S. aureus* were 34.17 ± 2.48 mm, 28.87 ± 0.75 mm, and 29.53 ± 1.06 mm, respectively (Table 4).

Table 4. Antibacterial activity of ethanolic extract of *C. javanicum* stem

Materials	Concentration (%)	Inhibition zone diameter (mm) (mean \pm SD; n=3)		
		<i>C. acnes</i>	<i>S. epidermidis</i>	<i>S. aureus</i>
Clindamycin gel	1	34.17 ± 2.48	28.87 ± 0.75	29.53 ± 1.06
Ethanolic extract of <i>C. javanicum</i> stem	1	4.20 ± 1.58	7.47 ± 3.87	4.33 ± 0.38
	5	5.10 ± 1.02	7.53 ± 1.80	4.80 ± 0.28
	10	4.00 ± 1.80	7.33 ± 0.38	4.03 ± 1.03
	15	3.17 ± 0.90	8.90 ± 1.50	5.83 ± 0.76

This study showed that *C. javanicum* extract was effective against the three bacteria tested and its inhibition zone was in the range of 3.17 ± 0.90 - 8.90 ± 1.50 mm (Figure 1). The highest antibacterial activity was found for *C. javanicum* extract against *S. epidermidis*, with inhibition zone diameters of 7.47 ± 3.87 mm, 7.53 ± 1.80 mm, 7.33 ± 0.38 mm and 8.90 ± 1.50 mm at concentrations of 1%, 5%, 10% and 15% (Table 4). The antibacterial activity of the extract can be divided into three levels: weak activity (inhibition zone less than 12 mm), moderate activity (inhibition zone 12-20 mm) and strong activity (inhibition zone greater than 20 mm)³².

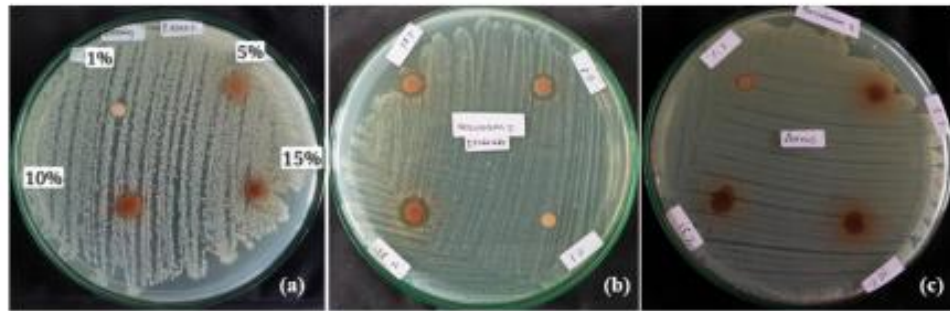


Figure 1. Antibacterial activity of ethanolic extract of *C. javanicum* stem against *C. acnes* (a), *S. epidermidis* (b), and *S. aureus* (c)

It can be concluded that the ethanol extract of the stem of *C. javanicum* has high potential antioxidant activity, but the antibacterial activity is classified as weak activity. The inhibition zones produced in this study fall on the concentration of extract of 10%. Further studies are needed to identify the factor that affects the rise and fall of the inhibition zone. In addition, negative control must be used in further study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING SOURCES

This research was funded by an internal grant from Muhammadiyah University of Palangkaraya and supported by a RisetMu grant batch 5 from Majelis Dikti-litbang PP Muhammadiyah for publication.

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DEWAN KOMITE ETIK PENELITIAN

Jl. Pramuka No.02 Banjarmasin Tlp. (0511) 3268105

Banjarmasin, 21 Juli 2020

No. SK : 074/KE-LPPM/UNISM/VII/2020
Lampiran : -
Perihal : Rekomendasi Penelitian

Peneliti yang disebutkan dibawah ini :

Ketua Peneliti : Susi Novaryatiin
NIP/NIK/NIM : 14.06.01.015
Anggota Peneliti : 1. Syahrida Dian Ardhany
2. Fahrudin Arfianto
Judul Penelitian : Aktivitas antioksidan dan antibakteri ekstrak etanol Sintok Lancang (*C. javanicum* Blume) asal Kalimantan Tengah

Berdasarkan pertimbangan Dewan Komite Etik Penelitian diputuskan bahwa Peneliti yang disebutkan diatas telah **DISETUJUI** untuk melanjutkan penelitiannya.

Demikian surat persetujuan ini diterbitkan untuk dipergunakan dengan penuh tanggung jawab.

Menyetujui,

An. Ketua

Sekretaris Dewan Komite Etik Penelitian

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