

Evaluation of Antibacterial Properties of *Averrhoa bilimbi* and *Averrhoa carambola* Using Different Solvent Extracts

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ABSTRACT

The ongoing research was conducted to compare the antibacterial properties of various solvent extracts of Averrhoa bilimbi and Averrhoa carambola against selected pathogenic bacteria. Freshly gathered plant materials were obtained, and extracted using ethanol, methanol, butanol, acetone and ethyl acetate solvents. The antibacterial activity of the extracts was evaluated by using the agar well diffusion approach to combat E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, B. subtilis, B. cereus and S. aureus. Streptomycin was utilized as the typical antibiotic control. The findings demonstrated that the antibacterial activity of each extract varied depending on the solvent and tested bacterial strain. Among the diverse solvent extracts, the butanol extract revealed the maximum antibacterial efficacy in both plants. In A. carambola, the butanol extract shown top inhibition against E. coli (35 mm), followed by K. pneumoniae (33 mm) and S. aureus (30 mm). In Averrhoa bilimbi, the chief growth restriction was detected against P. aeruginosa (30 mm) and E. coli (26 mm). Ethanol extracts likewise established substantial antibacterial activity, while methanol extracts exhibited sensible activity. Acetone also ethyl acetate extracted samples exhibited comparatively lower antibacterial effects. Comparative analysis revealed that Averrhoa carambola possessed stronger potential than Averrhoa bilimbi. The antibacterial activity may be credited to the occurrence of phytochemicals such as flavonoids, tannins, alkaloids, phenolics, and terpenoids. The study suggests that both plants can serve as promising natural sources of antibacterial agents for future pharmaceutical applications.

Keywords: Bioactive Compounds, Extraction, Pathogens, Phytochemicals, Solvents.

Introduction

Antibiotic-resistant microbes are becoming more and more common, which is a thoughtful risk to world health. There is a persistent essential for substitute healing mediator's derivative from natural sources because the overuse and abuse of synthetic antibiotics in human health, veterinary medicine, and agriculture have decreased the efficacy of many antimicrobial medications (Alvarez-Martínez et al., 2021). Researchers are looking into remedial plants as possible sources of new antimicrobial chemicals owing to the advent of germs that are resistant to drugs. Compared to manufactured medications, plant-derived bioactive compounds are thought to be safer, biodegradable, and less likely to cause negative

side effects. Phytochemicals are abundant in therapeutic shrubberies and have important antibacterial, antioxidant, anti-inflammatory, and therapeutic qualities (Luan et al. 2021). Through a variety of processes, including as rupture of the microbial cell wall, suppression of protein synthesis and interference with nucleic acid processing, these substances can prevent bacterial development.

Two significant therapeutic floras under the Oxalidaceae family are *Averrhoa carambola* and *Averrhoa bilimbi*. These sultry plants are usually grown in Asian nations and are valued for their dietary worth along with for therapeutic uses in conventional medical systems. Fruits, leaves, bark, and roots are among the several elements of these plants with documented historical use to treat fever, cough, inflammation, diabetes, skin conditions, digestive issues, and microbiological infections. Owing to the occurrence of phytochemicals and other secondary metabolites, both plants were reported in earlier research to have antimicrobial, antioxidant, anti-inflammatory, antidiabetic and hypoglycemic properties (Silva et al. 2021; Leliqia and Safitri 2021). Furthermore, these plants' antioxidant capacity may improve their therapeutic effectiveness in the ailment of illness and shield cells from oxidative stress.

Bioactive compounds extracted by a range of solvents, including ethyl alcohol, methyl alcohol, butanol, acetone, aqueous and ethyl acetate. The amount and quality of plant chemicals take out from plant parts are involved by the polarization of the solvent, extraction temperature and time, all of which have a substantial impact on extraction efficiency. While non-polar solvents are appropriate for extracting lipophilic molecules, polar solvents are typically more effective at extracting phenolic and flavonoid chemicals. Consequently, the antibacterial activity and general efficacy of plant extracts are greatly influenced by the solvent selection (Alvarez-Martínez et al. 2021). Comparative analysis of various solvent extracts can produce vital perceptions into the best extraction technique and the healing potential of therapeutic plants against pathogenic microbes.

Materials and Methodology

Collection and Preparation of fruit Extracts

Fresh and healthy fruits, *A. carambola* and *A. bilimbi* were obtained from Elavuvilai, Kanniyakumari District, and identified using standard botanical keys. Approximately 25g of fresh, healthy fruit was collected, thoroughly washed with distilled water, cut into small pieces, and homogenized using a sterile blender. The homogenized fruit material was separately mixed with 100 mL of ethanol, methanol, butanol, acetone and ethyl acetate. Each mixture was macerated for 48 hours under cool conditions with intermittent shaking to

facilitate the extraction of bioactive compounds. After maceration, the mixtures were filtered through Whatman No. 1 filter paper and the filtrates were concentrated by evaporating the respective solvents. The concentrated fruit extracts were collected in sterile, labelled containers and stored at 4° C until further analysis.

Test Microorganisms

E. coli, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *B. subtilis*, *B. cereus* and *Staphylococcus aureus* were among the harmful bacterial strains that were used to test the extracts' antibacterial efficacy. At 4°C, pure cultures of the bacteria were kept on nutrient agar slants.

Antibacterial Assay

The disc diffusion technique was handled to assess the solvent extracts antibacterial activity. Using sterile cotton swabs, bacterial cultures were evenly injected onto sterile Mueller-Hinton agar plates. Using sterile forceps, sterile filter paper discs (6 mm in diameter) were gently placed on the upper layer of the inoculated agar plates then being permeated with equivalent amounts (25µl) of various solvent extracts. The typical antibiotic control was a disc that contained streptomycin. After the plates were incubated at 37°C for a full day, the antibacterial activity was evaluated by measuring the diameter of the zone of inhibition surrounding each disc in millimeters (mm). The average values were recorded after the trials were conducted in triplicate. The average zone inhibition values of the triple experiments were employed to convey the findings. The antibacterial effects of different solvent extracts were analyzed by evaluating the diameters of the inhibition zones produced against the tested bacterial strains.

Results

Antibacterial potency of the *Averrhoa bilimbi* extracts

In comparison to the common antibiotic streptomycin, the antibacterial activity of several solvent fruit extracts of *Averrhoa bilimbi* was assessed against seven harmful bacterial strains. The findings demonstrated that, depending on the solvent utilized and the bacterial species examined, all extracts had differing levels of antibiotic activity (Table 1). The butanol extract had the strongest antibacterial activity against the majority of the microorganisms among the studied extracts. *P.s aeruginosa* had the major zone of growth resistance (30 mm), whereas *E. coli* had a zone of 26 mm. Additionally, strong antibacterial potential against *B. cereus* (18 mm) and *S. aureus* (19 mm) was noted. *Proteus mirabilis* (10 mm), *Bacillus subtilis* (9 mm), and *Klebsiella pneumoniae* (11 mm) all showed moderate inhibition. The ethanol extract also demonstrated considerable antimicrobial activity.

However, the peak growth resistance was distinguished against *E. coli* (18 mm) and *S. aureus* (15 mm). Moderate to low antibacterial efficiency was identified against *P. aeruginosa* (10 mm), *B. subtilis* (10 mm), *B. cereus* (9 mm), and *K. pneumoniae* (8 mm). The present findings demonstrate that the antibacterial efficacy varied contingent on the solvent used for extraction and the vulnerability of the bacterial species tested. No bacterial inhibitory activity was detected against *P. mirabilis*. The acetone and ethyl acetate isolates showed comparatively lower antibacterial activity.

Table 1: Antibacterial potency of the *Averrhoa bilimbi* extracts

Microbes	Ethanol	Methanol	Butanol	Acetone	Ethyl acetate	Streptomycin
<i>K. pneumoneae</i>	15	8	11	10	14	15
<i>P. mirabilis</i>	15	-	10	-	8	15
<i>E. coli</i>	24	18	26	13	12	18
<i>B. subtilis</i>	12	10	9	-	8	20
<i>P. aureginosa</i>	20	10	30	8	10	20
<i>B. cereus</i>	24	9	18	10	12	15
<i>S. aureus</i>	14	15	19	13	15	20

Antibacterial potency of the *Averrhoa carambola* extracts

Of all the solvent extracts, the butanol extract displayed the strongest antibacterial potency with majority of the tested bacteria. *E. coli* presented the major zone of hang-up (35 mm), indicating high susceptibility of the bacterium to the extract (Table 2). Considerable antibacterial potency was detected with *P. aeruginosa* and *B. cereus* (29 mm each), *S. aureus* (30 mm), and *K. pneumoniae* (33 mm). Additionally, the butanol extract displayed reasonable antibacterial potency against *B. subtilis* (20 mm) and strong activity against *P. mirabilis* (28 mm). These results imply that the butanol extract contains potent bioactive mixtures with broad-spectrum antibacterial properties. The ethanol extract demonstrated moderate to strong antibacterial efficacy against every species of bacteria tested. *E. coli* displayed the largest inhibition zone (25 mm), followed by *P. mirabilis* (24 mm). *B. subtilis* and *B. cereus* unveiled growth inhibition zones of 18 mm each, while *S. aureus* displayed a zone of moderate potency (20 mm). Comparatively, there was evidence of antibacterial potency lower against *P. aeruginosa* (15 mm) and *K. pneumoniae* (16 mm). The methanol extract exhibited moderate antibacterial activity. The greatest level of inhibition was distinguished against *B. subtilis* (22 mm) and *E. coli* (20 mm). Reasonable potency was detected against *B. cereus* (16 mm) and *S.*

aureus (15 mm), whereas lower inhibition was noted against *K. pneumoniae* and *P. mirabilis* (9 mm each). No bacterial growth resistance was detected against *P. aeruginosa*. The antibacterial capacity of the acetone and ethyl acetate isolates was comparatively low against the bacteria tested. The acetone extract showed no inhibitory activity against *P. mirabilis*, *P. aeruginosa* and *B. cereus*. These results denoted that the butanol extract of *A. carambola* possesses significant antibacterial efficiency and may aid as a promising natural gift of antimicrobial complexes.

Table 2: Antibacterial potency of the *Averrhoa carambola* extracts

Microbes	Ethanol	Methanol	Butanol	Acetone	Ethyl acetate	Streptomycin
<i>K. pneumoneae</i>	16	9	33	16	21	25
<i>P. mirabilis</i>	24	9	28	-	18	16
<i>E. coli</i>	25	20	35	20	16	23
<i>B. subtilis</i>	18	22	20	17	15	28
<i>P. aureginosa</i>	15	-	29	-	20	25
<i>B. cereus</i>	18	16	29	-	9	18
<i>S. aureus</i>	20	15	30	12	18	19

Discussion

The current investigation showed that several extracts of *A. bilimbi* and *A. carambola* have robust antibacterial efficacy against a number of harmful bacterial strains. The bacterial species examined and the extraction solvent utilized affected the antibacterial effectiveness. The butanol extract showed the most antibacterial activity against the majority of the tested microorganisms among the other solvent extracts. This could be because bioactive phytochemicals with potent antibacterial qualities, such as flavonoids (Panche et al. 2016), tannins (Cowan 1999), phenolic compounds (Daglia 2012), alkaloids (Wink 2012), and terpenoids (Bakkali et al., 2008) are effectively extracted (Luan et al. 2021).

In *A. carambola*, the butanol extract with the strongest antibacterial efficiency against *E. coli*, *K. pneumoniae*, and *S. aureus*. Similarly, in *A. bilimbi*, the butanol extract demonstrated better antibacterial efficiency against *P. aeruginosa* and *E. coli*. These findings are in agreement with earlier studies reporting that the presence of phenolic (Daglia 2012) and flavonoid (Panche et al. 2016) compounds in solvent extracts of *Averrhoa* species contributes to their broad-spectrum antibacterial efficacy against bacteria that are both Gram-positive and Gram-negative (Silva et al., 2021). Both plants' ethanol isolates displayed potent antibacterial

efficiency against a diverse of bacterial species. When extracting polar and moderately polar bioactive elements from medicinal plants, ethanol is thought to be an efficient solvent. Therefore, the inclusion of tannins, saponins, flavonoids and glycosides may be linked to the notable antibacterial efficiency of ethanol compounds found in the present investigation (Atiyah et al., 2022). Leliqia and Safitri (2021) reported similar findings, discovering that *A. bilimbi* ethanol extracts shown significant inhibitory potential against harmful microbes.

The antibacterial potency of the methanol extracts was moderate, whereas that of the extracts of acetone, ethyl acetate was relatively low. Variations in polarity of solvent and their ability to dissolve certain phytochemical elements may be accountable for the discrepancies in antibacterial activity between solvents. The efficiency of extracting antimicrobial chemicals from plant materials is significantly influenced by the solvent polarity (Alvarez-Martinez et al. 2021). In this current investigation, butanol and ethanol seemed to be better solvents than ethyl acetate and acetone for the extraction of antibacterial chemicals.

The antibacterial efficiency exhibited against Gram-positive bacteria *B. subtilis*, *B. cereus* and *S. aureus*, also Gram-negative bacteria including *E. coli*, *K.pneumoniae* and *P. aeruginosa*, demonstrates the broad-spectrum potency of the plant extracts. According to Cushnie and Lamb (2011), the antibacterial mechanism of phytochemicals may involve disruption of bacterial cell walls and cell membranes, inhibition of nucleic acid synthesis, interference with protein synthesis, and alteration of microbial metabolic pathways. Interestingly, in certain cases, the butanol extracts exhibited greater antibacterial activity than the standard antibiotic streptomycin. This finding suggests that these medicinal plants possess significant therapeutic potential as alternative natural antibacterial agents. The findings of the present investigation support earlier reports highlighting the medicinal and therapeutic importance of *A. bilimbi* and *A. carambola* in traditional healthcare systems (Luan et al., 2021; Leliqia and Safitri, 2021). The current investigation demonstrates that *A. bilimbi* and *A. carambola* possess significant antibacterial potential, particularly in their butanol and ethanol extracts. These findings suggest that both plants could serve as promising natural products for the growth of plant-based antibacterial agents. However, further studies are need to isolate, purify and elucidate the active bioactive complexes accountable for the antibacterial potency. In addition, detailed toxicity evaluation and pharmacological surveys are required to explore their safety, effectiveness and potential pharmaceutical applications.

Conclusion

The current investigation showed that *A. carambola* and *A. bilimbi* possess good antibacterial potency against different pathogenic bacteria. The antibacterial capacity varied reliant on the solvent used for extraction. Among all the extracts, the butanol extract showed the remarkable antibacterial capacity, followed by the ethanol extract. Methanol extracts showed moderate activity, while acetone and ethyl acetate extracts showed lower antibacterial activity. Compared to *A. bilimbi*, *A. carambola* exhibited stronger antibacterial activity. The antibacterial properties may be owing to the occurrence of phytochemicals and secondary metabolites. The study suggests that both plants can be used as natural sources of antibacterial compounds. Further investigation are essential to identify the functional groups and assess their therapeutic applications.

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