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A Direct Extraction Technique Enriched the Polyphenol Compositions and Their Biochemical Activities Obtained from *Garcinia* Species: An Evaluative Study

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ABSTRACT

Plant extraction has served as an initial and crucial procedure in ensuring the maintenance of the quality and safety of natural products for centuries. Extensive literature exploring the ideal extraction methods optimising the extractability of phytochemicals from plants, specifically *Garcinia,* was well defined. Yet, a lack of studies comparing the effects of direct and sequential extraction methods on these plants was indeed noticeable. This study evaluated the possible effects of various extraction procedures on the *Garcinia* species. Three *Garcinia* plants were selected, subjected to solvents under varying degrees, and extracted through direct and sequential techniques. The current results revealed that the direct extraction technique yields the highest in polyphenol compositions, including their biochemical activities, with ethanolic extract displaying the highest performance among all solvent polarities. In terms of antimicrobial activity, the ethanolic crude extract positively suppressed the inhibition of the growth of *Staphylococcus aureus* and *Bacillus subtilis* at specific concentrations. The findings suggested utilising a direct extraction technique to improve the extraction quality of Garcinia, signifying its relevance and efficiency in obtaining the phytochemicals with enhanced antioxidants and antimicrobial properties.

Keywords: Biochemical activities, extraction technique, Polyphenols, *Garcinia*

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INTRODUCTION

Traditional and natural products have been embraced and relied upon across the globe since ancient times, serving as primary healthcare to approximately 85% of the worldwide population (World Health Organisation [WHO], 2013). In many Asian countries, medicinal products are applied immensely due to their full and deep-rooted cultural practices for generations, creating the foundation of Traditional, Complementary, and Alternative Medicine (TCAM). Malaysia, in particular, has shown consistent reliability towards the adoption of TCAM, with nearly 70% of the population applying it in treating ailments and diseases (Siti et al., 2009). The increment in global dependency on traditional remedies consistently induced advancements in natural product development nowadays, emphasising the need for ongoing enhancement of innovation in fields such as food technology, pharmaceuticals, nutraceuticals, and agriculture.

Medicinal and aromatic plants, specifically those with ethnopharmacological importance, have served as natural reservoirs of TCAM for millennials (Chaachouay & Zidane, 2024). These plants are acknowledged to exhibit a notable spectrum of natural bioactive compounds with various chemical structures and challenging dynamic properties (Wernisch & Pennathur, 2016). Regardless, their multifaceted matrices limit the expansion of research on natural products, hindering the availability of the compounds and reducing the extraction efficiency. Considering these aforementioned issues, optimising the extraction process as the initial foundation step remains a critical priority in any intended research or applications.

In the extraction procedure, achieving the optimum extraction efficiency requires maximising the extractable yield of plants. Extractable matter, which consists of a wide range of secondary metabolites, essentially relies on the choice of an appropriate solvent, which feasibly influences the specificity of the compounds extracted. Generally, extraction methods are designed to target either polar or non-polar compounds using solvents of varying polarities. In most extensive studies previously, secondary metabolites and their bioactivities are often in the limelight, neglecting the possibility of gaps within the extraction methods impacting the quality of the plant extracts. In this sense, it is imperative to explore and assess all potential methods to bridge this research gap effectively.

The *Garcinia* genus, affiliated with the Clusiaceae family, is in the spotlight for having an abundance of bioactive compounds such as bioflavonoids, polyphenols, and xanthones possessing substantial therapeutic properties, including antioxidant and antimicrobial (Acuña et al., 2012; Santo et al., 2020). Attributable to their wide pharmacological potencies, numerous health and food products were developed, such as gels, creams, lotions, tablets, and syrups with *Garcinia* extracts as one of the ingredients, delivering a variety of health benefits to consumers (Krisanti et al., 2020; Paul & Zaman, 2022; Saptarini &

Hadisoebroto, 2020). Nevertheless, comparable to naturally produced products, therapeutic drugs that have been effectively developed pose several challenges (Harvey et al., 2015). It is worth mentioning that developing these natural products for direct consumption relies heavily on appropriate extraction procedures. With respect to *Garcinia*, wide-ranging research has been conducted to identify the best extraction technique yielding promising results over the years. Nevertheless, studies particularly comparing the effectiveness of sequential and direct extraction methods remain deficient.

The current study involved a comparative experiment analysing the variability of the polyphenolic compounds with few biological activities extracted from selected *Garcinia* plants, utilising two different solvent methods and analytical techniques (Figure 1). These plant species were chosen for their wide spectrum of pharmacological activities exhibited under various polarities. These materials were exposed to various methods with hexane, chloroform, and ethanol as extractants. Antioxidants with different scavenging assays and antimicrobials with various foodborne microorganisms were evaluated on the *Garcinia* extracts for their efficacies.

MATERIALS AND METHODS

Plant Preparations and Processing

For this study, three main *Garcinia* species were selected and collected at different areas within Universiti Putra Malaysia (Longitude: 101.7056 ° E, Latitude: 2.9995 ° N). *Garcinia mangostana* and *G. athroviridis* were harvested from Ladang Bersepadu, Ladang 10, whereas *G. hombroniana* was obtained from the Conservatory Garden of the Bioscience Institute. Leaves were collected from healthy plants at a comparable maturity stage to minimise variation in leaf age. Collection was performed within the same sampling period to minimise seasonal effects. The collected samples were first rinsed under running water to remove debris before being dried with tissue paper. Then, the samples were dried for 3 days consecutively at 30 °C in a conventional oven. Drying was conducted at a relatively low temperature of 30 °C to minimise thermal degradation of heat-sensitive compounds. Although some degradation cannot be fully excluded, the use of mild drying conditions was intended to reduce moisture while preserving phytochemical stability. After drying, the dried samples were ground into fine powders and stored properly at -20 °C for further research. The study was limited to three Garcinia species because these species are locally available, commonly used, and represent relevant members of the genus with reported phytochemical potential. Although the Garcinia genus is diverse, the selected species allowed a controlled comparative evaluation of extraction performance under the same experimental conditions.

Plant Extractions

A direct and sequential extraction process in this study was executed, in which fine-powdered *Garcinia* samples were precisely measured and macerated in various solvent polarities at a ratio of 1:10 (g/mL) (Figure 1). After 24 hours of macerating, the supernatant was obtained by filtering with Whatman filter No.1, demonstrating a direct extraction procedure. Proceeding to the sequential extraction required proper drying of the residue after the filtration by submerging it in the water bath at 40 °C, while the collected supernatants were concentrated at 40 °C with a rotary evaporator. The dried residue was correspondingly extracted through maceration with other solvents. All extracts were stored at -20 °C before they were used in the next analysis. In this study, maceration was selected because it is a simple, low-cost, and widely used extraction technique suitable for heat-sensitive plant bioactives such as phenolics and flavonoids. It also reflects a practical method that can be easily replicated without advanced equipment.

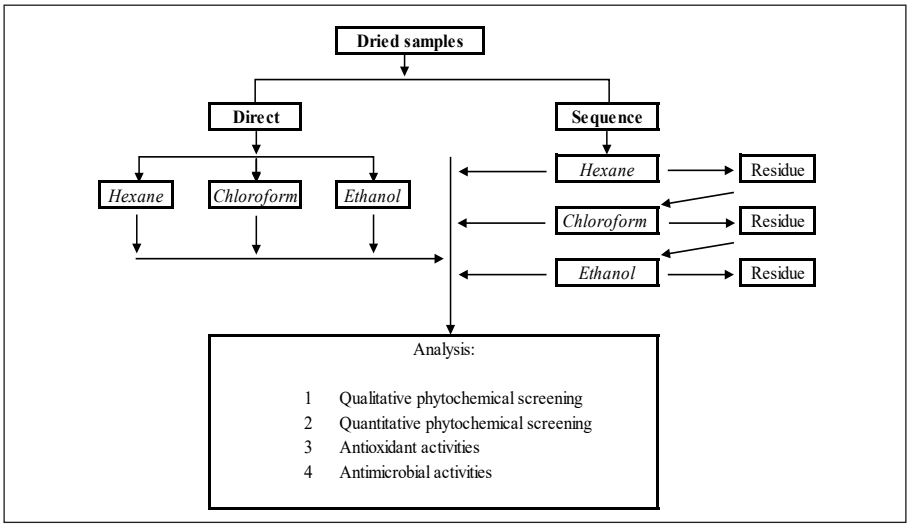

Figure 1. The flowchart of the extraction procedures subjected to the *Garcinia* samples

Extractable Matter and Phytochemical Screening Analysis

The extractable matter of Garcinia extracts was calculated as in Equation 1:

$$Extraction\ yield\ (\%) = \frac{Weight\ of\ crude\ extract}{Weight\ of\ powdered\ sample} \times 100 \qquad [1]$$

The phytochemical constituents, including phenolics, flavonoids, alkaloids, saponins, glycosides, tannins, and terpenoids in each plant crude extract were identified qualitatively according to the method described by Bisso et al. (2022).

Total Phenolic Content Determinations

The phenolic contents of *Garcinia* plant extracts were evaluated using the Folin-Ciocalteu method with slight adjustments (Samat et al., 2020). The samples with a volume of 200 µL were mixed with 2 mL of Folin-Ciocalteu's phenol reagent and allowed to react for 10 minutes. After that, the mixture was added with 1 mL of sodium carbonate solution and left aside with an incubation period of 30 min in dark conditions. The mixtures were then measured under a microplate reader with 765 nm as an absorbance reading. The phenolic values were calculated and presented as mg gallic acid equivalent/ g dry extract with Gallic acid as standard.

Total Flavonoid Content Determinations

The flavonoid compositions for each *Garcinia* sample were measured following Samat et al. (2020) with minor modifications. First, diluted samples were added with 0.3 mL of sodium nitrite solution and left aside for 6 minutes. Then, 0.3 mL of aluminium chloride solution was added to the working samples and set aside for 6 minutes. Finally, 2 mL of sodium hydroxide (1M) was added and left aside for another 20 minutes for the reaction. The samples were then measured under a microplate reader with an absorbance of 510 nm. The flavonoid values were quantified and presented as quercetin equivalent/ g dry extract.

DPPH Scavenging Activity

The radical scavenging activity exhibited by *Garcinia* samples was evaluated using the 2,2-diphenyl-picrylhydrazyl (DPPH) radical scavenging assay (Samat et al., 2020). About 1 mL of each sample was mixed with 1 mL of working DPPH solution prepared freshly with methanol (1M). The colour changes after 30 min dark incubations indicate the presence of antioxidants, and their values were measured at 517 nm using the following Equation 2:

$$DPPH\ scavenging\ inhibition\ (\%) = \frac{(Abs1_{517} - Abs0_{517})}{Abs0_{517}} \times 100 \qquad [2]$$

where, Abs0 and Abs1 were the absorbances for the control and sample, respectively.

ABTS Scavenging Activity

The scavenging activity through ABTS was done by preparing 7 mM ABTS in distilled water and 2.45 mM potassium persulfate in a ratio of 1 to 1, forming the ABTS working solution before being placed under a dark ambient room for 12-16 hours. Prior to usage, the mixture of ABTS was initially diluted with methanol until obtaining an absorbance of 0.700 at 734 nm. Then, 1 mL of diluted ABTS was mixed with 3 mL of each of the samples and incubated for 30 minutes. After the incubation, the reading was measured

using a microplate spectrophotometer. Percent inhibition of absorbance at 734 nm was calculated using Equation 3:

$$ABTS\ scavenging\ inhibition\ (\%) = \frac{(AB - AA)}{AB} \times 100 \quad [3]$$

where, AB is the absorbance of ABTS radical + methanol; AA is the absorbance of ABTS radical + sample extract/standard.

Antimicrobial Analysis

The antimicrobial activity of the *Garcinia* samples was evaluated on the microbial test strains, including *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa,* which were obtained from the Institute of Bioscience situated in the UPM. For the culturing media, nutrient agar and Mueller-Hinton Agar (MHA) were utilised to analyse the respective minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC). The assessment of the microbial assay of the samples was executed by using the agar dilution method. For the execution of MIC and MBC, various concentrations were tested until the concentration that completely suppressed the bacterial growth after 48 hours of incubation at 37 °C.

Statistical Analysis and Interpretation

The means of data collected in this study were analysed with one-way ANOVA (SAS 13.1) and the mean separation of the data was done using Tukey's Honestly Significant Difference (HSD) Test.

RESULTS

Extraction Yields and Qualitative Phytochemical Analysis

As presented in Figure 2, the extractable matter of *Garcinia* samples varied significantly following *Garcinia* species and solvent polarities. For plant species, *G. mangostana* produced the highest crude extract in comparison to *G. hombroniana* and *G. athroviridis*. Among the extraction methods, it was observed that the direct extraction technique effectively aids in yielding the extractable matter of *Garcinia,* approximately higher than the sequential method, about 30%, which varies across solvent polarities and *Garcinia* species categories. As we compared the effectiveness of the solvent polarities, it was demonstrated that crude extracts were influenced substantially by the type of solvents, with crude extract through ethanol as the most extractive sample, with a considerable average value of 20% in the direct extraction technique and 14% in the sequential extraction method.

From the perspective of phytochemicals in a qualitative sense, insignificant variations in the appearance of phytochemicals can be detected between *Garcinia* species in either direct or sequential extraction techniques. Most polar compounds were found in ethanol and chloroform, whereas non-polar compounds were detected heavily in hexane. With the exception of saponin, which was solely identified through the sequential method, the detection trend of phytochemical compounds was largely consistent across both extraction techniques (Table 1).

Quantitative Phytochemical Analysis

Figures 3 and 4 illustrate significant variations in phenolic and flavonoid contents across all species and solvent polarities when using direct and sequential extraction methods. According to the table, phenolic (Figure 3) and flavonoid (Figure 4) concentrations decreased by 15% to 35% during sequential extraction, regardless of solvent polarity. Similar to the extractive values, polyphenolic compounds were most abundant in *G. mangostana* when extracted with the most polar solvents, followed by *G. hombroniana* and *G. atroviridis*. In contrast, the lowest polyphenolic content was consistently observed in hexane across all species and extraction methods. In all species, flavonoid concentrations were approximately 30% to 60% lower than phenolic concentrations, irrespective of the solvent polarity or extraction method employed.

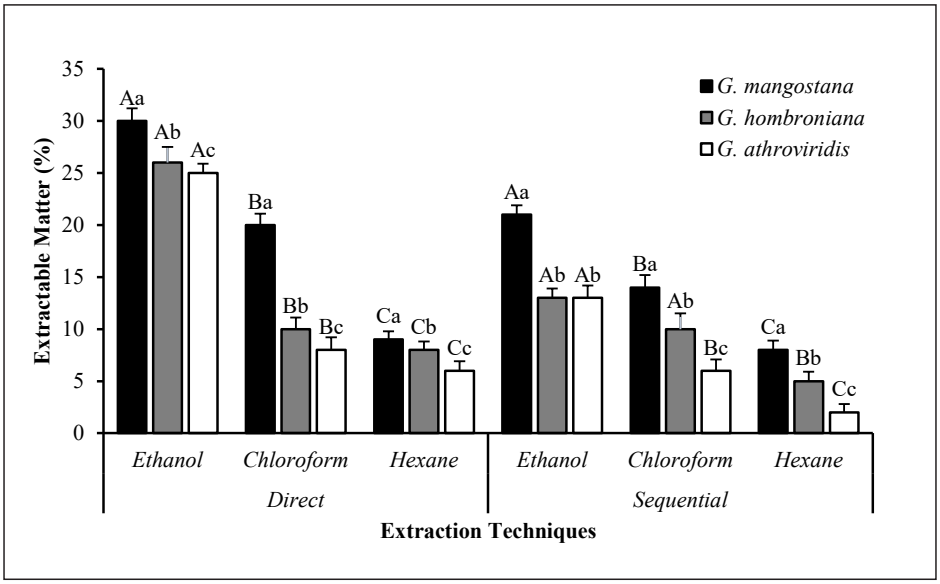

Figure 2. The extractable yield of *Garcinia* at different solvent polarities and extraction techniques
Note. Each bar chart represents the means ± standard error. Means followed by different letters (A, B, C) and (a, b, c) are significantly different at P<0.05 within the type of solvents and plant species, respectively

Table 1
Qualitative phytochemical assessment of Garcinia species subjected to direct and sequential extraction methods

Extraction Techniques	Extractants	*Garcinia* Species	Phytochemicals						
			Flavonoids	*Phenolics*	*Alkaloids*	*Saponins*	*Tannins*	*Terpenoids*	*Glycosides*
Direct	Ethanol	*Garcinia hombroniana*	+++	+++	+++	+++	+++	+	+
	Chloroform		++	++	+	++	++	+	++
	Hexane		-	-	-	-	-	++	++
Sequential	Ethanol		+++	+++	+++	+++	+++	-	+
	Chloroform		++	++	+	+	+	+	+
	Hexane		-	-	-	-	-	++	+
Direct	Ethanol	*Garcinia mangostana*	+++	+++	+++	+++	+++	+	+
	Chloroform		+++	++	++	++	++	+	++
	Hexane		-	-	-	-	-	+++	++
Sequential	Ethanol		+++	+++	+++	+++	+++	+	+
	Chloroform		++	++	++	++	++	++	++
	Hexane		-	-	-	-	-	++	++
Direct	Ethanol	*Garcinia athroviridis*	+++	+++	++	++	++	++	+
	Chloroform		+	+	+	+	+	++	+
	Hexane		-	-	-	-	-	++	+
Sequential	Ethanol		++	++	++	++	++	+	+
	Chloroform		+	+	+	+	+	++	+
	Hexane		-	-	-	-	-	++	+

Notes. (+) = Present in small concentration, (++) = Present in moderately high concentration, (+++) = Present in high concentration, (-) Absent

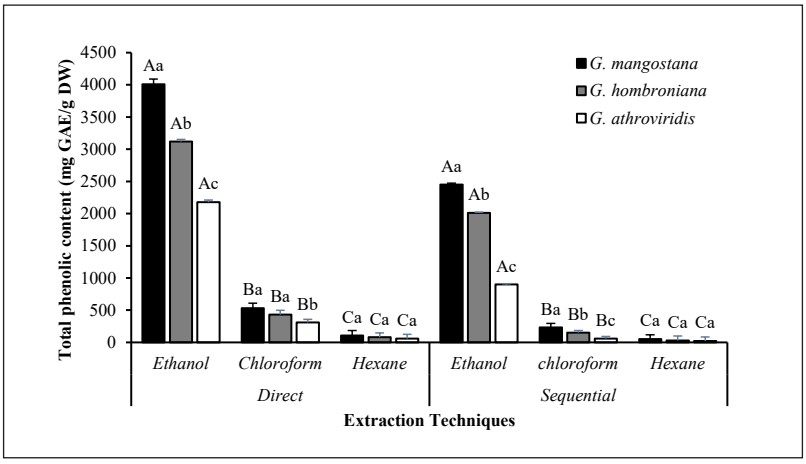

Figure 3. The phenolic contents of *Garcinia* at different solvent polarities and extraction techniques
Note. Each bar chart represents the means ± standard error. Means followed by different letters (A, B, C) and (a, b, c) are significantly different at P<0.05 within the type of solvents and plant species, respectively

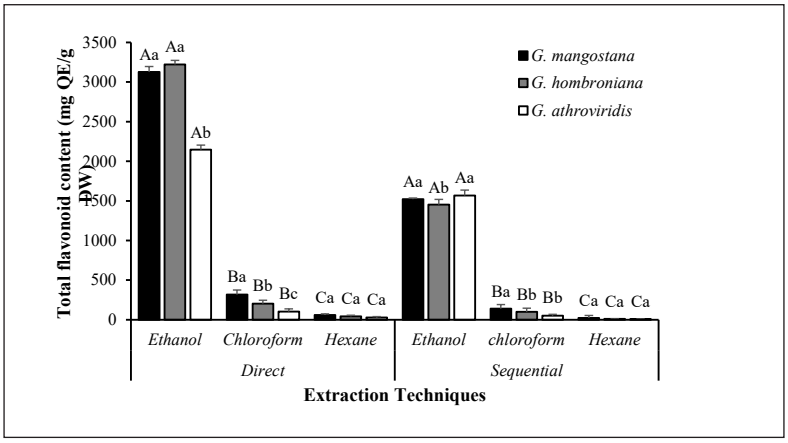

Figure 4. The flavonoid contents of *Garcinia* at different solvent polarities and extraction techniques
Note. Each bar chart represents the means ± standard error. Means followed by different letters (A, B, C) and (a, b, c) are significantly different at P<0.05 within the type of solvents and plant species, respectively

Radical Scavenging Activity

The antioxidant properties of plants can be evaluated through two primary mechanisms known as single electron transfer (SET) and hydrogen atom transfer (HAT). In this study, the antioxidant activity of the plants was assessed using DPPH and ABTS assays, as shown in Figures 5 and 6. These assays were used to investigate how the extraction methods and solvent polarities influenced the antioxidant capacity of the extracts.

The scavenging activity of all extracts differed depending on the extraction methods and solvent polarities, as depicted in Figures 5 and 6. Overall, the difference in antioxidant percentage between the DPPH and ABTS assays was minimal. A pattern similar to the polyphenolic compositions was observed, where extracts obtained directly from the plants exhibited higher antioxidant activity compared to those obtained using the sequential extraction method. Among the samples, *G. mangostana* showed the strongest scavenging activity when extracted with polar solvents, while *G. atroviridis* consistently displayed the lowest antioxidant activity across all extraction methods. Additionally, ethanolic extracts demonstrated significantly higher inhibition values, approximately 50% greater than those of chloroform extracts, irrespective of the species or extraction technique.

Antimicrobial Activity Test

According to Table 2, ethanolic extracts from all species were tested at a concentration of 100 mg/mL to evaluate their antimicrobial activity. The results revealed that the ethanolic extracts exhibited notable activity against *Staphylococcus aureus* and *Bacillus subtilis* compared to other microorganisms in the study, as shown in Figure 7. In contrast, extracts obtained using chloroform and hexane demonstrated no activity against any of the tested microorganisms at the same concentration.

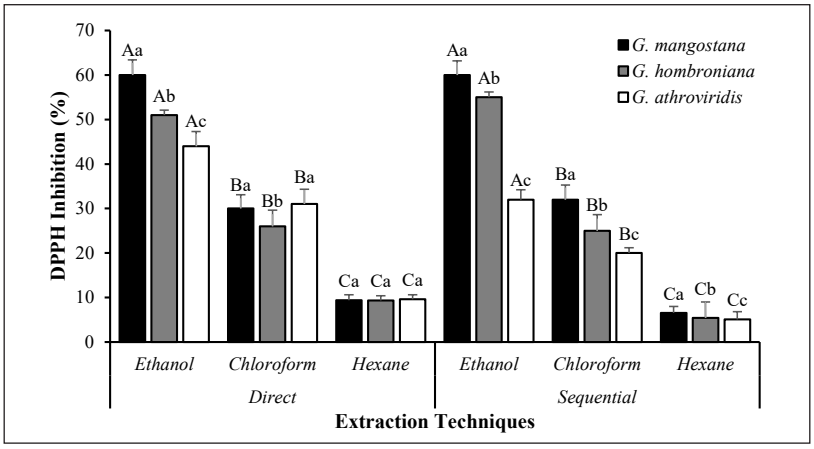

Figure 5. The DPPH scavenging activity of *Garcinia* at different solvent polarities and extraction techniques
Note. Each bar chart represents the means ± standard error. Means followed by different letters (A, B, C) and (a, b, c) are significantly different at P<0.05 within the type of solvents and plant species, respectively

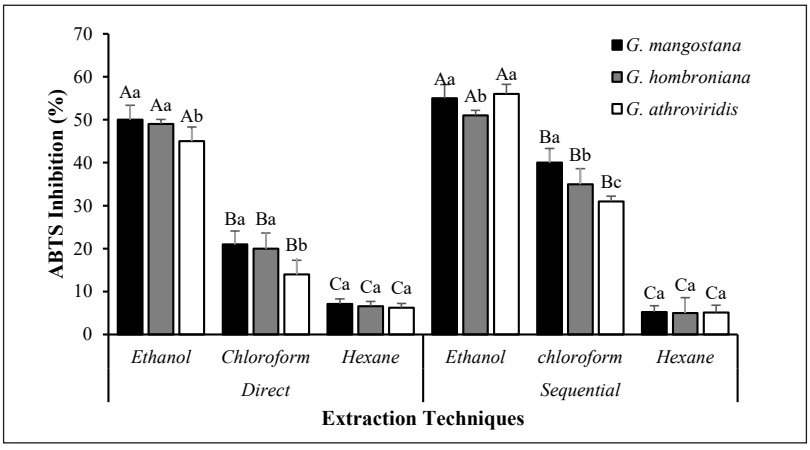

Figure 6. The ABTS scavenging activity of *Garcinia* at different solvent polarities and extraction techniques
Note. Each bar chart represents the means ± standard error. Means followed by different letters (A, B, C) and (a, b, c) are significantly different at P<0.05 within the type of solvents and plant species, respectively

Table 2
The antimicrobial properties of G. mangostana extracted through the direct method

Extractants	*Pseudomonas aeruginosa* (ATCC 15422)	*Escherichia coli* (ATCC 25922)	*Bacillus subtilis* (B29)	*Staphylococcus aereus* (ATCC 43300)
Ethanol	0	0	11.57 ± 2.14	11.37 ± 2.45
Chloroform	0	0	0	0
Hexane	0	0	0	0

Note. Data presented as mean with triplicate ± standard error. 0 value indicates no inhibition expressed upon solvent application

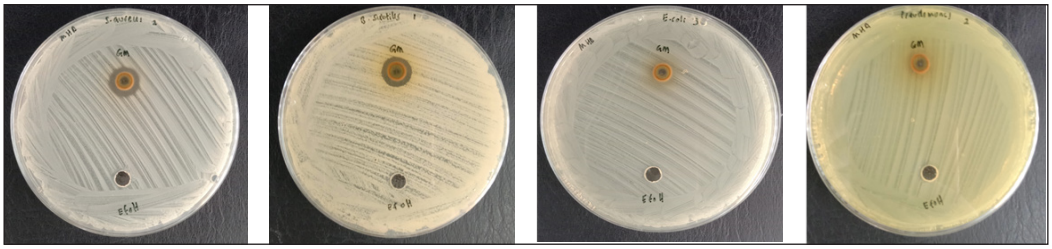

Figure 7. Well diffusion test of microbial suppression of *G. mangostana* extracts was exhibited only by *S. aureus* and *B. subtilis*

Table 3 presents the MIC values of the ethanolic extracts. Hexane and chloroform extracts showed no activity across all tested concentrations (data not shown). The ethanolic extract exhibited inhibitory effects against *S. aureus* at concentrations ranging from 25 to 100 mg/mL, while for *B. subtilis*, inhibition was observed between 50 and 100 mg/mL. The MBC results were consistent with the MIC, as no bacterial growth was detected at the lowest MIC values. Specifically, the ethanolic extract demonstrated the strongest suppression of *S. aureus* at 25 mg/mL, whereas the MBC for B. subtilis was determined to be 50 mg/mL.

Table 3
Minimum inhibition concentration (MIC) of the ethanolic extract of G. mangostana

Extractant	Concentration (mg/mL)	Inhibition Zone (mm)	
		S. aereus	***B. subtilis***
Ethanol	6.25	0	0
	12.5	0	0
	25	9 ± 1.6	0
	50	10 ± 1.8	11 ± 0.6
	100	12 ± 1.7	11 ± 1.2

Note. Data are means with triplicates ± standard error. 0 indicates no inhibition occurred upon solvent application

DISCUSSION

Since ancient times, the perspective of plants being the reservoir of valuable and natural bioactive phytochemicals has been fully embraced and incorporated into modern medicines for the treatment of various human diseases and ailments. In addressing chronic health conditions, herbal medicines offer substantial benefits, including potency effect, cost-effectiveness, minimal side effects, and ease of access. Regardless of their significance, these plants existed in multifaceted and complex matrices, which complicate the extraction process of specific bioactive compounds. Considering these issues, numerous approaches have been proposed, and it is plausible that emphasising the optimisation of the extraction

process will feasibly be the answer for the enhancement of the quality of the medicinal plant extracts.

Many exemplary works of literature successfully defined the substantial effect of extractant selection and extraction technique on the retrieval of polyphenols and their biochemical properties (Dirar et al., 2019; Ngo et al., 2017). In the recovery of the polyphenols, the impact of the type of extractants is more noticeable than the extraction technique itself in the present findings, regardless of the *Garcinia* species. These results were accurate since solvent polarity influences bioactive compound extraction efficiency (Wado et al., 2022). Arawande et al. (2018) also added that these solvents' ability to extract selected compounds can be evaluated based on the extractable crude matter collected from the extraction. Crude extract of chloroform, for instance, belongs to a non-polar solvent but exhibits an uneven charge distribution in its molecular structure, allowing it to attract polar compounds comparable to water (Martinsen & Heiskanen, 2023). This occurrence explained the lower polyphenolic compositions in the sequential technique when ethanol was used as the solvent, due to some compounds being feasibly extracted by chloroform beforehand. In contrast to chloroform, the presence of a polar hydroxyl group in the ethanol structure contributes to a high net dipole moment, increasing hydrogen bonding ability with other molecules. This property enables ethanol to attract more polar compounds, accounting for the significant increase in polyphenolic compound extraction observed in Figures 3 and 4.

Phytochemical analysis of *G. mangostana* leaf extract, along with other *Garcinia* species (data not shown), revealed the presence of various phytochemical compounds in both chloroform and ethanolic extracts. While both extracts demonstrated antioxidant properties, antimicrobial activity was observed only in the ethanolic extract, with hexane showing no activity against any tested microorganisms. These findings differ from those of Bhat and Al-Daihan (2013) and Janardhanan et al. (2020), who reported significant antimicrobial activity in the chloroform extract of *G. mangostana*. The inconsistency in findings could be attributable to the differences in extraction technique, given the low polarity of chloroform compared to ethanol. Accordingly, ethanol feasibly dissolved more active antimicrobial phytochemicals, reducing the effectiveness of the chloroform extract. Moreover, it is worth mentioning that the variability in polyphenol patterns in this study is probably due to the usage of crude extract form, which exhibits limitations. The potential for synergistic or antagonistic effects expressed by multiple bioactive compounds highlights the significance of evaluating the interactions between these compounds.

CONCLUSION

The direct extraction technique was revealed to improve the productivity of phytochemicals and extractable matter specifically for *G. mangostana*. Incomparable to the sequential technique, the direct extraction technique effectively enhanced the radical scavenging

activity of *Garcinia* plants, evidenced by the greater findings of DPPH and ABTS activity. Future studies emphasise identifying particular bioactive compounds accountable for the expression of antioxidants and antimicrobial activities in direct and sequential techniques, potentially offering a more comprehensive understanding of the overall variability of the results obtained.

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