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ORIGINAL PAPER

Antibacterial activity, phytochemical profiling, and toxicity evaluation of green extracts from *Garcinia Mangostana* pericarp for food antibacterial applications

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Abstract

This study investigated the antibacterial activity, phytochemical composition, and safety of *Garcinia mangostana* pericarp (GMP) extracts obtained using aqueous or ethanolic solvents via maceration–filtration or maceration–centrifugation–filtration. Extracts were tested against a panel of 13 bacterial strains, foodborne pathogens, spoilage bacteria, and food-contamination-associated microorganisms and analysed for phenolic and flavonoid content, gas chromatography–mass spectrometry (GC/MS) profiles, and toxicity to *Artemia salina*. Ethanolic GMP extract from maceration–filtration (GMPEF) inhibited all bacteria except *K. pneumoniae* and *S. sonnei*, showing the lowest MIC values (0.70–2.81 mg/mL), the highest extraction yield of 0.129 ± 0.002 g/100 g DW (w/w), and total phenolic content (238.44 ± 0.79 mg GAE/g DW). The upper fraction of ethanolic extract from maceration–centrifugation–filtration (GMPECA) had the highest flavonoid content (4.98 ± 0.001 mg QE/g DW). The GC/MS identified 33 putative compounds (≥90% match to the NIST 14 library), including β-sugars, phenolics, and stigmasterol, linked to antibacterial potency. All extracts were non-toxic to *A. salina* (LC_{50} > 1 mg/mL); aqueous extracts generally showed higher LC_{50} values, suggesting a greater safety margin. Principal Component Analysis (PCA) revealed correlations between specific phytochemicals and antibacterial performance, providing a possible chemical basis for selecting extracts. The GMPEF is well-suited in contexts where higher antibacterial activity is desired, and solvent removal is manageable, whereas GMPWCB offers a safer alternative when prioritising a conservative safety profile. While these findings indicate the potential of GMP extracts as sustainable, low-cost antibacterial agents from fruit by-products, further validation in mammalian systems and real food matrices is needed before considering practical applications.

Keywords *G. mangostana* pericarp · Antibacterial properties · Maceration and filtration · Maceration-filtration-centrifugation · Bioactive compounds

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Introduction

Garcinia mangostana, commonly known as mangosteen, belongs to the genus *Garcinia* and the family *Clusiaceae*. Native to Indonesia, it is widely cultivated in Southeast Asia, including Malaysia, Thailand, and Myanmar [1]. Often referred to as the "queen of fruits" for its luscious white flesh, mildly acidic–sweet taste, and popularity among tropical fruits [2], *G. mangostana* generates about 17 million tonnes of dark purple or reddish pericarp waste globally each year [3], which constitutes 60% of the fruit's weight [4] and is rich in bioactive compounds, but is usually discarded. Traditionally, the pericarp has been used to treat diarrhoea; however, its antibacterial potential remains underexplored in modern applications, leading to both environmental pollution and economic loss. Although previous studies have confirmed antibacterial activity against common pathogens such as *Staphylococcus aureus* and *Escherichia coli*, few have evaluated the effects of *Garcinia mangostana* pericarp (GMP) extract against a broad range of foodborne pathogens, food spoilage bacteria, and food-contamination-associated microorganisms. In this study, the tested bacteria comprised: (1) Gram-positive bacteria, namely *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Clostridium perfringens*, and *Listeria monocytogenes*; (2) Gram-negative foodborne bacteria, namely *Escherichia coli*, *Salmonella enterica* serovar Enteritidis, *Salmonella enterica* serovar Typhimurium, *Shigella sonnei*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*; and (3) food-contamination-associated Gram-negative bacteria, namely *Klebsiella pneumoniae* and *Proteus mirabilis*. Although *Klebsiella pneumoniae* and *Proteus mirabilis* are not recognised as primary foodborne pathogens or food spoilage organisms, *Klebsiella pneumoniae* has been sporadically isolated from food, e.g. chicken meat and salad [5], while *Proteus mirabilis* had been isolated from duck meat in China and dairy products in Egypt and India, commonly associated with poor hygiene and post-processing contamination [6]. Therefore, this study assessed the antibacterial activity of the GMP extract against a panel of 13 bacterial strains selected to represent foodborne pathogens, spoilage bacteria, and food-contamination-associated microorganisms, to evaluate the broad-spectrum antibacterial potential of the extract. In addition, valorising GMP, a fruit by-product typically discarded as waste, offers a sustainable approach that supports both food safety innovation and environmental sustainability [7].

Conventional extraction methods for GMP, such as Soxhlet, often use toxic solvents (e.g., methanol, acetone) that compromise food safety, require costly purification (e.g., distillation), and limit industrial scalability [8]. These solvents may also exhibit inherent antibacterial properties, which can complicate the interpretation of results. In response, researchers are increasingly favouring aqueous and ethanol solvents, along with green extraction methods such as maceration, microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE). Maceration offers energy efficiency and preserves heat-sensitive antibacterial compounds, whereas MAE can cause thermal degradation, and SFE demands costly high-pressure CO_2 systems. Filtration and centrifugation are also reported to enhance the recovery of antibacterial compounds from plants [9]. Here, we used aqueous and ethanol solvents, along with maceration, filtration, and centrifugation, to obtain GMP extracts.

The pericarp contains at least 50 bioactive compounds, including xanthones, catechins [10], flavonoids, tannins, saponins [1], procyanidins, and benzophenones [11], which contribute to its antibacterial properties [12] through mechanisms such as membrane disruption and concentration-dependent effects [13]. While bioactive diversity is well documented, the compounds responsible for antibacterial activity remain poorly characterised. Previous GC/MS studies focused on volatile constituents. In this work, we employed GC/MS with derivatisation to enhance the volatility of non-volatile compounds, thereby enabling cost-effective profiling compared to Liquid Chromatography – Mass Spectrometry (LC/MS), which often requires expensive chemical standards for compound validation [14].

An effective antibacterial agent should combine low toxicity with sufficient activity against target pathogens while maintaining chemical and physical stability. Thus, both ethanolic and aqueous GMP extracts require toxicity testing, as extraction, filtration, and centrifugation can influence production cost and consumer safety. Invertebrate assays such as the *A. salina* lethality test offer an alternative to rodent models, aligning with animal welfare principles [15]. We employed *A. salina* in this preliminary study because of its low cost and its strong correlation with in vitro mouse toxicity data [16]. However, it was used only as a rapid screen for general cytotoxicity, not as a definitive measure of mammalian or food safety [17].

The originality of this study lies in its combination of an expanded microbial panel comprising 13 foodborne pathogens, food spoilage bacteria, and food-contamination-associated microorganisms; a novel pairing of green solvent extraction with sequential maceration–filtration–centrifugation; and derivatised GC/MS profiling to capture both volatile and non-volatile compounds. Additionally, the integration of the *A. salina* lethality assay as a preliminary toxicity screen provides an early safety perspective, which has rarely been incorporated into similar studies on GMP extracts.

Furthermore, Principal Component Analysis (PCA) was applied as a multivariate statistical tool to explore correlations between antibacterial activity, phytochemical composition, and toxicity, providing a holistic view of how chemical constituents relate to bioactivity.

Most previous studies on *G. mangostana* extracts have primarily focused on antibacterial activity for medical, pharmaceutical, and cosmetic purposes. However, limited research has investigated their effects on food-associated microorganisms, although such pathogens are responsible for foodborne illnesses and spoilage that threaten food safety and quality. Hence, this study aimed to (1) evaluate the antibacterial properties of GMP extracts prepared using green solvents against 13 bacterial strains, (2) characterise their chemical composition using derivatised GC/MS, (3) assess their preliminary toxicity through the *A. salina* lethality assay, and (4) apply PCA to identify correlations between antibacterial activity, phytochemical composition, and toxicity. The findings aim to provide a comprehensive understanding of the bioactivity and safety of GMP extracts, supporting their potential development as safe and effective food preservatives derived from plant by-products.

Materials and methods

Experimental design

This study followed the experimental workflow shown in Fig. 1. The pericarp of *G. mangostana* was extracted separately with ethanol and water via maceration–filtration, producing crude ethanolic (GMPEF) and aqueous (GMPWF) extracts. GMPEF was further processed by maceration–filtration–centrifugation, generating an upper fraction (GMPECA) and a lower fraction (GMPECB). The same procedure applied to GMPWF yielded GMPWCA (upper) and GMPWCB (lower) fractions.

The antibacterial activity of all six extracts (GMPEF, GMPWF, GMPECA, GMPECB, GMPWCA, GMPWCB) was evaluated using the Disc Diffusion Test (DDT) and Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays.

Phytochemical characterisation included total phenolic content (TPC), total flavonoid content (TFC), and putative phytochemical profiling via gas chromatography–mass spectrometry (GC/MS). Toxicity screening was performed using the *A. salina* lethality assay to assess general cytotoxic potential. To explore relationships between antibacterial activity, phytochemical profile, toxicity, and extraction method, Principal Component Analysis (PCA) was conducted.

Plant material

G. mangostana fruits were purchased from Desaru Fruit Farm, Kota Tinggi, Johor, Malaysia. The plant material was taxonomically authenticated by the Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia, and a voucher specimen was deposited under voucher number KM0249/25. The pericarps were separated from the fruit, washed three times with tap water, and freeze-dried for 72 h (Christ Alpha, Germany). The dried pericarps were ground into a fine powder using an 800 g capacity grinder (China) and stored in a tightly sealed container, protected from light, until use.

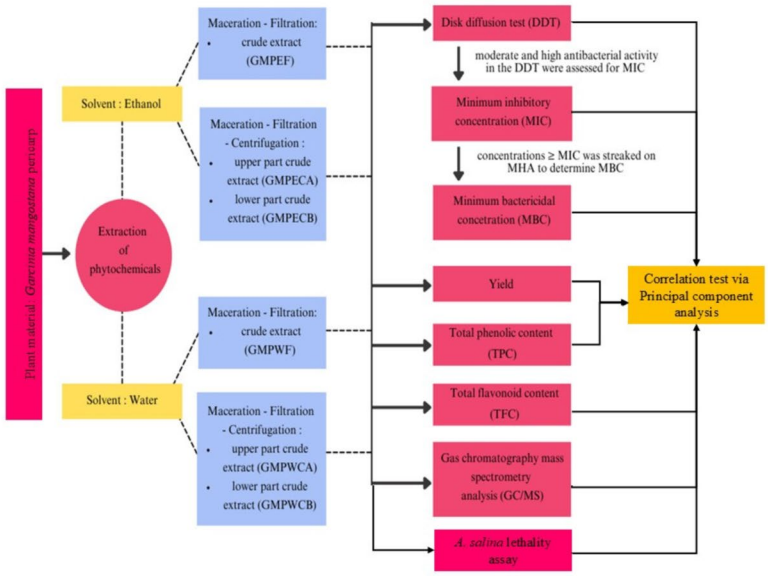

Fig. 1 Experimental design for the evaluation of antibacterial activity, phytochemical profiling, toxicity, and correlation analysis of *Garcinia mangostana* pericarp extracts

Extraction of phytochemicals

The freeze-dried G. *mangostana* pericarp (GMP) powder was extracted by maceration using ethanol or deionised water [18] with slight modifications. Maceration was selected for its simplicity, low thermal impact, and suitability for heat-sensitive antibacterial compounds [19]. Ethanol and water were chosen as food-grade solvents [20], and the ratio and extraction time were optimised through preliminary trials to maximise yield, clarity, and scalability [21].

Extraction was performed at a 1:20 solid-to-solvent ratio to ensure complete immersion of the pericarp. Briefly, 20 g of freeze-dried GMP powder was placed in a conical flask and mixed with 400 mL of solvent. Two approaches were applied: maceration–filtration and maceration–filtration–centrifugation (Fig. 1). For ethanolic maceration–filtration, GMP powder was macerated in a fume hood at 30 °C for 30 min, while aqueous maceration–filtration was performed in a water bath at 80 °C for 30 min. Both extracts were filtered through filter paper to obtain GMPEF and GMPWF, respectively.

In the maceration–filtration–centrifugation approach, GMPEF and GMPWF were centrifuged at 10,000 rpm for 30 min to separate upper (A) and lower (B) fractions, producing GMPECA and GMPECB from ethanolic extracts, and GMPWCA and GMPWCB from aqueous extracts.

The GMPEF, GMPECA, and GMPECB extracts were concentrated in pre-weighed flat-bottom flasks using a rotary vacuum evaporator at 40 °C, whereas GMPWF, GMPWCA, and GMPWCB extracts were freeze-dried. All dried extracts were stored at − 20 °C until further analysis. Extractions were performed in triplicate. The extraction yield was expressed as grams of dried GMP extract per 100 g of freeze-dried GMP powder (w/w) and calculated using the following formula [22]:

$$Yield\left(\frac{g}{100\,g\,dried\,weight\ (DW)},\ w/w\right) = \frac{Weight\ of\ dried\ GMP\ extract\ (g)}{100\,g\ of\ freeze-dried\ GMP\ powder}$$

Disc diffusion test (DDT)

The antibacterial activity of GMPEF, GMPWF, GMPECA, GMPECB, GMPWCA, and GMPWCB was evaluated using DDT [20] with a slide modification. The DDT was selected as a rapid, recommended method that produces clear, reproducible inhibition zones [21]. A concentration of 0.1 g/mL extract was chosen to optimise agar diffusion and minimise solvent-related artefacts [20].

An amount of 0.1 g of each extract was dissolved in 1 mL of 100% Dimethyl Sulfoxide (DMSO) and filtered through a 0.22 µm polyvinylidene difluoride (PVDF) syringe filter. The test was conducted against 13 bacteria: *Klebsiella pneumoniae* (ATCC 10031), *Bacillus cereus* (ATCC 10875), *Escherichia coli* (ATCC 11229), *Bacillus subtilis* (ATCC 11774), *Proteus mirabilis* (ATCC 12453), *Staphylococcus aureus* (ATCC 12600), *Salmonella enterica* subsp. *Enterica* serovar Enteritidis (ATCC 13076), *Clostridium perfringens* (ATCC 13124), *Salmonella enterica* subsp. *Enterica* serovar Typhimurium (ATCC 13311), *Vibrio parahaemolyticus* (ATCC 17802), *Listeria monocytogenes* (ATCC 19111), *Vibrio vulnificus* (ATCC 27562), and *Shigella sonnei* (ATCC 29930).

The bacterial strains were handled, stored, and subcultured following standard microbiological practices to maintain strain integrity and reproducibility. Stock cultures were preserved at −80 °C in appropriate cryoprotective media and revived by streaking onto selective agar media prior to experimentation. Working cultures were prepared by subculturing single, well-isolated colonies into fresh selective agar media and incubating at 37 °C for 4–16 h, specific to optimal growth conditions for each bacterium. All antibacterial assays were conducted using freshly prepared cultures in the logarithmic growth phase to ensure consistent physiological states across experiments.

Prior to DDT, a loopful of cells from the stock cultures maintained at 4 °C was inoculated into sterile Tryptone Soy Broths (TSB) and incubated at 37 °C for 4–16 h to obtain a 10^6 CFU/mL concentration. A volume of 0.1 mL of inoculum in the TSB was spread-plated on sterile Mueller-Hinton Agar (MHA) using a sterile cotton swab. A 6-mm sterile paper disc was placed on the spread-plated MHA, and 10 µL of the 0.1 g/mL extract solution was loaded onto the disc. A volume of 10 µL of 100% DMSO, serving as a negative control, and 10 mg/mL tetracycline hydrochloride, serving as a positive control, were loaded onto other sterile paper discs. The MHA plates were incubated for 24 h at 37 °C to allow the bacterial growth. The clear zone, indicating inhibition of bacterial growth, was measured, including 6 mm from the diameter of the paper disc [22], after the incubation period. This experiment was repeated three times with three replicates each time.

The inhibition zones were initially assessed against the Clinical and Laboratory Standards Institute (CLSI) guidelines [23] for the bacteria listed therein. However, as the CLSI guidelines do not provide inhibition zone criteria for all bacteria tested in the present study and guidelines are primarily intended for evaluating antibacterial activity against clinical isolates, this study adopted plant extract-based inhibition criteria commonly used for food antibacterial screening. Accordingly, antibacterial activity was categorised as slight (< 9 mm inhibition zone), moderate (9 mm ≤ inhibition zone < 10 mm), and strong (≥ 10 mm inhibition zone) [18]. All GMP extracts were subsequently subjected

to minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays.

Determination of minimum inhibitory concentration identification (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC tests were conducted using the microdilution method in Mueller-Hinton broth [20]. Determination of MIC and MBC provides quantitative confirmation of the inhibitory and bactericidal potency of extracts beyond the qualitative results from disc diffusion. These parameters are widely used benchmarks in food microbiology to compare antibacterial agents, assess dose-dependency, and inform potential application levels in food systems [24].

Extracts showing significant inhibition zones in the disc diffusion test were subjected to MIC determination using the two-fold microdilution method in 96-well microtiter plates. Each well was filled with 100 µL TSB, and 100 µL of 50 mg/mL crude extract in 100% DMSO was added to the first well. A 100 µL serial two-fold dilution was performed from the first to the eleventh well, discarding the final 100 µL from the last well. To achieve final concentrations of 22.50–0.02 mg/mL, 10 µL from each well was replaced with 10 µL of a 10^6 CFU/mL bacterial suspension. Optical density was measured at 600 nm at 37 °C before incubation (T_0) and after 24 h (T_{24}). A 10 mg/mL tetracycline hydrochloride solution in 100% DMSO, incorporated into TSB, served as the positive control. The negative control consisted of TSB dissolved in 100% DMSO, inoculated with the target bacterium without the extract. The MIC was determined as the lowest concentration of antibacterial agent capable of inhibiting the growth of the tested bacterial strain, as determined by a difference in absorbance of zero $= T_{24} - T_0 = 0$, i.e., $T_{24} = T_0$ or $T_{24} < T_0$. The MIC value of the studied GMP extract was expressed as mg/mL. The DMSO concentration was also computed in each well, corresponding with the GMP extract concentration.

A loopful from wells containing concentrations equal to or higher than the MIC was streaked onto MHA to determine the MBC and confirm the MIC. Plates were incubated at 37 °C for 24 h. The MBC was defined as the lowest concentration that produced no visible bacterial growth, indicating complete killing of the culture [25]. All determinations were carried out in triplicate.

Determination of total phenolic content (TPC)

The total phenol content (TPC) of GMP extracts was determined using a modified calorimetry assay with Folin-Ciocalteu [20]. The Folin–Ciocalteu method was chosen due to its robustness, simplicity, and wide acceptance in plant extract analysis, enabling direct comparison with literature values for *G. mangostana* and other fruit by-products [21].

Working standards ranging from 0 to 10 mg/L were prepared by pipetting corresponding volumes of 10 mg/L stock solution of gallic acid into a 5 mL volumetric flask and mixing with 1 mL of 1:10 diluted Folin-Ciocalteu reagent. The mixtures were covered with aluminium foil to protect them from light, vortexed for 10 s, incubated at 30 °C for 5 min, and then mixed with 1 mL of a 10% (w/v) sodium carbonate solution. The mixture was reconstituted to volume with ethanol, vortexed again for 10 s, and then incubated in the dark at 30 °C for 30 min.

The absorbance of the working standards was measured in triplicate using a spectrophotometer (Thermo Fisher Scientific, USA) at a wavelength of 747 nm against ethanol as a blank. A linear equation of the calibration curve was drawn using the working standards and expressed as $y = mx + c$, where y is the absorbance, x is the working standard concentration, m is the slope, and c is the intercept.

To determine the TPC in the extract, 0.05 g of the extract was diluted to 100 mL in a volumetric flask. A 1 mL aliquot of the diluted extract was then combined with 1 mL of 1:10 diluted Folin-Ciocalteu reagent and treated similarly to the working standards, including measurement of its absorbance. The equation of the calibration curve was used to quantify the TPC in the extract and express it as milligrams of Gallic Acid Equivalent (GAE) per gram of Dry Weight (DW). The results were reported as mean values with their corresponding standard deviations.

Determination of total flavonoid content (TFC)

Total Flavonoid Content (TFC) was determined to quantify bioactive flavonoids, which are known to contribute to antibacterial activity through multiple mechanisms. The TFC of GMP extracts was evaluated using a colourimetry assay [21].

Working standards ranging from 0 to 12.5 mg/L were prepared by pipetting appropriate volumes of 12.5 mg/L stock solution of quercetin into a 5 mL volumetric flask, followed by the addition of 0.5 mL of 10 M aluminium chloride solution and 0.5 mL of 1 M sodium acetate solution. The mixtures were marked to volume with ethanol, vortexed for 10 s, and wrapped with aluminium foil to prevent light-induced degradation. They were then incubated at 30 °C for 15 min to allow the flavonoids to react with the aluminium chloride solution.

The absorbance of the working standards was measured in triplicate using a spectrophotometer at a wavelength of 422 nm against ethanol as a blank. A linear equation of the calibration curve was drawn using the working standards and expressed as $y = mx + c$, where y is the absorbance, x is

the working standard concentration, m is the slope, and c is the intercept.

To determine the TFC in the extract, 0.05 g of the extract was diluted in a 100 mL volumetric flask. One millilitre of the diluted crude extract was then mixed with 0.5 mL of 10 M aluminium chloride solution and 0.5 mL of 1 M sodium acetate solution in an aluminium foil-wrapped 5 mL volumetric flask and treated similarly to the working standards, including measurement of its absorbance. The formation of a yellow-coloured complex indicated the presence of flavonoids. The TFC in the crude extracts was calculated using the equation of the calibration curve and expressed as milligram Quercetin Equivalent per gram Dry Weight (mg QE/g DW). The results were reported as mean values with their corresponding standard deviations.

Gas chromatography mass spectrometry (GC/MS) analysis

Gas chromatography–mass spectrometry (GC/MS) was used to profile phytochemicals and relate chemical composition to antibacterial effects. Derivatisation enabled the detection of non-volatile and thermolabile compounds in GMP extracts [14].

A quantity of 0.01 g of each GMPEF, GMPWF, GMPECA, GMPECB, GMPWCA, and GMPWCB extracts was derivatised with 0.5 mL of N, O-bistrimethylsilyl trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) in a 99:1 ratio. An additional 0.5 mL of anhydrous pyridine was added to create a mixture with a 0.01 g/mL concentration. The mixture was then heated at 60 °C for 30 min before injection into the GC/MS.

The analysis was conducted using an Agilent Technologies 7890 A GC system coupled with an Agilent Technologies 5975 mass selective detector. A volume of 1 µL of extract was injected into a 240 °C GC injector at a 10:1 ratio in split mode. The volatile extracts were carried by helium at 1.2 mL/min to the column.

The bioactive compounds in the extracts were separated using a 5%-phenyl-methylpolysiloxane (HP-MS) capillary column. The temperature programme consisted of an initial oven temperature of 70 °C, held for one min, followed by a ramp of 15 °C/min for 15 min, and then a further increase of 15 °C/min for 30 min to reach 300 °C.

The separated compounds were eluted into the MS detector via a 230 °C transfer line. The mass spectra were acquired in electron ionisation mode with an ionisation energy of 70 eV and a mass range detection of m/z 50–550. The mass spectra of each detected compound were compared to the mass fragmentation patterns of standards from the National Institute of Standards and Technology (NIST) 2014. Compounds showing ≥ 90% similarity were considered as putative identifications. These putatively identified compounds were then cross-referenced with the literature to explore their reported antibacterial activities.

Artemia Salina lethality assay

A brine shrimp (*A. salina*) lethality assay was assessed as a low-cost screen that correlates with general cytotoxicity. Interpretation was limited to screening purposes [17]. The assay was conducted to evaluate the toxicity of the GMP extracts using brine shrimp larvae [16]. The 50 mg extracts were dissolved in 1 mL DMSO to make stock extracts. The 50 mg/mL stock extracts were diluted with 0.021 g/mL artificial salt water and 1% (v/v) Tween 80 to prepare a series of seven working GMP extracts at concentrations ranging from 0.001 mg/mL to 10 mg/mL. A 1 mg/mL stock of vincristine sulphate in DMSO was diluted with artificial salt water to prepare a series of seven working positive controls at concentrations ranging from 0.001 mg/mL to 1 mg/mL. The 0.021 g/mL artificial seawater containing 1% (v/v) Tween 80 was prepared as the negative control.

A. salina eggs (Aquamaster, Malaysia) were hatched in 0.021 g/mL artificial seawater. After a 24-hr incubation period at 30 °C, 15 mL of a 4.86% yeast solution was added to the hatching chamber to feed the nauplii and left for another 24-hr incubation period at 30 °C. After hatching and maturation of *A. salina*, 30 nauplii were collected and placed into individual working extracts, positive and negative control vials, which were then incubated for another 24 h at 30 °C. The number of dead nauplii in each vial, including the negative control, was examined and counted. The percentage mortality at each working extract, positive and negative control concentrations, was computed as follows:

$$\%\,Mortality = \left[\frac{(number\ of\ nauplii\ death\ in\ working\ GMP\ extract\ vial)}{Total\ nauplii\ in\ vial}\right] \times 100$$

A plot of percentage mortality versus working GMP extract concentrations was established. The Lethal Concentration 50% (LC_{50}) and its 95% confidence interval were determined by probit analysis using XLSTAT 2025 (Lumivero, USA), following established toxicological procedures [26]. Mortality of *Artemia salina* nauplii was recorded for each concentration of GMP extract, including negative and positive controls. Percentage mortality was calculated and corrected for background mortality in the negative control using Abbott's formula [27]. To avoid infinite probit values at 0% and 100% mortality, extreme responses were adjusted using the Miller–Tainter correction [28]. Corrected mortality proportions were transformed into probit units and regressed against $\log_{10}$-transformed GMP extract concentrations. LC_{50} values were estimated at probit 5, and 95% confidence

intervals and range were derived from the regression standard error. The detailed determination of LC_{50} was highlighted in Supplementary 1. All assays were conducted in triplicate.

Statistical analysis

One-way analysis of variance (ANOVA)

Disc diffusion testing (DDT), total phenolic content (TPC), and total flavonoid content (TFC) data were expressed as means ± standard deviations. XLSTAT 2025 statistical software (Addinsoft, Paris, France) was employed to conduct a one-way analysis of variance (ANOVA) with Tukey's post hoc test to assess significant differences between the means for DDT, TPC, and TFC at a 95% confidence level ($p < 0.05$).

Principal component analysis (PCA)

Principal component analysis (PCA) was applied using XLSTAT 2025 statistical software (Addinsoft, USA) to integrate antibacterial, phytochemical, and toxicity data, enabling visualisation of relationships and identification of key contributing variables in this present study.

The dataset was arranged into antibacterial and phytochemical variables and toxicity. For the antibacterial variables, the DDT, MIC and MBC values for fifteen tested microorganisms were assigned accordingly. For instance, DDT, MIC and MBC for *B. cereus* were assigned as DDTBC, MICBC and MBCBC, respectively. All phytochemical variables were designated as Compound 1 (C1) until C33.

Missing values and outliers were identified and treated, followed by the Johnson transformation and the Shapiro-Wilk test, which were applied at a significant level (α) of 0.05 to ensure the dataset meets normality requirements. Pareto scaling was employed across the antibacterial and phytochemical variables to reduce the impact of variations between variables while maintaining the overall structure. Subsequently, the Kaiser-Meyer-Olkin (KMO) test was conducted to assess the adequacy of the dataset for PCA, evaluating the inter-variable and inter-sample correlations. The KMO with a value of < 0.5 indicated that the variables did not share sufficient common variance, suggesting that partial correlations among them were relatively high. Hence, PCA was used for exploratory analysis, as the dataset did not support a strong latent-factor structure.

The dataset was transformed into independent variables known as principal components (PCs) during PCA. The cumulative variance explained by the first two principal components, PC1 and PC2, was calculated to identify the significant variables contributing to each component. Variables were categorised based on their factor loadings (FL), where strong FL ($FL \geq 0.750$), moderate FL ($0.500 \leq |FL| \leq 0.740$), and weak FL ($FL \leq 0.499$) [19].

Since the KMO for the first PCA was < 0.5, another PCA was performed on variables with strong and moderate FL to investigate an improved variance explained by each PC1 and PC2, and the variance explained by the new PCA was reported. The relationships among variables were illustrated in a variable plot, while the variables' contributions to the different extraction treatments were illustrated in a biplot.

Result and discussion

Antibacterial assessment of disc diffusion test (DDT)

The disk diffusion test (DDT) was used to evaluate the antibacterial activity of GMPEF, GMPWF, GMPECA, GMPECB, GMPWCA, and GMPWCB extracts against Gram-positive bacteria (Table 1). The inhibition zones were initially assessed against the Clinical and Laboratory Standards Institute (CLSI) guidelines [23] for the bacteria listed therein. However, as the CLSI guidelines do not provide inhibition zone criteria for all bacteria tested in the present study and are primarily intended for evaluating antibacterial activity against clinical isolates, this study adopted plant extract-based inhibition criteria commonly used in food antibacterial screening [20]. Table 1 shows the inhibition zones of the GMP extracts against Gram-positive and Gram-negative bacteria, where the GMP extracts show slight antibacterial activity (inhibition zone < 9 mm) and strong antibacterial activity (inhibition zone ≥ 10 mm) [20]. No GMP extract had moderate antibacterial activity (9 mm ≤ inhibition zone < 10 mm). GMPEF had strong antibacterial activity against 11 tested bacteria, followed by 10 and nine tested bacteria by GMPECA and GMPECB, respectively. Meanwhile, GMPWCB, GMPWF, and GMPWCA exhibited strong antibacterial activity against nine, eight, and five tested bacteria, respectively. These results indicated that ethanolic extracts demonstrated greater antibacterial efficacy than aqueous extracts. This difference is likely due to ethanol's dual polar and non-polar properties, which enhance the solubility of antibacterial compounds, whereas water's high polarity limits its ability to extract non-polar compounds [29].

All GMP extracts exhibited strong antibacterial activity against Gram-positive bacteria, with the exception of GMPWCA, which showed no inhibitory effect against *B. subtilis*. Among the ethanolic extracts, the GMPEF exhibited the largest inhibition zones for *B. cereus*, *B. subtilis* and *L. monocytogenes*, followed by GMPECA. This result also

Table 1 Inhibition zone of *G. mangostana* Peel extracts on Gram-positive and Gram-negative bacteria using different solvents and extraction methods

Tested bacteria	Inhibition zone on Gram-positive and Gram-negative bacteria[2,3,4] (mm)							
	Ethanolic extract			Aqueous extracts			Controls	
	GMPEF	GMPECA	GMPECB	GMPWF	GMPWCA	GMPWCB	Positive control	Negative control
Gram-positive								
B. cereus	$22.00 \pm 7.00^{a}C$	$14.67 \pm 3.32^{bc}C$	$20.33 \pm 2.00^{a}C$	$11.33 \pm 1.50^{c}C$	$15.67 \pm 3.18^{b}C$	$15.33 \pm 1.00^{b}C$	$23.44 \pm 10.81^{a}C$	na
B. subtilis	$22.33 \pm 6.58^{a}C$	$17.00 \pm 3.42^{b}C$	$18.00 \pm 5.33^{ab}C$	$14.67 \pm 2.00^{b}C$	na	$14.67 \pm 2.80^{b}C$	$31.89 \pm 15.29^{a}C$	na
C. perfringens	$23.67 \pm 7.26^{a}C$	$23.67 \pm 6.92^{a}C$	$19.33 \pm 4.5^{ab}C$	$14.33 \pm 2.32^{bc}C$	$18.33 \pm 3.65^{bc}C$	$14.00 \pm 1.00^{c}C$	$22.11 \pm 4.08^{a}C$	na
L. monocytogenes	$21.33 \pm 4.00^{a}C$	$18.33 \pm 3.50^{ab}C$	$18.00 \pm 6.12^{ab}C$	$16.00 \pm 7.24^{b}C$	$19.00 \pm 2.50^{ab}C$	$15.67 \pm 2.00^{b}C$	$33.11 \pm 10.03^{a}C$	na
S. aureus	$14.67 \pm 3.32^{c}C$	$27.33 \pm 3.00^{a}C$	$24.33 \pm 2.80^{ab}C$	$23.00 \pm 7.06^{b}C$	$14.00 \pm 1.87^{c}C$	$15.00 \pm 2.10^{c}C$	$32.11 \pm 8.37^{a}C$	na
Gram-negative								
E. coli	$15.00 \pm 1.87^{a}C$	$12.00 \pm 1.87^{b}C$	na	na	na	$10.67 \pm 1.5^{c}C$	$24.44 \pm 4.56^{a}C$	na
S. Enteritidis	$12.33 \pm 1.50^{a}C$	na	na	na	na	$7.33 \pm 3.00^{b}A$	$23.44 \pm 3.50^{a}C$	na
S. sonnei	na	na	na	na	na	na	$14.67 \pm 17.56^{a}C$	na
S. Typhimurium	$13.00 \pm 2.50^{b}C$	$14.67 \pm 1.50^{a}C$	$14.67 \pm 1.50^{a}C$	na	na	na	$27.11 \pm 5.88^{a}C$	na
V. parahaemolyticus	$17.67 \pm 4.28^{ab}C$	$17.33 \pm 2.80^{ab}C$	$15.67 \pm 4.61^{ab}C$	$14.33 \pm 2.80^{b}C$	na	$20.67 \pm 8.05^{a}C$	$31 \pm 13.39^{a}C$	na
V. vulnificus	$25.00 \pm 3.30^{a}C$	$18.00 \pm 1.00^{b}C$	$13.00 \pm 2.73^{c}C$	$18.33 \pm 4.50^{b}C$	$10.33 \pm 1.50^{d}C$	$18.67 \pm 2.32^{b}C$	$30.78 \pm 15.28^{a}C$	na
K. pneumoniae	$7.00 \pm 0.60^{c}A$	$7.67 \pm 1.56^{b}A$	$7.67 \pm 1.56^{b}A$	$8.00 \pm 0.87^{b}A$	$8.00 \pm 0.78^{b}A$	$7.00 \pm 0.77^{c}A$	$34.00 \pm 1.32^{a}C$	$7.00 \pm 0.5^{c}A$
P. mirabilis	$12.67 \pm 2.00^{bc}C$	$13.67 \pm 2.32^{ab}C$	$11.33 \pm 1.5^{cd}C$	$10.33 \pm 4.28^{d}C$	na	$15.33 \pm 1.50^{a}C$	$21.33 \pm 3.08^{a}C$	na

[1]GMPWF: *G. mangostana* pericarp extract using aqueous as solvent and filtration method; GMPWCA: *G. mangostana* pericarp extract using aqueous as solvent and centrifuge method (upper part); GMPWCB: *G. mangostana* pericarp extract using aqueous as solvent and centrifuge method (lower part); GMPEF: *G. mangostana* pericarp extract using ethanol as solvent and filtration method; GMPECA: *G. mangostana* pericarp extract using ethanol as solvent and centrifuge method (upper part); GMPECB: *G. mangostana* pericarp extract using ethanol as solvent and centrifuge method (lower part); Positive control: 10 mg/mL tetracycline hydrochloride; and Negative control: 100% DMSO

[2]Means ± SD were calculated from triplicate data. Different superscripts demonstrate a significant difference between extracts in the inhibitory zone ($p < 0.05$)

[3]na- No antibacterial activity (inhibition zone < 7 mm)

[4]Different capital letters denoted the ranges of antibacterial activity; A: slight antibacterial activity (inhibition zone < 9 mm), B: moderate antibacterial activity (9 mm ≤ inhibition zone < 10 mm), and C: strong antibacterial activity (inhibition zone ≥ 10 mm)

indicated that maceration–filtration of ethanolic extract was more effective than maceration–filtration–centrifugation for Gram-positive bacteria. This may be due to the maceration–filtration retains more antibacterial compounds, whereas centrifugation can expose extracts to shear forces and heat [30], potentially degrading sensitive bioactive compounds [31]. Meanwhile, GMPWCA had larger inhibition zones than GMPWF, indicating that maceration-filtration-centrifugation was more effective for aqueous extraction. Based on the strongest antibacterial activities and the largest inhibition zone, the extract potency against Gram-positive bacteria was ranked as follows: GMPEF > GMPECA > GMPECB > GMPWF > GMPWCB > GMPWCA.

For Gram-negative bacteria (Table 1), based on the strong antibacterial activity of the extracts (inhibition zone ≥ 10 mm), *V. vulnificus* was the most sensitive, while *S. sonnei* was the least susceptible. The inhibition ranking was *V. vulnificus* (all extracts) > *V. parahaemolyticus* (five extracts) > *P. mirabilis* (five extracts) > *S.* Typhimurium (three extracts) > *E. coli* (three extracts) > *S.* Enteritidis (one extract) > *S. sonnei* (none).

The GMPEF showed no inhibitory activity against *Shigella sonnei* and *Klebsiella pneumoniae*; nevertheless, it remained the most potent extract among the ethanolic extracts, exhibiting strong antibacterial activity against six Gram-negative species, with inhibition zones ranging from 12.33 ± 1.50 mm to 25.00 ± 3.30 mm. Conversely, GMPECB was the least potent ethanolic extract, exhibiting strong antibacterial activity against only four Gram-negative bacteria.

Among aqueous extracts, the GMPWF, GMPWCA, and GMPWCB did not inhibit *S. sonnei and S.* Typhimurium. The GMPWCA was the least effective, strongly inhibiting only one Gram-negative *V. vulnificus*, whereas the GMPWCB was the most active aqueous extract, showing strong antibacterial activity against Gram-negative *E. coli*, *V. parahaemolyticus*, *V. vulnificus*, and *P. mirabilis.*

Based on the strong antibacterial activity, the potency ranking of the GMP extracts against the Gram-negative bacteria was as follows: GMPEF (six strains) > GMPECA (five) < GMPWCB and GMPECB (four each) > GMPWF (three) > GMPWCA (one).

The maceration–filtration method used for GMPEF demonstrated superior antibacterial activity against Gram-negative bacteria, exhibiting strong inhibition against 11 of the 13 tested strains, with the exceptions of *S. sonnei* and *K. pneumoniae*. In contrast, the maceration–filtration method applied to GMPWF showed lower antibacterial efficacy than the maceration–filtration–centrifugation approach used for GMPWCB. The enhanced inhibitory activity of GMPWCB against a broader range of bacteria may be attributed to the enrichment of denser or more aggregated polar compounds in the lower fraction following centrifugation. This fractionation effect has been reported as a useful strategy for concentrating bioactive compounds, including anthocyanins, which are associated with antibacterial activity [29].

Overall, Gram-positive bacteria were more susceptible to GMP extracts than Gram-negative ones. GMPEF was the most effective against both groups, consistent with previous findings reporting strong activity of ethanolic GMP extracts against *E. coli* and *S. aureus* [29]. Variations in inhibition zones between studies may be due to differences in media viscosity, compound diffusion rates, extract concentration, bacterial sensitivity, and test conditions. Gram-positive bacteria's simpler cell wall, mainly peptidoglycan, allows easier penetration of antibacterial agents, whereas Gram-negative bacteria's lipopolysaccharide-rich outer membrane provides greater resistance [32]. As GMP extracts were obtained using polar solvents, i.e. ethanol and water, which preferentially extract polar and moderately polar phytochemicals, this lipopolysaccharide-rich membrane limits the penetration of many hydrophobic antibacterial compounds and thereby confers increased resistance compared with Gram-positive bacteria [33]. Similar patterns have been observed in plant-based studies, such as *C. aurantium* pericarp extracts [34] and *M. domestica* pericarp extracts [35].

Based on Table 1, all tested bacteria were selected for MIC and MBC assays. As previously noted, DDT is a preliminary method, whereas MIC and MBC provide a more quantitative measure of antibacterial potency [24]. In addition, 10 mg/mL tetracycline hydrochloride was employed as a positive control and 100% dimethyl sulfoxide (DMSO) as a negative control to match the vehicle solvent of the tested extracts and to verify the suitability of the DDT for inhibiting the tested bacteria. As this study focused on investigating plant-derived extracts for food antibacterial applications, the use of clinical antibiotics as positive controls may not fully reflect real food preservation conditions. Therefore, future studies are encouraged to employ Generally Recognised as Safe (GRAS) food preservatives, such as nisin [36], potassium sorbate [37], or ethylenediaminetetraacetic acid (EDTA) [38], as positive controls to enhance the relevance of the findings to food systems.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

To validate the DDT findings and obtain quantitative data on extract efficacy, the minimum inhibitory concentrations (MICs) of the GMP extracts were determined. MIC testing was conducted on extracts that showed moderate to high antibacterial activity in the DDT, using thirteen selected microorganisms. A lower MIC value indicates higher antibacterial potency.

As shown in Table 2, S. Typhimurium was the most susceptible microorganism to the ethanolic extracts GMPEF and GMPECA, both with the lowest MIC value of 0.703 mg/mL. The aqueous-based GMPWCB extract also effectively inhibited *S. aureus* at this same MIC. These results indicate that GMPEF was the most effective extract overall, inhibiting bacterial growth at the lowest concentration.

For the ethanol-based GMP extract (GMPEF), indicator microorganisms based on MIC values were *B. cereus* (1.41 mg/mL), *C. perfringens* (1.41 mg/mL), *L. monocytogenes* (1.41 mg/mL), *S. aureus* (1.41 mg/mL), *E. coli* (1.41 mg/mL), and *V. vulnificus* (1.41 mg/mL). Ethanolic GMP extracts (GMPEF, GMPECA, and GMPECB) also showed lower MICs than ethanolic pomegranate peel extract, which recorded 25 mg/mL (*E. coli*), 6.25 mg/mL (*S. aureus*), and 3.125 mg/mL (*S.* Enteritidis and *S.* Typhimurium) [39]. They were also more potent than ethanolic grapefruit peel, with MICs of 3.125 mg/mL (*E. coli*) and 1.56 mg/mL (*S. aureus*) [40]. These findings suggest that ethanolic maceration–filtration–centrifugation can be an economical method for extracting antibacterial compounds from GMP.

The GMPWCB aqueous extract demonstrated the broadest antibacterial spectrum, inhibiting ten bacterial strains at 0.70–22.50 mg/mL, consistent with its strong DDT performance. This was superior to GMPWF and GMPWCA, which inhibited six and four strains at 2.81–22.50 mg/mL and 11.25 mg/mL, respectively. GMPWCB also inhibited *L. monocytogenes* (2.81 mg/mL) and *S. aureus* (0.70 mg/mL) more effectively than previously reported values of 4.17 mg/mL for *S. aureus* and 5.00 mg/mL for *L. monocytogenes* [41].

Furthermore, the GMPWF, GMPWCA, and GMPWCB showed greater antibacterial activity than *G. cambogia* and *G. indica* peel extracts, which recorded higher MICs for *B. subtilis* (6.25 mg/mL and 12.5 mg/mL) and *S. aureus* (12.5 mg/mL and 25.0 mg/mL) [42]. These lower MICs may be due to differences in bioactive compound profiles. However, compared with grapefruit peel aqueous extract, which had MICs of 12.5 mg/mL against *E. coli* and 3.125 mg/mL against *S. aureus* [40], GMPWF and GMPWCA were less potent. Notably, GMPWCB was significantly more potent, with an MIC of 0.70 mg/mL against *S. aureus*, likely due to its retention of more polar antibacterial compounds.

The concentrations of DMSO used in all MIC assays are explicitly stated in Table 2. Although it has been reported that high DMSO concentrations (e.g., 8%) can inhibit bacterial growth [43], other studies have shown that high DMSO concentrations may also inhibit the bactericidal action, while exerting little effect on MIC values [44]. As shown in Table 2, the DMSO concentration was 1.41% at 0.70 mg/mL GMPE and 2.81% at 1.41 mg/mL GMPE, with the same proportional trend across concentrations. Although previous studies have suggested that DMSO concentration may influence MIC determination, the present study measured optical density both before and after incubation to minimise any potential inhibitory effect of DMSO on the tested bacteria.

Table 2 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *G. mangostana* pericarp extracts on bacteria by different extraction approaches and solvents

Microorganisms	MIC and MBC (mg/mL)[1,2,3,4]							
	GMPEF	GMPECA	GMPECB	GMPWF	GMPWCA	GMPWCB	Positive control	Negative control
B. cereus	1.41 (1.41)	1.41 (1.41)	2.81 (2.81)	22.5 (22.5)	11.25 (11.25)	5.63 (5.63)	<0.02 (<0.02)	>22.50 (>22.50)
B. subtilis	2.81 (2.81)	2.81 (2.81)	1.41 (1.41)	nd	nd	5.63 (5.63)	<0.02 (<0.02)	>22.50 (>22.50)
C. diphtheria	2.81 (2.81)	2.81 (2.81)	5.63 (5.63)	nd	nd	2.81 (2.81)	<0.02 (<0.02)	>22.50 (>22.50)
L. monocytogenes	1.41 (1.41)	1.41 (2.81)	1.41 (1.41)	22.5 (22.5)	11.25 (11.25)	2.81	<0.02 (<0.02)	>22.50 (>22.50)
S. aureus	1.41 (1.41)	1.41 (1.41)	1.41 (1.41)	nd	11.25 (11.25)	0.70 (0.70)	<0.02 (<0.02)	>22.50 (>22.50)
E. coli	1.41 (2.81)	1.41 (5.63)	nd	nd	nd	22.5 (22.5)	<0.02 (<0.02)	>22.50 (>22.50)
S. Enteritidis	2.81 (2.81)	nd	nd	nd	nd	nd	<0.02 (<0.02)	>22.50 (>22.50)
S. sonnei	nd	nd	nd	nd	nd	nd	<0.02 (<0.02)	>22.50 (>22.50)
S. Typhimurium	1.41 (2.81)	0.70 (2.81)	22.5 (22.5)	nd	nd	nd	<0.02 (<0.02)	>22.50 (>22.50)
V. parahaemolyticus	2.81 (2.81)	2.81 (5.63)	2.81 (5.63)	22.5 (22.5)	nd	2.81 (5.63)	<0.02 (<0.02)	>22.50 (>22.50)
V. vulnificus	1.41 (1.41)	2.81 (2.81)	22.5 (22.5)	11.25 (22.5)	nd	22.5 (22.5)	<0.02 (<0.02)	>22.50 (>22.50)

[1]nd - Not determined due to slight antibacterial activity in the disk diffusion test

[2]Value in parentheses exhibits the minimum bactericidal concentration (MBC)

[3]GMPWF: *G. mangostana* pericarp extract using aqueous as solvent and filtration method; GMPWCA: *G. mangostana* pericarp extract using aqueous as solvent and centrifuge method (upper part); GMPWCB: *G. mangostana* pericarp extract using aqueous as solvent and centrifuge method (lower part); GMPEF: *G. mangostana* pericarp extract using ethanol as solvent and filtration method; GMPECA: *G. mangostana* pericarp extract using ethanol as solvent and centrifuge method (upper part); GMPECB: *G. mangostana* pericarp extract using ethanol as solvent and centrifuge method (lower part); Positive Control: 10 mg/mL tetracycline hydrochloride dissolved in 100% DMSO and incorporated into TSB; and Negative control: TSB dissolved in 100% DMSO inoculated with the target bacterium, without extract

[4]The DMSO concentration (%) for each GMPE concentration (mg/mL) is as follows: 45.00% for 22.50 mg/mL: 22.50% for 11.25 mg/mL; 11.25% for 5.63 mg/mL; 5.63% for 2.81 mg/mL; 2.81% for 1.41 mg/mL; 1.41% for 0.70 mg/mL; 0.70% for 0.35 mg/mL; 0.35% for 0.18 mg/mL; 0.18% for 0.09 mg/mL; 0.09% for 0.04 mg/mL; and 0.04% for 0.02 mg/mL

In addition, 100% DMSO was included as a negative control, for which the MIC was > 22.50 mg/mL, indicating that the inhibitory effect of DMSO under the experimental conditions was negligible.

The MIC assay for the positive control was performed using the same procedure as for the GMPE samples. To verify the assay's validity, a 10 mg/mL tetracycline hydrochloride solution in 100% DMSO was incorporated into TSB, yielding an MIC of <0.02 mg/mL. These results indicated that the GMPE exhibited higher MIC values than the control. Importantly, the higher MIC values of GMPE suggest its suitability for potential food applications, where excessively low MIC values may not be desirable. Because the present study targeted foodborne pathogens, spoilage bacteria, and food-contamination-associated microorganisms, future studies may benefit from employing food preservatives, such as nisin, potassium sorbate, and disodium ethylenediaminetetraacetate (Na_2EDTA), as positive controls rather than clinical antibiotics, to better reflect food preservation applications. This approach would allow a more relevant comparison of antibacterial efficacy within food preservation contexts.

Table 2 also presents MBC values of the GMP extracts, which ranged from 0.70 to 22.50 mg/mL. The MBC is the lowest concentration of an antibacterial agent capable of killing 99.9% of the bacterial population, while MIC reflects growth inhibition. The GMPWCB showed the lowest MBC against *S. aureus* at 0.70 mg/mL, where the MIC and MBC values were identical, indicating that the extracts could inhibit and kill bacteria at the same concentration, suggesting strong bactericidal effects [25].

While MIC and MBC values provide insights into antibacterial potency, identifying the bioactive compounds responsible is crucial. Variations in MIC among plant extracts are largely due to differences in chemical composition and the volatility of active compounds [45]. Therefore, chemical composition analyses were performed to identify the antibacterial constituents of the GMP extracts.

Extraction yield, total phenolic contents (TPC), and total flavonoid contents (TFC)

Table 3 presents the extraction yield, TPC, and TFC of GMP extracts to explore the chemical constituents potentially responsible for their antibacterial activity. TPC and TFC were determined from the calibration curves of gallic acid ($y=0.1107x+0.0129$, $R^2 = 0.9939$) and quercetin ($y=0.0981x+0.068$, $R^2 = 0.9992$), respectively. Relationships among yield, TPC, and TFC were also examined.

Extraction yields varied with solvent type and extraction procedure. Ethanol extracts yielded significantly more than aqueous extracts. GMPEF had the highest yield (0.129 ± 0.002 g/100 g DW, w/w), followed by GMPWF (0.056 ± 0.016 g/100 g DW, w/w) and GMPECA (0.042 ± 0.002 g/100 g DW, w/w). GMPWCB had the lowest yield (0.024 ± 0.008 g/100 g DW, w/w), not significantly different from GMPECB. These results indicated ethanol was more efficient than water in extracting phytochemicals from GMP.

Extraction yield depends on solvent type, efficiency, and the quantity of soluble components [46]. Solvent polarity plays a major role [36]; ethanol's polarity index is 5.2, while water's is 10.2 [47]. Different polarities extract different phytochemical classes, as no single solvent extracts all compounds uniformly [46]. Despite its higher polarity, water was less efficient than ethanol, supporting findings that moderate polarity solvents extract GMP compounds more effectively [48].

In terms of method, maceration–filtration produced higher yields than maceration–filtration–centrifugation. Although filtration and centrifugation are common in plant extraction [49], a single separation step appears more practical and efficient than two-step processes.

Table 3 also shows the TPC and TFC values. Ethanol was more effective for both phenolics and flavonoids than water. TPC values ranged from 103.87 to 238.44 mg GAE/g DW, with GMPEF having the highest (238.44 ± 0.79 mg GAE/g DW). Compared with earlier reports, these values were lower than those found 238.40 ± 5.48 mg GAE/g DW

Table 3 Extract yield, total phenolic content (TPC) and total flavonoid content (TFC) of *G. mangostana* pericarp extracts

G. mangostana pericarp extracts[1]	Yield (g/100 g dried GMP powder, w/w)[2]	TPC (mg GAE/g DW)[2,3]	TFC (mg QE/g DW)[2,4]
GMPEF	0.129 ± 0.002[a]	238.44 ± 0.79[a]	3.16 ± 0.004[b]
GMPECA	0.042 ± 0.002[c]	161.86 ± 0.24[b]	4.98 ± 0.001[a]
GMPECB	0.026 ± 0.006[d]	122.86 ± 0.05[e]	0.91 ± 0.015[e]
GMPWF	0.056 ± 0.016[b]	103.87 ± 0.08[f]	1.61 ± 0.017[c]
GMPWCA	0.041 ± 0.004[c]	143.89 ± 4.66[c]	0.36 ± 0.017[f]
GMPWCB	0.024 ± 0.008[d]	131.41 ± 0.16[d]	1.19 ± 0.020[d]

[1]GMPWF: *G. mangostana* pericarp extract using aqueous as solvent and filtration method; GMPWCA: *G. mangostana* pericarp extract using aqueous as solvent and centrifuge method (upper part); GMPWCB: *G. mangostana* pericarp extract using aqueous as solvent and centrifuge method (lower part); GMPEF: *G. mangostana* pericarp extract using ethanol as solvent and filtration method; GMPECA: *G. mangostana* pericarp extract using ethanol as solvent and centrifuge method (upper part); GMPECB: *G. mangostana* pericarp extract using ethanol as solvent and centrifuge method (lower part)

[2]Means ± SD was carried out for triplicate measurements. The value with different letters was significantly different ($p<0.05$)

[3]GAE: Gallic acid equivalent; DW: Dry weight; Calibration curve equation for TPC and coefficient determination (R^2) were $y=0.1107x+0.0129$ and $R^2=0.9939$

[4]QE: Quercetin equivalent; DW: Dry weight; Calibration curve equation for TFC and coefficient determination (R^2) were $y=0.0981x+0.068$ and $R^2=0.9992$

for aqueous and 377.01 ± 4.80 mg GAE/g DW for ethanolic GMP [50], but higher than the 112.05 ± 5.27 mg GAE/g DW reported for GMP [51]. Phenolics contribute to bacterial growth suppression due to their partially hydrophobic nature, which allows them to inhibit protease activity and interact with proteins and carbohydrates [52]. Solvent polarity can influence phenolic solubility, thereby affecting yield and bioactivity [53].

For ethanolic extracts, maceration–filtration produced higher TPC than maceration–filtration–centrifugation, likely because all soluble and suspended phenolics were retained. Meanwhile, the introduction of a centrifugation step may lead to the physical partitioning of phenolic compounds [54]. Furthermore, the phenolics may become entrapped, aggregated, or bind to proteins/polysaccharides during centrifugation, resulting in reduced reactivity in the Folin–Ciocalteu assay [55]. These reasons may explain the lower TPC in GMPECA and GMPECB compared with GMPEF.

For aqueous extracts, the trend differed. Filtrates likely contained proteins, sugars, organic acids, and reducing agents that interfered with the Folin–Ciocalteu assay. Centrifugation reduced these interferences, yielding a clearer extract and more accurate phenolic measurement. This was reflected in the higher TPC of GMPWCB (131.41 ± 0.16 mg GAE/g DW) compared with GMPWF (103.87 ± 0.08 mg GAE/g DW). This result is likely due to centrifugation, which was used to separate the plant extracts into distinct phases that may vary in phytochemical composition and bioactivity, as reported for the enrichment of phenolic compounds from citrus peels [56]. Centrifugation also removes insoluble particulates that could interfere with antibacterial testing [57]. Moderate-speed centrifugation (≤ 10,000 rpm) at ambient temperature enhances extract clarity and reproducibility [55], facilitating liquid phase separation based on polarity density, which results in the preferential enrichment of specific polyphenols [58]. The higher TPC in GMPWCB correlated with more potent antibacterial activity in the DDT and lower MIC and MBC values.

For TFC, ethanol extracts ranged from 0.91 ± 0.015 mg QE/g DW to 4.98 ± 0.001 mg QE/g DW, while aqueous extracts ranged from 0.36 ± 0.017 mg QE/g DW to 1.61 ± 0.017 mg QE/g DW. Flavonoids and glycosides are generally more soluble in ethanol [53], which aligns with the higher TFC in ethanol extracts. Flavonoids inhibit nucleic acid synthesis and disrupt microbial metabolism, with antibacterial effects linked to changes in membrane permeability.

Interestingly, GMPECA had the highest TFC (4.98 ± 0.001 mg QE/g DW), while GMPEF, despite its high TPC, had lower TFC. This result suggested phenolic and flavonoid contents do not necessarily correlate. The higher TFC in GMPECA did not translate into stronger antibacterial activity, suggesting that compounds other than flavonoids in the ethanolic extracts may play a greater inhibitory role.

For aqueous extracts, maceration–filtration resulted in higher TFC than maceration–filtration–centrifugation. This may be due to the poor water solubility of certain flavonoids, especially aglycones, which tend to sediment during centrifugation. This explains why GMPWCA had very low TFC (0.36 mg QE/g DW) and GMPWCB retained a moderate amount (1.19 mg QE/g DW), still less than GMPWF (1.61 mg QE/g DW). These TPC results are consistent with the phytochemicals identified in GMP extracts. High TPC may be due to phenolics such as catechin [59] and 3,4-dihydroxymandelic acid [60], both known to disrupt bacterial membranes and induce oxidative stress. Ethanolic extracts rich in flavonoids have also been reported to contain catechin and L-rhamnose glycosides, which enhance the antibacterial activity of aglycones [61]. These findings suggest that the higher TPC and TFC in certain extracts may be linked to specific phenolic and flavonoid compounds that directly contribute to antibacterial effects. This hypothesis is further explored in the next section using GC/MS-based profiling.

Chemical composition of green extracts from *Garcinia Mangostana* pericarp

The chemical composition of all GMP extracts was analysed using gas chromatography–mass spectrometry (GC/MS) to identify potential antibacterial components. As shown in Tables 4 and 33 compounds were identified, each with more than 90% similarity to spectra in the NIST 2014 library spectra. Since these bioactive compounds were putatively identified, further confirmation by authentic standards or retention index comparison is required in future studies. The extracts primarily contained 22 sugars and sugar alcohols, one fatty acid, four phenolic compounds, two sterols, two terpenes, one heterocycle, and one inorganic acid.

Table 4 also lists the number of trimethylsilyl (TMS) derivatives attached to each identified compound. These derivatives were produced by BSTFA–TMCS derivatisation and detected during GC/MS analysis. Derivatisation increases the volatility and sensitivity of bioactive compounds, allowing polar and higher-mass molecules to be detected [62]. Inorganic polar and non-volatile anions can also be converted into more GC/MS-suitable forms [63]. The silylation reaction replaces active hydrogen atoms in acids, alcohols, thiols, amines, amides, ketones, and aldehydes with a TMS group, producing stable derivatives identifiable in the NIST library [14]. BSTFA, a TMS donor, reacts with hydroxyl groups in fatty acids, phenolic compounds, sterols, and inorganic acids to form TMS ethers or esters. TMCS catalyses the reaction, especially for less

Table 4 Chemical composition and lethal concentration of the *Garcinia Mangostana* pericarp crude extracts

No.	Compound[1]/Lethal concentration (LC_{50})	Label	No. of TMS[2]	GMPEF[3]		GMPECA[4]		GMPECB[5]		GMPWF[6]		GMPWCA[7]		GMPWCB[8]	
				Rt[9]	Relative area (%)	Rt[9]	Relative area (%)	Rt[9]	Relative area (%)	Rt[9]	Relative area (%)	Rt[9]	Relative area (%)	Rt[9]	Relative area (%)
	Sugars and sugar alcohols														
1	α-D-(-)-Lyxopyranose	C1	1	nd	nd	27.10	0.63	nd	nd	nd	nd	nd	nd	nd	nd
2	α-D-(-)-Ribopyranose	C2	1	nd	nd	18.46	0.23	nd	nd	nd	nd	nd	nd	nd	nd
3	Sucrose	C3	8	nd	nd	32.39	4.34	nd	nd	nd	nd	32.39	1.83	nd	nd
4	α-L-(-)-Fucopyranose	C4	1	nd	nd	nd	nd	nd	nd	nd	nd	27.97	2.90	nd	nd
5	β-D(-)-Lyxopyranose	C5	1	nd	nd	nd	nd	27.11	1.29	nd	nd	nd	nd	nd	nd
6	β-D-(+)-Xylopyranose	C6	1	22.42	0.09	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	Methyl galactoside	C7	4	nd	nd	nd	nd	nd	nd	27.25	0.29	nd	nd	nd	nd
8	β-Gentiobiose	C8	1	nd	nd	nd	nd	32.55	0.40	nd	nd	nd	nd	nd	nd
9	β-L-Arabinopyranose	C9	1	nd	nd	nd	nd	18.41	0.23	nd	nd	nd	nd	nd	nd
10	Arabinose	C10	4	nd	nd	16.74	0.08	nd	nd	nd	nd	18.54	1.71	nd	nd
11	α-Fructofuranose	C11	1	25.71	4.85	25.73	7.38	nd	nd	nd	nd	nd	nd	nd	nd
12	α-Fructopyranose	C12	1	nd	nd	nd	nd	25.86	13.82	nd	nd	nd	nd	nd	nd
13	α-Tagatofuranose	C13	1	nd	nd	nd	nd	25.71	2.61	nd	nd	nd	nd	nd	nd
14	D-(+)-Galacturonic acid	C14	5	nd	nd	nd	nd	28.34	1.62	nd	nd	28.36	1.54	nd	nd
15	D-Arabinose	C15	1	nd	nd	24.71	0.26	nd	nd	nd	nd	nd	nd	nd	nd
16	L-Sorbose	C16	1	nd	nd	nd	nd	nd	nd	25.84	1.61	nd	nd	nd	nd
17	D-Galactose	C17	5	30.47	0.06	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
18	D-Xylopyranose	C18	4	27.60	0.11	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
19	D-Glucopyranose	C19	5	nd	nd	27.95	3.79	nd	nd	nd	nd	nd	nd	nd	nd
20	L-(-)-Arabitol	C20	1	nd	nd	nd	nd	nd	nd	nd	nd	23.61	0.78	nd	nd
21	α-Sorbofuranose	C21	1	nd	nd	nd	nd	nd	nd	nd	nd	25.86	10.20	nd	nd
22	L-Rhamnose	C22	4	26.97	3.84	nd	nd	27.94	6.22	nd	nd	nd	nd	27.60	0.75
	Fatty acids														
23	Hexanoic acid	C23	1	nd	nd	nd	nd	nd	nd	nd	nd	5.64	0.02	nd	nd
	Phenolic compounds (polyphenols and simple phenols)														
24	Phloroglucinol	C24	3	nd	nd	nd	nd	nd	nd	17.65	1.18	nd	nd	nd	nd
25	Catechin	C25	1	nd	nd	nd	nd	33.66	0.57	nd	nd	33.66	0.39	33.53	13.61
26	3,4-dihydroxymandelic acid	C26	4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	32.77	0.59
27	2,6-Bis(4-methoxybenzylidene)cyclohexanone	C27	0	nd	nd	nd	nd	nd	nd	nd	nd	28.30	0.34	nd	nd
	Sterols (phytosterols)														
28	β-Sitosterol	C28	1	nd	nd	38.66	0.45	nd	nd	nd	nd	nd	nd	38.65	0.46
29	Stigmasterol	C29	1	37.80	0.24	37.81	0.53	nd	nd	nd	nd	nd	nd	nd	nd
	Terpenes/Terpenoids (Sesquiterpenes)														
30	α-Copaene	C30	0	9.32	0.16	9.33	0.21	nd	nd	nd	nd	nd	nd	nd	nd
31	α-Cubebene	C31	0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	9.34	0.30
	Heterocycles														

Table 4 (continued)

No.	Compound[1]/Lethal concentration (LC_{50})	Label	No. of TMS[2]	GMPEF[3]		GMPECA[4]		GMPECB[5]		GMPWF[6]		GMPWCA[7]		GMPWCB[8]	
				Rt[9]	Relative area (%)	Rt[9]	Relative area (%)	Rt[9]	Relative area (%)	Rt[9]	Relative area (%)	Rt[9]	Relative area (%)	Rt[9]	Relative area (%)
32	2,4-dihydroxy-5-methylpyrimidine	C32	2	nd	nd	nd	nd	nd	nd	17.99	0.96	nd	nd	nd	nd
	Inorganic acid														
33	Phosphoric acid	C33	3	nd	nd	nd	nd	7.80	1.76	nd	nd	nd	nd	nd	nd
	Total relative area (%)			9.35		17.90		28.52		4.04		19.71		15.17	
	Number of identified compounds			7		10		9		4		9		5	
	Lethal concentration (LC_{50}), mg/mL[10,11]			2.75 ± 0.15^{a}		3.30 ± 0.30^{ab}		4.40 ± 0.50^{bc}		5.00 ± 0.50^{cd}		5.80 ± 0.60^{de}		6.40 ± 0.70^{e}	
	95% confidence interval for the (LC_{50}), mg/mL			2.75 ± 0.37		3.30 ± 0.75		4.40 ± 1.24		5.00 ± 1.24		5.80 ± 1.50		6.40 ± 1.74	
	95% confidence interval range for the (LC_{50}), mg/mL			2.38–3.12		2.55–4.05		3.16–5.64		3.76–6.24		4.31–7.29		4.66–8.14	

[1]Compounds detected at more than 90% similarity against the NIST library

[2]TMS: trimethylsilyl derivatives

[3]GMPEF: *G. mangostana* pericarp extract using ethanol as solvent and filtration method

[4]GMPECA: *G. mangostana* pericarp extract using ethanol as solvent and centrifuge method (upper part)

[5]GMPECB: *G. mangostana* pericarp extract using ethanol as solvent and centrifuge method (lower part)

[6]GMPWF: *G. mangostana* pericarp extract using aqueous as solvent and filtration method

[7]GMPWCA: *G. mangostana* pericarp extract using aqueous as solvent and centrifuge method (upper part)

[8]GMPWCB: *G. mangostana* pericarp extract using aqueous as solvent and centrifuge method (lower part)

[9]Rt: Retention time (min)

[10]Means ± SD was carried out for triplicate measurements. The value with different letters was significantly different ($p < 0.05$)

[11]Vincristine sulfate was used as the positive control, while artificial salt water with 1% (v/v) Tween 80 was used as the negative control. The LC_{50} of the positive control was 0.067 ± 0.005 mg/mL

reactive –OH groups such as those in phenols and enols [14]. This approach was chosen over liquid chromatography–mass spectrometry (LC/MS) because LC/MS would require multiple eluents and chemical standards with different polarities, increasing cost.

The 22 sugars and sugar alcohols detected in GMP extracts naturally occur as glycosides, with hydroxyl, carboxyl, or amino groups bound to phenolics, sterols, and terpene aglycones. Derivatisation made these glycosides volatile and detectable by breaking the glycosidic bonds, which leads to the release of sugars and sugar alcohols [14]. Some of these compounds have biological or indirect antibacterial relevance. Methyl galactoside, identified in GMPWF, may alter bioactivity and enhance hydrophobic interactions with bacterial membranes [64]. D-(+)-Galacturonic acid, found in GMPECB and GMPWCA, is a key pectin component with reported antibiofilm and matrix-disrupting activity [65]. L-Rhamnose, present in GMPEF, GMPECB, and GMPWCB, is commonly bound to flavonoids such as quercetin-rhamnoside and can enhance the bioactivity of aglycones [66]. L-Rhamnose in *Ochradenus baccatus* and *Aerva javanica*, both of which exhibited antibacterial activity against *Bacillus* species [61].

Hexanoic acid, detected only in GMPWCA, is a medium-chain fatty acid with moderate antibacterial activity against *S.* Typhimurium [67]. Its amphiphilic structure allows it to disrupt bacterial membranes, cause intracellular leakage, and inhibit enzymes through cytoplasmic acidification [68].

GC/MS also detected phenolic and flavonoid compounds, including phloroglucinol, catechin, 3,4-dihydroxymandelic acid, and 2,6-bis(4-methoxybenzylidene)cyclohexanone. Phloroglucinol disrupts bacterial membranes and was detected in GMPWF [69]. Catechin, found in GMPECB, GMPWCA, and GMPWCB, has broad-spectrum antibacterial activity, including against multidrug-resistant strains, through membrane disruption, enzyme inhibition, and nucleic acid interaction [70]. 3,4-Dihydroxymandelic acid, present in GMPWCB, has a catechol structure that facilitates antibacterial activity through membrane disruption, oxidative stress induction, and metal ion chelation [71]. The 2,6-Bis(4-methoxybenzylidene)cyclohexanone, detected in GMPWCA, has been shown to inhibit pathogens such as *S. aureus*, *E. coli*, *P. aeruginosa*, and *S.* Enteritidis [72].

The presence of catechin and 3,4-dihydroxymandelic acid in GMPWCB, absent in GMPWF, may explain its higher total phenolic content (TPC) and stronger antibacterial performance. Catechin is known for its ability to disrupt bacterial cell membranes, inhibit essential enzymatic activities, and scavenge free radicals, thereby enhancing antibacterial efficacy [70]. Their presence in GMPWCB is consistent with its strong antibacterial performance in the DDT and its lower MIC and MBC values compared to GMPWF. The higher catechin levels, a type of flavonoid present in GMPWCB compared to GMPWCA, correspond with the TFC values of both extracts. Although 3,4-dihydroxymandelic acid was reported to exert an antioxidative effect, its role in bacterial interactions is more related to promoting infection rather than inhibiting it [71]. Anthocyanins, due to their larger molecular mass, are more readily detected by LC/MS than GC/MS, which may explain their absence in this analysis despite being present in aqueous extracts.

Stigmasterol and β-sitosterol, both detected in the extracts, have reported antibacterial activity, potentially by inhibiting sortase enzymes and disrupting bacterial membranes [73]. α-Copaene, found in GMPEF and GMPECA, shows strong antibacterial activity against multiple pathogens [74], and may contribute to the superior performance of GMPEF. α-Cubebene, detected in GMPWCB, is known to reduce biofilm formation by up to 50% [75].

Among the extracts, GMPECB had the highest total relative peak area (28.52%), followed by GMPWCA (19.71%) and GMPECA (17.90%). GMPWCB, GMPEF, and GMPWF showed lower values (15.71%, 9.35%, and 4.04%, respectively). Although GMPECA did not have the largest percentage area, it contained the highest number of identified compounds (10). GMPECB and GMPWCA each had nine, while GMPEF, GMPWCB, and GMPWF had seven, five, and four, respectively. These results suggest that even small quantities of key bioactive compounds, such as L-rhamnose, stigmasterol, and α-copaene, may contribute significantly to antibacterial activity. These compounds could act directly or synergistically to enhance the antibacterial effectiveness of GMP extracts; however, the potential synergistic interactions were not experimentally validated in the present study and remain a subject for future investigation.

G. mangostana extracts toxicity (*Artemia salina*)

The toxicity test provided an initial indication of safety and helped prioritise extracts for further evaluation. Table 4 presents the LC_{50} values for GMP extracts in *A. salina*. GMPEF showed the lowest LC_{50} at 2.75 ± 0.15 mg/mL. The remaining extracts had LC_{50} values as follows: GMPECA at 3.30 ± 0.30 mg/mL, GMPECB at 4.40 ± 0.50 mg/mL, GMPWF at 5.00 ± 0.50 mg/mL, GMPWCA at 5.80 ± 0.60 mg/mL and GMPWCB at 6.40 ± 0.70 mg/mL. All extracts had LC_{50} values greater than 1 mg/mL and were therefore classified as non-toxic [76], according to the *A. salina* toxicity scale for plant extracts LC_{50}. This scale categorises extracts as non-toxic when $LC_{50} > 1$ mg/mL, low toxicity for $0.5 < LC_{50} \leq 1$ mg/mL, medium toxicity for $0.1 < LC_{50} \leq 0.5$ mg/mL, and highly toxic when $LC_{50} \leq 0.1$ mg/mL.

Overall, ethanolic extracts exhibited lower LC_{50} values than aqueous extracts, consistent with the enrichment of

lipophilic phenolics that interact with biological membranes in *A. salina*. Within the ethanolic group, GMPECA combined high flavonoid content with strong antibacterial performance, giving an LC_{50} close to GMPEF. The GMPECB, which contained more sugars and fewer prominent phytochemicals, had a higher LC_{50}. In contrast, aqueous extracts were richer in sugars, organic acids, and polysaccharides, which may dilute potency on a mass basis, resulting in higher LC_{50} values.

The *A. salina* lethality assay was chosen due to the preliminary stage of this research and the availability of a cost-effective in-house method. This assay provides a rapid indicator of general cytotoxic potential and has been shown to correlate with established cytotoxicity screens [16]. However, it does not replace mammalian or food safety testing, as it lacks metabolic relevance, chronic toxicity endpoints, and matrix-specific conditions [17]. The current findings therefore serve as an early safety signal, warranting confirmation in food-relevant systems and mammalian models.

From a safety-first perspective, GMPWCB may be a suitable candidate for food applications, given its higher LC_{50} compared to the ethanolic extracts, its strong antibacterial activity among aqueous fractions, and the absence of solvent-residue concerns. Ethanolic extracts, while also non-toxic in *A. salina*, remain attractive for their higher antibacterial potency. GMPECA offers broad-spectrum efficacy at a low effective dose, balancing strong antibacterial activity with a predicted LC_{50} close to GMPEF. Its use in food formulations would require confirmation of ethanol removal and compliance with food safety and halal standards.

While the mean ± standard deviation of the extract toxicity describes central tendency and experimental variability, 95% confidence interval (CI) provides precision of the LC_{50} estimate and supports statistical comparison between extracts. The 95% confidence interval represents the range within which the actual population LC_{50} is expected to lie with 95% certainty. The overlap or separation of 95% CIs provides inferential insight into whether observed differences in toxicity are statistically meaningful. Table 4 shows the LC_{50} values and corresponding 95% CI ranges. Although mean LC_{50} values indicated a gradual reduction in extract toxicity, comparison based on 95% confidence intervals supported a reliable assessment of toxicological differences. GMPEF exhibited the lowest LC_{50} value with a narrow confidence interval (2.38–3.12 mg/mL), indicating the highest toxicity, whereas GMPWCB showed the highest LC_{50} value and widest confidence interval (4.66–8.14 mg/mL), indicating the lowest toxicity.

The extracts can be ranked from most toxic to least toxic as follows:

GMPEF > GMPECA > GMPECB > GMPWF > GMPWCA > GMPWCB. This ranking is supported not only by the LC_{50} means but also by the progressive upward shift in CI ranges, indicating decreasing toxicity across the extract series. The GMPEF and GMPWCA; GMPECA, GMPECB and GMPWF; GMPECB, GMPWF and GMPWCA; GMPWF, GMPWCA and GMPWCB show an overlap interval range indicating no meaningful differences in toxicity, with GMPEF being significantly more toxic. Thus, the GMPEF and GMPWCB at the extremes show the most reliable differences in toxicity, whereas mid-range extracts exhibit more gradual transitions.

To further explore the relationships between antibacterial activity, phytochemical composition, and toxicity profiles, Principal Component Analysis (PCA) was conducted.

Correlation of antibacterial activities, phytochemicals, toxicity and extraction methods of *G. mangostana* pericarp

Figure 2 (a) presents the PCA' biplot showing the relationships and distribution patterns among antibacterial activities, phytochemicals, and toxicity of the GMP extracts. The PCA on 71 variables showed that the first two PCs explained 50.53% of the total variance, with PC1 explaining 28.35% and PC2 explaining 22.18%. The KMO value was 0.43, indicating that the variables did not share sufficient common variance, suggesting that partial correlations among them were relatively high. This result showed that only some variables exhibited strong or moderate correlations in PC1 and PC2; hence, this study focused on explaining the variables with these correlations [77]. Although the KMO value was low, PCA was used as an exploratory tool to visualise patterns and correlations among variables. Interpretation focused on variables with strong factor loadings ($FL \geq 0.750$) and moderate loadings ($0.500 \leq |FL| \leq 0.740$) in PC1 and PC2 [78].

Of the 71 variables, 27 variables, including DDTBS, DDTEC, DDTPM, DDTVV, MICBC, MICCP, MICLM, MICSA, MICPM, MICVP, MBCBC, MBCCP, MBCSA, MBCPM, MBCVP, MBCVV, TPC, TFC, C4, C7, C10, C20, C21, C23, C24, C27 and C32, had strong FL values, making them the primary contributors in this study. A further 19 variables, such as DDTKP, DDTSE, DDTST, DDTVP, MICBS, MICSE, MICVV, MBCBS, MBCLM, MBCSE, C3, C6, C11, C14, C17, C18, C29, C30 and LC_{50} had moderate FL values, while the remaining variables had weak loadings ($FL \leq 0.499$).

Figure 2 (b) shows the biplot of the second PCA with reduced dimensionality and improved cumulative variability at 68.97%, illustrating correlations among variables with strong and moderate FLs. The antibacterial activity of GMPEF and GMPECA was associated with inhibition zones against *S.* Typhimurium (DDTST), *S.* Enteritidis (DDTSE),

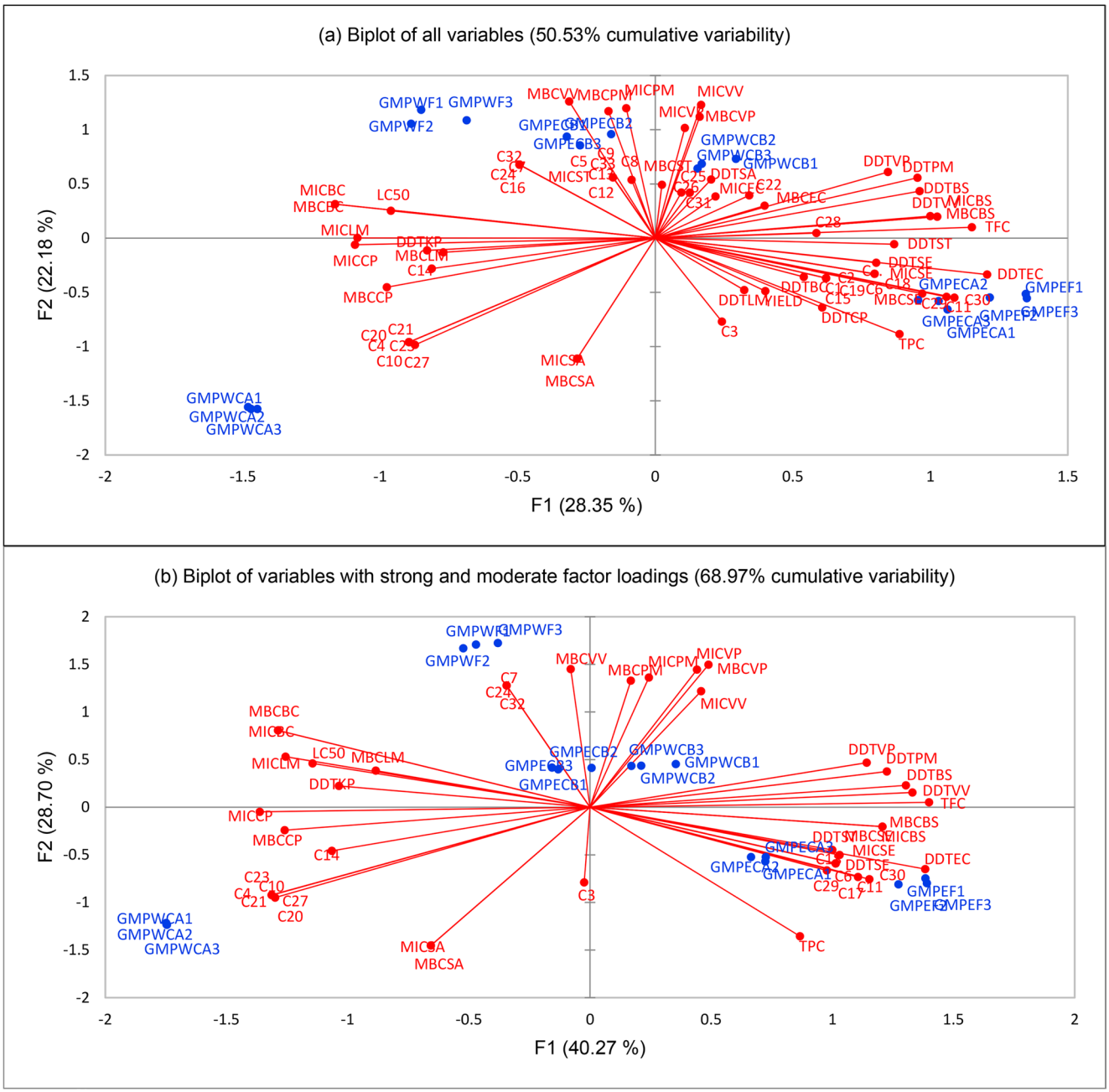

Fig. 2 (**a**) Biplot of all variables and (**b**) biplot of variables with strong and moderate factor loadings that shows the correlations among antibacterial activities, phytochemicals and toxicity of Garcinia *mangostana* pericarp extract

and *E. coli* (DDTEC), in the same vector direction. These extracts were also attributed to the inhibition of *B. cereus* (MBCBC and MICBC) and *L. monocytogenes* (MICLM and MBCLM) at the opposite vector. These inhibitions were probably contributed by positive correlations among flavonoids (TFC), phenolics (TPC), D-Xylopyranose (C18), β-D-(+)-Xylopyranose (C6), Stigmasterol (C29), D-Galactose (C17), α-Fructofuranose (C11), and α-Copaene (C30), and a negative correlation with the extract toxicity (LC_{50}). These results indicated that the presence of these phenolics, flavonoids and phytochemicals led to antibacterial activity of the GMPEF and GMPECA against *S.* Typhimurium, *S.* Enteritidis, *E. coli*, *B. cereus* and *L. monocytogenes*. However, D-Xylopyranose, β-D-(+)-Xylopyranose, D-Galactose, and α-Fructofuranose were sugars for which these phytochemicals did not synergistically enhance antibacterial potency. While sugars itself is not recognised as an antibacterial agent, this correlation suggests two non-mutually exclusive possibilities: first, that sugars may act as a marker for the co-extraction of potent, polar antibacterial

compounds present in the plant material; and second, that sugars could potentially exert a synergistic effect by imposing osmotic stress on bacterial cells, thereby increasing their susceptibility to other active constituents in the extract. Further targeted fractionation is warranted to isolate the true active principles responsible for the observed activity. Nevertheless, the presence of sugars was likely due to cleavage of the glycosidic bond linking the sugar to its aglycone [14], suggesting that sugars may not confer antibacterial activity. The presence of high sugar beets increased bacterial growth during storage, underscoring that sucrose serves as a nutrient for bacteria rather than an antibacterial agent [79]. Hence, studying the antibacterial effects of phytochemicals in the presence of glucose may confirm the impact of sugars on antibacterial activity.

Extracts such as GMPEF and GMPECA in Fig. 2 (b), which contain higher concentrations of these phytochemicals, also tended to exhibit lower LC_{50} values, indicating higher cytotoxicity. The negative orientation of flavonoids, phenolics, D-Xylopyranose, β-D-(+)-Xylopyranose, Stigmasterol, D-Galactose, α-Fructofuranose, and α-Copaene, relative to LC_{50} also suggested that higher levels of these phytochemicals were linked to lower LC_{50} values, indicating higher cytotoxicity in these extracts. This is a well-known phenomenon where potent antibacterial mechanisms, such as membrane disruption by stigmasterol and phenolics, also affect eukaryotic cells. This result underscores the dual nature of plant-derived antibacterials: the chemical features that confer strong antibacterial activity against foodborne pathogens often also contribute to broader cytotoxic effects, highlighting the importance of assessing their safety for potential use as natural food preservatives.

The vectors for C7, C24, and C32 were aligned with MBCVV, indicating positive associations, whereas negative associations were observed with TPC and C3 (Fig. 2 (b)). These associations contributed to the GMPECB and GMPWF; thus, these GMP extracts with higher levels of Methyl galactoside, Phloroglucinol, and 2,4-dihydroxy-5-methylpyrimidine, and lower levels of phenolics and sucrose, tended to show higher MICs for *V. vulnificus*, indicating lower antibacterial potency [21], and vice versa.

The MIC and MBC variables for *P. mirabilis*, *V. parahaemolyticus*, and *V. vulnificus* were clustered together in the opposite direction of GMPWCA (Fig. 2 (b)). They aligned with C4, C10, C14, C20, C21, C23 and C27, indicating positive associations. This indicated that extracts richer in α-L-(-)-Fucopyranose, Arabinose, D-(+)-Galacturonic acid, L-(-)-Arabitol, α-Sorbofuranose, Hexanoic acid *and* 2,6-Bis(4-methoxybenzylidene)cyclohexanone tended to show lower antibacterial potency against *P. mirabilis*, *V. parahaemolyticus*, and *V. vulnificus.* This study hypothesised that this pattern arises because D-(+)-Galacturonic acid, Hexanoic acid, and 2,6-Bis(4-methoxybenzylidene) cyclohexanone are either benign primary metabolites or possess high intrinsic antibacterial activity. Their high concentration in an extract may increase the potency of more effective, high-abundance antibacterials, thereby decreasing the required inhibitory concentration (MIC/MBC) of *P. mirabilis*, *V. parahaemolyticus*, and *V. vulnificus.* In contrast, the GMPWCA was also in the same vector direction as MIC and MBC of *C. perfringens* and *S. aureus.*

The MICCP, MBCCP, MICSA and MBCSA pointed in the opposite direction from GMPWCB (Fig. 2(b)). The MIC and MBC variables for *C. perfringens* and *S. aureus* along with α-L-(-)-Fucopyranose, Arabinose, D-(+)-Galacturonic acid, L-(-)-Arabitol, α-Sorbofuranose, Hexanoic acid *and* 2,6-Bis(4-methoxybenzylidene)cyclohexanone showed negative associations with GMPWCB, suggesting this pattern matched the higher antibacterial potency of GMPWCB, likely reflecting contributions from the low content of these sugars, fatty acid, and chalcone phytochemicals.

Conclusion

Extraction method and solvent choice appeared to influence the antibacterial potency, yield, and phytochemical composition of GMP extracts. Ethanolic maceration–filtration (GMPEF) tended to deliver the strongest broad-spectrum antibacterial activity and highest phenolic content, while aqueous maceration–centrifugation–filtration (GMPWCB) demonstrated a higher LC_{50}, suggesting a potentially greater safety margin. All extracts were non-toxic to *A. salina* ($LC_{50} > 1$ mg/mL), with ethanolic extracts generally showing stronger antibacterial effects but slightly lower LC_{50} values. The PCA associated specific sugars, phenolics, and stigmasterol with antibacterial potency, offering a possible chemical rationale for extract selection. GMPEF may be considered in applications where high efficacy is prioritised, and solvent removal is feasible. In contrast, GMPWCB may be more appropriate for settings where a conservative safety profile is desired. These findings indicate the potential of GMP extracts as sustainable, low-cost antibacterial agents from fruit by-products; however, their practical efficacy should be confirmed through further studies, including tests in mammalian systems and real food matrices, before commercial or industrial application.

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Declarations

Conflict of interest We declare no conflict of interest.

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