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Bioautography-Guided Thin Layer Chromatography and Fractionation of *Garcinia mangostana* Pericarp Extract for Antibacterial and Toxicity Evaluation

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***Abstract*— This study aims to explore the antibacterial activities of *Garcinia mangostana* pericarp (GMP) crude extract and partially purified extract. The antibacterial activity of both crude and partially purified extracts was assessed using Thin-Layer Chromatography (TLC) bioautography, Minimum Inhibitory Concentration (MIC), and toxicity assays. The GMP extract was separated via TLC using various eluents, including 5% ethyl acetate in petroleum ether, 1:3, 1:1, and 3:1 hexane:ethyl acetate, 1:1 hexane:diethyl ether, diethyl ether, 1:1 diethyl ether: methanol, Milli-Q water, and 0.1% formic acid in acetonitrile. A fractionation approach was used to evaluate the antibacterial activity of compounds present in the solvent fractions of the GMP extract. The antibacterial efficacy of the crude and partially purified GMP extracts was evaluated using MIC assays against *B. cereus*, *C. perfringens*, *E. coli*, *L. monocytogenes*, *S. aureus*, *S. pneumoniae*, and *V. vulnificus*. The GMP crude extract demonstrated the strongest antibacterial activity and the highest LC_{50} value (2.75 ± 0.15 mg/mL), indicating lower toxicity than partially purified GMP extracts. The *L. monocytogenes* was identified as the most susceptible microorganism to the GMP crude extract (MIC = 0.35 mg/mL), while *B. cereus* showed the least sensitivity (MIC = 11.25 mg/mL). These results support the potential use of GMP as a source of natural antibacterial agents, with possible applications in food preservation and safety.**

***Keywords*— *Garcinia mangostana* pericarp; antibacterial, thin-layer chromatography; toxicity**

I. INTRODUCTION

Garcinia mangostana is a fruit tree native to Indonesia and widely grown throughout Southeast Asia, including Malaysia, Thailand, Vietnam, Cambodia, the Moluccas, and Myanmar [1][2]. . Over the past 200 years, it has also been cultivated in various tropical regions beyond Asia, including India, Honduras, Brazil, and Australia [1]. This fruit is known for its valuable, rich phytochemical composition and diverse biological activities. The pericarp of *Garcinia mangostana* has been extensively studied for its bioactive compounds,

particularly phenolic, flavonoid, sugar, organic acid, and fatty acid, which exhibit potent antibacterial properties [3] . *Garcinia mangostana* pericarp (GMP) extract has long been used in traditional practices, and its various beneficial properties have been extensively validated by researchers through both in vitro and in vivo studies [4]. Several studies have demonstrated that GMP extract possesses strong antimicrobial activity, effectively inhibiting both Gram-positive and Gram-negative bacteria [4][5]. Despite reports of antibacterial activity against foodborne pathogens, spoilage bacteria, and food-contamination-associated microorganisms [6], the compounds in GMP extract that confer this activity remain underexplored.

The analysis of GMP extract using chromatographic techniques has provided fundamental insights into its chemical composition and pharmacological properties, aiding the identification of biologically active species [7][8]. Thin-layer chromatography (TLC) is a fundamental technique used to separate primarily non-volatile substances [9] and to screen complex plant extracts to identify bioactive compounds phytochemically. A direct bioautographic assay using TLC plates was selected for its role in identifying active compounds in the GMP extract. This method provided rapid insight into both the bioactivity and its precise localisation within complex plant matrices [10]. Bioautography was a technique that utilised chemical compounds to interact with substrates and chromogenic agents, producing distinct colour variations. This contrast in colouration enabled the visualisation of specific spots against a chromogenic background, allowing the tracking of active compounds [11][12]]. The combination of TLC and bioautography minimised the randomness in compound separation and highlighted differences in chemical composition between samples. They enabled the preliminary identification of biological activity within a mixture by linking it to specific compounds based on the spots they generated [12]. The TLC bioautography was a screening technique that combines TLC separation with biological activity assessment [12] . Bioautography utilised TLC plates to assess the biological activities of individual compounds in a crude extract after separation with an appropriate eluent. This technique is used to identify compounds with antibacterial activity against specific microorganisms [13][14][15] . The bioautography technique offered valuable insight into the role of individual compounds in the overall activity of an extract. It helps determine whether the antibacterial activity was primarily due to a dominant compound with strong effects or to the combined action of minor compounds [13] .

In this study, a bioassay-guided fractionation approach was employed to demonstrate the antibacterial properties of GMP extract compounds found in the tested solvent fractions. Fractionation and purification were employed to isolate the compounds and to analyse their cytotoxic properties [16] . Ethanol extraction solvent was considered a safe solvent for human consumption and was commonly used to extract natural compounds for both food and medicinal applications [17]; however, the use of organic solvents in extraction has raised concerns about potential toxicity for human use and their effects on human health [18] . Therefore, the solvent must be removed from the final extract, particularly when the product is intended for food applications [19] . Toxicity evaluation of crude and partially purified extracts was crucial before proceeding with further investigations, particularly for food applications. The Brine Shrimp Lethality Test (BSLT) is a widely used pre-screening method for assessing the bioactivity of compounds in plant extracts due to its affordability, speed, simplicity (eliminating the need for aseptic conditions), and reliability [20][21]. Furthermore, multiple studies have demonstrated a strong correlation between LC_{50} values derived from the BSLT and those obtained from acute toxicity tests in mice [20][22] .

Although several studies have investigated the antibacterial activity of GMP, there is limited information on the antibacterial activity of crude and partially purified extracts. This research aims to characterise the bioactive properties of GMP extract, evaluate its antibacterial activity, and assess its toxicity. The outcomes of this study may support the development of natural preservatives or antibacterial agents derived from *Garcinia mangostana*, highlighting its potential application in the food industry.

II. MATERIAL AND METHOD

A. Plant Material

Garcinia mangostana was bought from Desaru Fruit Farm, Kota Tinggi, Johor, Malaysia. The plant material was taxonomically verified by the Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia, and a voucher specimen was deposited with the reference number KM0249/25.. The fruit's pulps were removed, and the *Garcinia mangostana* pericarps were dried using freeze-drying for 72 hr. The freeze-dried pericarps were finely ground in a grinder and stored in a tightly sealed container protected from light until further use.

B. Extraction of Phytochemicals

Ultrasonic-assisted extraction was conducted on *Garcinia mangostana* crude using an electronic ultrasonic bath, specifically the CP2600D model by Crest Ultrasonic, Malaysia. The GMP crude extract was weighed and diluted with ethanol at a 1:40 solid-to-solvent ratio (SSR) based on the findings from the previous chapter. Then, the flask containing the GMP crude extract was submerged in an ultrasonic cleaner bath for 10 min and cooled to room temperature (25°C) for 20 min. The solution was filtered using a muslin cloth, transferred into a pre-weighed flat-bottom flask, and concentrated using a rotary vacuum evaporator (Heidolph, Germany) at 40 °C. The filtrate was stored at -20 °C in a refrigerator for further analysis.

C. Bioautography on Thin Layer Chromatography Plates

1:40 SSR of GMP extract was fractionated by thin-layer chromatography (TLC) over 10 x 20 cm silica gel 60 plates with pre-coated fluorescent indicator UV_{254} (Macherey-Nagel/Germany) using 5% ethyl acetate in petroleum ether, hexane: ethyl acetate (1:3, 1:1, 3:1), hexane diethyl ether (1:1), diethyl ether, diethyl ether: methanol (1:1), mili-q water and 0.1 % formic acid in acetonitrile as eluents. Eluents were selected based on their polarity increment. The fractions which were produced from eluents 5% ethyl acetate in petroleum ether (fraction A), hexane: ethyl acetate (1:3) (fraction B), hexane: ethyl acetate (1:1) (fraction C), hexane: ethyl acetate (3:1)

(fraction D), hexane diethyl ether (1:1) (fraction E), diethyl ether (fraction F), diethyl ether: methanol (1:1) (fraction G), mili-q water (fraction H) and 0.1 % formic acid in acetonitrile (fraction I), respectively on the TLC plates were then visualised under ultraviolet (UV). TLC plates were dried for 3 hr in a fume hood before being subjected to bioautography assay. The plates were dried to ensure the solvents evaporated, preventing them from inhibiting the bacteria. Otherwise, the solvent may inhibit the bacterial growth, not the GMP extract. Each fractionation was carried out in triplicate.

The antibacterial activity of the bioactive compounds on the TLC plates was assessed bioautographically after being dried for 3 hr using seven microorganisms, i.e., *Bacillus cereus* (ATCC 10875), *Clostridium perfringens* (ATCC 13124), *Escherichia coli* (ATCC 11229), *Listeria monocytogenes* (ATCC 19111), *Staphylococcus aureus* (ATCC 12600), *Streptococcus pneumoniae* (ATCC 10015) and *Vibrio vulnificus* (ATCC 27562). The developed TLC plates were sprayed with respective 10^6 CFU/mL microorganism suspension and continued incubated at 37°C for 24 hr. After incubation, the TLC plates were sprayed with a 2 mg/mL solution of iodonitrotetrazolium chloride (INT) and subsequently re-incubated for another 24 hr. Bacterial inhibition was visualised under UV light, and white spots against a pink background indicated bacterial growth inhibition by the antibacterial compound [23]. The retention factor (R_f) was determined using this formula [10]:

R_f = distance moved by the solute/ distance moved by the solvent.

D. Fractionation of Crude Garcinia Mangostana Pericarp Extract by Column Chromatography

To obtain larger quantities of the hexane and ethyl acetate (1:1) fraction (C), about 1 g of crude GMP extract was fractionated. This step was carried out using activated silica gel 60 (40 g, 0.040-0.063 mm, Merck) in a packed column with a 2 cm internal diameter, operated at a flow rate of 3 mL/min and a temperature of 30°C. The fractions were collected using 15 mL collectors. The R_f values were determined for each fraction via TLC, and the fractions with the same R_f values were combined.

E. Minimum Inhibitory Concentration (MIC)

The MIC were determined using a two-fold microdilution technique in a 96-well microtiter plate with a slight modification to the procedure as outlined by the Clinical and Laboratory Standards Institute [24]. Each well was filled with 100 µL TSB containing 1% (v/v) Tween 80. A volume of 100 µL of 50 mg/mL GMP crude extracts, sub-fractions C1 and C2 in DMSO, was then pipetted into the first well. A volume of 100 µL of scalar dilution was transferred from the first test well to the second well of each microtiter row, and then 100 µL of scalar dilution was pipetted from the second to the eleventh well. The last 100 µL from the last well were discarded. To obtain the concentration of 22.50 - 0.02 mg/mL, 90 µL from each well containing TSB mixed with extracts was combined with 10 µL of a bacterial suspension containing 10^6 CFU/mL of the *B. cereus*, *C. perfringens, E. coli*, *L. monocytogenes*, *S. aureus*, *S. pneumoniae,* and *V. vulnificus*. This adjustment resulted in an extract concentration of 22.50 mg/mL in the first well, progressively diluted to 0.02 mg/mL in the eleventh well, with the lowest concentration. The optical density was determined at 37°C before (T_0) and after 24 hr incubation (T_{24}) in an Ultra Microplate Reader at 600 nm. Tryptone Soy Broth (TSB) was employed as a positive control. The MIC was defined as the lowest concentration of the antibacterial agent capable of inhibiting the growth of the tested microorganisms, as indicated 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 was expressed as mg/mL. All determinations were carried out in triplicate.

F. Toxicity Assay

A brine shrimp lethality assay was carried out to determine the toxicity of the GMP extracts using brine shrimp larvae (*Artemia salina*) with a few modifications [25]. The 50mg/mL of GMP crude extract, along with 50mg/mL of sub-fractions C1 and C2 of hexane: ethyl acetate (1:1) extract, was combined with 1% (v/v) Tween 80 and then dissolved in dimethylsulphoxide (DMSO). Artificial seawater was prepared at 0.021 g/mL to dilute the mixtures of GMP crude extract, sub-fraction C1, and sub-fraction C2. Subsequently, a series of dilutions was performed to obtain extract concentrations ranging from 0.001 mg/mL to 10 mg/mL. Vincristine sulfate was prepared in DMSO to obtain an initial concentration of 1 mg/mL, followed by a series of dilutions to obtain concentrations between 0.001 mg/mL and 1 mg/mL. A complete series of vincristine sulfate concentrations was used as a positive control, whereas artificial sea salt water served as a negative control.

Brine shrimp or *Artemia Salina* eggs (Aquamaster, Malaysia) were hatched in artificial seawater prepared by dissolving 21 g of artificial sea salt in 1 L of distilled water. After 24 hr incubation period at 30°C, 15 mL of 4.86% yeast solution was added per litre of seawater to the hatching chamber to feed the nauplii. After hatching and maturation of *A. salina*, 10 nauplii were collected and placed into individual positive and negative control vials, and incubated for another 24 hr at 30 °C. The number of dead nauplii in each vial was examined and counted. The experiment was carried out three times. The percentage mortality at each dose and the control was determined via this formula [26]:

% Mortality: [(test death – control death) / total brine shrimp in each vial] x 100

The logarithm of concentrations was plotted against the mean percentage mortality rate. Lethal concentration (LC_{50}) was calculated from the best-fit line produced by linear regression analysis.

G. Statistical Analysis

The data were presented as mean ± standard deviation based on triplicate values for R_f and LC_{50}. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's test with XLSTAT-Pro (2025) software

(Addinsoft, Paris, France). A significance level of 95% ($p < 0.05$) was set to determine differences between mean values for bioautography and toxicity assay analyses.

III. RESULTS AND DISCUSSION

A. Bioautography on Thin Layer Chromatography (TLC) Plates

TLC was employed in the antibacterial study of the GMP extract to identify appropriate extractants and estimate the number of antibacterial compounds in the crude extract [13][27].

The eluents separated compounds on the TLC plates, covering a broad range of R_f values. The R_f values of the compounds varied from 0.13 to 0.80, as shown in Table I. Among the nine eluents tested for separating the compounds of GMP extract, eight eluents successfully separated a total of sixteen compounds. Diethyl ether demonstrated the highest separation efficiency, separating four compounds (sub-fractions F1, F2, F3, and F4) with R_f values ranging from 0.51 to 0.80. This result was followed by hexane: ethyl acetate (1:3) (sub-fractions B1 and B2), hexane: ethyl acetate (1:1) (sub-fractions C1 and C2), hexane: ethyl acetate (3:1) (sub--fractions D1 and D2), hexane: diethyl acetate (1:1) (sub-fractions E1 and E2), and diethyl ether: MeOH (1:1) (sub-fractions G1 and G2), which separated two compounds each with R_f values ranging from 0.32 to 0.80.

Meanwhile, the mixtures of 5% EtOAC in petroleum ether and 0.1% formic acid in acetonitrile could only separate one compound, each with R_f values 0.13 for sub-fraction A1 and 0.40 for sub-fraction H1. However, water was completely ineffective, failing to separate any compounds from the crude extracts. A study on the stem bark of *Barringtonia asiatica* successfully isolated three compounds with antibacterial properties using a combination of hexane and ethyl acetate as eluents, with R_f values ranging from 0.18 to 0.54 [28]. Studies have documented the various levels of this technology's ability to verify the presence of active natural compounds, including antibacterial, antifungal, anticancer, and antioxidant substances, as well as enzyme inhibitors. Continuous adsorption and desorption can be achieved throughout the mobile-phase elution process to separate distinct compounds by exploiting the different adsorption capabilities of each compound in the stationary phase [12]. The R_f values obtained for phytochemicals provide valuable insights into their polarity and serve as key indicators for their separation during the process. Different R_f values also provide insight into the compound's polarity, and using various solvent systems in TLC studies can be crucial for selecting the most suitable solvent for separation [29].

Of the compounds separated, only seven compounds from hexane: ethyl acetate (1:3), hexane: ethyl acetate (1:1), hexane: ethyl acetate (3:1), diethyl ether, and diethyl ether: MeOH (1:1) demonstrated antibacterial activity against *B. cereus*, *C. perfringens*, *L. monocytogenes*, *S. aureus*, *E. coli*, and *S. pneumoniae*, as indicated by the white spots on the TLC plates after sprayed with INT. Sub-fraction C2 was identified as the most potent compound, as it inhibited four microorganisms, i.e., *B. cereus*, *E. coli*, *L. monocytogenes*, and *S. pneumoniae*. In comparison, sub-fraction B1 showed antibacterial activity against two microorganisms, i.e. *C. perfringens* and *L. monocytogenes*. The remaining compounds each exhibited inhibition against only a single microorganism: sub-fraction C1 against *B. cereus*; sub-fraction D1 against *C. perfringens*; sub-fraction F1 against *E. coli*; sub-fraction F4 against *L. monocytogenes*; and sub-fraction G1 against *S. aureus*. An example of bioautography on a TLC plate for fraction C and fraction H against *L. monocytogenes* was presented in Table II. The TLC plates observed under UV light showed separated compounds with fluorescent or dark spots, indicating the presence of different compounds. After bioautography, the plates were treated with INT reagent. The INT was one of the tetrazolium salts introduced to highlight the zone of inhibition [12]. Metabolically, dehydrogenase enzymes in active microorganisms convert INT into a highly pigmented formazan. The INT was initially light in colour, such as pale yellow or colourless, and can be readily transformed into darker compounds by bacterial metabolites. As a result, antibacterial compounds were visualised as clear spots against the coloured background [10][12]. The presence of white spots (clear zone) on the post-bioautography plates signified antibacterial activity, as bacterial growth was inhibited in those areas. In contrast, the absence of white spots indicated that the compounds in those regions do not exhibit antibacterial effects.

The findings suggested that the antibacterial compounds in the GMP extract were effectively separated using the eluents, except water. The outcome confirmed that TLC-bioautography was a valuable method for screening bioactive compounds in complex plant extracts and aiding in the identification of potential antibacterial agents. The hexane-ethyl acetate mixture was the optimal eluent for separating the antibacterial compounds in the GMP extract. This finding was parallel to those reported in a previous study, in which the hexane-ethyl acetate mixture separated more compounds from a *Crescentia cujete* L. stem bark extract than other eluents [30]. However, to the best of our knowledge, the bioautography analysis of the antibacterial properties of the GMP extract has not been previously reported. This lack of prior research made it challenging to compare the present findings with existing data. After the separated antibacterial compounds were obtained, the next step involved fractionation using column chromatography.

B. Fractionation of Garcinia Mangostana Crude Extract by Column Chromatography

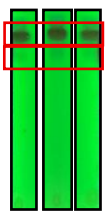
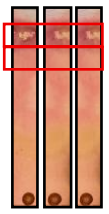
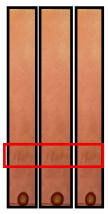
Fractionation was a technique used to separate plant extracts into distinct fractions, each containing multiple compounds. These fractions were then further divided into smaller portions, progressively isolating individual compounds until a pure compound was obtained [31]. Therefore, conducting column chromatography after bioautography was a crucial step in isolating, purifying, and understanding the individual and combined effects of bioactive compounds in GMP extract. Two sub-fractions (sub-fractions C1 and C2) were obtained from the GMP extract based on their R_f values determined via TLC. Sub-fraction C1 had a recovery of 3.1 g, accounting for 15.5% of the total extract, while sub-fraction C2 was the largest, with a recovery of 8.60 g, corresponding to 86% of the total extract.

TABLE I. THE RETENTION FACTOR (R_F) OF *GARCINIA MANGOSTANA* CRUDE EXTRACT SEPARATED USING VARIOUS ELUENTS ON TLC PLATES

Eluent/Fraction	No. of detected compound[1]	Compound detected (sub-fraction)[2,4]	Rf[3,4]	No. of compounds with antibacterial activity	Antibacterial activity detected[4]	Sensitive microorganism[3,4]
5% EtOAC in petroleum ether (Fraction A)	1	Compound 1 (A1)	0.13 ± 0.01^{m}	0	No	Na
Hexane: ethyl acetate (1:3) (Fraction B)	2	Compound 1 (B1)	0.32 ± 0.02^{k}	1	Yes	*C. perfringens* and *L. monocytogenes*
		Compound 2 (B2)	0.77 ± 0.02^{b}	0	No	Na
Hexane: ethyl acetate (1:1) (Fraction C)	2	Compound 1 (C1)	0.38 ± 0.01^{i}	1	Yes	*B.cereus*
		Compound 2 (C2)	0.75 ± 0.01^{c}	1	Yes	*B. cereus, E. coli, L. monocytogenes* and *S. pneumoniae*
Hexane: ethyl acetate (3:1) (Fraction D)	2	Compound 1 (D1)	0.37 ± 0.01^{j}	1	Yes	*C. perfringens*
		Compound 2 (D2)	0.53 ± 0.01^{f}	0	No	Na
Hexane: diethyl acetate (1:1) (Fraction E)	2	Compound1 (E1)	0.30 ± 0.01^{l}	0	No	Na
		Compound 2 (E2)	0.40 ± 0.01^{h}	0	No	Na
Diethyl ether (Fraction F)	4	Compound 1 (F1)	0.51 ± 0.01^{g}	1	Yes	*E. coli*
		Compound 2 (F2)	0.67 ± 0.01^{e}	0	No	Na
		Compound 3 (F3)	0.77 ± 0.01^{c}	0	No	Na
		Compound 4 (F4)	0.80 ± 0.01^{a}	1	Yes	*L. monocytogenes*
Diethyl ether: MeOH (1:1) (Fraction G)	2	Compound 1 (G1)	0.68 ± 0.01^{d}	1	Yes	*S. aureus*
		Compound 2 (G2)	0.80 ± 0.01^{a}	0	No	Na
0.1% formic acid in acetonitrile (Fraction H)	1	Compound 1 (H1)	0.40 ± 0.01^{h}	0	Na	Na
Water (Fraction I)	0	Na	Na	0	Na	Na

[1] [1]Identified compound produced a purple spot under UV-VIS observation.
[2] [2]Compound exhibited a white spot against the pink background of the TLC plate.
[3] [3]Mean of Rf with different superscript alphabet is significantly different ($p < 0.05$).
[4] [4]Na = not available.

TABLE III. BIOAUTOGRAPHY OF *L. MONOCYTOGENES* ON TLC PLATES FRACTIONATED BY HEXANE: ETHYL ACETATE (1:1) AND 0.1% FORMIC ACID IN ACETONITRILE

Observation of TLC	**Fraction**			
	Hexane: ethyl acetate (1:1) (Fraction C)		**0.1 % of formic acid in acetonitrile (Fraction H)**	
UV light observation	Sub-fraction C2 Sub-fraction C1		Sub-fraction H1	
Post autobiography	Sub-fraction C2 Sub-fraction C1 (both presence white spot)		Sub-fraction H1 (absence of white spot)	

C. *Minimum Inhibitory Concentration and Toxicity Assay*

The MIC test was conducted to identify the lowest concentration of GMP extract and its sub-fractions to prevent bacterial growth. This quantification was crucial for assessing the potency of the antibacterial compounds identified during bioautography and fractionation. A brine shrimp lethality test was essential to ensure the safety of the GMP extract and its subfractions. It was performed to determine the toxicity of various substances, including plant extracts and bioactive compounds, to assess potential risks associated with plant consumption, and to detect toxic compounds [32]. The toxicity test provided an indication and helped prioritise compounds with favourable safety profiles for further testing. Table III shows the MIC of crude GMP extract and its sub-fractions (C1 and C2) against *B. cereus*, *C. perfringens*, *L. monocytogenes*, *S. aureus*, *E. coli*, *S. pneumoniae*, and *V. vulnificus*.

Based on the MIC, the GMP crude extract exhibited the most potent inhibitory activity against all tested microorganisms, compared with the partially purified extracts hexane:ethyl acetate (1:1), sub-fraction C1, and sub-fraction C2, as indicated by their MIC values. The GMP crude extract exhibited varying antibacterial activity, with *L. monocytogenes* being the most sensitive, yielding the lowest MIC of 0.35 mg/mL, while *B. cereus* showed the least sensitivity, with a

MIC of 11.25 mg/mL. The GMP crude extract showed greater sensitivity than sub-fractions C1 and C2 against all tested microorganisms except *B. cereus.*

Interestingly, the tested Gram-positive *B. cereus* showed the greatest susceptibility to sub-fractions C1 and C2, with MIC values of 5.63 mg/mL for both. The more significant inhibition of sub-fractions C1 and C2 was indicated by multiple mechanisms and synergistic effects among various bioactive compounds [33]. Sub-fraction C1 and sub-fraction C2 also shared similar MIC values for *C. perfringens* (11.25 mg/mL), *S. aureus* (5.63 mg/mL), and *V. vulnificus* (22.5 mg/mL). However, sub-fraction C2 was more potent against *L. monocytogenes*, *E. coli*, and *S. pneumoniae*, requiring lower concentrations to inhibit bacterial growth than sub-fraction C1. Based on the MIC results, they were consistent with many research studies that have found that Gram-positive microorganisms tend to be more susceptible to phytochemical compounds than Gram-negative microorganisms [34]. Gram-positive and Gram-negative microorganisms may exhibit varying sensitivities to different bioactive compounds in plants due to differences in their membrane structures and cell walls [35]. The cell wall of Gram-positive microorganisms primarily consists of a thick peptidoglycan layer with minimal lipid content and water-soluble polysaccharides such as teichoic acids.

In contrast, Gram-negative microorganisms possess a more complex cell wall structure composed of multiple layers, including a plasma membrane, periplasmic space, a thinner peptidoglycan layer, and an outer membrane rich in lipids, proteins, and oligosaccharides. Due to this structural complexity, Gram-negative bacteria are generally more resistant to inhibition than Gram-positive bacteria, which have a simpler cell wall structure [36][37][38][39]. For bioactive compounds to exert antimicrobial activity, they must interact at the lipid-water interface, which requires partial hydrophobicity. This hydrophobic characteristic enabled the compounds to associate with bacterial membranes, especially those of Gram-positive bacteria, leading to membrane disruption. Such interference compromises membrane integrity and flexibility, disrupts vital transport processes, and ultimately weakens the bacterial cell structure [35].

The decrease in MIC values for subfractions C1 and C2 compared with the GMP crude extract suggested that fractionation enhanced antibacterial potency, likely due to the isolation of bioactive compounds. This phenomenon can be explained by the presence of various antibacterial bioactive compounds in the crude extract, as some microorganisms may be more susceptible to multiple compounds than to a single one. Furthermore, the complex mixture of secondary metabolites in the crude extract may act synergistically, enhancing antibacterial effects against specific microorganisms [40]. Therefore, the antibacterial activity observed in the GMPE extract in this study may result from a synergistic interaction among multiple compounds, which could have been lost following the fractionation process [41]. The MIC outcome of this study was aligned with the previous finding of crude roselle (*Hibiscus sabdariffa L.*) with a lower MIC value (0.47 mg/mL) against *S. aureus* [33]. In comparison, its partially purified fraction (F28) showed a slightly higher MIC value (0.78 mg/mL). Conversely, in another study on *Paullinia pinnata Linn* leaf extract, the crude extract exhibited slightly higher MIC values (8.75 mg/mL) against *S. aureus* than its sub-fractions, which demonstrated lower MIC values (2.19 mg/mL) [36].

TABLE IIIII. MINIMUM INHIBITORY CONCENTRATION (MIC) OF CRUDE *GARCINIA MANGOSTANA* PERICARP EXTRACT AND SUB-FRACTIONS (C1 AND C2)

Microorganisms	Sample / MIC (mg/mL)		
	GMP crude extract	Sub-fraction C1	Sub-fraction C2
B. cereus	11.25	5.63	5.63
C. perfringens	5.63	11.25	11.25
L. monocytogenes	0.35	11.25	5.63
S. aureus	1.41	5.63	5.63
E. coli	2.81	11.25	2.81
S. pneumoniae	1.41	5.63	2.81
V. vulnificus	2.81	22.5	22.5

A brine shrimp lethality test was essential to ensure the safety of the GMP extract and its subfractions. It was performed to determine the toxicity of various substances, including plant extracts and bioactive compounds, to assess potential risks linked to plant consumption, and to detect toxic compounds [32]. The toxicity test provided an indication and helped prioritise compounds with favourable safety profiles for further testing. The LC_{50} values for crude GMP extract, sub-fraction C1, and sub-fraction C2 were determined by performing a linear regression analysis of the mortality percentage in relation to the different extracts (GMP extract and sub-fractions C1 and C2) concentrations (Figure 1). The compounds derived from natural products are considered toxic if the LC_{50} is less than or equal to 1.0 mg/mL [42]. According to Clarkson's toxicity guidelines, extracts were classified based on their LC_{50} values as follows: non-toxic if LC_{50} is greater than 1 mg/mL, low toxicity for LC_{50} between 0.5-1 mg/mL, medium toxicity for LC50 between 0.1-0.5 mg/mL, and highly toxic for LC_{50} below 0.1 mg/mL [22].

In a brine shrimp lethality assay, cytotoxicity increased with increasing extract concentration. As shown in Table IV, all three extracts showed a significant increase in mortality percentage with increasing concentration. Evidently, at concentrations of 0.001, 0.01, 0.1, 1, and 10 mg/mL, the crude GMP extract caused 16.67%, 40%, 53.33%, 76.67% and 90% mortality, respectively, while the sub-fraction C2 showed 23.33%, 53.33%, 76.67%, 90% and 100% mortality, respectively. It is worth mentioning that sub-fraction C1 exhibited 50% mortality at a concentration of 0.001 mg/mL. Both sub-fractions C1 and C2 extracts showed similarity when more than 70% mortality occurred at a concentration of 0.1 mg/mL and 100% mortality at a concentration of 10 mg/mL, respectively. From the logarithmic correlation curves in Figure 1 (a) to 1 (c), the log concentrations of crude GMP extract, sub-fraction C1, and sub-fraction C2 exhibited regression values (R^2) ranging from 0.9522 to 0.9911, indicating clearly defined linear relationship patterns. A higher regression value signified a stronger correlation between the logarithmic concentration and the percentage mortality of nauplii [43].

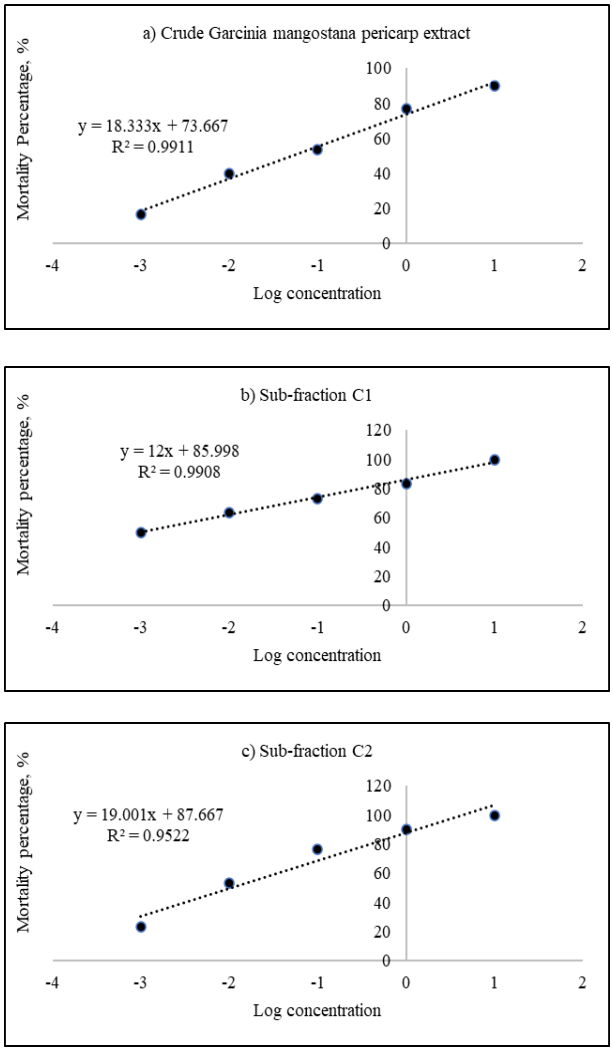


Figure 1. Calibration curve of *Artemia salina* percentage mortality for a) crude *Garcinia mangostana* pericarp extract, b) Sub-fraction C1, and c) Sub-fraction C2

In this assay, the crude GMP extract and sub-fraction C2 exhibited lethal concentration 50 (LC_{50}) values of 2.75 ± 0.15 and 2.02 ± 0.07 mg/mL, respectively. They were considered non-toxic because their LC_{50} values were greater than 1 mg/mL. However, the sub-fraction C1 extract exhibited low toxicity, with an LC_{50} of 0.5–1 mg/mL (0.99 ± 0.27 mg/mL). Among the sub-fractions (C1 and C2) derived from the same fraction (fraction C), sub-fraction C2 exhibited non-toxic properties, while sub-fraction C1 demonstrated a low degree of toxicity. These results indicated that fractionation may selectively concentrate certain bioactive compounds, leading to variations in toxicity between sub-fractions [44]. A similar trend was observed in a previous study where the crude *Carica papaya* seed extract (CPSE) exhibited a higher LC_{50} value (5.505 ± 0.718 mg/mL) compared to the hexane: ethyl acetate (1:1) fraction (1.797 ± 0.305 mg/mL) and sub-fraction 3C (0.332 ± 0.059 mg/mL) [45]. This result aligned with the findings of the present study, in which the crude GMP extract showed the highest LC_{50} value compared to the subfractions C1 and C2. Although both the crude GMP extract and sub-fraction C2 were classified as non-toxic, the crude GMP extract showed a higher LC_{50}, indicating lower toxicity. This result suggested that the crude extract was safer for application, making it a more favourable choice for food-related use [46], and even suggested that the crude extract was generally less effective than the purified compound in inhibiting microorganisms. This study selected the crude GMP extract over the sub-fraction C1 and sub-fraction C2 for further investigation due to its lower toxicity and MIC. Additionally, the production of subfractions from hexane:ethyl acetate (1:1) (fraction C) was expensive, and hexane was toxic to human cells [45]. Moreover, column chromatography techniques were labour-intensive, required large amounts of solvents, and lacked reproducibility [47].

TABLE IVV. TOXICITY OF CRUDE *GARCINIA MANGOSTANA* PERICARP EXTRACT, HEXANE: ETHYL ACETATE (1:1)

Samples	Concentration (mg/mL)	Mortality (%)	LC_{50} (mg/mL)[1]
GMP crude extract	10	90	2.75 ± 0.15[a]
	1	76.67	
	0.1	53.33	
	0.01	40	
	0.001	16.67	
Sub-fraction C1	10	100	0.99 ± 0.27[c]
	1	83.33	
	0.1	73.33	
	0.01	63.33	
	0.001	50	
Sub-fraction C2	10	100	2.02 ± 0.07[b]
	1	90	
	0.1	76.67	
	0.01	53.33	
	0.001	23.33	

[1]Mean of LC_{50} with different superscript alphabet is significantly different ($p < 0.05$).

IV. CONCLUSIONS

The crude ethanolic extract of *Garcinia mangostana* pericarp (GMP) exhibited antibacterial activity against *B. cereus*, *C. perfringens*, *L. monocytogenes*, *S. aureus*, *E. coli*, *S. pneumoniae*, and *V. vulnificus*. Additionally, it had the lowest toxicity (LC_{50} = 2.75 ± 0.15 mg/mL) compared to the hexane: ethyl acetate (1:1) sub-fraction C1 and C2 extracts. Both hexane:ethyl acetate (1:1) sub-fraction A and B extracts displayed a white antibacterial spot on the TLC bioautography and exhibited the highest antibacterial activity against *B. cereus*, *E. coli*, *L. monocytogenes*, and *S. pneumoniae* for sub-fraction B. The crude extract was suitable for antibacterial application studies, as its fractionated and purified forms did not show any additional advantages in terms of microbial inhibition. Both the crude and partially purified extracts demonstrated antibacterial activity, but the partially purified extract showed lower toxicity to *Artemia salina*. Therefore, the crude extract was the most practical option compared to the partially purified extract, as it did not require further processing.

The crude GMP extract's antibacterial properties make it highly relevant for commercial applications, particularly as a natural food preservative against foodborne pathogens such as *B. cereus*, *C. perfringens*, *L. monocytogenes*, *S. aureus*, *E. coli*, *S. pneumoniae*, and *V. vulnificus*. Although some studies have previously examined the antibacterial activity of GMP extracts,

to the best of our knowledge, this study was the first to evaluate the antibacterial effects of both crude and partially purified extracts, as well as their toxicity. These findings may encourage further research into the potential of GMP extract to enhance food safety and extend product shelf life by preventing the growth of contaminating microorganisms. To validate the effectiveness of the GMP extract, it may be tested in a food model system.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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