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Antimicrobial Properties of Ethanolic Extract from Gac Fruit (*Momordica cochinchinensis* Spreng.) Powder Composed of Pulp and Aril

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The growing prevalence of antimicrobial-resistant pathogens necessitates the exploration of safer and more sustainable natural alternatives to conventional therapeutics. Gac fruit (*Momordica cochinchinensis* Spreng), noted for its phenolic-rich composition and inherent antimicrobial potential, offers a promising source of bioactive compounds whose functional properties can be preserved through appropriate post-harvest processing such as lyophilization. This study investigates the antimicrobial properties of ethanolic extracts obtained from lyophilized-powder and paste of Gac fruit pulp and aril, with a particular emphasis on the sample exhibiting the highest total phenolic content (TPC). Both the pulp-aril paste and the powder forms of Gac fruit underwent Soxhlet extraction, and their TPC was determined using the Folin-Ciocalteau assay. The powdered form demonstrated a slightly higher TPC (21.901 µg GAE/mg) compared to the paste counterpart (18.750 ± 0.019 µg GAE/mg). Minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) measurements were used to evaluate the antibacterial properties of the extract against nine opportunistic pathogens. The results indicated variable levels of susceptibility: *Escherichia coli* required the highest extract concentration (50 mg/mL) for bactericidal activity, whereas *Aspergillus oryzae* was the most susceptible, with both MIC and MBC at 8.33 mg/mL. These findings demonstrate that the phenolic content and antimicrobial activity of Gac fruit are largely retained following lyophilization, highlighting the relevance of this post-harvest technique for preserving bioactive quality in industrial processing. The preserved antibacterial properties underscore the potential of Gac fruit as a source of plant-based antimicrobial agents. Further work is needed to refine extraction conditions and explore synergistic interactions with existing antimicrobials to enhance overall efficacy.

Keywords: Gac fruit, antimicrobial activity, ethanolic extract, Soxhlet extraction

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The World Health Organization has identified antimicrobial resistance (AMR) as one of the top ten global public health threats, highlighting its significance in the 21st century [1]. In essence, AMR arises when pathogenic bacteria, viruses, fungi, and parasites develop the ability to evade the effects of antimicrobial drugs that were previously effective against them. As a result, treatments become less effective or even useless, leading to persistent infections, longer illness durations, and, unfortunately, higher mortality rates. The escalation of resistant strains is largely fueled by the overuse and misuse of antimicrobials, both in clinical settings and in agriculture. This trend has complicated the treatment of even common infections, which were once considered easily manageable. In response to the growing threat and the anxiety surrounding potential future pandemics, many individuals are seeking additional strategies for self-protection. Beyond standard hygiene practices and vaccination, there is increasing interest in natural and holistic health approaches. This has notably driven attention toward plant and fruit-derived medications and supplements, which are recognized for their rich content of bioactive compounds with potential antimicrobial properties [2].

Gac fruit, scientifically known as *Momordica cochinchinensis* Spreng. Remains somewhat obscure

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outside its native regions, despite its striking appearance and notable nutritional profile. Originating from Southeast Asia, this vivid orange, spiky fruit has attracted scholarly attention for its botanical history. In 1790, the Portuguese missionary Loreiro first identified it as *Muricia cochinchinensis* during his travels in Vietnam. Later, in 1826, Sprengel reclassified the species under the Linnaean genus Momordica, reflecting advances in botanical taxonomy [3]. The epithet "cochinchinensis" refers specifically to the Cochinchina region in northern Vietnam [4], though the fruit's natural habitat extends across Northeastern Australia and much of South and Southeast Asia. Commonly referred to as baby jackfruit, spiny bitter gourd, sweet gourd, or cochinchin gourd, Gac thrives in the tropical climates of countries such as Thailand, Vietnam, Laos, and China. Despite its widespread cultivation and regional popularity, Gac fruit remains a relatively underappreciated resource in the global context, meriting further academic and nutritional exploration.

Gac fruit is genuinely a fascinating perennial vine. The species is dioecious, a reproductive system in which male and female flowers are found on separate individuals. The vines can go the distance, stretching up to six meters along a fence. Usually, the flowering starts about two months after planting. Female flowers can be readily identified by the prominent swelling at the base of the floral structure, indicating the initial development of the fruit. In contrast, male flowers typically exhibit lighter-coloured petals and a more open floral morphology [5]. The fruit is initially green and gradually transitions to a deep orange or red upon maturation. The edible portion is the aril (Figure 1), a red, lipid-rich membrane that envelopes the seeds. The outer shell of the fruit is inedible and therefore unsuitable for consumption. Both the pulp and aril exhibit a soft, mushy texture and possess only a mildly flavoured taste profile [6]. Over the last few decades, Gac fruit has gotten a lot of attention for being packed with carotenoids, which are considerably higher than in most other fruits. This is mainly due to the high concentrations of lycopene and β-carotene in that oily seed covering [7,8]. Current investigations into the antimicrobial activity of Gac fruit are still in their early phases, but initial findings suggest considerable potential. The fruit itself contains substantial levels of bioactive compounds, carotenoids, flavonoids, and phenolic acids, which, according to Thavamany et al. [9], may position Gac as a promising natural antimicrobial agent.

Various post-harvest techniques have been employed in the industry to preserve the natural phytochemical profile of horticultural products, with lyophilization being one of the most effective approaches [8]. Lyophilization minimizes thermal degradation and oxidative loss by removing water under low temperature and vacuum conditions, thereby maintaining the stability of heat-sensitive compounds such as phenolics, carotenoids, and other bioactives. This technique not only extends shelf life but also helps retain the functional properties of the fruit for subsequent processing, formulation, or therapeutic applications. However, it remains unclear whether lyophilization preserves the antimicrobial properties of Gac fruit, and no study has directly addressed this question.

In this context, the present study specifically investigates whether the antimicrobial activity of Gac fruit is retained after lyophilization, alongside identifying which ethanolic extract derived from either fresh fruit paste or lyophilized powder containing both pulp and aril exhibits the highest total phenolic content (TPC). Additionally, the study determines the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract with the highest TPC against selected opportunistic microbes.

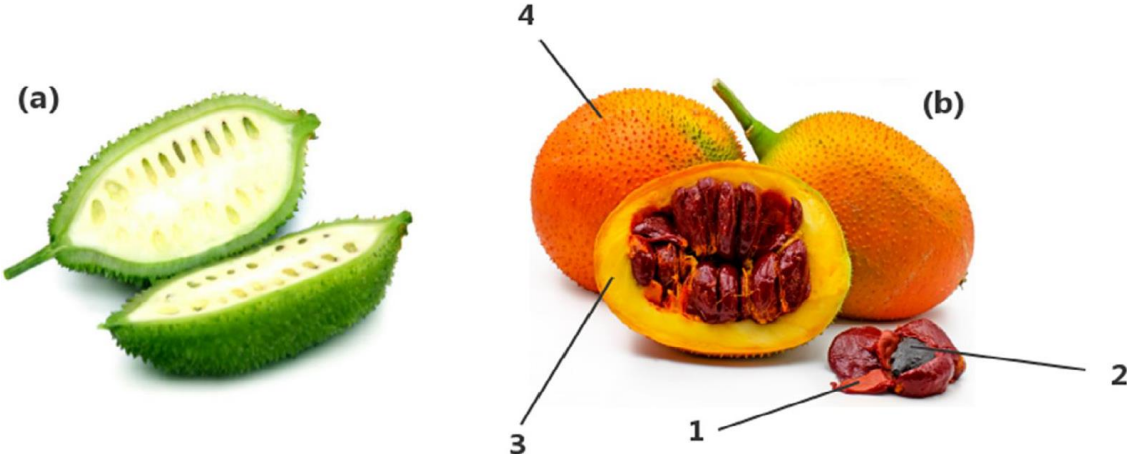

Figure 1. The structure of the Gac fruit at two stages: (a) young and (b) moderately ripe. Labels indicate: 1. Aril, 2. Seed, 3. Pulp, 4. Peel with spines.

EXPERIMENTAL

Chemicals and Materials

In this study, two forms of Gac fruit were obtained: a paste and a powder, both derived from the aril and pulp components. The samples were supplied by GLF Capital Holdings Sdn. Bhd. (Johor Bahru, Johor, Malaysia). The powder was produced using lyophilization (freeze-drying) of the aril and pulp, while the paste was obtained by thoroughly mashing fresh aril, and pulp to achieve a uniform, viscous texture.

Characterization Methods

Extraction of Gac Fruit Samples

In this study, the extraction of Gac fruit samples followed a modified version of the method described by Motong et al. [10]. Briefly, samples were placed in cellulose extraction thimbles and loaded into the extraction chamber of a Soxhlet apparatus. Approximately 200 mL of ethanol was added to the boiling flask. The extraction proceeded at a sample-to-solvent ratio of 20:1 for 11 hours at 78°C. Following extraction, ethanol was removed from the Gac fruit extract using a rotary evaporator. The resulting crude extract was collected in 5 mL microcentrifuge tubes and stored at 4°C until further analysis.

Total Phenolic Content (TPC)

The total phenolic content (TPC) of the ethanolic extracts, prepared from a combination of Gac fruit pulp and aril, was determined using a method adapted from Yusoff et al. [11] via the Folin-Ciocalteu assay. A 15% sodium carbonate solution was prepared by dissolving 7.5 g of sodium carbonate (Na_2CO_3) in 50 mL of distilled water. Gac fruit extract was prepared at a concentration of 1 mg/mL by dissolving 5 mg of extract in 5 mL of ethanol. For the calibration curve, gallic acid standards ranging from 25 to 200 µg/mL were prepared in distilled water. For the assay, 1 mL of 1 mg/mL Gac fruit extract was mixed with 1 mL of Folin & Ciocalteu reagent and vortexed for 5 minutes. Next, 1 mL of the 15% sodium carbonate solution and 5 mL of distilled water were added. The mixture was left to stand for 90 minutes, after which absorbance was measured at 760 nm, using 1 mL of distilled water as the blank. A linear regression model ($R^2 = 0.9964$) was used to construct the calibration curve for gallic acid, with the equation $Y = 0.0128x - 0.035$, where Y represents absorbance and x is the gallic acid equivalent (GAE) in µg per mg of dried extract. The Gac fruit sample with the highest TPC was selected for subsequent MIC and MBC determination against various opportunistic microorganisms.

Cultivation Media and Growth Parameters

To cultivate the seven bacterial strains, *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhimurium, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus,* and *Enterobacter aerogenes*, Mueller-Hinton Broth (MHB) and Mueller-Hinton Agar (MHA) were utilized to ensure consistency in growth conditions. For fungal cultures, specifically *Candida albicans* and *Aspergillus oryzae*, Potato Dextrose Broth (PDB) and Potato Dextrose Agar (PDA) were selected, given their suitability for fungal propagation. Before experimental procedures, pre-cultivation was performed to optimize the physiological state of microorganisms. All strains were activated and incubated at 35 °C for 24 hours, except for *Aspergillus oryzae*, which required a 48-hour incubation period before inoculum preparation. These pre-cultures were then used to prepare inoculum broths and agar plates for further experimentation.

Minimum Inhibitory Concentration (MIC)

For the determination of the Minimum Inhibitory Concentration (MIC) of the Gac fruit extract, the study adhered to the Clinical and Laboratory Standards Institute (CLSI) guidelines, employing the two-fold broth microdilution technique. The assay was conducted in 96-well microplates. Microbial suspensions were prepared to an OD600 of 0.5 (corresponding to approximately 108–109 CFU/ml) using a spectrophotometer. The Gac fruit extract was prepared at a concentration of 100 mg/mL and subjected to serial two-fold dilutions within the microplate. The tested concentrations ranged from 0.195 mg/mL to 100 mg/mL. The negative controls consisted of medium alone and medium inoculated with the test microorganism. The plates were incubated at 35 ± 2°C for 24–48 hours, and all assays were performed in triplicate. The MIC was defined as the lowest concentration of Gac fruit extract at which no visible turbidity was observed in the wells after incubation.

Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) refers to the lowest concentration of an antibacterial agent that can kill bacteria, as described by Cole et al. [12]. In this experiment, after determining the MIC, 10 µL from each well of the microplate was transferred onto Mueller-Hinton agar (MHA) plates, with each plate set up for the specific pathogen being tested. Both negative and positive controls were included, these came from columns 1 and 2, respectively. After plating, the samples were incubated at 35 ± 2°C for between 24 and 48 hours. After incubation, the plates were checked, and the MBC was identified as the lowest concentration where no visible bacterial growth was observed. All tests were run in triplicate to keep results reliable. The MIC and MBC values came from the classic two-fold dilution method,

starting at 100 mg/mL in column 12 and cutting the concentration in half for each following column.

Statistical Analysis

All measurements were performed in triplicate, ensuring reliability, and the results were reported as mean ± standard deviation for TPC, MIC, and MBC in the Gac fruit samples. Statistical analysis was carried out using ANOVA within MS Excel (Microsoft Office 365) to assess significant differences between the pulp-aril powder and pulp-aril paste extracts. Descriptive statistics were also utilized to summarize the antimicrobial activity data of the Gac fruit samples against the selected opportunistic microorganism.

RESULTS AND DISCUSSION

Extraction of Gac Fruit Samples

For extracting bioactive compounds from Gac fruit, the Soxhlet extraction method was employed due to its notable efficiency. This technique works by continuously cycling the solvent, ethanol, through the sample, which enhances the extraction of target compounds. The Soxhlet apparatus also enables solvent recovery and reuse. This is particularly beneficial when using ethanol, making it both cost-effective and environmentally sustainable compared to procedures that require frequent use of fresh solvent [13]. The primary drawback is the time required: extraction of Gac fruit powder and paste took approximately 11 hours. Post-extraction, ethanol was removed via rotary evaporation, which added further processing time to concentrate the extracts and isolate the bioactive components [14].

Ethanol was selected as the solvent because it is polar and capable of dissolving a wide range of both polar and non-polar compounds. This property is essential for extracting various bioactive constituents from both the pulp and aril of Gac fruit. Ethanol is also generally safer to handle and less toxic compared to other common solvents such as methanol or acetone [15], and is biodegradable, meaning it has a lower environmental impact [16]. Its boiling point of 78.4 °C is ideal for Soxhlet extraction, allowing efficient extraction without excessive heating. Furthermore, ethanol is relatively inexpensive and widely available. Thus, it is suitable for large-scale extractions that making it an optimal solvent for isolating valuable compounds from Gac fruit powder and paste in an effective, safe, and environmentally responsible manner.

Determination of Total Phenolic Content (TPC)

Table 1 presents the total phenolic content (TPC) values for ethanolic extracts from Gac fruit powder and paste, both incorporating pulp and aril. The TPC in the freeze-dried (lyophilized) pulp-aril powder (21.901 ± 0.007 µg GAE/mg) is higher than that in the pulp-aril paste (18.750 ± 0.019 µg GAE/mg). This suggests that lyophilization is more effective at preserving the phenolic compounds in Gac fruit than concentration into a paste. Statistical analysis demonstrates a significant difference between these two groups ($p = 0.026185$, $p < 0.05$). Kubola and Siriamornpun [17] reported that the aril exhibited the highest TPC value (4.29 ± 0.15 µg GAE/mg), with the pulp and peel following, and the seed having the lowest. Their work also confirmed a strong correlation between antioxidant activity, TPC, and total flavonoid content (TFC).

Table 1. The total phenolic content (TPC) of the ethanolic extract of Gac fruit paste and lyophilized powder derived from the combination of pulp-aril.

Gac Fruit Sample	Total Phenolic Content (µg GAE/mg)
Pulp-Aril Paste	18.750 ± 0.019^{a}
Pulp-Aril Powder	21.901 ± 0.007^{b}

Values are expressed as mean ± standard deviation (n=3). Means with columns followed by different small letters (a,b) are significantly at the level $P<0.05$. GAE denotes gallic acid equivalent.

A recent review by Vadiveloo et al. (2024) compared polyphenol content across various drying and extraction methods, highlighting the influence of heat and different solvents on phenolic retention. For example, ripe Gac aril extracted with ethanol showed a TPC of 0.191 µg GAE/mg, and ripe pulp extracted with ethanol yielded 0.205 µg GAE/mg, higher than those in many paste preparations, but still subject to variation depending on fruit freshness and ripeness [18]. Tinrat et al. [19] also found that TPC is

influenced by both fruit maturity and the type of solvent used. The highest TPC (0.416 ± 0.0024 µg GAE/mg) was obtained from acetone extraction of unripe pulp, whereas lower values were observed in ripe samples or when using less efficient solvents like hexane. The TPC value obtained from the lyophilized pulp-aril powder (21.901 ± 0.007 µg GAE/mg) in this study is higher compared to those studies. The variation of data can be due to several factors, such as different sample preparation and extraction method [20], the maturity stage of the fruit, and the quality of the raw material of the fruit. In another study, Tinrat et al. [21] reported the TPC of 0.205 µg GAE/mg for the ethanolic extract from pulp fraction. These findings reinforce that freeze-drying combined with ethanol extraction, as used in this study, provides a balanced and food-safe approach that preserves an appreciable number of polyphenols.

Antimicrobial Activity of Gac Fruit Pulp-Aril Powder

The antimicrobial activity of the ethanolic extract derived from the pulp-aril of Gac fruit powder, as assessed by both minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values (Table 2), reveals a considerable degree of variability across different microbial species. This variation is particularly noteworthy, as it underscores the nuanced potential of Gac fruit pulp-aril powder extract as an antimicrobial agent, evidencing a spectrum of sensitivities among the tested microorganisms. Correspondingly, Figure 2 provides a visual representation of these findings, allowing for a comparative analysis of MIC and MBC values, and thereby offering further insight into the relative susceptibility of each microorganism to the extract.

Table 2. The antimicrobial activity of the ethanolic extract from the pulp-aril of Gac fruit powder.

Type of Microorganisms	MIC (mg/mL)	MBC (mg/mL)
Gram-positive bacteria		
Staphylococcus aureus	41.67 ± 14.43	41.67 ± 14.43
Staphylococcus epidermidis	33.33 ± 14.43	33.33 ± 14.43
Bacillus cereus	12.50 ± 0.00	16.67 ± 7.22
Gram-negative bacteria		
Salmonella typhimurium	20.83 ± 7.22	25.00 ± 0.00
Pseudomonas aeruginosa	12.50 ± 0.00	25.00 ± 0.00
Escherichia coli	25.00 ± 0.00	50.00 ± 0.00
Enterobacter aerogenes	12.50 ± 0.00	25.00 ± 0.00
Fungi		
Candida albicans	20.83 ± 7.22	33.33 ± 14.43
Aspergillus oryzae	8.33 ± 3.61	8.33 ± 3.61

Values are expressed as mean ± standard deviation (n=3).

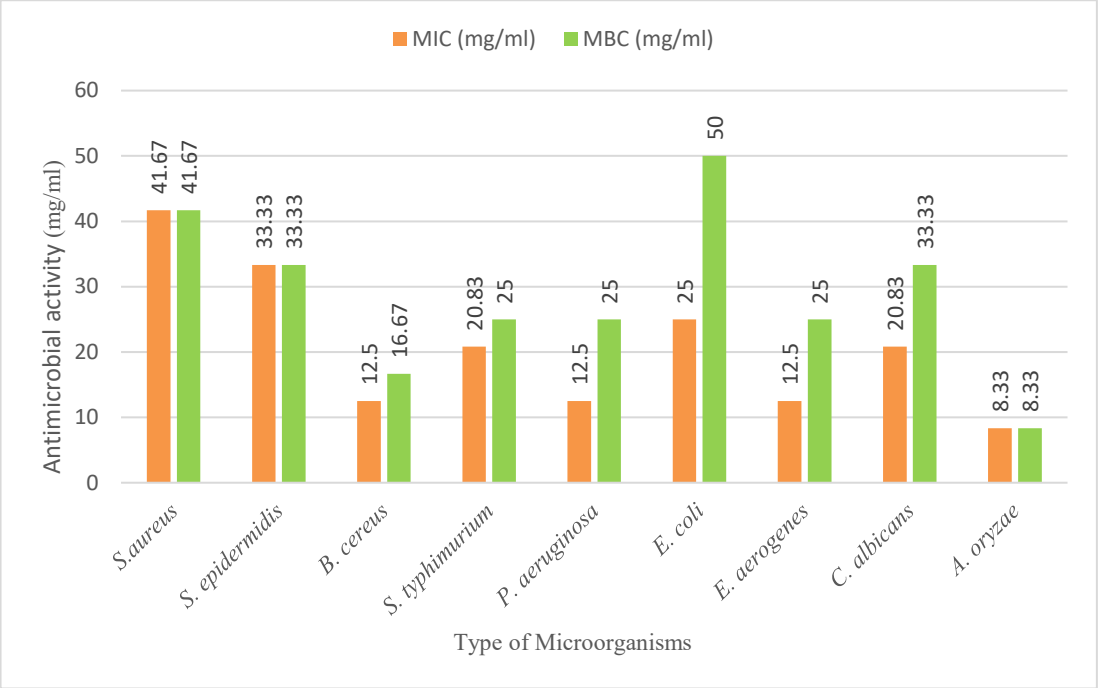

Figure 2. Overall comparison of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of Gac fruit pulp-aril powder extract against various opportunistic microorganisms.

In the case of *Staphylococcus aureus*, for instance, the MIC and MBC values were identical, measured at 41.67 ± 14.43 mg/mL. This observation implies that the concentration required to inhibit the growth of *S. aureus* is also sufficient to exert a bactericidal effect, suggesting a robust dual-action potential at this dosage. Such a finding is significant, as agents capable of both inhibiting and killing pathogens at the same concentration may offer a therapeutic advantage, especially in clinical contexts where both bacteriostatic and bactericidal activities are desirable.

The gram-positive bacterium *Staphylococcus epidermidis* demonstrated similar results, albeit with slightly lower MIC and MBC values of 33.33 ± 14.43 mg/mL. The lower concentration required for both inhibition and killing, compared to *S. aureus*, indicates a heightened sensitivity of *S. epidermidis* to the Gac fruit extract. Nevertheless, the concentration involved remains substantial, suggesting that while the extract is effective, it may require further refinement or combination with other agents to achieve maximal efficacy in practical applications. *Bacillus cereus*, another gram-positive and spore-forming bacterium, presented with a MIC of 12.50 ± 0.00 mg/mL and an MBC of 16.67 ± 7.22 mg/mL. The relatively narrow gap between these values highlights the extract's pronounced efficacy against *B. cereus*, particularly when contrasted with the other gram-positive bacteria included in the study. The lower concentrations necessary for both inhibitory and bactericidal effects suggest that *B. cereus* is especially vulnerable to the bioactive compounds present in the Gac fruit pulp-aril extract.

The results for gram-negative bacteria also merit attention. For *Salmonella typhimurium*, the MIC was recorded at 20.83 ± 7.22 mg/mL, while the MBC was 25.00 ± 0.00 mg/mL. The proximity of these two values indicates that the extract transitions efficiently from exerting a bacteriostatic effect to achieving bactericidal action in *S. typhimurium*, making it an effective antimicrobial agent at moderate concentrations for this organism. *Pseudomonas aeruginosa* and *Enterobacter aerogenes* exhibited similar patterns of sensitivity. Both species had MICs of 12.50 ± 0.00 mg/mL and MBCs of 25.00 ± 0.00 mg/mL. This suggests that the Gac fruit extract can inhibit these bacteria at relatively low concentrations, but a higher dose is necessary to ensure bactericidal activity. This finding points to the potential of the extract as a preventative measure in controlling bacterial growth, while also highlighting the need for higher concentrations for complete eradication. *Escherichia coli*, however, demonstrated a higher level of resistance. The MIC for *E. coli* was observed at 25.00 ± 0.00 mg/mL, but the MBC was markedly higher at 50.00 ± 0.00 mg/mL. This considerable difference between the concentrations needed for inhibition and killing indicates that while the extract can suppress *E. coli* at moderate doses, much greater amounts are needed to achieve bactericidal effects. Such a result may be indicative of intrinsic resistance mechanisms within

E. coli and suggests that the efficacy of the Gac fruit extract varies significantly depending on the bacterial species in question.

Fungal were also included in the study, with results indicating moderate to strong antifungal activity. For *Candida albicans*, the MIC was 20.83 ± 7.22 mg/mL, and the MBC was 33.33 ± 14.43 mg/mL. The fact that a higher concentration is required for killing than for inhibition suggests moderate antifungal efficacy and may point to the potential for the extract to be used in conjunction with other antifungal agents for enhanced outcomes. Of note is *Aspergillus oryzae*, which demonstrated the highest susceptibility among all tested microorganisms. Both the MIC and MBC were the lowest recorded in the study, at 8.33 ± 3.61 mg/mL. The congruence of these values underscores the potent antifungal activity of the Gac fruit pulp-aril extract, suggesting its promise as a natural antifungal agent, especially against *A. oryzae*.

A comprehensive investigation of the antimicrobial properties of fully ripe red Gac fruit, focusing on three distinct anatomical parts: the peel, pulp, and aril [20]. By employing an ethanolic solvent extraction technique, they aimed to determine the extent to which these separate fruit components could inhibit or eradicate pathogenic microorganisms. The study targeted six specific pathogens: *Staphylococcus aureus* ATCC 1216, *Bacillus cereus* DMST 5040, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Salmonella typhimurium* ATCC 13311, each representing a clinically relevant threat. The minimum inhibitory concentration (MIC) values for the extracts were, in most cases, notably low, suggesting that the ethanolic extracts derived from Gac fruit parts can effectively suppress microbial proliferation. This indicates a promising potential for these natural extracts in inhibiting the growth of various pathogens, which could be of considerable interest for food safety and medical applications. However, even at the highest concentration tested (50 mg/mL), no minimum bactericidal concentration (MBC) was observed for any of the microorganisms. In other words, while the extracts were able to halt the growth of these pathogens, they did not kill them under the conditions tested. This is in stark contrast to the outcomes observed with the Gac fruit pulp-aril powder extract, which demonstrated significant bactericidal effects. Such a discrepancy underscores the importance of both the extraction method and the physical form of the fruit material, as these can fundamentally influence the spectrum and strength of antimicrobial activity observed.

The efficacy of Gac fruit as an antimicrobial agent varies across microbial species and extraction methods. The ethanolic extract from the pulp-aril exhibited broad-spectrum activity, showing notable effectiveness against *Aspergillus oryzae* and *Bacillus cereus*, which were inhibited at comparatively low concentrations. In contrast, organisms such as *Staphylococcus aureus* and *Escherichia coli* required higher extract concentrations for both inhibitory and bactericidal effects. These results indicate that the antimicrobial potential of Gac fruit extracts is strongly dependent on the target organism and the method of extraction. Overall, the pulp-aril powder extract demonstrates significant antimicrobial and antifungal activity, particularly against certain gram-positive bacteria and fungi, highlighting its potential as a natural antimicrobial agent or food preservative. Further studies are warranted to isolate the active compounds and optimize their concentrations for practical applications.

Antimicrobial Efficacy and Potential

While showing activity, the MIC values generally range from 8.33 mg/mL to 41.67 mg/mL. Comparable findings have been reported in previous studies, where MIC values for Gac fruit peel, pulp, and aril extracts ranged from 3.125 to 12.5 mg/mL [21], and aril extracts showed MICs ranging from 12.5 to 50 mg/mL against various microorganisms [22], aligning with the range observed in the present work. These concentrations are relatively high when compared to synthetic antibiotics (ampicillin), but they still demonstrate a clear antimicrobial effect for a natural extract. When compared to the MIC values of other plant derived antimicrobials reported in Table 3, the Gac fruit extract demonstrates similar or moderately higher activity. For instance, plant extracts commonly exhibit MICs of 0.2–12.4 mg/mL against *Staphylococcus aureus,* around 6 mg/mL for *Staphylococcus epidermidis*, 1–8 mg/mL for *Bacillus cereus*, 0.156–2.5 mg/mL for *Salmonella typhimurium*, 6.25 mg/mL for *Pseudomonas aeruginosa*, 0.02–0.52 mg/mL for *Escherichia coli*, and approximately 32 mg/mL for *Enterobacter aerogenes*. For fungal species, plant extracts typically show MIC ranges of 0.125–4 mg/mL against *Candida albicans*, while active fractions have demonstrated MIC values around 0.125–0.5 mg/mL against *Aspergillus oryzae*. These comparative observations indicate that although Gac fruit extract requires higher concentration than some potent plant extracts, its antimicrobial potential remains within the expected range for crude phytochemical rich materials.

Table 3. The minimum inhibitory concentration (MIC) values of plant extracts.

Microorganism	MIC (mg/mL)	Reference
S. aureus	0.20–12.4	[25]
S. aureus	1.67	[26]
S. epidermidis	6.00	[27]
B. cereus	1.00–8.00	[28]
S. typhimurium	0.156–2.50	[29]
P. aeruginosa	6.25	[30]
E. coli	0.02–0.52	[31]
E. aerogenes	32.00	[32]
C. albicans	0.125–4.00	[33]
A. oryzae	0.125–0.50	[34]

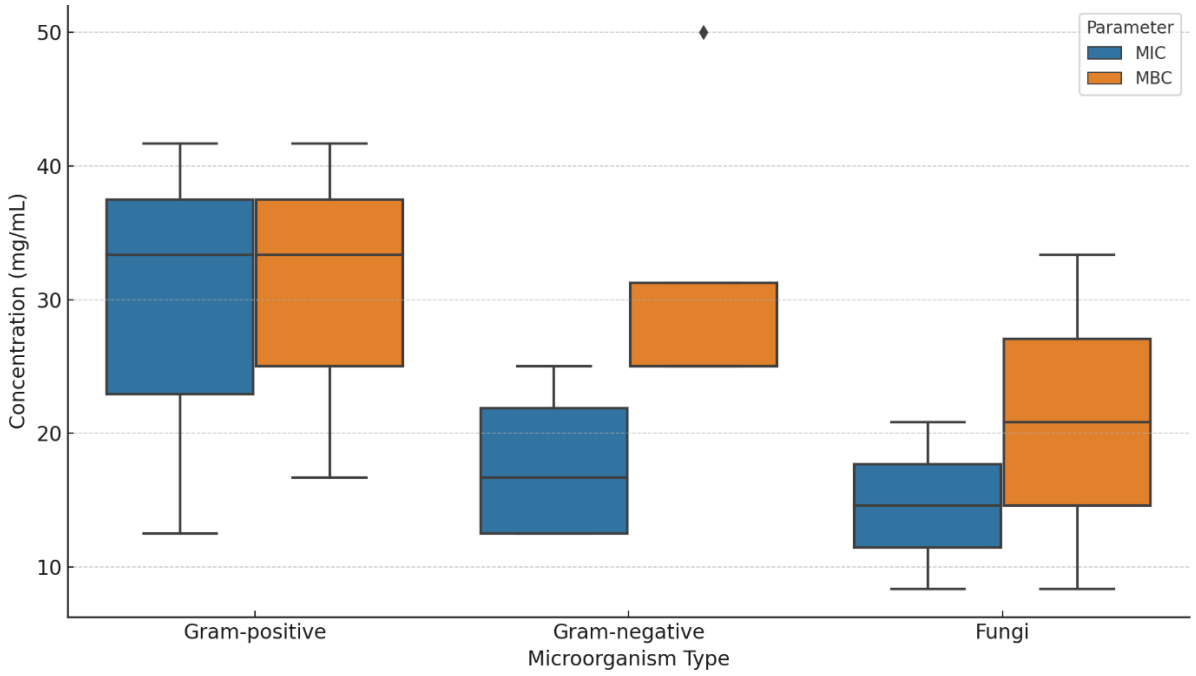

Figure 3. Box plot of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) range values by microorganism type.

Figure 3 shows the box plot illustrating the distribution of MIC and MBC values for Gac fruit extract across different microorganism types, revealing clear trends in susceptibility. Fungal strains, particularly *Aspergillus oryzae* and *Candida albicans*, showed the lowest and most consistent MIC and MBC values, indicating strong sensitivity to the extract. In contrast, Gram-positive bacteria, including *Staphylococcus aureus* and *Staphylococcus epidermidis*, exhibited the highest median values and the widest range. This suggests relatively lower susceptibility and higher variability in response. Gram-negative bacteria displayed intermediate activity, with moderate MIC and MBC values and less variability compared to Gram-positive organisms. To statistically validate these observations, a one-way

ANOVA was performed, which confirmed significant differences in MIC and MBC values among the tested species. The analysis revealed that *A. oryzae* exhibited significantly lower MIC/MBC values compared to all bacterial strains ($p < 0.05$), while *S. aureus* and *S. epidermidis* showed significantly higher values. Additionally, demonstrated a markedly higher MBC relative to its MIC, indicating reduced bactericidal susceptibility. These statistical outcomes support the species-dependent differences in antimicrobial response. These findings suggest that Gac fruit extract is particularly effective against fungal pathogens and selected Gram-negative bacteria, while higher concentrations are required to inhibit or kill Gram-positive strains.

The observed antimicrobial pattern can be explained by differences in cell wall and membrane structures among microorganisms. Fungal pathogens, such as *Aspergillus oryzae* and *Candida albicans*, possess cell walls composed primarily of chitin and β-glucans, which may be more susceptible to disruption by phenolic and terpenoid compounds present in plant extracts [23]. Unlike bacteria, fungi lack peptidoglycan, possibly allowing better penetration of these bioactive. Gram-negative bacteria, despite having an additional outer membrane, sometimes exhibit greater susceptibility to plant extracts due to the presence of porins and variability in outer membrane permeability [24]. For instance, *Pseudomonas aeruginosa* and *Enterobacter aerogenes* may be more sensitive due to lower efflux capacity or membrane composition that permits bioactive entry. In contrast, Gram-positive bacteria, such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, possess a thick peptidoglycan layer, which acts as a robust physical barrier and may inhibit the diffusion of antimicrobial compounds [25]. These structural differences, coupled with microbial-specific resistance mechanisms (e.g., efflux pumps, enzymatic degradation), likely account for the higher MIC and MBC values required to inhibit Gram-positive organisms.

The implications of these findings are significant. The observed antimicrobial activity at higher concentrations suggests that Gac fruit extracts contain bioactive compounds capable of inhibiting microbial growth, positioning them as promising candidates for alternative or complementary antimicrobial agents. This is particularly relevant in the context of rising antibiotic resistance and increasing consumer demand for natural products. However, their application must be carefully tailored to the specific microbial threats and contexts, as effectiveness varies depending on the extraction method and the type of microbial inhibition bacteriostatic versus bactericidal. Further purification and optimization could enhance efficacy while reducing the concentration required. Additionally, investigating the underlying mechanisms of action and potential synergistic effects with established antimicrobial agents could facilitate more practical and versatile applications of Gac fruit in food preservation and clinical settings. Ultimately, while this study establishes a solid foundation, a deeper understanding of the factors influencing antimicrobial performance is essential for reliable real-world use.

This study advances previous research on Gac fruit antimicrobial activity in several key ways. Unlike earlier studies that focused solely on crude extracts, we directly compared two processing techniques, fresh paste and freeze-dried powder, to evaluate their impact on the stability of bioactive compounds. Lyophilization was shown to effectively preserve phytochemicals, achieving 21.901 µg GAE/mg of total phenolic content, highlighting its value as a post-harvest technique for maintaining bioactivity with practical implications for industrial and medicinal applications. Furthermore, whereas prior studies primarily reported only MIC values, our work provides a comprehensive MIC/MBC profile against nine clinically relevant opportunistic pathogens, allowing differentiation between bacteriostatic and bactericidal effects, which is critical for therapeutic decision-making, particularly in immunocompromised patients. By including seven bacterial and two fungal species not extensively evaluated in earlier research, our findings further broaden the understanding of Gac fruit's antimicrobial spectrum and offer a more complete picture of its potential applications.

A limitation of the present study is the absence of a standard antibiotic control, which would have enabled direct benchmarking of antimicrobial activity. Nevertheless, comparison with published MIC values provides an indirect reference, allowing an informed assessment of the relative antimicrobial potency of the Gac fruit extract despite the lack of a direct positive control.

CONCLUSION

The antimicrobial investigation of Gac fruit pulp–aril extract demonstrates strong antibacterial and antifungal activity, with efficacy varying markedly among the tested pathogens. The extract was most effective against Aspergillus oryzae, exhibiting the lowest MIC and MBC values, highlighting its considerable antifungal potential at very low concentrations. In contrast, Staphylococcus aureus and Escherichia coli required substantially higher concentrations for inhibition and bactericidal effects, reflecting organism-specific resistance and lower susceptibility. These results further demonstrate that freeze-drying effectively preserves both the phytochemical content and bioactivity of Gac fruit. Overall, the findings underscore the potential of Gac fruit extracts as natural antimicrobial agents, particularly against sensitive fungal pathogens, and provide a foundation for future work on extraction optimization, formulation development, and the

identification of the specific bioactive compounds responsible for the observed antimicrobial properties.

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REFERENCES

1. World Health Organization, W. (2023) Anti-microbial Resistance (AMR). Retrieved from https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.

2. Mishra, N. P., Das, S. S., Yadav, S., Khan, W., Afzal, M., Alarifi, A., Ansari, M. T., Hasnain, M. S. and Nayak, A. K. (2020) Global impacts of pre-and post-COVID-19 pandemic: Focus on socio-economic consequences. *Sensors International*, **1**, 100042.

3. Vuong, L. T. (2000) Underutilized β-carotene–rich crops of Vietnam. *Food and Nutrition Bulletin*, **21(2)**, 173–181.

4. Franke, A. A., Custer, L. J. and Murphy, S. P. (2006) Momordica cochinchinensis Spreng (gac) Fruit carotenoids reevaluated. *Journal of Food Composition and Analysis*, **19(6-7)**, 664–668.

5. Parks, S. E., Murray, C. T., Gale, D. L., Al-Khawaldeh, B. and Spohr, L. J. (2013) Propagation and production of Gac (Momordica cochinchinensis Spreng.), A Greenhouse Case Study. *Experimental Agriculture*, **49(2)**, 234–243.

6. Pinthong, S., Judprasong, K., Tangsuphoom, N., Jittinandana, S., and Naknga manong, Y. (2019) Effect of different drying processes on physical properties and carotenoid content of Gac fruit (Momordica cochinchinensis Spreng.). *Journal of Food Science and Agricultural Technology*, **5**, 61–70.

7. Chuyen, H. V., Nguyen, M. H., Roach, P. D., Golding, J. B. and Parks, S. E. (2015) Gac fruit (Momordica cochinchinensis Spreng.): a rich source of bioactive compounds and its potential health benefits. *International Journal of Food Science & Technology*, **50(3)**, 567–577.

8. Mat, S. A., El Enshasy, H. A., Rahim, N. A., Chong, X. N., Vadiveloo, S. D., Ya'akob, H., Dailin, D. J., Chew, D. S. T. and Abdul Manas, N. H. (2024) Gac fruit (Momordica cochinchinensis Spreng): From nutritional value to food processing technology. *Biocatalysis and Agricultural Biotechnology*, **62**, 103444.

9. Thavamany, P. J., Chew, H. L., Sreeramanan, S., Chew, B. L. and Ong, M. -T. (2020) 'Momordica cochinchinensis' Spreng (Gac fruit): An abundant source of nutrients, phytochemicals, and its pharmacological activities. *Australian Journal of Crop Science*, **14(12)**, 1844–1854.

10. Motong, N., Boonnoun, P., Saksuntor, S., Sooknuan, K. and Thongsri, S. (2022) Determination of Suitable Solvent and Extraction Conditions for Lycopene Isolation from Gac Fruit (Momordica cochinchinensis Spreng.). *Naresuan University Engineering Journal*, **17(1)**, 1–5.

11. Yusoff, I. M., Azelee, N. I. W., Chua, L. S. and Mustafa, R. R. (2024) A comparison of phytoconstituent and functionally loaded low-moisture food from Averrhoa bilimbi using freeze drying and oven drying methods. *Journal of Food Measurement and Characterization*, **18**, 7468–7479.

12. Cole, S. D., Rankin, S. C. and Sykes, J. E. (2021) Isolation and identification of aerobic and anaerobic bacteria. In *Greene's Infectious Diseases of the Dog and Cat*, *5th ed. W.B. Saunders*, 19–30.

13. Peanparkdee, M. and Iwamoto, S. (2019) Bioactive compounds from by-products of rice cultivation and rice processing: Extraction and application in the food and pharmaceutical industries. *Trends in Food Science & Technology*, **86**, 109–117.

14. Saini, R. K. and Keum, Y. S. (2018) Carotenoid extraction methods: A review of recent developments. *Food Chemistry*, **240**, 90–103.

15. Dai, J. and Mumper, R. J. (2010) Plant phenolics: extraction, analysis, and their antioxidant and anticancer properties. *Molecules*, **15(10)**, 7313–7352.

16. Chemat, F., Vian, M. A., and Cravotto, G. (2012) Green extraction of natural products: Concept and principles. *International Journal of Molecular Sciences*, **13(7)**, 8615–8627.

17. Kubola, J. and Siriamornpun, S. (2011) Phyto-chemicals and antioxidant activity of different fruit fractions (peel, pulp, aril, and seed) of Thai Gac (Momordica cochinchinensis Spreng.). *Food Chemistry*, **127(3)**, 1138–1145.

18. Vadiveloo, S. D., El-Enshasy, H. A., Dailin, D. J., Teong, D. C. S. (2024) Phytochemical compounds, therapeutic effects, and anti-microbial properties of Gac fruit (Momordica cochinchinensis Spreng). *Bioprocessing and Biomass Technology*, **3(2)**, 97–105.

19. Tinrat, S. (2014) Comparison of antioxidant and antimicrobial activities of unripe and ripe fruit extracts of Momordica cochinchinensis Spreng (Gac fruit). *International Journal of Pharmaceutical Sciences Review and Research*, **28(1)**, 75–82.

20. Raposo, F., Borja, R. and Gutiérrez-González, J. A. (2024) A comprehensive and critical review of the unstandardized Folin-Ciocalteu assay to determine the total content of polyphenols: The conundrum of the experimental factors and method validation. *Talanta*, **272**, 125771

21. Tinrat, S., Akkarachaneeyakorn, S. and Singhapol, C. (2014) Evaluation of antioxidant and anti-microbial activities of Momordica cochinchinensis Spreng (Gac fruit) ethanolic extract. *International Journal of Pharmaceutical Sciences and Research*, **5(8)**, 3163.

22. Chanpeng, P. and Khajorncheep, S. (2021) Antibacterial property of Gac fruit aril extract and para rubber film mixed with Gac fruit aril extract. *Journal of Engineering and Innovation*, **14(2)**, 1–9.

23. Chao-Wei, Z., Xiao-Jun, Z., Yun-Shi, Z., Muhammad, S. R. R., Muhammad, H. H., Peng, Z., Xun, S. (2023) Antifungal natural products and their derivatives: A review of their activity and mechanism of actions. *Pharmacological Research - Modern Chinese Medicine*, **7**, 100262.

24. Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R. and De Feo, V. (2013) Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*, **6(12)**, 1451–1474.

25. Barbarossa, A., Rosato, A., Tardugno, R., Carrieri, A., Corbo, F., Limongelli, F., Fumarola, L., Fracchiolla, G. and Carocci, A. (2025) Antibiofilm Effects of Plant Extracts Against Staphylococcus aureus. *Microorganisms*, **13(2)**, 454.

26. Zeleke, B., Mekonnen, Z., Bireda, M., Yitbarek, M. and Dendir, A. (2024) Phytochemical Screening and Antimicrobial Activity of Polygala Sadebeckiana GüRke Extracts on Bacterial Isolates from Wound Samples of Patients with "Shimetere". *BMC Complementary Medicine and Therapies*, **24**, 72.

27. Kamonwannasit, S., Nantapong, N., Kumkrai, P., Luecha, P., Kupittayanant, S. and Chudapongse, N. (2013) Antibacterial Activity of Aquilaria Crassna Leaf Extract against Staphylococcus Epidermidis by Disruption of Cell Wall. *Annals of Clinical Microbiology and Antimicrobials*, **12**, 20.

28. Thongphichai, W., Pongkittiphan, V., Laorpaksa, A., Wiwatcharakornkul, W. and Sukrong, S. (2023) Antimicrobial Activity against Foodborne Pathogens and Antioxidant Activity of Plant Leaves Traditionally Used as Food Packaging. *Foods*, **12(12)**, 2409.

29. Dehghani, M, and Saeidi, S. (2023) Anti-microbial Effects of Medicinal Plant Species on Salmonella typhimurium Strains Isolated from Poultry Feces Samples. *Jentashapir Journal of Cellular and Molecular Biology*, **14(4)**, e135947.

30. Sasidharan, S., Darah, I. and Noordin, M. K. M. J. (2010) In Vitro Antimicrobial Activity against Pseudomonas Aeruginosa and Acute Oral Toxicity of Marine Algae Gracilaria Changii. *New Biotechnology*, **27(4)**, 390–96.

31. Elisha, I. L., Botha, F. S., McGaw, L. J. and 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.

32. Sitthan, V. K., Abdallah, M. S., Nallappan, M., Choi, S. H., Paik, J. H. and Go, R. (2023) Antioxidant and Antibacterial Activity of Different Solvent Extracts of Leaves and Stem of Alyxia reinwardtii Blume. *Malaysian Applied Biology*, **52(6)**, 67–80.

33. Prabhakar, K., Kumar, L. S., Rajendran, S., Chandrasekaran, M., Bhaskar, K. and Sajit Khan, A. K. (2025) Antifungal Activity of Plant Extracts against Candida Species from Oral Lesions. *Indian Journal of Pharmaceutical Sciences*, **70(6)**, 801.

34. Karray, A., Alonazi, M., Smaoui, S., Michaud, P., Soliman, D. and Ben Bacha, A. (2020) Purification and Biochemical Characterization of a New Protease Inhibitor from Conyza Dioscoridis with Antimicrobial, Antifungal and Cytotoxic Effects. *Molecules*, **25(22)**, 5452.