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Anti-pathogenic bacterial activity from *Garcinia cowa* leaves extract

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Abstract

Garcinia cowa Roxb., a tropical plant traditionally used in Southeast Asian ethnomedicine, is known for its diverse biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects. The leaves of *G. cowa* contain a variety of bioactive phytochemicals that have garnered interest as potential natural agents against pathogenic microorganisms, particularly amid the global challenge of increasing antibiotic resistance. This study aimed to evaluate the antibacterial efficacy of *G. cowa* leaf extracts obtained using different solvents; methanol, ethyl acetate, dichloromethane, and hexane against six pathogenic bacterial strains including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. Antibacterial activity was assessed by measuring inhibition zones, and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using spread plate assays. The methanol extract exhibited the most potent antibacterial activity, particularly against *S. aureus*, with the largest inhibition zone (10 mm), the lowest MIC (0.0037 mg/mL), and a significant MBC (0.15 mg/mL). Ethyl acetate extracts showed moderate activity against several strains, while dichloromethane and hexane extracts demonstrated minimal to no antibacterial effects. Notably, the methanol extract's efficacy approached that of the reference antibiotic kanamycin for certain bacteria. In conclusion, *G. cowa* leaf methanol extract holds strong potential as a natural antibacterial agent, offering a promising alternative for combating bacterial infections and addressing antibiotic resistance. This research supports further investigation into isolating and characterizing the active compounds responsible for these effects, potentially advancing the development of novel phytotherapeutics for clinical and pharmaceutical applications.

Keywords: *Garcinia cowa* extract; Antibacterial activity; Phytochemical extracts

Introduction

The escalating prevalence of antibiotic-resistant bacteria presents a critical challenge to global healthcare systems by compromising the efficacy of existing antimicrobial agents and increasing patient morbidity and mortality (Rattanasuk et al., 2025; Sawisit et al., 2025). The rapid emergence of resistance mechanisms in pathogenic microorganisms necessitates the urgent discovery and development of novel antibiotics (Sharma et al., 2024). Medicinal plants, abundant in diverse bioactive secondary metabolites, represent a valuable and largely untapped resource for identifying new antimicrobial compounds with unique modes of action (Newman & Cragg, 2020). Extensive research has highlighted the antibacterial potential of phytochemicals including alkaloids, flavonoids, and terpenoids against multidrug-resistant bacterial strains, underscoring their promise as alternatives or adjuncts to conventional antibiotics (Cushnie

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& Lamb, 2011). Consequently, the exploration of plant-derived natural products is imperative to mitigate the growing threat of antibiotic resistance and to advance the development of effective therapeutic agents.

Garcinia cowa, known locally in Thailand as Cha Muang, is a member of the Clusiaceae family and a rich source of diverse secondary metabolites. Its crude extracts exhibit a wide range of pharmacological activities, including antitumor-promoting, anti-platelet, antioxidant, antimutagenic, antibacterial, anti-inflammatory, antimalarial, and α-glucosidase inhibitory effects (Siwaporn et al., 2024). The major bioactive constituents xanthones, phloroglucinols, benzophenones, biphenyls, and bioflavonoids, underpin these therapeutic properties (Rahman & Panichayupakaranant, 2024). Notably, compounds such as morelloflavone, daucosterol, p-coumaric acid, cowaxanthone, cowanin, α-mangostin, cambogin, and guttiferone K have been successfully isolated from *G. cowa* fruits, highlighting its potential as a valuable source for drug discovery and development (Gupta et al., 2021).

Previous studies have established the significant antibacterial potential of *G. cowa* extracts against a broad spectrum of pathogenic bacteria. Notably, ethanolic extracts of *G. cowa* have exhibited strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, as demonstrated by disc diffusion assays. In addition, crude hexane and chloroform extracts obtained from the fruit rinds of *G. cowa* were evaluated for their antimicrobial efficacy against various foodborne pathogens and spoilage bacteria, including *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. The minimum inhibitory concentrations (MICs), determined through the agar dilution method, varied considerably, ranging from 15 to 500 μg/mL for the more active extracts and from 300 to 1250 μg/mL for those exhibiting lower activity, highlighting the diverse yet substantial antibacterial properties inherent in these extracts.

Based on these previous findings, this study aimed to evaluate the antibacterial activity of *G. cowa* leaf extracts against six pathogenic bacterial strains: *Staphylococcus aureus* TISTR 1446, *Staphylococcus epidermidis* TISTR 518, *Bacillus subtilis* TISTR 008, *Pseudomonas aeruginosa* TISTR 2370, *Escherichia coli* TISTR 780, and *Klebsiella pneumoniae* TISTR 1383. This study presented the antibacterial properties of *G. cowa* leaf extracts, revealing their potential as effective natural alternatives to fight harmful bacteria. The results support the growing interest in plant-based treatments that could help tackle antibiotic resistance while offering more sustainable healthcare options.

Material and methods

Plant sample collection and preparation

G. cowa leaves were collected from Thamuang, Selaphum District, Roi Et Province, Thailand. The samples were thoroughly rinsed with tap water, subsequently cut into small pieces, and dried in a hot air oven (POL-EKO-APARATURA, Wodzisław Śląski, Poland). at 50°C until completely dehydrated. The dried leaf samples were ground into powder using a herbal grinder (WF-20B THAIGRINDER, Thailand). Subsequently, 100 g of the powdered leaves were separately extracted with methanol, ethyl acetate, dichloromethane, and hexane solvents. The extraction process was conducted by shaking the samples at 150 rpm overnight at room temperature. The resulting extract was collected by filtration, and the filtrate was subsequently evaporated at 50°C to remove the extraction solvent. The crude extracts were diluted and adjusted to a final concentration of 50 mg/mL using dimethyl sulfoxide (DMSO) (Rattanasuk & Phiwthong, 2021).

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Bacterial cultivation and preparation

The bacterial strains *Staphylococcus aureus* TISTR 1446, *Staphylococcus epidermidis* TISTR 518, *Bacillus subtilis* TISTR 008, *Pseudomonas aeruginosa* TISTR 2370, *Escherichia coli* TISTR 780, and *Klebsiella pneumoniae* TISTR 1383 were obtained from the culture collection of the Thailand Institute of Scientific and Technological Research (TISTR), Thailand. A single colony of each strain was inoculated into 5 mL of nutrient broth and incubated with shaking at 37°C for 18 hours. The bacterial suspensions were then standardized to an optical density of 0.1 at 600 nm (OD600) for subsequent assays (Armassa et al., 2025; Boongapim et al., 2021).

Agar disc diffusion assay

Nutrient agar plates were prepared as the solid growth medium. A bacterial suspension was diluted and adjusted to an optical density of 0.1 at 600 nm, and 100 µL was spread evenly across the surface of the nutrient agar plates. Sterile paper discs (5 mm in diameter) were then placed onto the agar surface. Each paper disc was loaded with 10 µL of the test extract, while dimethyl sulfoxide (DMSO) was used as the solvent control. The plates were incubated at 37°C for 24 hours. Following incubation, the diameter of the inhibition zones round each disc was measured in millimeters to assess the antibacterial activity.

MIC and MBC assay

One hundred microliters of nutrient broth (NB) were added to each well of a 96-well microplate. Subsequently, 100 µL of the test extract was added to the first well, followed by a two-fold serial dilution across the plate. Then, 100 µL of bacterial suspension, adjusted to an optical density of 0.1 at 600 nm (OD600), was added to each well. The microplate was incubated at 37°C for 24 hours. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the extract that completely inhibited visible bacterial growth in the wells.

Following the MIC assay, 100 µL of the bacterial suspension was aseptically withdrawn from the wells corresponding to the MIC and the two preceding higher concentrations (total of three wells showing no visible turbidity). These aliquots were then spread evenly onto nutrient agar (NA) plates. The plates were incubated at 37°C for 24 hours. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of the extract at which no bacterial growth was observed on the agar plates (Rattanasuk et al., 2021).

Data Analysis

The inhibition zone diameters were measured and analyzed using SPSS version 28. The study was conducted using a Completely Randomized Design (CRD) with three replicates per treatment, each replicate including five plates. To assess differences between treatments, analysis of variance (ANOVA) was performed, followed by Duncan's Multiple Range Test (DMRT) for pairwise comparisons. Differences were considered statistically significant at p-values below 0.05.

Results and Discussion

Inhibition zone of *Garcinia cowa* leave extracts

The antimicrobial activity of *G. cowa* leaf extracts against six tested pathogenic bacteria were evaluated by measuring the diameter of the inhibition zones (Table 1). The methanol extract exhibited the strongest inhibitory effect against *S. aureus* TISTR 1446, with a clear zone of 10 mm, indicating significant antibacterial potential. This extract also showed moderate activity against *S. epidermidis* TISTR 518 (6 mm),

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B. subtilis TISTR 008 (7 mm), *E. coli* TISTR 780 (7 mm), and *K. pneumoniae* TISTR 1383 (6 mm). The ethyl acetate extract demonstrated antibacterial activity primarily against *S. aureus* TISTR 1466 (6 mm), *B. subtilis* TISTR 008 (6 mm), *P. aeruginosa* TISTR 2370 (6 mm), and *E. coli* TISTR 780 (7 mm) (Table 1). Notably, no inhibition was observed with dichloromethane extracts across all tested bacteria. The hexane extract showed limited activity, only inhibiting *S. epidermidis* TISTR 518 with a zone of 6 mm. Overall, the methanol extract of *G. cowa* leaves exhibited the broadest spectrum and strongest antibacterial efficacy among the solvents tested.

Table 1 Diameter of inhibition zone of *Garcinia cowa* leave extracts against tested pathogenic bacteria

Bacteria	Diameter of inhibition zone (mm)*			
	Methanol	Ethly acetate	Dichloromethane	Hexane
Staphylococcus aureus TISTR 1446	10a	6a	0	0
Staphylococcus epidermidis TISTR 518	6b	0b	0	6a
Bacillus subtilis TISTR 008	7b	6a	0	0
Pseudomonas aeruginosa TISTR 2370	0c	6a	0	0
Escherichia coli TISTR 780	7b	7a	0	0
Klebsiella pneumoniae TISTR 1383	6b	0b	0	0

*Means (n=3) in the column followed by the same common letter were not significantly different (DMRT, p>0.05).

MIC and MBC values

The minimum inhibitory concentration (MIC) values of *G. cowa* leaf extracts against six pathogenic bacteria are presented in Table 2. The methanol extract exhibited the strongest inhibitory effect, particularly against *S. aureus* TISTR 1446, with an MIC value of 0.0037 mg/mL, which is notably lower than that of the standard antibiotic kanamycin (0.02 mg/mL). For *S. epidermidis* TISTR 518, *Bacillus subtilis* TISTR 008, *E. coli* TISTR 780, and *K. pneumoniae* TISTR 1383, the methanol extract showed moderate antibacterial activity with MIC values of 0.62 mg/mL. The ethyl acetate extract demonstrated inhibitory effects against *S. aureus* TISTR 1466, *B. subtilis* TISTR 008, *P. aeruginosa* TISTR 2370, and *E. coli* TISTR 780 with MIC values of 1.25 and 0.62 mg/mL, respectively (Table 2). No inhibitory activity was detected for dichloromethane extracts across all tested strains. The hexane extract showed limited activity only against *S. epidermidis* TISTR 518 with an MIC of 1.25 mg/mL. Overall, these results indicate that the methanol extract of *G. cowa* leaves possesses potent antibacterial activity, especially against *S. aureus* TISTR 1466, and compares favorably with the reference antibiotic kanamycin.

The minimum bactericidal concentration (MBC) values of *G. cowa* leaf extracts against the tested pathogenic bacteria are summarized in Table 3. The methanol extract exhibited bactericidal activity against *S. aureus* TISTR 1466 with an MBC of 0.15 mg/mL, indicating strong killing ability, although higher than the reference antibiotic kanamycin (0.048 mg/mL). It also showed bactericidal effects against *B. subtilis* TISTR 780 and *K. pneumoniae* TISTR 1383 at 1.25 mg/mL and 2.5 mg/mL, respectively. The ethyl acetate extract demonstrated bactericidal activity against *S. aureus* TISTR 1466, *P. aeruginosa* TISTR 2370, and *E. coli* TISTR 780, each at an MBC of 2.5 mg/mL. No bactericidal activity was detected for dichloromethane and hexane extracts against any tested bacteria at the concentrations tested. *S. epidermidis* TISTR 518 was only susceptible to kanamycin, with no bactericidal effect observed from any plant extracts. These findings

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suggest that the methanol extract of *G. cowa* leaves possesses notable bactericidal potential, particularly against gram-positive bacteria, albeit at higher concentrations compared to standard antibiotics.

Table 2 The MIC values of *Garcinia cowa* leave extracts against pathogenic bacteria

Bacteria	MIC (mg/mL)				
	Methanol	Ethly acetate	Dichloro-methane	Hexane	kanamycin
Staphylococcus aureus TISTR 1446	0.0037	1.25	-	-	0.02
Staphylococcus epidermidis TISTR 518	0.62	-	-	1.25	0.02
Bacillus subtilis TISTR 008	0.62	0.62	-	-	0.02
Pseudomonas aeruginosa TISTR 2370	-	1.25	-	-	3.125
Escherichia coli TISTR 780	0.62	0.62	-	-	0.048
Klebsiella pneumoniae TISTR 1383	0.62	-	-	-	0.02

Table 3 The MBC values of *Garcinia cowa* leave extracts against pathogenic bacteria

Bacteria	MBC (mg/mL)				
	Methanol	Ethly acetate	Dichloro-methane	Hexane	kanamycin
Staphylococcus aureus TISTR 1446	0.15	2.5	-	-	0.048
Staphylococcus epidermidis TISTR 518	-	-	-	-	0.048
Bacillus subtilis TISTR 008	1.25	-	-	-	0.048
Pseudomonas aeruginosa TISTR 2370	-	2.5	-	-	12.5
Escherichia coli TISTR 780	-	2.5	-	-	1.56
Klebsiella pneumoniae TISTR 1383	2.5	-	-	-	0.048

The results of this study clearly indicate that methanol extracts of *Garcinia cowa* leaves possess potent antibacterial activity, particularly against *Staphylococcus aureus* TISTR 1446. These findings are consistent with those of Sakunpak and Panichayupakaranant (2012), who reported significant inhibition of *S. aureus* by the ethyl acetate extract of *G. cowa*. Their study demonstrated that *G. cowa* leaf extracts exhibited antibacterial effects against *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus* species, with minimum inhibitory concentrations (MICs) of 31.2 μg/mL (Sakunpak & Panichayupakaranant, 2012). Similarly, Tayana et al. (2017) demonstrated that β-mangostin exhibited potent antibacterial activity against *Bacillus cereus* TISTR 687 and methicillin-resistant *Staphylococcus aureus* (MRSA) SK1, with a MIC of 4 mg/mL (Tayana et al., 2017).

The exceptionally low MIC value against *Staphylococcus aureus* TISTR 1446 observed in this study (0.0037 mg/mL) indicates a highly potent extract, which may be attributed to differences in extraction methods or the source of plant material. Notably, the antimicrobial activity reported here surpasses that documented by Nguyen et al. (2021). In related studies, the ethyl acetate extract of *Garcinia gaudichaudii* exhibited efficacy primarily against Gram-positive bacteria, with MIC values ranging from 15.625 to 25 μg/mL, whereas the methanol extract of *Garcinia planchonii* demonstrated activity against both Gram-positive (MIC = 160 μg/mL) and Gram-negative bacteria (MIC = 75 μg/mL) (Nguyen et al., 2021).

The bactericidal activity indicated by the MBC results, particularly against Gram-positive bacteria such as *S. aureus* and *Bacillus subtilis*, supports the potential of *G. cowa* methanol extracts as viable antibacterial agents. However, the higher concentrations required compared to kanamycin reflect a need for further purification or combination therapies. Taken together, these results reinforce the significant antibacterial potential of *G. cowa* leaf extracts and highlight their promise as natural alternatives or complements to conventional antibiotics, especially in an era challenged by increasing antimicrobial resistance.

Conclusion

This study demonstrates that *G. cowa* leaf extracts, particularly the methanol fraction, possess significant antibacterial activity against a range of pathogenic bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella pneumoniae*. The methanol extract exhibited the lowest MIC and notable MBC values, highlighting its strong inhibitory and bactericidal potential, which in some cases approaches the efficacy of standard antibiotics such as kanamycin. In contrast, dichloromethane and hexane extracts showed limited or no antibacterial effects. These findings validate the potential use of *G. cowa* leaves as a natural source of antimicrobial agents.

The identification of potent antibacterial activity in *G. cowa* leaf extracts underscores their potential as alternative or complementary therapies to conventional antibiotics, especially in the face of rising antimicrobial resistance. This research provides a scientific basis for further phytochemical analysis and bioactive compound isolation from *G. cowa*, facilitating the development of novel plant-based antimicrobial drugs. Moreover, utilizing such natural extracts could reduce reliance on synthetic antibiotics, thereby contributing to sustainable approaches in infection control and public health.

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