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Influence of Solvent Type, Extraction Time, and Fruit Fraction on Phenolic Yield and Antibacterial Activity of *Momordica cochinchinensis* (Gac) Extracts

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Abstract: Plant-derived phenolic compounds have attracted increasing attention as natural antimicrobial agents due to growing concerns over antimicrobial resistance and consumer demand for safer, naturally sourced alternatives. This study investigated how solvent polarity and extraction duration influence the phenolic content and antibacterial activity of *Momordica cochinchinensis* (gac) extracts obtained from aril, pulp, and seeds. Three solvents (ethyl acetate, 95% ethanol, and water) and three extraction times (24, 48, and 72 h) were evaluated. Total phenolic content (TPC) was quantified using the Folin–Ciocalteu method, and antibacterial activity was assessed against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* through disk diffusion, MIC, and MBC assays. The ethyl acetate seed extract yielded the highest TPC (565 ± 1.15 µg GAE/mL at 72 h) and produced the most bioactive fractions across all sample types. Notably, antibacterial activity peaked at 48 h even though the TPC continued to increase up to 72 h, suggesting that prolonged extraction may reduce the stability or functional availability of certain phenolic constituents. The 48-hour ethyl acetate extracts exhibited broad-spectrum inhibition of both Gram-positive and Gram-negative bacteria, including strains typically more tolerant to plant-derived antimicrobials. Although the phytochemical composition of the aril, pulp, and seeds differs, they all provide meaningful antibacterial effects. The recovery of bioactive compounds from the seed, a commonly discarded byproduct, underscores the potential for valorizing underutilized biomass. These findings highlight the importance of solvent–time optimization and support the development of gac-derived natural antimicrobial agents for future food, cosmetic, and biomedical applications.

Keywords: Pulp, Aril, Seed, Plant-derived antimicrobials, Solvent polarity

INTRODUCTION

Plant-derived phenolic compounds are recognized for their diverse bioactivities, including antioxidant, antimicrobial, and anti-inflammatory effects (1, 2). In recent years, increasing awareness of microbial resistance and consumer demand for natural food preservatives have renewed scientific interest in phenolic compounds as safe and sustainable alternatives to synthetic agents (3, 4).

Momordica cochinchinensis (commonly known as gac) is a tropical fruit native to Southeast Asia rich in carotenoids and polyphenols (5). Distinct fruit parts exhibit unique phytochemical profiles: the aril is rich in carotenoids, the pulp contains abundant flavonoids and phenolics, and the seeds are rich in saponins and bioactive metabolites (6-8). These compositional differences suggest that extraction behavior and biological activity may vary substantially across fruit parts.

Gac fruit is distinguished by exceptionally high carotenoid levels—especially lycopene and β-carotene—sometimes exceeding carrot levels tenfold (9, 10). In addition to carotenoids, gac also contains phenolic acids and flavonoids capable of free-radical scavenging and metal chelation (6), compounds that also contribute to antibacterial activity through membrane disruption and enzyme inhibition (11).

However, previous studies have reported inconsistent results regarding the bioactivities of gac extracts due to variations in solvent polarity, extraction duration, and fruit part used (7). Extraction efficiency and the resulting biological activity are strongly influenced by solvent polarity and extraction time (12). Polar solvents such as ethanol and water typically extract hydrophilic phenolics, whereas mid-polar solvents such as ethyl acetate effectively isolate moderately polar compounds that may exhibit stronger antibacterial potential (13, 14). Extended extraction may increase phenolic yield but may also promote oxidative instability or structural alteration of sensitive compounds, even at ambient temperature, which may reduce biological activity (15).

Accordingly, this study aimed to clarify the relationships among extraction time, solvent polarity, phenolic yield, and antibacterial activity in gac fruit extracts. The objectives were (i) to identify the solvent–time combination with the highest antibacterial activity, (ii) to evaluate the effect of extraction duration on the TPC, and (iii) to examine whether the TPC correlates directly with antibacterial potency.

Although previous studies have reported total phenolic content (TPC) and antibacterial activity of gac extracts, systematic investigations integrating solvent polarity, extraction duration, and fruit fraction (aril, pulp, and seed) within a single experimental framework remain limited. The present study addresses this gap by comparatively evaluating these three key extraction variables and examining their combined influence on phenolic yield and antibacterial potency, with particular emphasis on underutilized fruit fractions.

MATERIALS AND METHODS

Plant Material and Extraction

The fully ripe gac fruits (*Momordica cochinchinensis* Spreng.) were harvested from a local farm in Nakhon Pathom Province, Thailand. The collection was conducted during the peak harvesting season between December 2024 and February 2025. Only fruits exhibiting a uniform dark orange to deep red pericarp, indicating a fully ripe stage, were selected for this study.

Extraction was performed under static conditions with occasional manual agitation to enhance solvent–sample contact for 24, 48, or 72 h at room temperature (25 ± 2 °C). Fresh aril, pulp, and seed samples were chopped into small pieces prior to extraction. Each sample (200 g fresh weight) was immersed in 200 mL of solvent (water, 95% ethanol, or ethyl acetate), ensuring complete solvent coverage of the plant material. After extraction, the mixtures were filtered, and the filtrates were concentrated under reduced pressure and dried to constant weight. Dried extracts were stored at 4 °C until analysis. Extraction yield (%) was calculated as the mass of dried extract relative to the fresh weight of the plant material used for extraction.

Bacterial Strains

The bacterial strains used in this study were *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC6633, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 2785, which are commonly used as reference strains for antimicrobial testing. All strains were recovered from glycerol stocks and subsequently cultured in tryptic soy broth (TSB) before use. Cell density was standardized to 10^8 CFU/mL for all assays.

Determination of Total Phenolic Content (TPC)

TPC was quantified using the Folin–Ciocalteu assay (16) and expressed as μg GAE/mL (mean ± SD, n = 3). Differences in TPC among extraction times within each solvent–fruit fraction group were analyzed using one-way ANOVA followed by Tukey's test ($p < 0.05$).

Antibacterial Assays

Antibacterial activity was evaluated using agar-disk diffusion following CLSI guidelines (17). Extracts were prepared at a concentration of 10 mg/mL (dry extract weight per volume), as this range is widely used for preliminary screening of crude plant extracts and allows for observable inhibition zones without exceeding solvent tolerance limits. Sterile 6 mm disks were loaded with 10 µL of extract and placed on tryptic soy agar (TSA) plates inoculated with 10^8 CFU/mL of each bacterial strain. To verify that inhibitory effects were not caused by the solvents, disks containing 5 % dimethyl sulfoxide (DMSO) and 10 % ethanol were used as negative controls. Although 5 % DMSO stock solutions were used as solvent controls, the final DMSO concentration on agar plates after diffusion was below levels reported to inhibit bacterial growth and produced no observable inhibition zones. The plates were incubated at 37 °C for 24 h, after which inhibition zones were measured.

MIC and MBC values were determined by twofold serial dilutions in 96-well microplates (18) and are expressed as µg/mL of dry extract and were determined by two-fold serial dilution of the same stock solutions used in the disk diffusion assay. The MIC was defined as the lowest extract concentration that inhibited visible growth, and the MBC as the lowest concentration preventing colony formation. Statistical analyses were performed using one-way ANOVA with Tukey's multiple comparison test ($p < 0.05$).

Identification of the Most Active Extract

The solvent–fruit fraction–time combination with the highest antibacterial activity was identified based on inhibition zones, MIC values, and MBC values. The TPC data were compared with antibacterial parameters to determine whether phenolic concentration correlated with antibacterial potency.

Statistical Analysis

All experiments were conducted in triplicate. Data are expressed as mean ± standard deviation (SD). Statistical differences among extraction times within the same solvent–fruit fraction group were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Extraction Yield

The extraction yield varied significantly among solvents and extraction durations ($p < 0.05$). The highest yield was obtained from the water extract of

Table 1. Extraction yield (%) of *Momordica cochinchinensis* extracts obtained using different solvents and extraction durations.

Solvent	Fruit fraction	Yield (%) (mean ± SD)		
		Extraction time 24 h	Extraction time 48 h	Extraction time 72 h
Ethyl acetate	Aril	3.31 ± 0.15^{b}	2.08 ± 0.09^{a}	1.53 ± 0.11^{c}
	Pulp	0.28 ± 0.02^{b}	0.55 ± 0.04^{a}	0.90 ± 0.06^{c}
	Seed	3.98 ± 0.18^{a}	4.41 ± 0.23^{a}	3.85 ± 0.14^{b}
Ethanol (95%)	Aril	1.85 ± 0.08^{a}	2.14 ± 0.11^{a}	3.20 ± 0.19^{b}
	Pulp	2.16 ± 0.13^{b}	3.07 ± 0.21^{a}	2.48 ± 0.04^{b}
	Seed	4.30 ± 0.23^{b}	1.55 ± 0.10^{a}	1.96 ± 0.15^{a}
Water	Aril	3.83 ± 0.16^{b}	2.57 ± 0.11^{a}	4.88 ± 0.17^{c}
	Pulp	3.25 ± 0.14^{a}	3.31 ± 0.13^{a}	7.11 ± 0.31^{b}
	Seed	2.80 ± 0.09^{b}	3.21 ± 0.15^{a}	1.58 ± 0.08^{c}

Note: Values are expressed as the mean ± standard deviations (n = 3). Different superscript letters (a–c) within the same solvent–fruit part group indicate significant differences among extraction times according to one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

the pulp at 72 h (7.11 ± 0.31 %). Water and ethanol generally produced higher yields than ethyl acetate, consistent with their greater ability to extract hydrophilic matrix components. The solvent polarity had a marked influence on the extraction efficiency (Table 1).

Antibacterial Activity

The antibacterial activities of the *Momordica cochinchinensis* extracts are summarized in Table 2. Notably, the ethyl acetate extracts exhibited the strongest inhibitory effects on both Gram-positive and Gram-negative bacteria, whereas the aqueous extracts showed no detectable inhibitory effects. Furthermore, the antibacterial potency increased from 24 to 48 h and then decreased at 72 h, suggesting possible degradation or oxidation of the active phenolics.

At 48 h, the ethyl acetate seed extract displayed the greatest potency, producing inhibition zones of 20.0 ± 1.0 mm against *E. coli* and 13.3 ± 0.6 mm against *S. aureus*, with MICs of 1.6 µg/mL and 3.1 µg/mL, respectively. The aril extract showed comparable activity (*E. coli* 19.0 ± 2.0 mm; MIC = 1.6 µg/mL). The ethanolic extracts demonstrated moderate inhibition (10–14 mm; MIC = 3.1–25 µg/mL), whereas the water extracts remained inactive.

Statistical analysis confirmed that solvent polarity had a significant effect on antibacterial potency, with ethyl acetate consistently yielding stronger inhibition than ethanol and water. Typically, Gram-negative bacteria are less susceptible than Gram-positive bacteria, likely due to differences in cell-wall architecture and permeability to bioactive compounds (19). However, in this case, *E. coli* exhibited pronounced sensitivity to ethyl acetate extracts at 48 h,

Table 2. Antibacterial activity (Zone of Inhibition, MIC, and MBC) of *Momordica cochinchinensis* extracts obtained using different solvents, fruit parts, and extraction times.

Solvent + Fruit Part	Time (h)	*S. aureus*		*B. subtilis*		*E. coli*		*P. aeruginosa*	
		Inhibition Zone (mm)	MIC (µg/mL)/ MBC (µg/mL)	Inhibition Zone (mm)	MIC (µg/mL)/ MBC (µg/mL)	Inhibition Zone (mm)	MIC (µg/mL)/ MBC (µg/mL)	Inhibition Zone (mm)	MIC (µg/mL)/ MBC (µg/mL)
Ethyl acetate + Aril	24	9.8±1^{c}	50/ 100	8.5±0.7^{b}	50/ 100	11±0^{c}	25/ 50	10±1^{c}	25/ 50
	48	20±1^{a}	1.6/ 6.3	11.3±0.6^{a}	25/ 50	19±2^{a}	1.6/ 6.3	14±1^{a}	3.1/ 6.3
	72	9±0^{c}	50/ 100	10.5±0.7^{ab}	25/ 50	9.3±1.5^{bc}	50/ 100	9.3±0.6^{bc}	50/ 100
Ethyl acetate + Pulp	24	11.3±1.2^{b}	25/ 50	7±1^{b}	50/ 100	11.3±1.2^{b}	25/ 50	12.7±1.5^{ab}	3.1/ 12.5
	48	18.3±2.1^{a}	1.6/ 3.1	10±1^{a}	25/ 50	16±1^{a}	3.1/ 6.3	12±2^{ab}	3.1/ 12.5
	72	9.2±0.8^{c}	50/ 100	11.5±0.7^{a}	25/ 50	9±0^{c}	50/ 100	9.3±0.6^{b}	50/ 100
Ethyl acetate + Seed	24	12±0^{b}	12.5/ 25	7.2±0.6^{b}	50/ 100	10.3±0.6^{c}	25/ 50	13±1^{a}	3.1/ 6.3
	48	13.3^{a}±0.6	3.1/ 6.3	9±1^{ab}	50/ 100	20±1^{a}	1.6/ 3.1	10.7±1.5^{ab}	25/ 50
	72	9.7±0.6^{c}	50/ 100	9.5±0.7^{ab}	50/ 100	9±0^{c}	50/ 100	8.2±1.3^{b}	50/ 100
95% Ethanol + Aril	24	10.5±0.7^{bc}	25/ 50	ND	NA	6.5±0.7^{b}	10/ 20	7.5±0.7^{c}	50/ 100
	48	11±1.4^{b}	25/ 50	ND	NA	9.5±0.7^{a}	50/ 100	9±1.4^{b}	50/ 100
	72	13±1.4^{a}	3.1/ 6.3	ND	NA	14±1.4^{a}	3.1/ 12.5	10.5±0.7^{a}	25/ 50
95% Ethanol + Pulp	24	ND	NA	ND	NA	ND	NA	8±0^{c}	50/ 100
	48	ND	NA	ND	NA	ND	NA	9.5±0.7^{b}	50/ 100
	72	11.5±0.7^{a}	25/ 50	ND	NA	ND	NA	12±1.4^{a}	3.1/ 12.5
95% Ethanol + Seed	24	ND	NA	ND	NA	ND	NA	ND	NA
	48	ND	NA	ND	NA	ND	NA	ND	NA
	72	8.5±0.7	50/ 100	ND	NA	ND	NA	ND	NA
Water Extracts (all parts)	24–72	ND	NA	ND	NA	ND	NA	ND	NA

Note: Different superscript letters (a–c) within the same solvent–fruit part group indicate significant differences among extraction times for the same bacterial strain according to one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$). Means sharing at least one common letter (e.g., "a" and "ab") are not significantly different, whereas those with no common letters (e.g., "a" vs. "c") differ significantly. ND = no detectable inhibition; NA = not applicable

indicating that susceptibility varied depending on both bacterial species and extraction conditions.

TPC and Its Relationship to Antibacterial Activity

The TPC increased significantly with extraction duration for all solvent–fruit fraction combinations ($p < 0.05$). Data are expressed as the mean ± SD ($n = 3$) and presented in Table 3, as clearly illustrated in Figure 1. The highest TPC (565 ± 1.15 µg GAE/mL) was obtained from the ethyl acetate seed extract at 72 h. Although the total phenolic content increased with increasing extraction duration, antibacterial activity did not increase proportionally beyond 48 h. This observation suggests that antibacterial potency does not solely depend on total phenolic concentration. Data from Tables 2 and 3 were used to summarize the relationship between TPC and antibacterial efficacy in Table 4.

Table 3. The TPC of the *M. cochinchinensis* extracts expressed as µg gallic acid equivalents (GAE) per mL extract.

Solvent + fruit part	24 h (µg GAE/mL ± SD)	48 h (µg GAE/mL ± SD)	72 h (µg GAE/mL ± SD)
Ethyl acetate + Aril	165.33 ± 1.15^{c}	250 ± 0.0^{b}	260.67 ± 1.15^{a}
Ethyl acetate + Pulp	47.33 ± 1.15^{c}	61.33 ± 1.15^{b}	109.33 ± 1.15^{a}
Ethyl acetate + Seed	360.00 ± 2.00^{c}	470.67 ± 1.15^{b}	565.33 ± 1.15^{a}
95% Ethanol + Aril	84.00 ± 2.00^{c}	96.00 ± 0.00^{b}	140.67 ± 1.15^{a}
95% Ethanol + Pulp	91.33 ± 2.31^{c}	156.00 ± 2.00^{b}	170.00 ± 2.00^{a}
95% Ethanol + Seed	92.67 ± 2.31^{c}	271.33 ± 1.15^{b}	350.67 ± 1.15^{a}
Water + Aril	96.00 ± 2.00^{c}	150.00 ± 0.00^{b}	164.67 ± 1.15^{a}
Water + Pulp	74.00 ± 2.00^{c}	124.67 ± 1.15^{b}	139.33 ± 1.15^{a}
Water + Seed	82.00 ± 2.00^{c}	120.00 ± 2.00^{b}	159.33 ± 1.15^{a}

Note: Different superscript letters (a–c) within the same solvent–fruit part group indicate significant differences among extraction times according to one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

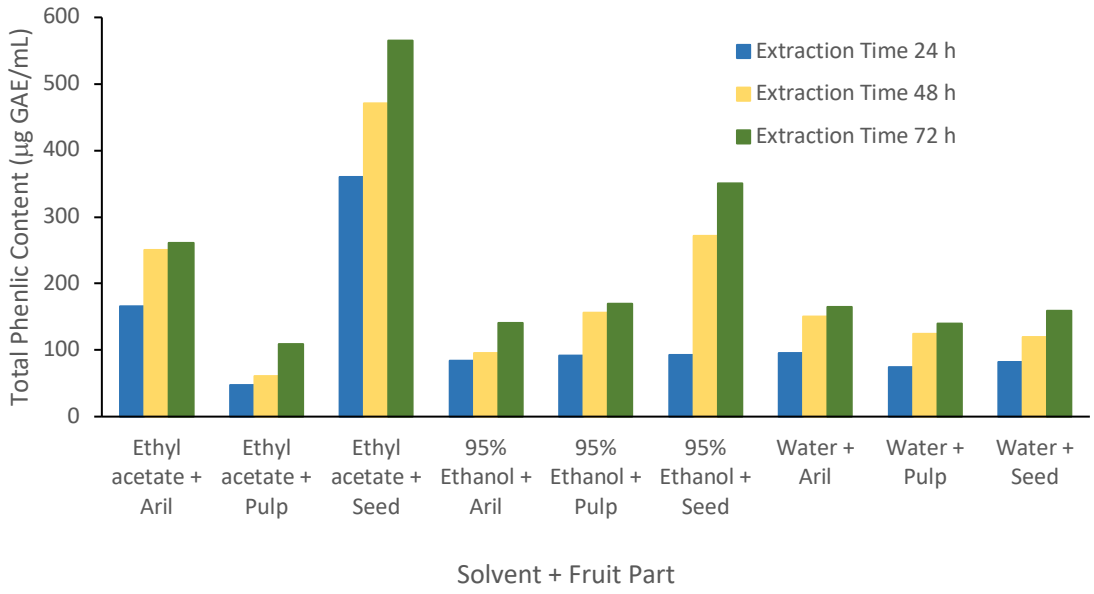

Figure 1. TPC of gac fruit extracts at various extraction times (expressed as µg GAE/mL)

Table 4. Summary of the most active gac (*Momordica cochinchinensis*) extracts and their corresponding TPC and antibacterial parameters

Solvent	Fruit Part	Extraction Time (h)	TPC (µg GAE/mL±SD)	Zone (mm)/ MIC (µg/mL)/ MBC (µg/mL)			
				S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
Ethyl acetate	Aril	48	250 ± 0.0	20±1[a]/ 1.6/ 6.3	11.3[a]±0.6/ 25/ 50	19±2[a]/ 1.6/ 6.3	14±1[a]/ 3.1/ 6.3
Ethyl acetate	Pulp	48	61.33 ± 1.15	18.3±2.1[a]/ 1.6/ 3.1	10±1[a]/ 25/ 50	16±1[a]/ 3.1/ 6.3	12±2[ab]/ 3.1/ 12.5
Ethyl acetate	seed	48	470.67 ± 1.15	13.3[a]±0.6/ 3.1/ 6.3	9±1[ab]/ 50/ 100	20±1[a]/ 1.6/ 3.1	10.7±1.5[ab]/ 25/ 50

Note: Entries are selected time-points showing the strongest antibacterial activity (largest zones and/or lowest MIC) per solvent–fruit part. The TPC values shown correspond to the same time-points; no additional statistical testing was performed for this summary. The full statistics are reported in Tables 2–3.

DISCUSSION

A comparison of TPC and antibacterial activity indicated that *Momordica cochinchinensis* contains antibacterial constituents distributed across the aril, pulp, and seeds, and that the efficiency of their recovery is governed by solvent polarity and extraction duration. Ethyl acetate has been shown to be the most effective solvent, producing bioactive extracts from all fruit parts, in agreement with the established capacity of mid-polarity solvents to solubilize moderately lipophilic phenolics and aglycones associated with antimicrobial mechanisms (12, 20, 21). Although the TPC increased with longer extraction times, the antibacterial activity reached its maximum at 48 h, indicating that extended extraction may favor oxidative or structural alterations that diminish biological function (15, 22). These observations suggest that antibacterial activity is not determined solely by the total quantity of phenolics but is likely influenced by phenolic composition, potential synergistic interactions with non-phenolic constituents, and changes in compound stability during prolonged extraction. Consistent with this interpretation, although aqueous extracts yielded higher extraction efficiencies, they exhibited no detectable antibacterial activity. This discrepancy likely reflects the co-extraction of non-bioactive hydrophilic matrix components in water, which dilute antibacterial constituents, whereas ethyl acetate selectively enriches moderately polar compounds with greater antimicrobial potency despite lower overall yields.

A notable outcome of this work was the consistent inhibition of both Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria by the 48-hour ethyl acetate extracts from all fruit parts. Gram-negative bacteria typically exhibit greater tolerance to plant-derived compounds due to their protective outer membrane (19), yet the extracts overcome this barrier, suggesting the presence of compounds with membrane-permeabilizing or intracellular-targeting capabilities. The phytochemical diversity of gac—including phenolic acids, flavonoids, carotenoids, saponins, and glycosides—provides multiple modes of action that likely contribute to the broad-spectrum activity observed (11, 23, 24). References to these compound classes are based on previously published phytochemical analyses of gac and are cited here to provide a mechanistic context; however, as no compound-level characterization was performed in the present study, these assignments should be regarded as indicative rather than definitive.

Previous phytochemical investigations of *Momordica cochinchinensis* have further documented the presence of these bioactive classes, including phenolic acids, flavonoids, saponins, and carotenoid-related compounds, identified using chromatographic techniques such as GC-MS, HPLC, and LC-MS. In particular, gac seeds have been shown to contain higher levels of saponins and moderately polar secondary metabolites compared with the aril and pulp, many of which have been associated with antibacterial mechanisms such as membrane disruption, increased permeability, and inhibition of intracellular enzymes. The pronounced antibacterial

activity observed for the ethyl acetate seed extracts at 48 h in the present study is therefore consistent with these earlier chemical profiling reports, suggesting that mid-polar solvents preferentially recover antibacterial constituents from the seed fraction. Nevertheless, as compound-level identification was not performed in this study, the contribution of individual metabolites should be regarded as literature-supported and warrants confirmation through targeted chemical analyses in future work.

In addition to their biological properties, seed extracts are particularly relevant from a sustainability perspective. Seeds are typically discarded during processing, yet the present findings demonstrate that they contain recoverable bioactive compounds with measurable antibacterial activity. Their utilization supports circular bioeconomic approaches by transforming underused biomass into functional value-added ingredients (25). Taken together, these results emphasize that optimized extraction—particularly with ethyl acetate for 48 h—enables effective recovery of bioactive constituents from all major fruit parts and underscores the potential of gac as a natural source of antibacterial agents.

Despite the clear trends observed in extraction efficiency and antibacterial activity, it should be acknowledged that total phenolic content was used only as a global indicator of phenolic recovery, and no chromatographic profiling (e.g., HPLC or LC-MS) was performed in the present study. Therefore, interpretations regarding specific phenolic acids, flavonoids, carotenoids, or saponins are based on previous reports on gac phytochemistry and should be regarded as tentative. Future studies incorporating targeted chemical profiling will be necessary to identify the dominant antibacterial constituents and clarify structure–activity relationships.

CONCLUSION

This study revealed that optimized extraction conditions are essential for recovering antibacterial constituents from *Momordica cochinchinensis*. Ethyl acetate extraction for 48 h provided a favorable balance between phenolic stability and bioactivity, yielding extracts from the aril, pulp, and seeds capable of inhibiting both Gram-positive and Gram-negative bacteria. These results confirm that the antimicrobial potential of gac is distributed across multiple fruit parts and is not determined by TPC alone. These findings provide practical guidance for optimizing solvent extraction conditions in the development of gac-derived natural antibacterial agents for food, cosmetic, and biomedical applications.

The recovery of bioactive compounds from the seed—a commonly discarded byproduct—also highlights an opportunity to convert underutilized biomass into natural antimicrobial ingredients, supporting sustainable resource management. Future research should characterize individual active metabolites, evaluate their mechanisms and stability, and explore their applications in food, cosmetic, and biomedical systems.

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