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Antibacterial Potential of *Cinnamomum culilaban* Bark Ethanolic Extract Prepared by Ultrasound-Assisted Extraction against Oral Pathogens

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ABSTRACT

Cinnamomum culilaban is one of the native Indonesian plants that has been used as medicinal plant. The local community on Seram Island uses the stem bark to treat toothaches. Eugenol, a chemical compound found in *Cinnamomum culilaban*, is used in dental practice and has antibacterial activity. Research on *Cinnamomum culilaban* is very limited, its activity against bacteria that cause oral infections has not been reported. This study aims to investigate the antibacterial activity of *Cinnamomum culilaban* bark extract against oral pathogens *Porphyromonas gingivalis*, *Streptpcoccus mutans,* and *Enterococcus faecalis*. The extraction was carried out using 96% ethanol with ultrasound-assisted extraction method. Disc diffusion assay was conducted to investigate the antibacterial activity. The concentration of extract used were 50%, 25%, and 12.5% (b/v) in DMSO. Eugenol (50% v/v in DMSO) was used as positive control. The phytochemicals screening was carried out to investigate the chemical compounds contained in *Cinnamomum culilaban* bark extract. Extraction of *Cinnamomum culilaban* bark using 96% ethanol with the ultrasound-assisted extraction method obtained a yield of 23.36±0.49%. The extract contains alkaloids, flavonoids, tannins, saponins, phenolics, and steroids/terpenoids. The ethanolic extract of *Cinnamomum culilaban* bark has inhibitory activity against *Streptpcoccus mutans* and *Enterococcus faecalis*. *Cinnamomum culilaban* bark has the potential as an antibacterial agent.

***Keywords*:** *Cinnamomum culilaban*; Antimicrobial; Oral pathogens; Ultrasound assisted extraction

INTRODUCTION

Indonesia is the highest mega-biodiversity country in the world due to its vast array of biological diversity. A significant number of native Indonesian plants have the potential for use as medicinal plants.[1] One such plant utilized in traditional medicine is *Cinnamomum culilaban* (L.) J. Presl., an endemic species found in eastern Indonesia and known as lawang tree, particularly in Papua and Maluku. The part of the plant most commonly used is the bark, known as kayu lawang. The local community on Seram Island uses the bark to treat toothaches. The people of Papua use oil from kayu lawang to treat bone pain, as a tonic, and as a massage oil.[2]

The chemical constituents of kayu lawang include propanoic acid, naphthalene, sparthulenol, terpinol, calamenene, cuminol, methyl eugenol, myrtenol, rubean, verbanol, and verbenone.[3] Many plants from the genus *Cinnamomum* exhibit antibacterial activity. Eugenol and cinnamaldehyde, compounds

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found in *Cinnamomum*, are responsible for their antibacterial properties.[4]

Eugenol, a compound found in *Cinnamomum culilaban* (L.) J. Presl., is a volatile monoterpenoid phenolic compound.[5] Eugenol can be extracted using the Ultrasound Assisted Extraction (UAE) method. Pilot-scale extraction of clove using the UAE method with ethanol as the solvent produced an extract containing eugenol.[6] The UAE extraction method has been reported to produced high extract yields from *Cinnamomum zeylanicum* Blume leaves, showing potential as an antibacterial agent.[7] Cinnamon extracted using the UAE method could inhibit bacterial growth in food samples, with lower microbial growth observed in meat samples containing cinnamon extract compared to others.[8]

Eugenol is widely used in the pharmaceutical industry, particularly for dental and oral care.[9] Antibacterial tests on eugenol against several bacteria causing dental infections have been conducted. Eugenol could inhibit the growth of *Streptococcus mutans*, the primary cause of dental caries, with a minimum inhibitory concentration (MIC) of 322.54 µg/ml.[10] Additionally, eugenol could inhibit the growth of *Porphyromonas gingivalis*, which causes periodontitis, with an MIC of 31.25 µM.[4] The inhibition of *Enterococcus faecalis*, which causes endodontic infections, by zinc oxide-eugenol has also been reported.[4]

In this study, the extraction of *Cinnamomum culilaban* bark was conducted using the UAE method with 96% ethanol as the solvent. The extract was tested for antibacterial activity against Streptococcus mutans, Porphyromonas gingivalis, and Enterococcus faecalis. Eugenol used as a positive control.

METHODS

Equipment and Materials

The instruments used in this study were an ultrasonicator (Krisbow, Indonesia), rotary vacuum evaporator (Buchi, Swiss), autoclave (TOMY, Jepang), incubator (Memmert), laminar air flow (Esco, Indonesia), analytical balance (OHAUS, USA). vortex (Barnstead, USA), hot plate with stirrer (Corning, USA), caliper (Vernier Caliper, Perancis), blanc disc (Macherey-Nagel, Jerman), and petri dish (Biologix, USA).

The materials used were 96% ethanol technical grade (Bratachem, Indonesia), eugenol (Sigma Aldrich, USA), Folin-ciocalteu (Merck), zinc powder (Sigma Aldrich, USA), magnesium powder (Sigma Aldrich, USA), Dragendroff reagent, Mayer reagent, Lieberman-Bouchard reagent, dimethylsulfoxide (Merck, Jerman), $FeCl_3$ (Merck, Jerman), Mc Farland III standard (Sigma Aldrich, USA), distilled water (OneMed, Indonesia), nutrient agar (Merck, Jerman), and 0.9% NaCl solution (Otsuka, Indonesia).

The samples used in this research were *Cinnamomum culilaban* (L.) J. Presl. bark or kayu lawang, which were taken from Manado, North Sulawesi. The plant has been identified and authenticated in Laboratorium FMIPA, Universitas Lambung Mangkurat with the testing report certification number 132/L.B.LABDASAR/V/2023.

The microbes used were *Streptococcus mutans* ATCC 35668, *Porphyromonas gingivalis* ATCC 33277, and *Enterococcus faecalis* that were obtained from the Pharmaceutical Microbiology and Biotechnology Laboratory at Universitas Indonesia (Depok, Indonesia).

Extract Preparation

Extraction was carried out on the *Cinnamomum culilaban* bark simplicia using the UAE method with ultrasonicator and 96% ethanol as a solvent. The ratio of sample and solvent used was 1:10. Ultrasonicator was set for 30 minutes. After extraction was complete, the extract was filtered, the filtrate was collected and the solvent was evaporated using a vacuum rotary evaporator at a temperature of 50°C. The extract was transferred to an evaporator dish to be dried using a water bath.[11]

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Phytochemical screening

A phytochemical screening test was carried out on the extract. The screening test included alkaloid, flavonoid, saponin, tannin, phenolic, and terpenoid/steroid. Reagents that were used for the alkaloid test was Mayer, Dragendorff, and Bouchardat. Shinoda reagent was used for flavonoid test. NaCl-gelatin test was used for the tannin test, FeCl3 for the phenolic test, foam test for the saponin test, dan Liebermann-Burchard reagent for the terpenoid/steroid test.[11]

Antibacterial Activity Test

The extract used were prepared in concentration of 12.5% b/v, 25% b/v, and 50% b/v in DMSO. Eugenol standard 50% in DMSO was used as a positive control. The antibacterial activity test was carried out using disc diffusion method. Each disc of sample was infused with 10 µl of extract, disc of positive control was infused with 10 µl of eugenol (50% v/v), and disc of negative control was infused with 10 µl of DMSO.

The bacterial stocks used were 24-h-old bacteria that were cultured in nutrient agar, which had been incubated at 37° C for 24 h. The preparation of microbial inoculum was determined using the McFarland turbidity standard. The inoculum was prepared by adding a 24-hour-old bacterial culture into a tube containing 3 ml of NaCl solution (0.9%). The turbidity of the inoculum suspension was compared to the McFarland III standard solution (equivalent to 10^9 microbes/mL), which was then diluted 1000 times using physiological NaCl solution to obtain an inoculum with a concentration equivalent to 10^6 microbes/mL. They were aseptically inoculated on the surface of the nutrient agar media that were placed in a sterile petri dish by swabbing at 60° rotation to uniformly distribute bacteria throughout media surface using a cotton swab. Each disc contains of sample, positive control, and negative control were place on the surface of the swabbed nutrient agar. Subsequently, they were incubated at 37° C for 24 h, with petri dish placed upside down. The *S. mutans* and *P. gingivalis* were incubated in anaerobic condition using anaerobic jar. The *E. faecalis* was incubated in aerobic condition. The zone of inhibition against bacteria after incubation were determined and recorded. Antibacterial activity was evaluated by measuring the diameter of the inhibitory zone around the disc using a calliper. The experiment was done in triplicate.[12]

RESULTS AND DISCUSSION

Extraction of *Cinnamomum culilaban* bark using 96% ethanol solvent with the ultrasound-assisted extraction (UAE) method resulted in a dry extract that is dark brown in color (Figure 1). The extract has a distinctive clove-like aroma. The extraction yield obtained was 23.36±0.49%.

Figure 1. *Cinnamomum culilaban* bark simplicia (a), *Cinnamomum culilaban* bark ethanolic extract (b)

In another study, the extraction of clove (*Syzygium aromaticum*) leaves using the UAE method with ethanol solvent was more effective compared to the maceration method, where the extraction yield and eugenol content in the obtained extract were higher.[13] UAE is one of the non-conventional extraction methods that utilizes ultrasonic waves with a frequency of 20-100 kHz to enhance the permeability of plant cells and induce cavitation.[14] The mechanism in UAE extraction involves diffusion through the cell wall and dissolving cell contents after breaking the cell wall, allowing the contents to be released easily.[15] The advantages of UAE include shorter extraction time, higher extraction efficiency, less solvent usage, and lower extraction temperature. Additionally, UAE is easy to apply, has lower costs compared to other non-conventional extraction methods, and can be used for thermolabile compounds.[11]

Based on phytochemical tests, *Cinnamomum culilaban* bark ethanolic extract showed positive results in testing the content of alkaloids, flavonoids, tannins, saponins, phenolics, and steroids/terpenoids (Table 1).

The results of the disc diffusion assay of the ethanolic extract of *Cinnamomum culilaban* bark showed antibacterial activity (Figure 2). Ethanolic extract of *Cinnamomum culilaban* bark at concentrations of 50%, 25%, and 12.5% showed inhibition zones on media inoculated with *S. mutans* and *E. faecalis* bacteria. No inhibition zone was observed on media inoculated with *P. gingivalis* bacteria. Eugenol (positive control) showed inhibition zones against all test bacteria, which is consistent with previous studies mentioned earlier. The measurement results of the inhibition zones (Table 2) indicated that the lower the extract concentration, the smaller the inhibition zone formed.

Table 1. Phytochemical screening of *Cinnamomum culilaban* bark ethanolic extract

The Secondary Metabolites	**Result**	**Description**
Alkaloid	+	White precipitate
Flavonoid	+	Red color
Phenolic	+	Blackish green color
Tannin		White precipitate
Saponin	+	Foam
Steroids/Terpenoid	+	Red color

Table 2. Diameter of inhibition zone of *Cinnamomum culilaban* bark extract against *S. mutans, E. faecalis,* and *P. gingivalis*

Samples	**Diameter of inhibition zone (mm)**		
	S. mutans	***E. faecalis***	***P. gingivalis***
50% (b/v) extract	7.79±1.01	10.16±0.31	-
25% (b/v) extract	6.85±1.30	8.08±0.33	-
12.5% (b/v) extract	4.87±0.81	6.48±0.69	-
Positive control (50% v/v eugenol)	13.06±0.29	9.58±0.16	10.06±0.22
Negative control (100% v/v DMSO)	-	-	-

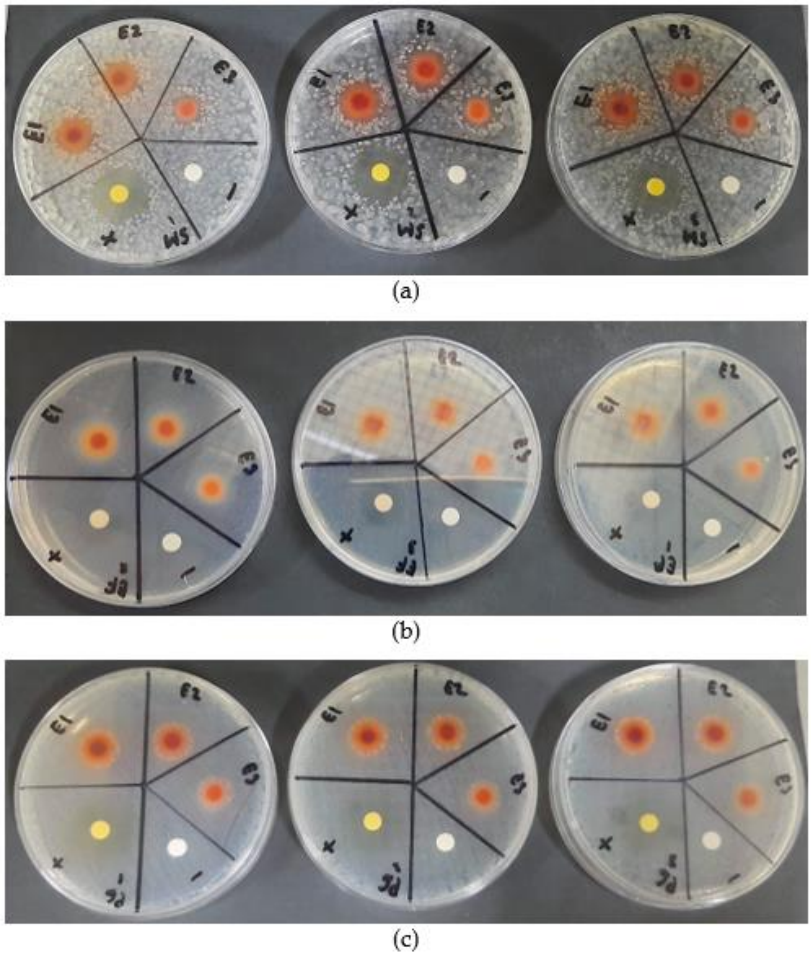

Figure 2. Disc diffusion antibacterial test of *Cinnamomum culilaban* bark ethanolic extract against *S. mutans* (a), *E. faecalis* (b), and *P. gingivalis* (c). E1: 50% extract, E2: 25% extract, E3: 12.5% extract, (+) positive control, (-) negative control.

The antimicrobial study of *Cinnamomum culilaban* bark extract, which was extracted using a stepwise maceration method with hexane, ethyl acetate, methanol, and water solvents, was conducted by Hapsari et al. The results showed that only the water extract exhibited antimicrobial activity against *Escherichia coli* with an inhibition zone of 0.8 cm. The water extract showed negative results against *Staphylococcus aureus* and *Candida albicans*.[16]

Ethanolic extract of *Cinnamomum culilaban* bark can inhibit bacteria which are influenced by the presence of chemical compounds contained. Alkaloids play a vital role in the effects of numerous Chinese herbal medicines. Their antibacterial properties have been widely studied in biomedical research. Investigations into the antibacterial mechanisms of natural alkaloids reveal that they can disrupt bacterial cell membranes, interfere with DNA function, and inhibit protein synthesis. As a result, they are being utilized as lead compounds in the development of new antimicrobial drugs.[17] Flavonoids are recognized for their ability to disrupt the permeability of bacterial cell walls, microsomes, and lysosomes. This disruption occurs due to the interaction between flavonoids and bacterial DNA, which inhibits the incorporation of non-crosslinked glucan chains into the peptidoglycan of the cell membrane, resulting in a weakened structure.[18]

Phenolic compounds, which are a part of the human diet, offer numerous health benefits. These compounds exhibit specific chemical reactivity that leads to various

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biological activities.[19] Regarding their antibacterial properties, plant polyphenols combat bacterial cells through multiple mechanisms. These include interacting with proteins and bacterial cell walls, altering cytoplasmic functions and membrane permeability, inhibiting energy metabolism, and causing DNA damage or inhibiting nucleic acid synthesis. At the DNA level, the planarity and hydrophobic core of polyphenols allow them to penetrate the DNA helix during replication, recombination, repair, and transcription processes. Additionally, the hydroxyl groups of phenolic compounds enable the formation of hydrogen bonds with nucleic acid bases. Phenolics also impact synthetic pathways, such as by inhibiting topoisomerase or DNA gyrase activity. Furthermore, polyphenols can form complexes with metals like Cu2+, which alter DNA stability. The mechanism of inhibition varies depending on the structure of the polyphenols and the bacterial species. The molecule's hydrophilic or hydrophobic nature, influenced by its action sites, highlights the significant role of the amphipathic character of phenolic compounds in their antibacterial activity.[20]

Tannins possess notable antibacterial properties, largely due to their structural characteristics. As macromolecular polyphenols, tannins contain numerous phenolic hydroxyl groups, which contribute to their potent antibacterial activity. Various clinical trials have assessed the effectiveness of tannins in combating bacterial infections. One such trial, a double-blind, randomized parallel-group study, evaluated the efficacy of a 0.6% cranberry (tannin-rich plant) mouthwash against *S. mutans*. The results indicated that the cranberry mouthwash reduced the *S. mutans* bacterial count by 68%, proving to be as effective as chlorhexidine mouthwash, while also providing beneficial local and systemic effects.[21]

Saponins, which are triterpene and sterol glycosides, are commonly found in plants. Saponins exhibit antimicrobial effects by inhibiting microbial growth and killing microbes through interactions with sterols. The primary impact of saponins on bacteria involves the release of proteins and enzymes from within the cell, thus disrupting the permeability of bacterial cell membranes. The hydrophobic end of saponins binds to membrane proteins through polar group bonds, while the non-polar groups bind to cell membrane fats, causing membrane damage and the release of crucial cellular components such as proteins, nucleic acids, and nucleotides. This disruption indirectly prevents bacteria from attaching to host cells.[22] Extracts from water hyacinth leaves, which contain alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols, have demonstrated antibacterial activity against plaque bacteria in gingivitis patients, effectively killing 91.5% (> 90%) of bacterial colonies.[21]

CONCLUSION

The ethanolic extract of *Cinnamomum culilaban* bark has inhibitory activity against *S. mutans* and *E. faecalis*. Further research can be conducted to develop the potential of *Cinnamomum culilaban* bark as an antibacterial agent to combat oral pathogens.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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