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Antioxidant and antimicrobial properties of *Artocarpus lakoocha* Roxb. leaves and heartwood for natural food preservative

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Abstract - *Artocarpus lakoocha* Roxb. (Moraceae) is highly regarded for its properties. The bioactive composition (total phenolic and flavonoid contents) of *A. lakoocha* leaves and heartwood extracts, as well as the antioxidant activity against 2,2-Diphenyl-1-picrylhydrazyl (DPPH), were evaluated. Furthermore, the antibacterial properties of these extracts were evaluated against strains of *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus,* and *Salmonella typhimurium.* Our study showed that the heartwood extracts had high total phenolic content (53.93 mg GAE/g dry extract), total flavonoid content (1459.73 µg QE/g dry extract), and strong antioxidant activity (119.37 µg VCE C/g dry extract). The antibacterial results also demonstrated that the heartwood extracts showed a maximum inhibition zone of 15.55 mm against *B. cereus*. While the leaf extracts' inhibitory zone against *B. cereus* was 4.86 mm. Both extracts were sensitive to *E. coli* with the lowest minimum inhibitory concentration (MIC) (1.953 mg/mL). However, the extracts were insensitive to *S. typhimurium,* with the highest MIC value of 125 mg/mL and 3.906 mg/mL from leaves and heartwood extracts, respectively. The result's findings underscore the bioactive composition, antioxidant capacity, and antimicrobial properties of *A. lakoocha* heartwood extract,

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suggesting its potential as a possible natural antibacterial source for food preservation applications.

Keywords: Antimicrobial property, antioxidant activity, *Artocarpus lakoocha*, bioactive compound

1. Introduction

Plants are the most effective natural source of polyphenol compounds, which are classified into two major categories (flavonoids and phenolic acids) These compounds have been demonstrating antioxidant properties due to their capacity to provide free radicals with hydrogen atoms. Consuming plants high in phenolic and flavonoid chemicals, which have strong antioxidant properties, has been associated with a decreased risk of diseases, such as diabetes, cancer, cardiovascular disease, and neurological disorders. In addition to their antioxidant properties, researchers have been investigating plant polyphenols as natural preservatives for the past few decades. They have been regarded as safe Generally Recognized as Safe, affordable, and effective against food pathogens in food products. Many medicinal plants, including tamarind, kaffir lime, mint, lemongrass, and cinnamon, have been applied as natural antibacterial agents to reduce the requirement for chemical preservatives in food (Nanasombat & Lohasupthawee, 2005).

Many countries in South and Southeast Asia, including Nepal, India, Sri Lanka, Myanmar, Southern China, Vietnam, Thailand, Malaysia, and Indonesia use *Artocarpus lakoocha* Roxb. (*A. lakoocha*) as a medicinal herb (Biswas & Chakraborty, 2013; Gardner et al., 2000). Various parts of *A. lakoocha* have been shown to possess exceptional. The seeds are employed for relieving gastrointestinal hepatic diseases (Gautam & Patel, 2014). Heartwood has shown potential as an anti-diabetic medication (Muti et al., 2021). Traditional medicine uses the bark and heartwood to treat diarrhea and sore throats (Nair et al., 2019). Oxyresveratrol extract from the stem bark has demonstrated antiviral properties *in vitro* and is utilized as a vermifuge for the treatment of tapeworm infestations (Kumar et al., 2010; Senapong et al., 2014). The fruit contains antioxidants and vitamins, including beta-carotene and vitamin C (Jahan et al., 2011). Furthermore, many studies have utilized *A. lakoocha* extracts to study the inhibition of pathogenic microorganisms, especially bacteria associated with the oral cavity (Nath & Boruah, 2019; Palanuvej et al., 2007; Teanpaisan et al., 2014), and in cosmetics (Teanpaisan et al., 2014; Teeranachaideekul et al., 2013). However, no studies have used *A. lakoocha* extract as food preservative to inhibit pathogenic microorganisms because organic solvents (ethanol, acetonitrile, ethyl acetate, isopropanol, petroleum ether, chloroform, and hexane) used in the extraction processes are not environmentally sustainable (Pandey & Bhatnagar, 2009; Prashanthi et al., 2016; Teanpaisan et al., 2014).

The requirement to increase process safety and product quality, as well as an increasing concern for environmental preservation, prompted this research into more environmentally friendly techniques by using water in the extraction process. Thus,

the purpose of this study was to investigate the bioactive composition, antioxidant, and antimicrobial properties against foodborne pathogens using the most commonly available components of *A. lakoocha* (leaves and heartwood) for potential application as a food preservative.

2. Materials and methods

2.1 Plant material and crude extracts preparation

(A) (B)

(C) (D)

Figure 1. Physical appearance of A. lakoocha leaves (A) and heartwood (B); crude extract of *A. lakoocha* leaves (C) and heartwood (D)

The leaves of *A. lakoocha* are collected in the province of Kalasin (latitude 16.432510 and longitude 103.506920), while the heartwood powder is collected in the province of Nakhonratchasima (latitude 14.862630 and longitude 101.978113). *A. lakoocha* leaves were washed and desiccated, then cut into small pieces and mixed with deionized water in a ratio of 1:3. The leaves were mixed using a blender for 3 minutes, filtered through cheesecloth and then shaken at 150 rpm for 24 hours. The leaf extracts were dried using a freeze dryer (Labconco™ FreeZone™ Bulk Tray Dryers, US) and stored at -20 °C for further experimentation figure 1. The 10 g of heartwood powder was mixed with 100 mL of deionized water and shaken at 150 rpm for 24 hours, then filtered using a vacuum pump with filter paper No. 2. The extract was dried using a freeze dryer. The extract was ground using a blender for 1 minute and stored at a temperature of -20 °C for further experiment.

2.2 Chemical composition and antioxidant of extract

2.2.1 Preparation of extracts

A gram of the extract was dissolved in 10 mL of 80% methanol. The tube was agitated in an UltraRocker™ Rocking Platform (Hercules, CA) at 120 rpm for 60 minutes at room temperature. Centrifugation (ALC 4239R high speed refrigerated centrifuge, International PBI, Milan, Italy) at 9000 rpm for 15 minutes separated the aqueous and organic phases. The organic phase was then collected and filtered with a 0.45 µm nylon syringe.

2.2.2 Determination of total phenolic content (TPC)

The TPC in *A. lakoocha* leaves and heartwood extracts was determined using the Folin-Ciocalteu reagent based on the method of Salih et al. (2021) with some modifications. Briefly, 5 µL of the extract was mixed with 50 µL of the Folin-Ciocalteu reagent and 1.5 mL of deionized water. The solution was left at room temperature for 8 minutes before being added to 50 µL of a 20% sodium carbonate solution. After a 30 minutes reaction time, the resulting color absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Biochrom Libra S22, Cambridge, UK). A calibration curve was generated by using different concentrations of gallic acid (ranging from 0.15 to 10 mg/mL) to obtain data on the content of the phenolic component. The concentration of the total phenolic components was determined as milligrams of gallic acid equivalent (GAE) per gram of dry extract.

2.2.3 Determination of total flavonoid content (TFC)

The TFC of *A. lakoocha* leaves and heartwood extracts was determined by the colorimetric method (Nabi & Shrivastava, 2016). In brief, 1.5 mL of each extract was mixed with 1.5 mL of a 10% solution of aluminum chloride. It was then incubated in the dark for 30 minutes at room temperature. The measurement of absorbance was conducted using a UV-Vis spectrophotometer at a wavelength of 425 nm. A calibration curve was established using the quercetin reference standard from 3 to 100 µg/mL. The total concentration of flavonoid was measured and reported as micrograms of quercetin (QE) per gram of dry extract.

2.2.4 Determination of DPPH radical scavenging capacity

The plant extracts were assessed for their antioxidant activity against DPPH using the methodology described by Chaves et al. (2020). A 1.5 mL solution of DPPH radical with a concentration of 0.1 mM combined with 1 mL of extract solutions with a concentration ranging from 0.1 to 2 mg/mL. Following a 30 minutes incubation period in the absence of light at ambient temperature, the absorbance was measured at wavelength 517 nm using a UV-Vis spectrophotometer. The calibration line was established using vitamin C concentrations ranging from 7 to 125 µg/mL. The results were represented as micrograms of vitamin C (Vit C) equivatent (VCE) per gram of dry extract**.**

2.3 Antibacterial method

2.3.1 The agar disc diffusion method

The extract powder was tested for its antimicrobial activity, following the method published by Heatley (1944). The bacteria indicators, *E. coli (*ATCC25922*), B. subtilis* (TISTR1248*), B. cereus* (TISTR1449*), S. aureus* (TISTR746), and *S. typhimurium* (TISTR1472), were cultured in a nutrient broth and incubated overnight at 37 ºC, and the OD_{600} was adjusted from 0.1 to 0.12, about 10^8 CFU/mL. The 90 µL of bacteria indicators were spread into nutrient agar, and a 6 mm disc of sterile filter paper (disc) was placed on the medium. 10 µL of *A. lakoocha* extract (250 mg/mL) was spotted onto a disc and incubated at 37 °C for 24 hours. The clear zone was determined by using a vernier caliper (clear zone = diameter of clear zone - diameter of the disc). The positive control was the antibiotic vancomycin (30 µg/disc), and the negative control was DMSO and sterile distilled water.

2.3.2 The minimal inhibitory concentration (MIC)

The minimal inhibitory concentration of *A. lakoocha* extract was determined using a resazurin assay. *A. lakoocha* extract was prepared at 250 mg/mL with muller hinton broth, diluted in 96 well plates for 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 15.625 mg/mL, 7.812 mg/mL, 3.906 mg/mL, and 1.953 mg/mL. The bacteria indicators, such as *E.coli* ATCC25922*, B. subtilis* TISTR1248*, B. cereus* TISTR1449*, S. aureus* TISTR746, and *S. typhimurium* TISTR1472 were cultured in a nutrient broth and incubated overnight at 37 ºC for 18 hours and the OD_{600} was adjusted to 0.1 to 0.12 about 10^8 CFU/mL. Then 50 µL of bacteria culture was added to each well, incubated at 37 °C for 18 hours, then 40 µL of 0.015% resazurin was added, incubated at 37 °C for 3 hours, and the results were measured by observing the color change of the solution in each well. The solution in each well was pink or light purple which indicated that bacteria were still growing. If the color did not change, it suggests that we could inhibit the pathogen's growth

2.3.3 The minimal bactericidal concentration (MBC)

The dilution in resazurin assay from the MIC test was used in the MBC test by the steak plate technique. The loops were immersed in intact 96 well plates, then steaked in nutrient agar (NA) and incubated at 37 °C for 24 hours. The results showed that the concentration of *A. lakoocha* extract can kill bacteria but not grow on nutrient agar.

2.4 Statistical analysis

For all experiments, the findings were presented as mean values and standard deviations from triplicate samples of each treatment. The data collected were analyzed by two-way analysis of variance in a completely randomized design (CRD) using the SPSS trial version.

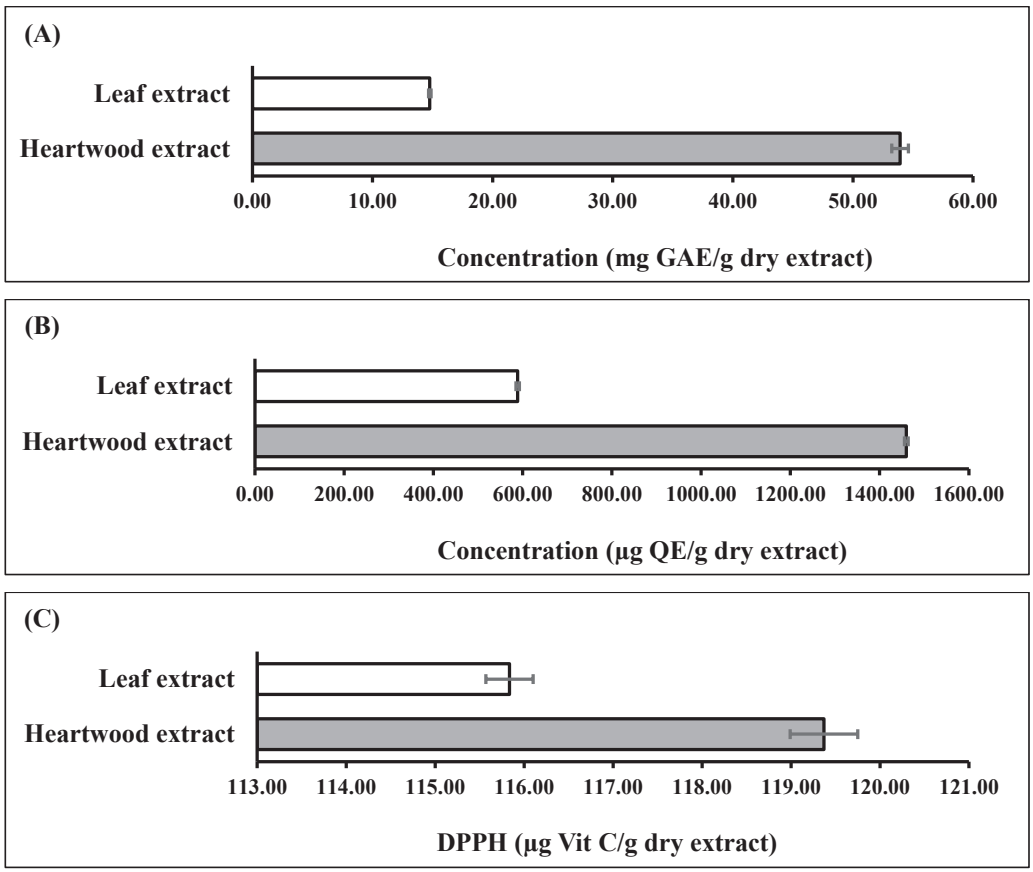

Figure 2. Total phenolic content (A), total flavonoid content (B), and antioxidant activity (C) of *A. lakoocha* crude extract from leaf and heartwood.

3. Results and discussion

3.1 Chemical composition and antioxidant of the extract

The chemical composition of the different extracts revealed that the leaves and heartwood of *A. lakoocha* had a high total phenolic and total flavonoid content. Figure 2(A) showed that the heartwood of *A. lakoocha* had a total phenolic content up to 53.93 mg GAE/g dry extract, which was about three times greater than the leaves (14.77 mg GAE/g dry extract). Nevertheless, the total phenolic content detected in the heartwood and leaves of *A. lakoocha* in this study was lower than that recorded by Charirak and Ratananikom (2022) at 318.42 mg GAE/g extract and by Bhattacharya et al. (2019) at 83.33 to 3,175.21 mg GAE/g dry extract,

respectively. Figure 2(B) revealed that the highest total flavonoid content was also revealed in the heartwood of *A. lakoocha* with 1,459.73 µg QE/g dry extract. While the leaves showed 588.76 µg QE/g dry extract, other studies (Bhattacharya et al., 2019; Singhatong et al., 2010) had more than 212.96 mg QE/g dry extract.

Previous studies showed that phytochemical substances were impacted by a variety of factors, including varietals, extract process, and ambient conditions. state that different clones of the same variety may have different chemical compositions. Moreover, cultivar and harvesting year interaction may also be significant. showed that the extraction method has a major impact on the phytochemical content and bioactivity. Additionally, Charirak & Ratananikom (2022) showed that the total phenolic content of the acetonitrile extract peaked at 455.29 mg GAE/g, indicating that the type of solvent extraction was a significant factor in the separation of bioactive components from *A. lakoocha*. Conversely, the extract containing hexane (33.33 mg GAE/g) exhibited the least amount of total phenolic content and antioxidant activity. When compared to water-based approaches, organic solvents often showed greater efficiency in extracting active chemicals for antibacterial activity (Lima-Filho et al., 2002; Shanmughapriya et al., 2008).

The antioxidant activity of *A. lakoocha* leaves and heartwood extracts is shown in Figure 2(C). The heartwood still showed the highest DPPH radical scavenging capacity (119.37 µg VCE/g dry extract), followed by leaves (115.84 µg Vit C/g dry extract). It is conceivable to propose that the antioxidant activity of the heartwood is mostly due to phenolic and flavonoid groups. Due to their capacity to provide free radicals with hydrogen atoms, phenolic and flavonoid molecules are responsible for deactivating free radicals. Additionally, their structural features make them perfect for scavenging free radicals (Amarowicz et al., 2004). Flavonoids scavenge reactive species, inhibit the production of reactive oxygen, chelate trace elements involved in the production of free radicals, and enhance and safeguard antioxidant defenses (Agati et al., 2012). Comparably, phenolic compounds react with different types of free radicals to provide an antioxidant effect. Either hydrogen atom transfer, single electron transfer, sequential proton loss electron transfer, or transition metal chelation were the mechanisms of antioxidant effects. phenolics also promote plants' resistance to oxidative stress (Baba & Malik, 2015). The total phenolic and flavonoid content has a linear relationship with antioxidant ability, according to many literature publications (Shrestha & Dhillion, 2006).

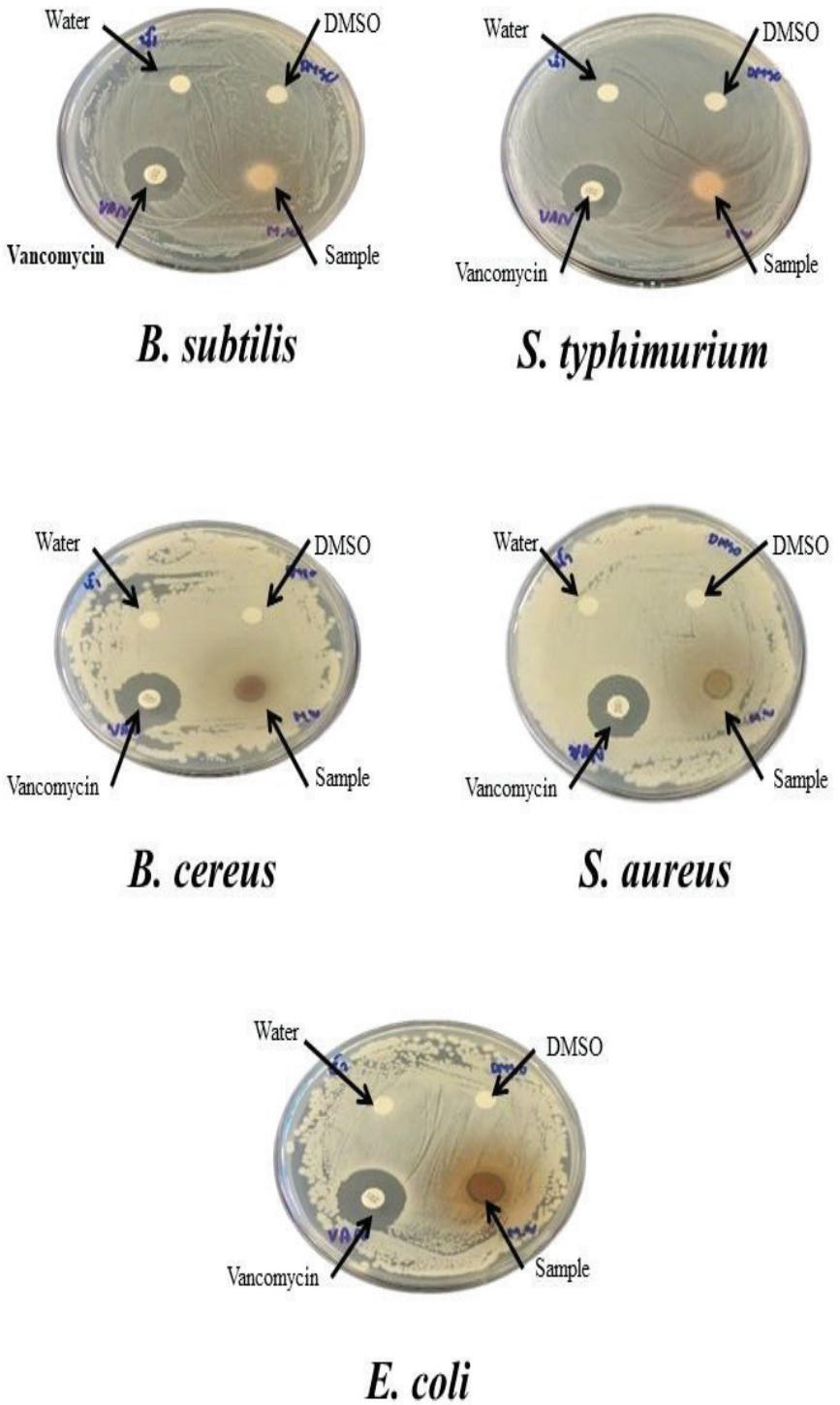

Figure 3. Inhibition zone of *A. lakoocha* extract (leaves) against *B. subtilis* TISTR1248, *S. typhimurium* TISTR1472, *B. cereus* TISTR1449, *S. aureus* TISTR746 and *E. coli* TISTR117. The positive control is vancomycin (30 µg/disc), and the negative control is DMSO and sterile distilled water.

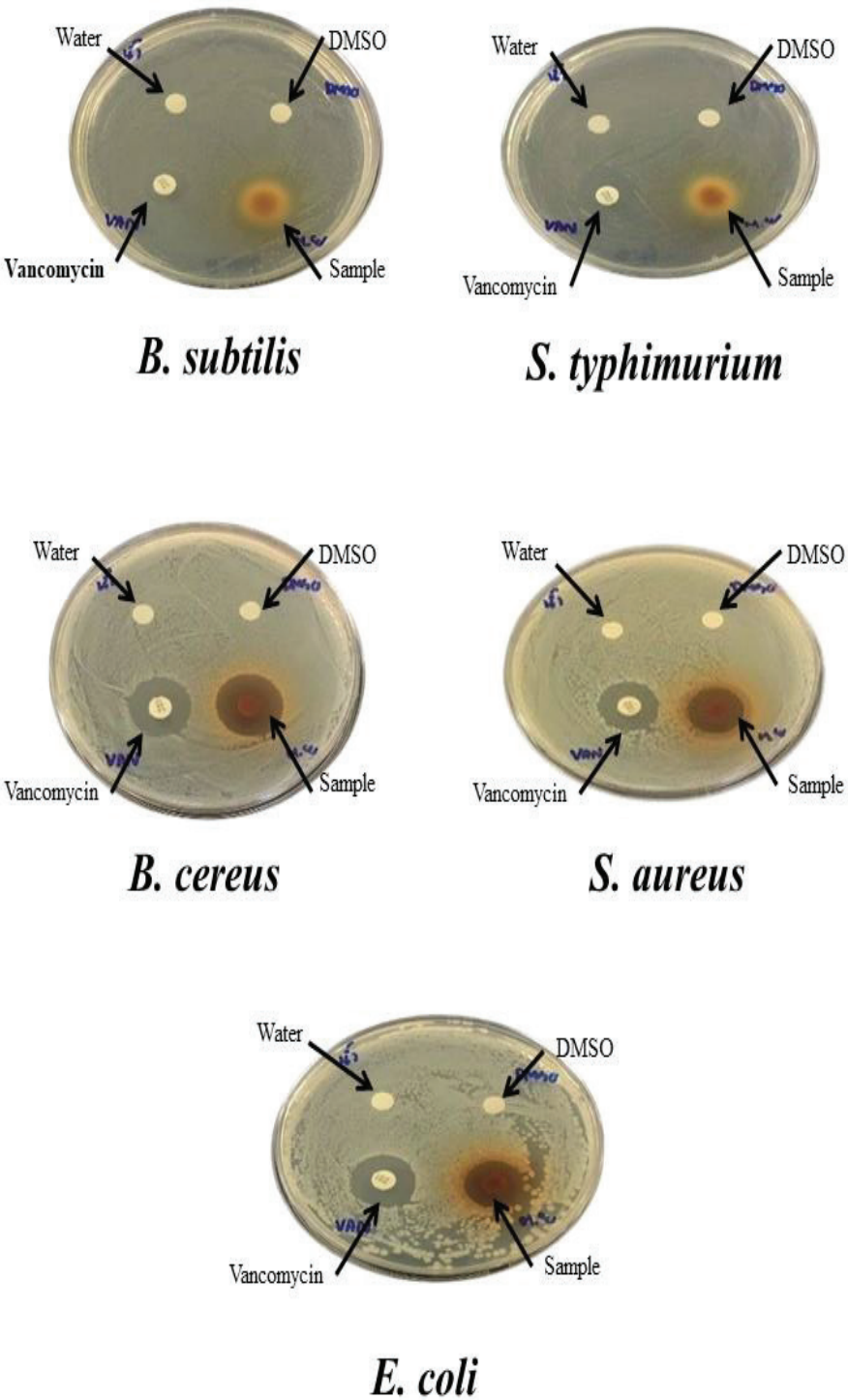

Figure 4. Inhibition zone of *A. lakoocha* extract (heartwood) against *B. subtilis* TISTR1248, *S. typhimurium* TISTR1472, *B. cereus* TISTR1449, *S. aureus* TISTR746 and *E. coli* TISTR117. The positive control is vancomycin (30 µg/disc), and the negative control is DMSO and sterile distilled water.

3.2 Antibacterial method

Table 1. Antimicrobial activity of *A. lakoocha* extract (leaves and heartwood extract) against different bacterial stains.

Bacteria strain	Diameter of the zone of inhibition (in mm)	
	Leaf extract	Heartwood extract
Gram negative bacteria		
S. typhimurium TISTR1472	0.00±0.00^{d}	0.00±0.00^{c}
E. coli ATCC25922	4.86±0.75^{a}	11.38±1.66^{b}
Gram positive bacteria		
S. aureus TISTR746	1.98 ±0.17^{c}	10.98±1.13^{b}
B. cereus TISTR1449	3.66±0.30^{b}	15.55±2.15^{a}
B. subtilis TISTR1248	0.00±0.00^{d}	12.51±0.25^{b}

Values within a column represent the mean ± standard deviation of triplicate experiments (n = 3) with different letters indicating a significant difference (P<0.05)

3.2.1 The agar disc diffusion method

A. lakoocha extract (leaves and heartwood) was evaluated against five pathogenic bacteria, *i.e., S. aureus* (TISTR746), *S. typhimurium* (TISTR1472), *E. coli* (ATCC25922), *B. cereus* (TISTR1449), and *B. subtilis* (TISTR1248). The antimicrobial activity of leaves and heartwood extracts of *A. lakoocha* is shown in Table 1. The results were recorded as the zone of inhibition formed of each disk. The leaf extract was found to be effective against *E. coli* ATCC25922 (4.86±0.75 mm), whereas it shows the lowest antimicrobial activity against *S. typhimurium* TISTR1472 and *B. subtilis* TISTR1248 (0.00±0.00 mm), as shown in Figure 3. Results of our study also clarified that gram-negative bacteria (*E. coli* ATCC25922) were found to be more susceptible than gram-positive bacteria (*S. aureus* TISTR746, *B. cereus* TISTR1449, and *B. subtilis* TISTR1248) to leaf extract. The reason for this could be that gram-negative bacteria are surrounded by a thinner cell wall than gram-positive bacteria, which are composed of a relatively thick cell wall (Chapot-Chartier & Kulakauskas, 2014). The result of this investigation correlates with previous studies on the antimicrobial effects of *A. lakoocha* extract on oral infections. This research revealed that gram-negative bacteria were more susceptible to the extract than gram-positive bacteria (Nath & Boruah, 2019; Teanpaisan et al., 2014). Furthermore, gram-negative bacteria were more suppressed by Thai medicinal plant extract than gram-positive bacteria (Teanpaisan et al., 2017). *Citrus bergamia, Woodfordia fruticosa,* and *Piper betle* L. also demonstrated similar outcomes suggesting that gram-negative bacteria exhibited greater susceptibility to specific plant extracts compared to gram-positive bacteria (Mandalari et al., 2007; Parekh & Chanda, 2007; Teanpaisan et al., 2017). However, the susceptibility of the foodborne bacteria is also affected by the bacterial species, purity, and polyphenol structure of the extract, together with the experimental procedures.

In comparison, heartwood extract of *A. lakoocha* is highly effective against *B. cereus* TISTR1449 (15.55±2.15mm), while its efficacy was lower in the inhibition of *S. typhimurium* TISTR1472 (0.00±0.00 mm), as shown in Table 1 and Figure 4. Additionally, the result also shows that the heartwood extract was able to destroy both gram-negative bacteria *(E. coli* ATCC25922) and gram-positive bacteria (*S. aureus* TISTR746, *B. cereus* TISTR1449 and *B. subtilis* TISTR1248). Previous studies by Ratananikom et al. (2019) reported lower antimicrobial activity against *S. aureus* TISTR746 of the heartwood extract, which shows a zone of inhibition of 6.00±0.00 mm, while our study showed a larger inhibition zone of 10.98±1.1 mm. It has been mentioned that although the extraction procedure in both trials used only water, the drying of the heartwood extract was done using two different techniques (a freeze dryer and a rotary evaporator), which may have contributed to the loss of various active components during the drying process (Hu et al., 2017).

It could be proven that heartwood extracts have a greater antibacterial effect than leaf extracts on four bacterial species (*S. aureus* TISTR746, *E. coli* ATCC25922, *B. cereus* TISTR1449, and *B. subtilis* TISTR1248) with inhibition zones > 10 mm, while leaf extracts showed a smaller inhibition zone (1.98 to 4.86 mm). However, both extracts showed no antimicrobial activity for *S. typhimurium* TISTR1472. The resistance of *S. typhimurium* TISTR472 has been demonstrated by earlier studies. According to research by Aliero & Ibrahim (2012), *S. typhimurium* was the least sensitive to extracts of *Commelina bengalensis*. *S. typhimurium* was also shown to be more resistant to the extracts of seven plant species than *Pseudomonas aeruginosa* and *E. coli* (Elisha et al., 2017). Furthermore, observed that *S. typhimurium* expresses blaTEM, fimA, fimZ, and integrons at considerably greater levels, which may be related to the development of resistance. The resistance of *S. typhimurium* is concerning for public health, since *Salmonella* spp. that are resistant to antibiotics occur because of the use of antimicrobial growth promoters in animals raised for food (Aliero & Ibrahim, 2012).

Table 2. Minimal inhibitory concentration and minimal bactericidal concentration of A. lakoocha extract (leaves and heartwood extract)

Bacteria strain	Leaf extract		Control	Heartwood extract		Control
	MIC (mg/mL)	MBC (mg/mL)	VA. (µg/mL)	MIC (mg/mL)	MBC (mg/mL)	VA (µg/mL)
Gram negative bacteria						
S. typhimurium TISTR1472	125±0.00	250±0.00	≤50	3.906±0.00	31.25±0.00	≤50
E. coli ATCC25922	1.953±0.00	7.812±0.00	≤50	1.953±0.00	7.812±0.00	≤50
Gram positive bacteria						
S. aureus TISTR746	3.906±0.00	1.953±0.00	≤50	3.906±0.00	15.625±0.00	≤50
B. cereus TISTR1449	7.812±0.00	31.25±0.00	≤50	3.906±0.00	15.625±0.00	≤50
B. subtilis TISTR1248	7.812±0.00	62.5±0.00	≤50	3.906±0.00	62.5±0.00	≤50

MIC = *minimal inhibitory concentration*
MBC = *minimal bactericidal concentration*
VA = vancomycin

Minimum inhibitory concentration (MIC)

The capability of *A. lakoocha* crude extract (leaves) to inhibit bacterial growth was investigated using the resazurin test. The extract was diluted to give concentrations of 250 mg/mL, 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 15.625 mg/mL, 7.812 mg/mL, 3.906 mg/mL, and 1.953 mg/mL, as indicated in Table 2.

The crude extracts from *A. lakoocha* leaves were active against all the pathogens with average MICs ranging from 1.953 to 125 mg/mL. As expected, the extracts had the greatest potential antibacterial action against *E. coli* ATCC25922, with a minimum inhibitory concentration (MIC) of 1.953 mg/mL, followed by *S. aureus* (MIC = 3.906 mg/mL), *B. cereus* TISTR1449 (MIC = 7.812 mg/mL), *B. subtilis* TISTR1248 (MIC = 7.812 mg/mL), and *S. typhimurium* TISTR1472 (MIC = 125 mg/mL). The sensitivity of *E. coli* was also shown in the previous investigations (Elisha et al., 2017). Makhafola (2012) early assessment of the antibacterial activity of crude acetone extracts of Ochna species indicated that *E. coli* was the most sensitive kind of bacterium studied. In addition, Elisha et al. (2017) discovered that *E. coli* was sensitive to the extracts of nine plant species (MICs of 0.09 mg/mL) whereas *S. typhimurium* showed greater resistance to the extracts (average MICs of 0.22 mg/mL). According to our research, the MIC values of the leaf extracts against *S. typhimurium* (MIC = 125 mg/mL) were significantly higher than the MIC value of the other bacteria (MIC = 1.953 to 7.812 mg/mL).

There were similarities between the minimal inhibitory concentrations of leaf and heartwood extracts. Heartwood extract was also effective against all the pathogens. *E. coli* was still the most sensitive to the heartwood extract, with MIC values of 1.953 mg/mL. The gram-positive bacteria were inhibited by the heartwood extract, with MIC values of 3.906 mg/mL. There were some major differences in the minimal inhibitory concentration of both extracts; the leaf extract had the highest MIC values against *S. typhimurium* TISTR1472 (125 mg/mL), while the heartwood extract had a lower MIC of 3.906 mg/mL.

These findings showed that the heartwood extracts had better bactericidal effects than the leaf extracts. The antibacterial activity was found to be compatible with the number of phenolic compounds and flavonoids present in the crude extract of *A. lakoocha* when comparing the total phenolic content and total flavonoid content (Figure 2) with the inhibitory zones (Table 1). For instance, the heartwood extracts had the biggest inhibitory zones (0 to15.55±2.15 mm) and the highest phenolic and total flavonoid contents. On the other hand, the inhibition zones were lower in the leaf extracts (0 to 4.86±0.75 mm). Therefore, the results of this study suggest that chemicals, including flavonoids and phenolics, may be crucial in inhibiting the development of bacteria.

According to the previous reports, the antibacterial action of plant extracts is caused by their polyphenols, including flavonoids, phenolic acids, tannins, and stilbenoids. These compounds inhibit the growth of a variety of microorganisms including viruses, bacteria, and fungi. Choi et al. (2006) reported that one of the primary constituents of *A. lakoocha* flavonoids has been demonstrated to possess several

advantageous qualities, such as the capacity to inhibit mitochondrial adhesion, act as an antibacterial, act as an antiulcer, act as an antiarthritic, act as an antiangiogenic, and act as an anticancer agent. Antibacterial mechanisms of action of flavonoids include decreasing the fluidity of the cytoplasmic membrane, blocking the synthesis of nucleic acids by inhibiting DNA gyrase and/or topoisomerase, paralyzing energy metabolism by inhibiting ATP synthase and the respiratory chain, preventing cell wall synthesis by acting as competitive inhibitors of the D-alanine-D-alanine ligase, and preventing cell membrane synthesis by inhibiting enzymes of the fatty acid synthesis pathway

Antimicrobial properties of phenolic compounds have also been documented often (Bouarab-Chibane et al., 2019; Lobiuc et al., 2023; Vittaya et al., 2022). It has been suggested that the hydrophobic properties of its phenolic components contribute to their antibacterial effect. The mechanism behind polyphenols' toxicity to microorganisms may involve the suppression of hydrolytic enzymes (proteases) or other interactions that render cell envelope transport proteins, microbial adhesins, and non-specific interactions with carbohydrates inactive (Baba & Malik, 2015; Pyla et al., 2010). Furthermore, it has been reported by that the site and number of hydroxyl groups on the benzoic ring may be related to an increase in hydroxylation, which enhances the toxicity to microorganisms.

Minimal bactericidal concentration (MBC)

All crude extracts of *A. lakoocha*. under investigation exhibited exceptional concentration-dependent antimicrobial activity against all five bacteria. The most promising activity of leaf extract was displayed against the gram-positive bacteria *S. aureus* TISTR746 with the MBC value of 1.953 mg/mL. The leaf extracts were also bactericidal against *B. subtilis* TISTR1248, *B. cereus* TISTR1449 and *E. coli* ATCC25922, with the MBC value of 62.5, 31.25 and 7.812 mg/mL, respectively. However, the leaf extracts showed inhibition against *S. typhimurium* TISTR1472, with the MBC value of 250 mg/mL. The heartwood extract demonstrated the highest antibacterial activity against the gram-negative bacteria *E. coli* ATCC25922, with the MBC value of 7.812 mg/mL. The extract of heartwood also resulted in the MBC value of 15.625 to 62.5 mg/mL against gram-positive bacteria.

According to the previous results, leaves and heartwood extracts showed no inhibition against *S. typhimurium* TISTR1472 with the agar spot technique. Nevertheless, it was discovered that the extracts can both inhibit and kill microorganisms by testing the minimal inhibitory concentration (MIC) and minimum concentration (MBC). It can be described that the agar spot technique test was used for initial pathogen selection and the amount of active material utilized in the test was molested (Rios et al., 1988). The minimum inhibitory concentration (MIC) is the lowest concentration of a bacteriostatic antimicrobial agent, and the minimum bactericidal concentration (MBC) is the lowest antibacterial agent concentration required to kill a bacterium under specific conditions. Both evaluations can be quite useful for measuring the effect of decreasing concentrations of extract over a defined period in terms of inhibiting microbial population growth and resulting

in microbial death (Rodríguez-Melcón et al., 2021).

According to the findings of the research, the heartwood extract of *A. lakoocha* showed a greater antibacterial effect than leaf extracts in the antibacterial inhibition test using the agar disc diffusion method. The heartwood extracts also showed potential antibacterial effects against five bacterial species with minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC).

4. Conclusion

Overall, this investigation revealed that the crude extract from the heartwood of *A. lakoocha* exhibited higher levels of total phenolic content, total flavonoid content, and antioxidant activity compared to leaf extract. Both extracts have bactericidal effects against four microorganisms, including *S. aureus* (TISTR746), *E. coli* (ATCC25922), *B. cereus* (TISTR1449), and *B. subtilis* (TISTR1248). However, heartwood extracts showed higher antibacterial activity. This study indicates that chemical substances derived from *A. lakoocha* heartwood extract have the potential to be used as a basis for developing future antimicrobial agent

Declaration

The authors declare that there are no conflicts of interest.

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References

Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science, 196*, 67-76. https://doi.org/10.1016/j.plantsci.2012.07.014

Aliero, A. A., & Ibrahim, A. D. (2012). Antibiotic resistance and the prospects of medicinal plants in the treatment of salmonellosis. In *Salmonella-A Diversified Superbug* (pp. 65-90). IntechOpen. https://doi.org/10.5772/29241

Amarowicz, R., Pegg, R. B., Rahimi-Moghaddam, P., Barl, B., & Weil, J. A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry, 84*(4), 551-562. https://doi.org/10.1016/S0308-8146(03)00278-4

Baba, S. A., & Malik, S. A. (2015). Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *Journal of Taibah University for Science, 9*(4), 449-454. https://doi.org/10.1016/j.jtusci.2014.11.001

Bhattacharya, E., Dutta, R., Chakraborty, S., & Biswas, S. M. (2019). Phytochemical profiling of *Artocarpus lakoocha* Roxb. leaf methanol extract and its antioxidant, antimicrobial and antioxidative activities. *Asian Pacific Journal of Tropical Biomedicine, 9*(11), 484-492. https://doi.org/10.4103/2221-1691.270984

Biswas, S. M., & Chakraborty, N. (2013). Shedded *Artocarpus* leaves—good plant sources of natural squalene with potent antioxidant and antimicrobial activity—alternative to marine animals. *Journal of Natural Pharmaceuticals, 4*(1), 21-27. https://doi.org/10.4103/2229-5119.110344

Bouarab-Chibane, L., Forquet, V., Lantéri, P., Clément, Y., Léonard-Akkari, L., Oulahal, N., Degraeve, P., & Bordes, C. (2019). Antibacterial properties of polyphenols: Characterization and QSAR (Quantitative structure-activity relationship) models. *Frontiers in Microbiology, 10*, 829. https://doi.org/10.3389/fmicb.2019.00829

Chapot-Chartier, M.-P., & Kulakauskas, S. (2014). Cell wall structure and function in lactic acid bacteria. *Microbial Cell Factories, 13*(1), 1-23. https://doi.org/10.1186/1475-2859-13-S1-S9

Charirak, P., & Ratananikom, K. (2022). Anti-Methicillin-Resistant *Staphylococcus aureus* activities of *Artocarpus lakoocha* Roxb. extract and its mode of action. *Scientific World Journal, 2022*, 1839356. https://doi.org/10.1155/2022/1839356

Chaves, N., Santiago, A., & Alías, J. C. (2020). Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. *Antioxidants, 9*(1), 76. https://doi.org/10.3390/antiox9010076

Choi, Y. M., Noh, D. O., Cho, S. Y., Suh, H. J., Kim, K. M., & Kim, J. M. (2006). Antioxidant and antimicrobial activities of propolis from several regions of Korea. *LWT - Food Science and Technology, 39*(7), 756-761. https://doi.org/10.1016/j.lwt.2005.05.015

Elisha, I. L., Botha, F. S., McGaw, L. J., & Eloff, J. N. (2017). The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts. *BMC Complementary and Alternative Medicine, 17*(1), 1-10. https://doi.org/10.1186/s12906-017-1645-z

Gardner, S., Sidisunthorn, P., & Anusarnsunthorn, V. (2000). *A field guide to forest trees of northern Thailand*. Kobfai Publishing Project.

Gautam, P., & Patel, R. (2014). *Artocarpus lakoocha* Roxb: An overview. *Journal of Complementary and Alternative Medicine, 1*(1), 10-14.

Heatley, N. G. (1944). A method for the assay of penicillin. *Biochemical Journal, 38*(1), 61-65. https://doi.org/10.1042/bj0380061

Hu, S., Zhao, G., Zheng, Y., Qu, M., Jin, Q., Tong, C., & Li, W. (2017). Effect of drying procedures on the physicochemical properties and antioxidant activities of polysaccharides from *Crassostrea gigas*. *PLoS ONE, 12*(11), e0188536. https://doi.org/10.1371/journal.pone.0188536

Jahan, S., Gosh, T., Begum, M., & Saha, B. K. (2011). Nutritional profile of some tropical fruits in Bangladesh: Specially anti-oxidant vitamins and minerals. *Bangladesh Journal of Medical Science, 10*(2), 95-103. https://doi.org/10.3329/bjms.v10i2.7804

Kumar, M. B. S., Kumar, M. C. R., Bharath, A. C., Kumar, H. R. V., Kekuda, T. R. P., Nandini, K. C., Rakshitha, M. N., & Raghavendra, H. L. (2010). Screening of selected biological activities of *Artocarpus lakoocha* Roxb. (Moraceae) fruit pericarp. *Journal of Basic and Clinical Pharmacy, 1*(4), 239-245.

Lima-Filho, J. V. M., Carvalho, A. F. F. U., Freitas, S. M., & Melo, V. M. M. (2002). Antibacterial activity of extracts of six macroalgae from the northeastern Brazilian coast. *Brazilian Journal of Microbiology, 33*, 311-314. https://doi.org/10.1590/S1517-83822002000400006

Lobiuc, A., Pavăl, N.-E., Mangalagiu, I. I., Gheorghiță, R., Teliban, G.-C., Amăriucăi-Mantu, D., & Stoleru, V. (2023). Future antimicrobials: Natural and functionalized phenolics. *Molecules, 28*(3), 1114. https://doi.org/10.3390/molecules28031114

Makhafola, T. J. (2012). Five *Ochna* species have high antibacterial activity and more than ten antibacterial compounds. *South African Journal of Science, 108*(1), 1-6. https://doi.org/10.4102/sajs.v108i1/2.689

Mandalari, G., Bennett, R. N., Bisignano, G., Trombetta, D., Saija, A., Faulds, C. B., Gasson, M. J., & Narbad, A. (2007). Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry. *Journal of Applied Microbiology, 103*(6), 2056-2064. https://doi.org/10.1111/j.1365-2672.2007.03456.x

Muti, A. F., Pradana, D. L. C., & Rahmi, E. P. (2021). Extract of *Caesalpinia sappan* L. heartwood as food treatment anti-diabetic: A narrative review. *IOP Conference Series: Earth and Environmental Science, 755*(1), 012042. https://doi.org/10.1088/1755-1315/755/1/012042

Nabi, N., & Shrivastava, M. (2016). Estimation of total flavonoids and antioxidant activity of *Spilanthes acmella* leaves. *UK Journal of Pharmaceutical and Biosciences, 4*, 29-34. https://doi.org/10.20510/ukjpb/4/i6/134657

Nair, A., Chattopadhyay, D., & Saha, B. (2019). Plant-derived immunomodulators. In M. S. Ahmad Khan, I. Ahmad, & D. Chattopadhyay (Eds.), *New look to phytomedicine* (pp. 435-499). Academic Press. https://doi.org/10.1016/B978-0-12-814619-4.00018-5

Nanasombat, S., & Lohasupthawee, P. (2005). Antibacterial activity of crude ethanolic extracts and essential oils of spices against salmonellae and other enterobacteria. *KMITL Science and Technology Journal, 5*(3), 527-538.

Nath, P. C., & Boruah, S. (2019). Antimicrobial activity of ethanolic and methanolic extract of *Artocarpus lakoocha* Wall. Ex Roxb. (Moraceae) against five different oral bacterial strains. *International Journal of Current Microbiology and Applied Sciences, 8*(3), 1321-1325. https://doi.org/10.20546/ijcmas.2019.803.156

Palanuvej, C., Issaravanich, S., Tunsaringkarn, T., Rungsiyothin, A., Vipunngeun, N., Ruangrungsi, N., & Likhitwitayawuid, K. (2007). Pharmacognostic study of *Artocarpus lakoocha* heartwood. *Journal of Health Research, 21*(4), 257-262.

Pandey, A., & Bhatnagar, S. P. (2009). Preliminary phytochemical screening and antimicrobial studies on *Artocarpus lakoocha* Roxb. *Ancient Science of Life, 28*(4), 21-24.

Parekh, J., & Chanda, S. (2007). In vitro antibacterial activity of the crude methanol extract of *Woodfordia fruticosa* Kurz. flower (Lythraceae). *Brazilian Journal of Microbiology, 38*, 204-207. https://doi.org/10.1590/S1517-83822007000200004

Prashanthi, P., Rajamma, A. J., Sateesha, S. B., Chandan, K., Tiwari, S. N., & Ghosh, S. K. (2016). Pharmacognostical and larvicidal evaluation of *Artocarpus lakoocha* Roxb. from Western Ghats. *International Journal of Natural Products Research, 7*(2), 141-149.

Pyla, R., Kim, T.-J., Silva, J. L., & Jung, Y.-S. (2010). Enhanced antimicrobial activity of starch-based film impregnated with thermally processed tannic acid, a strong antioxidant. *International Journal of Food Microbiology, 137*(2-3), 154-160. https://doi.org/10.1016/j.ijfoodmicro.2009.12.011

Ratananikom, K., Srikacha, N., & Khannalao, Y. (2019). Antibacterial activity of *Artocarpus lakoocha* Roxb. crude extract towards common human pathogens. *Journal of Thai Traditional and Alternative Medicine, 17*(1), 54-62.

Rios, J. L., Recio, M. C., & Villar, A. (1988). Screening methods for natural products with antimicrobial activity: A review of the literature. *Journal of Ethnopharmacology, 23*(2), 127-149. https://doi.org/10.1016/0378-8741(88)90001-3

Rodríguez-Melcón, C., Alonso-Calleja, C., García-Fernández, C., Carballo, J., & Capita, R. (2021). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for twelve antimicrobials (biocides and antibiotics) in eight strains of *Listeria monocytogenes*. *Biology, 11*(1), 46. https://doi.org/10.3390/biology11010046

Salih, A. M., Al-Qurainy, F., Nadeem, M., Tarroum, M., Khan, S., Shaikhaldein, H. O., Al-Hashimi, A., Alfagham, A., & Alkahtani, J. (2021). Optimization method for phenolic compounds extraction from medicinal plant (*Juniperus procera*) and phytochemicals screening. *Molecules, 26*(24), 7454. https://doi.org/10.3390/molecules26247454

Senapong, S., Puripattanavong, J., & Teanpaisan, R. (2014). Anticandidal and antibiofilm activity of *Artocarpus lakoocha* extract. *Songklanakarin Journal of Science and Technology, 36*(4), 451-457.

Shanmughapriya, S., Manilal, A., Sujith, S., Selvin, J., Kiran, G. S., & Natarajaseenivasan, K. (2008). Antimicrobial activity of seaweed extracts against multiresistant pathogens. *Annals of Microbiology, 58*, 535-541. https://doi.org/10.1007/BF03175554

Shrestha, P. M., & Dhillion, S. S. (2006). Diversity and traditional knowledge concerning wild food species in a locally managed forest in Nepal. *Agroforestry Systems, 66*, 55-63. https://doi.org/10.1007/s10457-005-6642-4

Singhatong, S., Leelarungrayub, D., & Chaiyasut, C. (2010). Antioxidant and toxicity activities of *Artocarpus lakoocha* Roxb. heartwood extract. *Journal of Medicinal Plant Research, 4*(10), 947-953. https://doi.org/10.5897/JMPR10.133

Teanpaisan, R., Kawsud, P., Pahumunto, N., & Puripattanavong, J. (2017). Screening for antibacterial and antibiofilm activity in Thai medicinal plant extracts against oral microorganisms. *Journal of Traditional and Complementary Medicine, 7*(2), 172-177. https://doi.org/10.1016/j.jtcme.2016.06.007

Teanpaisan, R., Senapong, S., & Puripattanavong, J. (2014). In vitro antimicrobial and antibiofilm activity of *Artocarpus lakoocha* (Moraceae) extract against some oral pathogens. *Tropical Journal of Pharmaceutical Research, 13*(7), 1149-1155. https://doi.org/10.4314/tjpr.v13i7.20

Teeranachaideekul, V., Nithitanakool, S., Junhunkit, T., Ponpanich, L., Nopporn, N., Detamornrat, U., & Chulasiri, M. (2013). Liposomes: A novel carrier system for *Artocarpus lakoocha* extract to improve skin whitening. *Journal of Applied and Advanced Science and Pharmacy, 2*, 243-253.

Vittaya, L., Charoendat, U., Ui-eng, J., & Leesakul, N. (2022). Effect of extraction solvents on phenolic compounds and flavonoids from *Pongame oiltree* (*Derris indica* [Lamk.] Bennet) aerial parts and their growth inhibition of aquatic pathogenic bacteria. *Agriculture and Natural Resources, 56*(3), 569-582. https://doi.org/10.34044/j.anres.2022.56.3.13