Antimicrobial activity of *Amomum krervanh* seed extract against *Escherichia coli* and *Staphylococcus aureus*

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Abstract

The purpose of this of this study was to evaluate the antimicrobial activities of *Amomum krervanh* seed extract against two bacterial pathogens, *Escherichia coli* and *Staphylococcus aureus*. The disc diffusion method was employed to measure the inhibition zones produced by the seed extract. The extract exhibited its largest inhibition zone with an average diameter of 7.33 ± 0.6 mm. Comparative analysis was conducted using standard antimicrobial agents, including the antibiotics Amikacin, Ampicillin, and Clotrimazole. The results demonstrated that the inhibition zones produced by *A. krervanh* seed extract were smaller than those of Amikacin and Ampicillin, indicating lower antibacterial efficacy compared to these antibiotics. However, the extract outperformed Clotrimazole, showing a larger inhibition zone, particularly against *E. coli*. This finding highlights the extract's relatively stronger antimicrobial potential in this context. Additionally, the study found that the extract was more effective against *E. coli* than against *S. aureus* when compared to Clotrimazole. These findings indicate that *A. krervanh* seed extract possesses significant antimicrobial properties, particularly in inhibiting the growth of *E. coli* and *S. aureus*. The extract's effectiveness suggests its potential as a natural antimicrobial agent for developing alternative treatments or products to manage bacterial infections. The extract's demonstrated effectiveness highlights its potential as a natural antimicrobial agent, offering opportunities to develop alternative treatments or products for managing bacterial infections. This makes it a promising candidate for innovative antimicrobial solutions to control infections and combat antibiotic resistance in the future.

Keywords: *Amomum krervanh*, *Escherichia coli*, *Staphylococcus aureus,* Antibiotic drugs

1. Introduction

Currently, countries around the world are emphasizing the use of medicinal plants in the pharmaceutical industry. In Thailand, traditional herbal medicine, which has been passed down through generations, is utilized for treating diseases and maintaining health (Jangklang et al., 2022). Various diseases caused by microorganisms pose significant threats to life and public health. Developing methods to prevent these pathogens from entering the body could

significantly reduce the costs associated with purchasing medicines and treatment (Burakorn, 2011).

The aromatic fruits of several plants in the genus Amomum [Zingiberaceae] are used worldwide as the spice cardamon. The most widespread cardamom species in Thailand is *Amomum krevanh* Pierre, commonly called "Kra-Waan" in Thai or "Round Siam Cardamom" in the international spice market. Possibly because cardamon has been a household item since ancient times, chemical investigations of this genus were mostly conducted in the early days of organic chemistry and focused on simple monoterpenes, which were isolated from steam volatile oils (Kamchonwongpaisan et al., 1995).

Presently, infectious diseases are a major public health issue, with a rising trend every year. Skin infections are caused by various microorganisms, including bacteria, fungi, and viruses (Rangseepanurat et al., 2013). Bacteria are the cause of infectious diseases that adapt over time. The use of antibiotics in excessive doses or without proper justification leads to antibiotic resistance in microorganisms, resulting in significant challenges in medical treatment (Jaratpongpasut & Laappra, 2018). Therefore, this study investigates the antibacterial activity of crude extracts from *A. krervanh* seeds to determine the potential applications of *A. krervanh* seed extract for maximizing efficacy. The aim is to promote the use of medicinal plants and increase the agricultural value of *A. krervanh* seed extract. Furthermore, this research serves as a foundational scientific database that can be developed and applied in the creation of medical products in the future.

1.1 Research Objective

The objective of this research was to evaluate the potential of A. krervanh seed extract on standard microorganism strains and to compare its antimicrobial activity against antibiotics.

2. Literature Review

Scientific name: *Amomum krevanh Pierre, Amomum cardamomum L.*

Family: *ZINGBERACEAE*

Common names: Camphor Seed, Siam Cardamom, Best Cardamom, Round Siam Cardamom, Clustered Cardamon

Characteristics: *A. krervanh* is an herbaceous plant that grows 1-3 meters tall, typically found in moist forests on high mountain slopes. It has underground rhizomes. The leaves are single, alternately arranged, and oblong in shape, measuring 8-15 cm wide and 40-50 cm long, with no petioles. The flowers grow in clusters from the rhizomes, with white petals that form tubes and swell into pouches. The plant blooms when it is 2-3 years old. The fruit is smooth and round, measuring 6-15 mm in size. When mature, the fruit's skin becomes dry and hard. Each fruit contains 12-18 small seeds, grouped into 3 clusters separated by thin membranes. The seeds are fragrant and have a spicy taste (Promchit, 2000).

Herbal Benefits: According to Thai traditional medicine, the fruit is used as a carminative to treat bloating, flatulence, and indigestion. The recommended dosage is 1-2 grams, brewed in water as a drink. It is also used as a spice to add fragrance to food (Sudjaroen et al., 2022).

Key Chemicals and Nutrients: The essential oil obtained through steam distillation is popular in the food and beverage industry, especially in liqueurs and perfumes. Oleoresin products, extracted from seeds using organic solvents, are commonly used in meat and sausage products. Dried cardamom fruits contain approximately 3.5-7% essential oil, primarily in the seeds, which is a pale-yellow liquid. The main components are 1.8-cineole (20-60%) and α-terpinyl acetate (20-53%). *A. krervanh* has about 5% volatile oil, consisting mainly of borneol and camphor in similar amounts. Additionally, it contains terpenes like pinene, caryophyllene, and diterpene peroxide (Promchit, S., 2000). As part of a multidisciplinary research program on antimalarial natural products, we are screening Thai medicinal plants that are alleged to have antimalarial activity and are used in primary health care. This study uses selected plant extracts in a biological assay against Plasmodium falciparum (EC_{50} values). The crude hexane extract of the fruit of *A. krervanh* Pierre shows high potency ($EC_{50} = 8 \times 10^{-7}$ g/mL) and has been investigated in detail employing the same bioassay to guide the isolation and purification procedure. (Busaba, n.d.; Kamchonwongpaisan et al., 1995)

Bacterial Information

Staphylococcus bacteria are round and form clusters resembling grape bunches. They belong to the group of aerobes or facultative anaerobes. The major pathogenic species is *Staphylococcus aureus*, which causes abscesses, toxic shock syndrome, and post-surgical wound infections. Additionally, this bacterium can produce enterotoxins, leading to food poisoning. *S. aureus* is a facultative anaerobe pathogen in the Family Micrococcaceae, capable of growing in both aerobic and anaerobic conditions, but thrives better in the presence of air. It is spherical (coccus) in shape, measuring 0.5 - 1.0 microns, Gram-positive, and typically forms grape-like clusters, pairs, or short chains. It is non-motile, mostly lacks a capsule, and can be found in air, dust, waste, respiratory tract, throat, hair, skin, urinary tract, and wounds. This bacterium is commonly found in low quantities in animal-derived foods or foods handled by humans. Infections caused by *S. aureus* can lead to Staphylococcal Scalded Skin Syndrome (SSSS) and Toxic Shock Syndrome (TSS) (Sittisak, 2015).

Escherichia coli (*E. coli*) is a Gram-negative, rod-shaped bacterium belonging to the family Enterobacteriaceae. It can grow in both aerobic and anaerobic conditions (facultative anaerobe) and does not form spores (non-spore forming). Most strains are motile due to the presence of peritrichous flagella. *E. coli* can ferment glucose to produce acid and catalase but does not produce oxidase. It can also reduce nitrate to nitrite through reduction reactions. *E. coli* is part of the normal flora in the intestines of humans and animals and can be found in the environment, such as in soil, water, and food. Typically, it does not harm or cause serious disease when residing in the intestines, where it aids in digesting food. However, if *E. coli* invades other systems of the body, it can cause severe infections, such as traveler's diarrhea, urinary tract infections, meningitis, and septicemia. Some strains of *E. coli* can cause diarrhea (Jaratpongpasut & Laappra, 2018).

3. Methodology

Plant material

Amomum krervanh seed extract were collected from Thai traditional drug stores. The sample was identified and compare specimen with the herbarium at Department of Applied Thai Traditional Medicine, College of Allied Health Sciences, Suan Sunandha Rajabhat University, Thailand.

Extraction

The air-dried and powdered plant material were extracted successively by maceration under room temperature with 95 % ethanol for 7 day. The extract was filtrated and evaporated under reduced pressure at 50 °C by the rotary evaporator to obtain the crude extract. The extract yield was weighed, recorded, and stored at -20 °C for further antimicrobial testing. The crude extract was dissolved in Dimethyl sulfoxide (DMSO) to obtain a concentration of 600 mg/ml for Disc diffusion. The percentage yield of the crude extract was calculated using the formula:

% Yieid= weight of extract recovered / weight of fresh dry plant

Bacterial strains

Bacterial strains in this study obtained from stock culture from College of Allied Health Sciences, Suan Sunandha Rajabhat University, Thailand. Strains used included *Staphylococcus aureus* ATCC 6538P and *Escherichia coli* ATCC 25922

Preparation of the inoculum

Bacterial strains were maintained on Muller-Hinton agar (MHA). They were inoculated at 37 °C, for 18-24 hrs. Four to five of isolated colonies from the overnight culture were suspended in 0.85% of normal saline. The turbidity of the suspension was measured by using a spectrophotometer at 625 nm to obtain the absorbance of 0.08-0.13, comparable to 0.5 McFarland's (CLSI., 2018).

Testing the Effect of Inhibiting the Growth of Microorganisms Using the Disc Diffusion Method.

A micro pipette was used to add the prepared culture and drop it into 20 µl of MHA culture medium. A cotton swab was used to spread it evenly over the surface of the culture medium. A 6 mm filter disc, dripped with *A. krervanh* seed extract at a concentration of 600 mg/ml, was placed onto the surface of the agar medium. The samples were incubated at 37 °C for 18-24 hours. The zones of inhibition were measured in millimeters, and the experiment was carried out in triplicates.

4. Dataanalysis

Descriptive statistics, including mean and standard deviation (SD), were used to analyze the data to test the effect of inhibiting the growth of microorganisms using the Disc diffusion method.

5. Result

For the preparation, it was prepared by maceration with 95% ethanol for this study. The percent yield was 3.17% as shown in Table 1. The characteristic of the *A. krervanh* seed extract was sticky texture with dark brown and aromatic odor.

Table 1: Thai name, scientific name, part used and percent yields.

Thai name	scientific name	part of used	% Yield (w/w)
			95% Ethanol
Krevanh	*Amomum krevanh Pierre*	Seed	3.17%

The results of this test showed that *A. krervanh* seed extract produced an inhibition zone of 7.33 ± 0.6 mm against both *S. aureus* and *E. coli*. Positive control antibiotic Ampicillin produced an inhibition zone of 46.67±0.6 mm against *S. aureus* and *E. coli*, while Amikacin produced an inhibition zone of 11.33 ± 0.6 mm against *S. aureus* and 23.33 ± 0.6 mm against *E. coli*. Clotrimazole produced an inhibition zone of 20.67 ± 0.6 mm against *S. aureus* but no inhibition against *E. coli*. In the negative control, there was no inhibition of either strain. The results are shown in Table 2.

Table 2: Zone of inhibition of Amomum krervanh seed extract, negative and positive controls.

Tested microorganisms	Zone of Inhibition (mm)				
	K	Ampicillin	Amikacin	Clotrimazole	DMSO
Staphylococcus aureus	7.33±0.6	46.67±0.6	11.33±0.6	20.67±0.6	NA
Escherichia coli	7.33±0.6	25.33±0.6	23.33±0.6	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate. K = *Amomum krervanh* seed extract

6. Discussion

A. krervanh seed extract produced an inhibition zone against both *S. aureus* and *E. coli*. When comparing the antimicrobial activity of *A. krervanh* seed extract to several antibiotics such as Amikacin and Ampicillin, it was found that the inhibition zones caused by the seed extract were smaller than those of the antibiotics. These findings indicate that while *A. krervanh* seed extract possesses antimicrobial properties against *S. aureus* and *E. coli*, its effectiveness is still lower than that of conventional antibiotics. However, compared with the antibiotic Clotrimazole, the extract was found to have a better inhibitory effect. The results of this experiment were consistent with the results of the ethanolic extract of *Jasminum sambac* flower, which was able to inhibit *E. coli* better than Clotrimazole as well (Chansukh, 2016). Therefore, this study suggests that *A. krervanh* seed extract can control the growth of *S. aureus* and *E. coli* and may be beneficial as a potential option for developing antimicrobial agents or infection control products in the future.

7. Conclusion

Amomum krervanh seed extract possesses potential antibacterial activity against gram-positive and gram-negative bacteria. The results revealed that the *A. krervanh* seed extract showed the largest inhibition zone of 7.33 ± 0.6 mm against both *S. aureus* and *E. coli*. Moreover, *A. krervanh* seed extract has a better effect in inhibiting *E. coli* than the antibiotic Clotrimazole.

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