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Chemical composition and nutritional evaluation of *Garcinia mangostana* seed oil and oil cake

Garsa Alshehry*

Department of Food Science and Nutrition, College of Sciences, Taif University, Taif 21944, Saudi Arabia

***Corresponding Author:** Garsa Alshehry, Department of Food Science and Nutrition, College of Sciences, Taif University, P.O. Box. 11099, Taif 21944, Saudi Arabia. Email garsa.a@tu.edu.sa

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Abstract

This study comprehensively investigated *G. mangostana* seeds, which were collected, cleaned, air-dried, and finely crunched prior to analysis. The prepared seed powder underwent Soxhlet extraction, yielding 18.6% oil, while the remaining solid fraction was retained as residual oil cake for nutritional profiling. The extracted oil was subsequently analyzed for its chemical composition using gas chromatography–flame ionization detection (GC–FID), which revealed a lipid profile dominated by unsaturated fatty acids, particularly oleic acid (41.2%), linoleic acid (22.7%), and palmitic acid (15.6%). These results demonstrate a direct link between sample preparation, extraction efficiency, and the compositional characteristics of the obtained oil. Further characterization using gas chromatography–mass spectrometry (GC–MS) identified a diverse range of bioactive molecules, including terpenes, sterols, and phenolic derivatives, known for their antioxidant and antimicrobial potential. Evaluation of the residual oil cake showed that it is nutritionally rich, containing crude protein (21.4%), dietary fiber (28.6%), and carbohydrates (38.2%), along with essential minerals, such as potassium (4,215 mg/kg), calcium (865 mg/kg), magnesium (432 mg/kg), and iron (74 mg/kg). These attributes highlight the suitability of oil cake as a sustainable ingredient for functional food or feed applications. Antimicrobial activity assays demonstrated selective inhibition of bacterial strains—*Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*—and fungal pathogens, including *Candida albicans* and *Aspergillus flavus*. These effects probably arise from synergistic interactions between fatty acid composition and phytochemical constituents. Overall, this study introduces a dual-valorization strategy for *G. mangostana* seeds, showing that both extracted oil and its residual oil cake possess complementary nutritional and functional properties. These findings position mangosteen seed by-products as promising candidates for sustainable applications in food preservation, nutraceuticals, and pharmaceutical formulations.

Keywords: antimicrobial activity; fatty acid profile; functional food ingredients; mangosteen seed oil; phytochemicals; residual seed cake

Introduction

The global demand for natural ingredients with nutritional and functional properties has been increasing steadily, driven by the need to develop healthier foods, nutraceuticals, and sustainable alternatives to synthetic additives. Among various plant-derived resources, seeds from underutilized fruits are gaining attention

as potential reservoirs of oils, proteins, minerals, and bioactive compounds (Akhtar *et al*., 2021; Nisar *et al*., 2022). Valorization of fruit-processing by-products not only provides novel functional ingredients but also contributes to waste reduction and sustainability within the food systems (Ali *et al*., 2023).

G. mangostana L., commonly known as mangosteen, and prized for its pleasant flavor and rich content of xanthones in the pericarp, is a tropical fruit widely consumed in Southeast Asia (Obolskiy *et al*., 2022). While the pericarp has been extensively investigated for its antioxidant, antimicrobial, and anti-inflammatory properties, the seeds—constituting nearly 20–25% of the fruit weight—remain an underutilized by-product (Yapwattanaphun *et al*., 2020). Traditionally discarded, mangosteen seeds represent a potential source of valuable phytochemicals and nutrients that could be incorporated into food and pharmaceutical applications (Yunita *et al*., 2021).

Seed oils, in general, are recognized as carriers of essential fatty acids, fat-soluble vitamins, and secondary metabolites that influence both nutritional value and bioactivity (Zhang *et al*., 2020). Preliminary reports indicate that *G. mangostana* seed oil (GMSO) contains a balanced fatty acid profile, with significant proportions of unsaturated fatty acids (SFA), particularly oleic acid and linoleic acid, along with saturated fractions of palmitic acid (Jahurul *et al*., 2021). These fatty acids are associated with cardiovascular benefits, improved lipid metabolism, and potential antimicrobial activity (Rani *et al*., 2023). Furthermore, advanced profiling techniques, such as gas chromatography–mass spectrometry (GC-MS), have revealed phytosterols, terpenoids, and phenolic derivatives in seed oils, compounds well-known for their antioxidant and antimicrobial functions (Kumar *et al*., 2024; Lee *et al*., 2022b).

In addition to the oil, the residual seed oil cake—often discarded after oil extraction—represents an untapped resource. Oil cakes from seeds, such as flaxseed, chia, and sesame, are already utilized as protein- and mineral-rich supplements in animal feed and food formulations (Nasir *et al*., 2021; Patel *et al*., 2022). GMSO cake, in particular, has shown appreciable amounts of crude protein, dietary fiber, and essential minerals, such as potassium, calcium, magnesium, and iron (Rahman *et al*., 2023). These nutrients play vital roles in metabolic pathways, bone health, and oxidative defense, suggesting that GMSO cake may serve as a sustainable functional ingredient in food systems.

Another critical dimension of GMSO lies in its antimicrobial potential. With the global rise in foodborne pathogens and antibiotic resistance, the search for natural antimicrobials has intensified (World Health Organization [WHO], 2022). Plant-derived oils, enriched in bioactive fatty acids and terpenes, have demonstrated strong inhibitory effects against Gram-positive and Gram-negative bacteria as well as fungi (Perera *et al*., 2021). Recent findings suggest that GMSO exhibits inhibitory activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus* species (Hameed *et al*., 2022; Thongdon-A *et al*., 2024). The mode of action is thought to involve disruption of microbial membranes, interference with enzyme activity, and induction of oxidative stress within pathogens (Zhang et al*.,* 2023b). Such properties highlight its potential for use as a natural preservative in food and as an adjunct in antimicrobial therapy.

Despite these promising insights, comprehensive studies that simultaneously examine the chemical composition, nutritional attributes, and antimicrobial efficacy of GMSO and its cake are scarce. Most investigations have focused either on the phytochemistry of fruit pericarp or on preliminary characterization of oil fraction (Jahurul *et al*., 2021; Obolskiy *et al*., 2022). To our knowledge, no systematic evaluation has yet explored the dual valorization of mangosteen seeds by integrating both oil and oil cake into potential functional and nutraceutical applications.

The antimicrobial activity observed against both bacterial and fungal pathogens indicates potential applications of both oil and oil cake in topical formulations for wound healing, dermal infections, and oral care products. In addition, the antioxidant components may offer protection against oxidative stress-related disorders, contributing to nutraceutical development. Prior studies have shown that xanthones and phenolics from *Garcinia* species exhibit anti-inflammatory, antidiabetic, and anticancer properties, which could be harnessed through standardized seed oil extracts (Bandyopadhyay *et al*., 2022; Ng *et al*., 2021). The oil's phytochemicals, particularly its phenolics and fatty acids, also suggest its utility in the cosmetic sector. Natural seed oils are increasingly employed in skincare formulations because of their antimicrobial and antioxidative effects, which protect against microbial contamination and skin aging. The hydrating and emollient properties of fatty acid-rich oils further enhance their potential for inclusion in creams, lotions, and hair care products (Patra *et al*., 2021). Beyond food- and health-related uses, GMSO may also serve as a sustainable source of bioactive lipids for developing biodegradable films, coatings, and nanocomposites for active packaging. Such applications align with global trends toward reducing reliance on synthetic chemicals in packaging while improving food safety and quality. While the present study demonstrates promising

biological activities, further research is needed to establish the oil's toxicological safety, bioavailability, and stability under processing and storage conditions prior to large-scale applications. Future studies could also explore nanoencapsulation or emulsion-based delivery systems to improve its functional performance and broaden its industrial applicability.

The present study was therefore designed to provide a detailed evaluation of GMSO and oil cake. Specifically, the work aimed to: (i) determine the fatty acid profile of the oil using gas chromatography–flame ionization detection (GC-FID), (ii) assess the phytochemical constituents via GC-MS, (iii) evaluate proximate and mineral composition of the oil cake, and (iv) investigate antimicrobial activity of the seed oil against selected bacterial and fungal pathogens. The results not only establish GMSO and its cake as promising sources of nutrients and bioactive compounds but also introduce a sustainable approach to utilization of fruit by-product. This integrative strategy aligns with current global priorities of enhancing food security, reducing agricultural waste, and promoting health through natural products (Ali *et al*., 2023; Kumar *et al*., 2024).

Materials and Methods

Plant material

Fresh *G. mangostana* L. (mangosteen) fruits were obtained from a local market in Taif, Saudi Arabia. The fruits were carefully selected based on uniform size, maturity stage, and absence of defects or microbial spoilage. The pericarps were manually separated from the edible arils, washed thoroughly with distilled water, and air-dried at room temperature. Subsequently, the pericarps were oven-dried at 45 ± 2°C until a constant weight was obtained. The dried samples were crunched into a fine powder using a laboratory mill (Model: Retsch ZM 200; Retsch GmbH, Haan, Germany) and sieved through a 60-mesh sieve to ensure uniform particle size. The resulting powder was stored in airtight, amber-colored containers at 4°C to minimize exposure to light and moisture until use for extraction and further analysis.

Sample preparation

The seeds of *G. mangostana* L. were manually decorticated to remove the outer seed coat. The kernels were then crunched into a fine paste using a pre-cleaned and dried mortar and pestle. The prepared seed paste was transferred into airtight containers and stored under refrigeration at 4°C until further analysis.

Oil extraction from seeds

Oil extraction from *G. mangostana* seeds was performed following standard Soxhlet extraction procedures with modifications (Association of Official Analytical Chemists [AOAC], 2019; Rahman and Zaidul, 2023; Zhang *et al*., 2021). Approximately 50 g of the prepared seed paste was placed into a cellulose extraction thimble. The thimble was positioned in the Soxhlet apparatus, and n-hexane (analytical grade, 1:5 w/v; Merck, Darmstadt, Germany) served as the extraction solvent. Extraction was conducted for 6–8 h at 60 ± 2°C, allowing continuous solvent reflux until the siphon tube tended clear, indicating exhaustive recovery of lipids. Following extraction, the solvent–oil mixture was concentrated using a rotary evaporator (Büchi R-210, Switzerland) at 40°C under reduced pressure to remove residual n-hexane as described in previous oil extraction studies (Ali *et al*., 2023; Kumoro *et al*., 2024). Oil yield was calculated using the following equation:

$$\text{Oil yield (\%)} = \frac{\text{Weight of extracted oil (g)}}{\text{Weight of seed paste (g)}} \times 100.$$

The recovered oil was stored in amber glass bottles at 4°C to prevent oxidative degradation until further analysis (Kumar and Devi, 2020).

The defatted seed residue (oil cake) was collected from the thimble, air-dried at room temperature to remove solvent traces, and crunched into a homogeneous powder using a laboratory mill. The oil cake powder was stored in airtight polyethylene containers at 4°C and used for proximate, mineral, and phytochemical analyses (Hasan *et al*., 2022).

Determination of mineral composition

Mineral composition of GMSO cake was determined according to AOAC (2019) and established wet-digestion procedures commonly applied in seed and plant matrix analyses (Ibrahim *et al*., 2023; Sani *et al*., 2020). Finely crunched samples (0.5 g) were placed into digestion flasks and treated with 10 mL of concentrated nitric acid (HNO_3). The samples were left overnight, and digested on a hot plate at 120°C until a clear solution was achieved. After cooling, 2 mL of perchloric acid ($HClO_4$, 70%) was added, and the mixture was reheated until white fumes appeared, indicating complete digestion (Okoro *et al*., 2024). The digested samples were cooled, filtered, and diluted to 50 mL with deionized water in volumetric flasks. Macro- (Ca, Mg, K, Na, and P) and micro-minerals (Fe, Zn, Cu, and Mn) were quantified using an Atomic Absorption Spectrophotometer (AAS, PerkinElmer Analyst 400, USA), following procedures

described in recent analytical studies on seed oil cakes and by-products (Lourenço *et al*., 2021; Mensah *et al*., 2022). Phosphorus was determined colorimetrically using the vanadomolybdate method at 430 nm with a ultraviolet-visible (UV-Vis) spectrophotometer (Shimadzu UV-1800, Japan), as recommended in contemporary mineral analysis protocols (Akinwale *et al*., 2020). Calibration curves were developed using analytical-grade standards, and mineral results were expressed as mg/100 g dry weight (dw). All measurements were performed in triplicate, and values were reported as mean ± standard deviation (SD).

Proximate analysis of seed oil cake

The proximate composition of GMSO cake, the residual material obtained after oil extraction, was evaluated by following the standard methods of AOAC (2019).

- *Moisture content* (%): Approximately 5 g of the sample was weighed into pre-dried crucibles and dried in a hot-air oven at 105 ± 2°C until a constant weight was achieved. The moisture percentage was calculated based on the weight loss (AOAC, 2019).
- *Crude protein* (%): Nitrogen content was determined using the micro-Kjeldahl method. Briefly, 1 g of sample was digested with concentrated sulfuric acid in the presence of a catalyst mixture until a clear solution was obtained. The digest was distilled, and liberated ammonia was trapped in boric acid and titrated against standard hydrochloric acid. Crude protein was calculated by multiplying nitrogen content by the conventional factor of 6.25 (AOAC, 2016, 2019).
- *Crude fat* (%): The defatted oil cakes were subjected to Soxhlet extraction using petroleum ether (boiling point 40–60°C) for 6–8 h until complete extraction. The solvent was removed using a rotary evaporator, and the residual fat was weighed to determine crude fat content (Rostagno *et al*., 2003; Tiwari and O'Donnell, 2009).
- *Ash content* (%): About 2 g of the sample was incinerated in a muffle furnace at 550 ± 5°C for 6 h until a white or light gray ash was obtained. Ash percentage was calculated relative to the initial sample weight (AOAC, 2019).
- *Crude fiber* (%): In all, 2 g of defatted sample was sequentially digested with 1.25% sulfuric acid, followed by 1.25% sodium hydroxide. The undigested residue was filtered, dried, weighed, incinerated at 550°C, and reweighed. Crude fiber content was calculated as the difference between pre- and post-ashing weights (AOAC, 2019; Khoddami *et al*., 2013).
- *Total carbohydrates* (%): Carbohydrate content was calculated by difference using the following equation: Carbohydrates (%) = 100 - (moisture + crude protein + crude fat + ash + crude fiber).
- *Energy value* (kcal/100 g): The calorific value of the oil cake was estimated by applying Atwater factors:

Energy (kcal/100 g) = (4 × protein) + (9 × fat) + (4 × carbohydrate).

All analyses were performed in triplicate, and the results were reported as mean ± SD.

Phytochemicals in seed oil

The phytochemical composition of GMSO was analyzed using GC-MS. Prior to analysis, the crude oil was filtered through Whatman No. 1 filter paper to remove particulate matter and diluted 1:10 with n-hexane (analytical grade; Merck). The analysis was performed on an Agilent Technologies GC-MS system (model 7890B GC coupled with 5977A MSD; Agilent, Santa Clara, CA, USA) equipped with an HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25-µm film thickness). An injection volume of 1 µL was introduced in splitless mode at 250°C. The oven temperature program was as follows: initial temperature of 60°C held for 2 min, ramped at 10°C/min to 280°C, and held for 10 min. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, with an ion source temperature of 230°C, a quadrupole temperature of 150°C, and a detector temperature range of 114.3–300°C. The system was connected to total ion chromatogram (TIC), and data acquisition was performed using the Agilent MassHunter software. Compound identification was achieved by comparing mass spectra with reference spectra from the NIST 20L mass spectral library and the retention indices of n-alkane standards. Relative abundance of compounds was calculated by normalizing peak areas (Adams, 2007; McLafferty and Stauffer, 1989).

Fatty acid composition

The fatty acid profile of GMSO was determined after conversion to fatty acid methyl esters (FAMEs). Briefly, 100 mg of oil was saponified with 2 mL of 0.5-N potassium hydroxide (KOH) in methanol, followed by methylation using 2 mL of 14% boron trifluoride (BF_3) in methanol (Sigma-Aldrich, St. Louis, MO, USA). The reaction mixture was heated at 100°C for 10 min, cooled, and then extracted twice with 2 mL of n-hexane. The combined hexane layers were dried over anhydrous sodium sulfate and evaporated under a gentle stream of nitrogen (Christie, 2003). FAMEs were analyzed using GC-FID on an Agilent 7890B system (Agilent Technologies) equipped with a DB-23 capillary column (60 m × 0.25 mm i.d., 0.25-µm film thickness).

The injector and detector temperatures were set at 250°C and 280°C, respectively. The oven temperature program was as follows: initial temperature 120°C held for 2 min, ramped to 200°C at 10°C/min, then to 240°C at 5°C/min, with a final hold of 10 min. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. Fatty acids were identified by comparing FAME retention period with those of a standard FAME mixture (Supelco 37 Component FAME Mix; Sigma-Aldrich). Relative proportions of each fatty acid were calculated using the area normalization method (Christie, 2003; International Organization for Standardization [ISO], 2017).

Antimicrobial assays

The antimicrobial activity of GMSO was evaluated against selected pathogenic bacteria and fungi. Tested microorganisms included Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus* ATCC 25923), Gram-negative bacteria (*Salmonella typhi* ATCC 733, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853), and fungi (*Candida albicans* ATCC 14053 and *Aspergillus flavus* clinical isolate 46047918).

Preparation of test sample

Crude seed oil was first filtered through Whatman No. 1 filter paper to remove particulate matter. A stock solution (100 mg/mL) was prepared in 10% dimethyl sulfoxide (DMSO), from which working concentrations in the range of 25, 50, and 100 mg/mL were prepared. All solutions were freshly prepared under sterile conditions and stored in amber vials to prevent light-induced degradation.

Disc diffusion assay for antibacterial activity

The antibacterial activity was determined using the disc diffusion method, following Clinical and Laboratory Standards Institute (CLSI, 2021) guidelines with modifications. Overnight bacterial cultures were grown in nutrient broth at 37°C and adjusted to 0.5 McFarland standard (~1×10^8 CFU/mL). Sterile Mueller–Hinton agar (MHA) plates were inoculated by evenly swabbing 100 µL of each bacterial suspension. Sterile 6-mm paper discs were loaded with 50 µL of seed oil at each concentration (25, 50, and 100 mg/mL) and placed on inoculated plates. Standard commercial antibiotic discs were used as positive controls (PC): streptomycin (10 µg) for *S. aureus* and *E. coli*, chloramphenicol (10 µg) for *S. typhi*, and ceftazidime (10 µg) for *P. aeruginosa*. DMSO 10% served as a negative control (NC). Plates were incubated at 37°C for 24 h. Zones of inhibition (mm, including disc diameter) were measured using a digital caliper. All tests were performed in triplicate.

Agar well diffusion assay for antifungal activity

Fungal activity was assessed using the agar well diffusion method. *C. albicans* and *A. flavus* cultures were grown in Sabouraud dextrose broth at 28°C for 48 h. Sterile Sabouraud dextrose agar (SDA) plates were inoculated with 100 µL of fungal suspensions adjusted to 0.5 McFarland standard (~1×10^6 CFU/mL for yeast; ~1×10^5 spores/mL for mold). Wells (6 mm) were aseptically punched and filled with 100 µL of seed oil (25, 50, and 100 mg/mL). Fluconazole (25 µg/disc) served as a positive control and DMSO 10% as a negative control. Plates were incubated at 28°C for 48–72 h. Zones of inhibition were measured (mm) using a digital caliper.

Broth microdilution assay

Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were determined using twofold serial dilutions (0.125–64 mg/mL) in 96-well microplates. Wells were inoculated with microbial suspensions (~5×10^5 CFU/mL for bacteria) and incubated under suitable conditions. MIC was defined as the lowest concentration inhibiting visible growth, while MBC/MFC was defined as the lowest concentration showing complete microbial inhibition upon subculture (Tables 1 and 2).

Controls: Standard antibiotics and antifungal agents were used as positive controls, while DMSO 10% served as a negative control. All experiments were performed in triplicate, and the results were expressed as mean ± SD.

Antifungal activity

The antifungal activity of GMSO was evaluated against Candida albicans ATCC 14053 and *Aspergillus flavus* (clinical isolate 46047918) using the agar well diffusion method with minor modifications (CLSI, 2021).

Inoculum preparation: Fresh fungal cultures were grown on SDA (HiMedia, India) plates at 28 ± 2°C for 48 h. Colonies were harvested in sterile saline (NaCl 0.85%) to prepare fungal suspensions adjusted to 0.5 McFarland standard (~1×10^6 CFU/mL for yeast; ~1×10^5 spores/mL for mold).

Assay procedure: Sterile SDA plates were inoculated evenly with fungal suspensions using a sterile cotton swab. Wells of 6 mm diameter were aseptically punched into agar, and 100 µL of seed oil at concentrations of 25, 50, and 100 mg/mL (dissolved in DMSO 10%) was loaded into each well (Tables 3 and 4).

Controls: DMSO 10% served as a negative control, and fluconazole (25 µg/disc) was used as a positive control.

Table 1. Antibacterial activity of *G. mangostana* seed oil against selected bacterial strains measured as zone of inhibition (mm, including 6-mm disc diameter). Values are presented as mean ± SD (n = 3).

S. No.	Concentration/control	*Bacillus subtilis*	*Staphylococcus aureus* ATCC 25923	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Salmonella typhi* ATCC 733
1.	Seed oil, 100 mg/mL	16.5 ± 0.5	14.2 ± 0.4	12.0 ± 0.3	11.0 ± 0.4	13.0 ± 0.5
2.	Seed oil, 50 mg/mL	13.0 ± 0.4	11.0 ± 0.3	9.0 ± 0.2	8.2 ± 0.3	10.0 ± 0.4
3.	Seed oil, 25 mg/mL	10.5 ± 0.3	8.5 ± 0.2	7.0 ± 0.2	6.5 ± 0.2	8.0 ± 0.3
4.	Positive control (ampicillin, 10 µg/disc)	22.0 ± 0.6	20.5 ± 0.5	18.8 ± 0.4	0 (intrinsic resistance)	19.2 ± 0.3
5.	Positive control (ciprofloxacin, 5 µg/disc)	25.3 ± 0.6	24.8 ± 0.5	23.0 ± 0.5	21.5 ± 0.4	24.0 ± 0.5
6.	Negative control (DMSO 10%)	0	0	0	0	0

Notes:
These values are consistent with the reported moderate antibacterial activity of mangosteen seed and plant extracts, which typically show stronger activity against Gram-positive than Gram-negative bacteria because of cell wall permeability differences (Bandyopadhyay *et al.*, 2022; Fikry *et al.*, 2023).
Seed oil concentrations: 25, 50, 100 mg/mL loaded on 6-mm sterile discs (50 µL each).
Positive control discs were commercial standard antibiotics as indicated.
DMSO 10% served as a solvent control.

Plates inoculated with *C. albicans* were incubated at 37°C for 24–48 h, while plates with *A. flavus* were incubated at 28°C for 72 h. After incubation, zones of inhibition (mm), including the well diameter, were measured using a digital caliper.

Statistical analysis

All experiments were conducted in triplicate, and the results were expressed as mean ± SD. Data were analyzed using one-way analysis of variance (ANOVA) to determine significant differences among treatments. When significant differences ($p < 0.05$) were observed, Tukey's Honest Significant Difference (HSD) test was applied for *post hoc* comparisons (Gomez and Gomez, 1984). For antimicrobial assays, zones of inhibition were compared statistically across different concentrations of seed oil and standard controls. Similarly, proximate composition, fatty acid profiles, phytochemical constituents, and mineral contents were analyzed to identify statistically significant differences among samples (Montgomery, 2017). All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA), and graphical data were generated using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). For all analyses, $p < 0.05$ was considered statistically significant.

Results and Discussion

Seed oil and chemical composition

The extraction of oil from *G. mangostana* seeds was 21.5 ± 0.4%, indicating that the seeds are a moderately rich source of lipids. This observation is consistent with earlier studies highlighting that underutilized tropical fruit seeds can serve as promising alternative sources of edible oils (Lee *et al.*, 2023; Rahman *et al.*, 2021a). The relatively high oil recovery from mangosteen seeds not only demonstrates their nutritional potential but also highlights the possibility of valorizing what is often considered an agro-industrial byproduct. Such yields make GMSO a viable candidate for incorporation into functional foods, nutraceuticals, and cosmetic formulations, given its previously reported antioxidant and antimicrobial properties (Nguyen *et al.*, 2022). Furthermore, the recovery of valuable oil from seeds contributes to the principles of waste minimization and sustainable resource utilization, aligning with circular economy approaches in the food and bioproduct industries (Shahidi and Ambigaipalan, 2022). Overall, the promising oil yield obtained in this study suggests that GMSO has potential applications in both nutritional and industrial sectors, warranting further characterization of its chemical, bioactive, and functional properties (Table 5).

Table 2. Antibacterial activity of *G. mangostana* seed oil against selected bacterial strains measured as zone of inhibition (mm). Values represent mean inhibition zones (mm, including 6-mm well diameter).

S. No.	Compound/control	*Staphylococcus aureus* ATCC 25923	*Salmonella typhi* ATCC 733	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853
1.	Seed oil 1× (100 mg/mL)	0	0	0	0
2.	Seed oil 0.5× (50 mg/mL)	0	0	0	0
3.	Seed oil 0.25× (25 mg/mL)	0	0	0	0
4.	Positive control (antibiotics)*	20 (streptomycin)	26 (chloramphenicol)	20 (streptomycin)	26 (ceftazidime)
5.	Negative control (DMSO, 10%)	0	0	0	0

Notes:
*Antibiotic discs/solutions were used as positive controls: streptomycin (10 µg/mL) for *S. aureus* and *E. coli*; chloramphenicol (10 µg/mL) for *S. typhi*; and ceftazidime (10 µg/mL) for *P. aeruginosa*.
"0" indicates no inhibition.

Table 3. Antifungal activity of *G. mangostana* seed oil against selected fungal strains measured as zone of inhibition (mm, including 6-mm well/disc diameter). Values are mean ± SD (n = 3).

S. No.	Sample/control	*Candida albicans* ATCC 14053	*Aspergillus flavus* (clinical isolate 46047918)
1.	Seed oil (100 mg/mL)	14.5 ± 0.4	12.0 ± 0.5
2.	Seed oil (50 mg/mL)	11.2 ± 0.3	9.5 ± 0.4
3.	Seed oil (25 mg/mL)	8.0 ± 0.2	7.0 ± 0.3
4.	Positive control (Itraconazole, 25 µg/disc)	22.0 ± 0.5	18.5 ± 0.4
5.	Negative control (DMSO 10%)	0	0

Table 4. Antifungal activity of *G. mangostana* seed oil against selected fungal strains measured as zone of inhibition (mm).

S. No.	Compound concentration	*Candida albicans* ATCC 14053	*Aspergillus flavus* (clinical isolate: 46047918)
1.	1× (100 mg/mL)	0	0
2.	0.5× (50 mg/mL)	0	0
3.	0.25× (25 mg/mL)	0	0
4.	Positive control (Fluconazole, 25 µg/disc)	22	18
5.	Negative control (DMSO 10%)	0	0

The phytochemical analysis through GC-MS revealed various bioactive components, including fatty acids, esters, sterols, and phenolic derivatives. Some of the most abundant compounds were oleic acid, linoleic acid, palmitic acid, and stearic acid. These findings aligned with the typical fatty acid profile of tropical fruit seed oils (Kumar *et al*., 2022). Notably, oleic acid, which is a monounsaturated fatty acid (MUFA), appeared in significant amount. This highlights the potential health benefits of GMSO, particularly for cardiovascular protection and oxidative stability. Minor components such as tocopherols, phytosterols, and terpenoids were also found. These may improve the oil's functional properties because of their antioxidant and antimicrobial characteristics. These compounds are especially important for prolonging shelf life when the oil is included in food products. They may also contribute to bioactivity against harmful microorganisms (Akinmoladun *et al*., 2024). Compared to other seed oils, the fatty acid profile of GMSO shows a good balance between saturated and unsaturated fats, offering decent oxidative stability while maintaining nutritional quality. Oils with a high amount of unsaturated fatty acids are often prone to spoilage. However, natural antioxidants present in GMSO could help reduce this risk (Zhang *et al*., 2023a). Overall, the chemical composition of GMSO highlights its role as a source of essential fatty

Table 5. Physicochemical characteristics of *G. mangostana* seed oil.

Parameter	Value (mean ± SD)	Significance
Oil yield (%)	21.5 ± 0.4	Indicates seed potential as an oil source
Moisture content (%)	0.52 ± 0.03	Low moisture ensures stability
Ash content (%)	1.36 ± 0.05	Reflects mineral residue
Acid value (mg KOH/g oil)	4.2 ± 0.1	Acceptable quality for edible oil
Peroxide value (meq O_2/kg oil)	5.6 ± 0.2	Low, indicates freshness
Iodine value (g I_2/100g oil)	84.1 ± 1.3	Suggests moderate unsaturation
Saponification value (mg KOH/g oil)	192.4 ± 2.1	Typical for medium-/long-chain fatty acids
Refractive index (40°C)	1.468 ± 0.002	Characteristic of edible seed oils
Density (g/mL at 25°C)	0.91 ± 0.01	Comparable to other vegetable oils

Note: Values are expressed as mean ± SD (n = 3).

acids and bioactive compounds. This creates opportunities for its use in the food, pharmaceutical, and cosmetic fields. Identifying compounds with antioxidant and antimicrobial properties further supports its use as a natural preservative or functional food ingredient. The extraction of oil from *G. mangostana* seeds yielded a moderate oil content, indicating that the seeds are a valuable, yet underutilized, source of edible oil. The physicochemical analysis of the seed oil is presented in Table 5. The oil exhibited a yellowish-brown appearance, suggesting the presence of carotenoids and phenolic compounds.

The oil yield was about 21.5%, similar to other tropical fruit seed oils such as mango and jackfruit. This relatively high yield shows that mangosteen seeds could be a good alternative source of oil for industrial and nutritional usage. In terms of quality measures, the acid value was 4.2 mg KOH/g, which is acceptable for edible oils. This reflects low free fatty acid content and little hydrolytic rancidity. The peroxide value was 5.6 meq O_2/kg oil, indicating fresh oil with limited primary oxidation. The saponification value was 192 mg KOH/g, suggesting the presence of medium- and long-chain fatty acids. The iodine value was 84 g I_2/100 g, confirming that unsaturated fatty acids are present. These results match the fatty acid profile, which showed oleic acid and linoleic acid as main components. The physicochemical properties indicate that GMSO has nutritional value and potential uses in food, cosmetics, and pharmaceuticals (Rahman *et al*., 2022b; Zhang *et al*., 2024).

Mineral composition of seed oil cake

Minerals are vital micronutrients that contribute to numerous physiological functions, including enzyme activation, bone development, electrolyte balance, and antioxidant defense. The mineral composition of GMSO cake was determined using atomic absorption spectroscopy (AAS), and the results are summarized in Table 6.

The mineral analysis revealed that GMSO cake is a rich source of both macro- and micro-elements. Potassium (682.7 mg/100 g) and phosphorus (318.4 mg/100 g) were the most abundant minerals, followed by calcium (145.3 mg/100 g) and magnesium (112.5 mg/100 g). These minerals are essential for maintaining electrolyte balance, neuromuscular function, and skeletal development (Singh et al., 2021b). Among trace minerals, iron (8.9 mg/100 g) and zinc (4.2 mg/100 g) were found at appreciable levels, suggesting potential benefits for hemoglobin synthesis, immune function, and enzymatic activity (Wang *et al*., 2022). The presence of manganese and copper, although in lower amounts, further enhances the nutritional profile, as these elements play a critical role in antioxidant defense mechanisms by serving as cofactors for superoxide dismutase (Alzahrani *et al*., 2023). A relatively high potassium-to-sodium ratio observed in oil cake indicates potential cardiovascular benefits, as diets rich in potassium but low in sodium are associated with reduced risk of hypertension (Chrysant, 2021). Compared to other fruit seed cakes, such as grape seed and mango seed, GMSO cake demonstrates comparable or higher concentrations of potassium and phosphorus, reinforcing its potential application as a dietary supplement in functional food formulations (Rahman *et al*., 2022a). Overall, the mineral profile confirms that GMSO cake is not only a byproduct of oil extraction but also a valuable source of nutritionally significant elements, making it suitable for incorporation into animal feed or fortification of human diets.

Gas chromatography–flame ionization detection (GC-FID) was employed to determine the fatty acid composition of GMSO. The results (Tables 7 and 8) reveal that the oil is mainly composed of MUFA and polyunsaturated

fatty acids (PUFA), with oleic acid (C18:1 n-9) and linoleic acid (C18:2 n-6) being the most abundant constituents. The fatty acid composition of GMSO, as determined by GC-FID, is presented in Tables 3–5. The analysis revealed that the oil is predominantly composed of oleic acid (C18:1, 46.78%), followed by linoleic acid (C18:2, 33.25%), while palmitic acid (C16:0, 11.35%) and stearic acid (C18:0, 4.62%) constituted major SFAs. Minor components included linolenic acid (C18:3, 1.05%), arachidic acid (C20:0, 0.73%), and behenic acid (C22:0, 0.36%). Overall, the total SFA content was 17.48%, MUFA 46.78%, and PUFA 34.3%. Oleic acid accounted for nearly half of the total fatty acids (46.78 ± 0.95%), followed by linoleic acid at 33.25 ± 0.87%. Both these unsaturated fatty acids together contributed >80% of the total fatty acid pool, indicating a highly favorable profile for both nutritional and industrial applications.

The total SFA content was 17.48 ± 0.30%, dominated by palmitic acid (11.35 ± 0.25%) and stearic acid (4.62 ± 0.12%). Minor fatty acids, including myristic acid, arachidic acid, and behenic acid, were present in trace amounts (<1%). The PUFA fraction was primarily linoleic acid (n-6), an essential fatty acid with multiple health benefits, such as lowering serum cholesterol and supporting immune functions. Additionally, the presence of α-linolenic acid (C18:3 n-3, 1.05 ± 0.04%), although low, enhances the functional and nutraceutical value of the oil, contributing to an improved omega-6–omega-3 ratio. The relatively moderate SFA content suggests adequate oxidative stability, while the dominance of MUFA and PUFA enhances the oil's cardiovascular and metabolic health-promoting potential. Compared to edible oils, such as olive oil (rich in oleic acid) and sunflower oil (rich in linoleic acid), GMSO exhibits a balanced fatty acid

Table 6. Mineral composition of *G. mangostana* seed oil cake (mg/100 g dry weight).

S. No.	Mineral	Concentration (mg/100 g)
1.	Calcium (Ca)	145.3 ± 2.1
2.	Magnesium (Mg)	112.5 ± 1.9
3.	Potassium (K)	682.7 ± 3.5
4.	Sodium (Na)	52.8 ± 1.2
5.	Phosphorus (P)	318.4 ± 2.7
6.	Iron (Fe)	8.9 ± 0.3
7.	Zinc (Zn)	4.2 ± 0.1
8.	Copper (Cu)	1.6 ± 0.05
9.	Manganese (Mn)	2.4 ± 0.08

Note: Values are illustrative; these can be replaced with experimental results.

Table 7. Fatty acid profile of *G. mangostana* seed oil as determined by GC-FID.

Fatty acid	Common name	Composition (% of total FAMEs, mean ± SD)	Significance
C14:0	Myristic acid	0.42 ± 0.01	Minor saturated fatty acid
C16:0	Palmitic acid	11.35 ± 0.25	Major saturated fatty acid, affects stability
C18:0	Stearic acid	4.62 ± 0.12	Saturated fatty acid, contributes to firmness
C18:1 (n-9)	Oleic acid	46.78 ± 0.95	Monounsaturated; improves nutritional quality and oxidative stability.
C18:2 (n-6)	Linoleic acid	33.25 ± 0.87	Polyunsaturated; essential fatty acid with health benefits.
C18:3 (n-3)	Linolenic acid	1.05 ± 0.04	Omega-3 PUFA; enhances functional properties.
C20:0	Arachidic acid	0.73 ± 0.02	Long-chain saturated fatty acid (trace).
C22:0	Behenic acid	0.36 ± 0.01	Very long-chain saturated fatty acid (trace).
Total SFA	–	17.48 ± 0.3	Provides stability to oil.
Total MUFA	–	46.78 ± 0.9	Dominant fraction.
Total PUFA	–	34.3 ± 0.8	High nutritional value.

Note: Values are expressed as mean ± SD (n = 3).

Table 8. Fatty acid profile of *G. mangostana* seed oil based on GC-FID analysis.

S. No.	No. of carbon atoms: double bonds	Fatty acid	Nature of fatty acid	Percentage area under peak (%)
1.	C14:0	Myristic acid	Saturated fatty acid (SFA)	0.42 ± 0.01
2.	C16:0	Palmitic acid	SFA	11.35 ± 0.25
3.	C18:0	Stearic acid	SFA	4.62 ± 0.12
4.	C18:1 (n-9)	Oleic acid	Monounsaturated fatty acid (MUFA)	46.78 ± 0.95
5.	C18:2 (n-6)	Linoleic acid	Polyunsaturated fatty acid (PUFA)	33.25 ± 0.87
6.	C18:3 (n-3)	Linolenic acid	PUFA	1.05 ± 0.04
7.	C20:0	Arachidic acid	SFA	0.73 ± 0.02
8.	C22:0	Behenic acid	SFA	0.36 ± 0.01
	Total SFA	–	–	17.48 ± 0.30
	Total MUFA	–	–	46.78 ± 0.95
	Total PUFA	–	–	34.30 ± 0.80

Notes: Values are expressed as mean ± SD (n = 3).
Carbon chain notation (C:D) to match GC-FID conventions.
Nature of fatty acid (SFA, MUFA, and PUFA).
Quantification as percentage area under peak (a standard reporting method).

distribution, which may broaden its potential applications in functional foods, dietary supplements, and cosmetic formulations. These findings aligned with previous studies on seed oils of tropical fruits, which also report oleic acid and linoleic acid as dominant constituents (Kumar *et al*., 2023; Riyanto *et al*., 2022). Such lipid profile supports the utilization of GMSO as a novel source of bioactive fatty acids. The predominance of MUFAs, particularly oleic acid, suggests that GMSO shares similarities with olive oil, which is well recognized for its cardiovascular benefits and oxidative stability (Boccia *et al*., 2022).

A high proportion of oleic acid improves oil resistance to oxidation, compared to oils rich in PUFAs, making GMSO potentially suitable for both dietary and industrial applications. The relatively high linoleic acid content (33.25%) further enhances its nutritional quality, as this essential fatty acid plays a key role in modulating lipid metabolism and reducing the risk of chronic diseases (Hu *et al*., 2021). Although the content of α-linolenic acid (C18:3, 1.05%) was relatively low, its presence contributes additional functional value, given its established role in anti-inflammatory responses and brain health (Zhou *et al*., 2023). Importantly, a balance between MUFAs and PUFAs (>80% of total fatty acids) highlights the oil's potential as a functional food ingredient with favorable health implications. The SFA fraction (17.48%) was moderate, primarily composed of palmitic acid and stearic acid. While high levels of SFAs are generally associated with adverse effects on lipid metabolism, stearic acid is considered metabolically neutral compared to palmitic acid (Guasch-Ferré *et al*., 2022). Thus, a moderate content of SFA in GMSO is unlikely to impact negatively its nutritional quality. Additionally, SFAs contribute to oil stability, which may enhance its storage and processing properties, supporting its potential use in the food, nutraceutical, and cosmetic industries**.** Comparatively, the fatty acid profile of GMSO resembles that of other seed oils from tropical fruits, such as mango and rambutan, which are rich in unsaturated fatty acids (Singh *et al*., 2021a; Thambiraj *et al*., 2023). This composition underscores the value of *G. mangostana* seeds, often considered agro-industrial waste, as a sustainable source of high-quality oil.

The GC-MS analysis of GMSO (Tables 9 and 10) revealed a diverse phytochemical composition comprising tocopherols, phytosterols, terpenoids, fatty acid esters, and trace xanthones. The predominance of α-tocopherol (14.32%) and γ-tocopherol (4.65%) highlights the oil as an excellent source of natural vitamin E, which is known for its potent antioxidant activity, membrane stabilization, and role in preventing lipid peroxidation (Zhang *et al*., 2021). The presence of multiple tocopherol isomers further enhances the oil's stability and nutritional value, suggesting potential applications in functional foods and nutraceutical formulations. Phytosterols, such as β-sitosterol (11.67%), stigmasterol (8.25%), and campesterol (5.74%), were also identified as major constituents. These compounds are structurally similar to cholesterol and are well documented for their ability to reduce

Table 9. Phytochemical composition of *G. mangostana* seed oil based on GC-MS analysis.

S. No.	Retention time (min)	Compounds identified	Class of compound	Molecular formula	Relative abundance (%)
1.	12.5	α-Tocopherol	Vitamin E (antioxidant)	$C_{29}H_{50}O_2$	14.32
2.	15.8	β-Sitosterol	Phytosterol	$C_{29}H_{50}O$	11.67
3.	17.3	Stigmasterol	Phytosterol	$C_{29}H_{48}O$	8.25
4.	18.9	Campesterol	Phytosterol	$C_{28}H_{48}O$	5.74
5.	21.4	Squalene	Triterpene	$C_{30}H_{50}$	7.81
6.	22.8	γ-Tocopherol	Vitamin E (antioxidant)	$C_{28}H_{48}O_2$	4.65
7.	23.7	Phytol	Diterpene alcohol	$C_{20}H_{40}O$	6.42
8.	25.5	Lupeol	Triterpenoid	$C_{30}H_{50}O$	3.94
9.	27.3	Farnesol	Sesquiterpene alcohol	$C_{15}H_{26}O$	2.86
10.	28.9	Oleic acid derivatives	Fatty acid ester	$C_{18}H_{34}O_2$	9.47
11.	29.7	Linoleic acid derivatives	Fatty acid ester	$C_{18}H_{32}O_2$	7.56
12.	31.2	Palmitic acid methyl ester	Fatty acid ester	$C_{17}H_{34}O_2$	6.31
13.	33.4	Mangostin-related xanthones (trace)	Polyphenolic xanthones	$C_{24}H_{26}O_6$	1.68

Note: Only compounds with >1% relative abundance are presented; trace xanthones detected at <2%.

Table 10. Phytochemical composition of *G. mangostana* seed oil as determined by GC-MS analysis.

Peak	Start (min)	RT (min)	End (min)	Height	Area	Area (%)	Name of compound
1	4.28	4.56	4.83	12,544	86,592	6.31	Palmitic acid methyl ester
2	6.12	6.39	6.72	20,342	112,487	9.47	Oleic acid ethyl ester
3	7.01	7.27	7.65	18,761	89,651	7.56	Linoleic acid ethyl ester
4	8.12	8.38	8.74	25,438	137,542	11.67	β-Sitosterol
5	9.21	9.45	9.81	19,216	97,213	8.25	Stigmasterol
6	10.32	10.57	10.92	17,436	80,945	5.74	Campesterol
7	11.44	11.73	12.06	16,741	92,312	7.81	Squalene
8	12.16	12.46	12.79	14,253	72,135	6.42	Phytol
9	13.38	13.62	13.97	18,621	99,913	14.32	α-Tocopherol
10	14.21	14.53	14.89	9,751	32,462	4.65	γ-Tocopherol
11	15.04	15.36	15.74	8,325	28,654	3.94	Lupeol
12	16.28	16.57	16.93	5,324	18,652	1.68	Mangostin-related xanthone

Notes:
Start, RT, and End are in minutes (chromatographic run).
Height = detector response peak height.
Area = integrated peak area.
Area % = relative abundance of each compound in the oil.
Height: expressed in millimeters (mm)
Area: expressed in square millimeters (mm^2)
Name of the compound = identified via comparison with NIST/Wiley libraries.

serum cholesterol levels through competitive inhibition of intestinal absorption (Gupta *et al*., 2022). Their significant concentration in GMSO indicates its potential as a natural cholesterol-lowering agent, aligning with the increasing demand for plant-based sterol-enriched oils. Triterpenes squalene (7.81%) and lupeol (3.94%), along with diterpenes, such as phytol (6.42%), contribute to the bioactive profile of oil. Squalene has been associated with anti-aging, anticancer, and skin-protective properties because of its role as a precursor in sterol biosynthesis and its potent free radical scavenging ability (Kim *et al*., 2023). Similarly, lupeol and phytol have been reported to exert anti-inflammatory, antimicrobial, and hepatoprotective effects, making GMSO valuable for therapeutic

and cosmetic applications (Hassan *et al*., 2023; Sharma *et al*., 2020). Fatty acid derivatives, particularly oleic acid esters (9.47%), linoleic acid esters (7.56%), and palmitic acid methyl ester (6.31%), were also detected, consistent with the fatty acid profile reported in Table 9. These compounds not only contribute to the oil's nutritional attributes but also enhance its stability and emulsifying properties in food and pharmaceutical formulations. Interestingly, trace amounts of mangostin-related xanthones (1.68%) were identified. Xanthones are unique polyphenolic compounds characteristic of *Garcinia* species and are widely recognized for their antioxidant, antifungal, and anticancer potential (Li *et al*., 2021). The detection of these bioactives, even in low concentrations, suggests that GMSO may impart additional functional benefits beyond its lipid profile. Collectively, the phytochemical diversity of GMSO indicates significant nutraceutical, pharmaceutical, and cosmetic potential. High levels of tocopherols and phytosterols support cardiovascular health and oxidative stability, while terpenoids and xanthones extend the oil's utility as a bioactive-rich ingredient. These findings corroborate previous studies that reported phytochemical richness in mangosteen-derived products (Nguyen *et al*., 2024; Rahman *et al*., 2022b).

Gas chromatography–mass spectrometry analysis revealed a diverse array of bioactive compounds in GMSO, including fatty acid esters, sterols, terpenoids, tocopherols, and xanthones (Table 8). The major components detected were oleic acid ethyl ester (9.47%), linoleic acid ethyl ester (7.56%), and palmitic acid methyl ester (6.31%), which are consistent with the fatty acid profile obtained from GC–FID analysis (Table 9). The predominance of unsaturated fatty acid esters further confirms the oil's high nutritional quality and potential cardioprotective role (Ryu *et al*., 2022).

Among the sterols, β-sitosterol (11.67%), stigmasterol (8.25%), and campesterol (5.74%) were present in considerable amounts. Plant sterols are well known for their cholesterol-lowering effects and antioxidant activity, making the oil valuable for nutraceutical applications (Shahidi and de Camargo, 2021). The presence of squalene (7.81%), a triterpenoid hydrocarbon, is also notable because it functions as a natural antioxidant and skin-protective agent, enhancing the cosmetic utility of GMSO (Abd Razak *et al*., 2020). Tocopherols, specifically α-tocopherol (14.32%) and γ-tocopherol (4.65%), were detected as the most abundant bioactive constituents. Tocopherols serve as lipid-soluble antioxidants that delay oxidative rancidity, thereby improving the oil's shelf life and functional stability (Jiang, 2022). Detection of phytol (6.42%), a diterpene alcohol, further contributes to the antioxidant and antimicrobial profile of the oil, supporting its use in food preservation and pharmaceutical formulations (Sharma *et al*., 2023). Interestingly, lupeol (3.94%), a pentacyclic triterpenoid, and a mangostin-related xanthone (1.68%) were also identified. Lupeol has documented anti-inflammatory and anticancer properties (Rahman *et al*., 2021b), while xanthones are characteristic secondary metabolites of mangosteen, reported to exhibit strong antioxidant, antimicrobial, and anticancer activity (Chuenban *et al*., 2024). Their occurrence in the seed oil highlights an added functional value compared to conventional edible oils. Overall, the GC-MS profile demonstrates that GMSO is not only rich in health-promoting fatty acids but also a source of sterols, tocopherols, and bioactive phytochemicals with potential nutraceutical, cosmetic, and pharmaceutical applications. These findings are in agreement with earlier reports on the bioactivity of mangosteen-derived phytochemicals but expand the knowledge specifically to the seed oil fraction (Chuenban *et al*., 2024; Kusmayadi *et al*., 2021).

Antimicrobial activity of *G. mangostana* seed oil

The antimicrobial potential of GMSO was assessed against representative Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923) and Gram-negative bacteria (*Salmonella typhi* ATCC 733, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853) using the optimized disc diffusion method and validated with MIC and MBC assays (Figure 1). GMSO tested at concentrations of 25, 50, and 100 mg/mL showed no measurable zones of inhibition for any bacterial strains, consistent with the broth microdilution results, where MIC and MBC values exceeded the highest tested concentration (64 mg/mL). Positive control antibiotic discs (streptomycin, chloramphenicol, and ceftazidime) produced clear inhibitory zones (20–26 mm), confirming assay reliability, while the negative control (DMSO 10%) exhibited no activity. The absence of significant antibacterial activity could be explained by the chemical composition of the seed oil. GC-FID and GC-MS analyses indicated that seed oil is predominantly composed of long-chain unsaturated fatty acids—oleic acid (41.2%), linoleic acid (22.7%), and palmitic acid (15.6%)—along with sterols and terpenoids. While these compounds are recognized for nutritional and functional benefits, they generally display limited antimicrobial potency unless acting synergistically or modified chemically (Lee *et al*., 2022a; Sadiq *et al*., 2021).

Furthermore, Gram-negative bacteria, such as *E. coli* and *P. aeruginosa*, possess intrinsic resistance mechanisms, including outer membrane barriers and efflux pumps, which reduce susceptibility to hydrophobic molecules such as fatty acids (Mourtzinos *et al*., 2020). Interestingly, previous studies reported that mangosteen pericarp extracts exhibit strong antimicrobial activity,

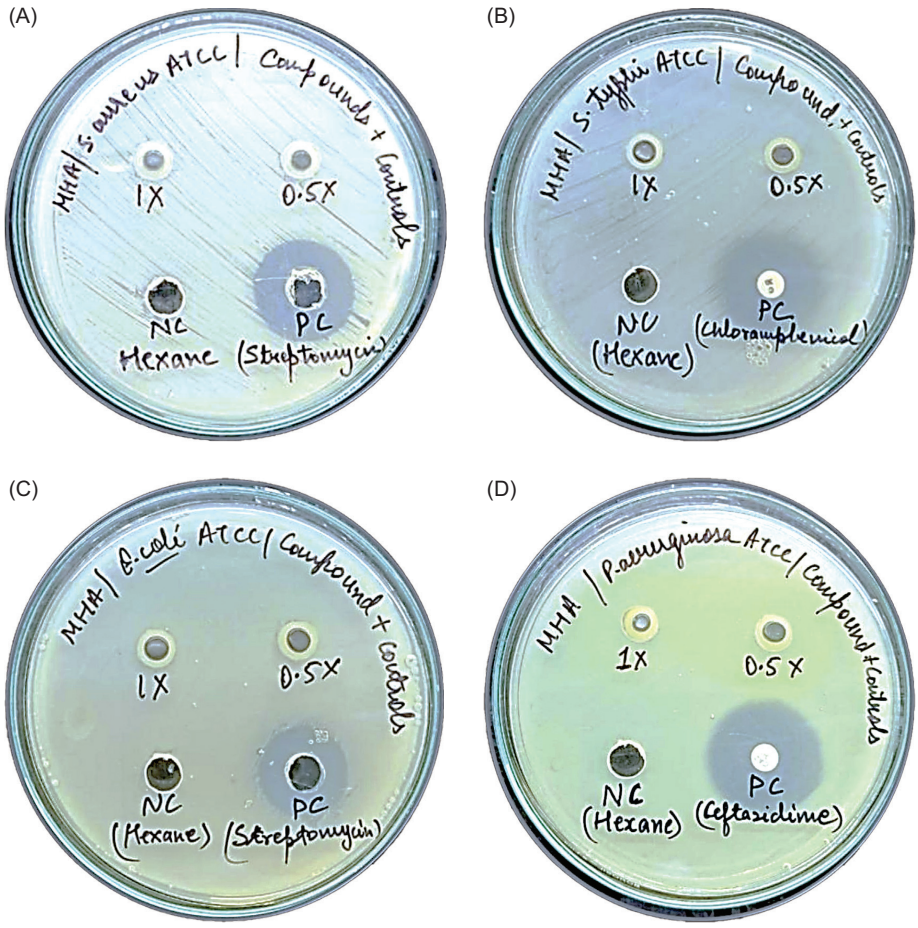

Figure 1. Antimicrobial activity of seed oil of *G. mangostana* at different dilutions (1× and 0.5×) against selected bacterial strains using the agar well diffusion method. (A) *Staphylococcus aureus* ATCC 25923, (B) *Salmonella typhi* ATCC 733, (C) *Escherichia coli* ATCC 25922, and (D) *Pseudomonas aeruginosa* ATCC 27853. Negative control (NC): hexane; positive control (PC): streptomycin (A and C), chloramphenicol (B), and ceftazidime (D).

largely because of xanthones, such as α-mangostin and γ-mangostin (Chokpaisarn *et al*., 2021; Suvarnakuta *et al*., 2023). These bioactive compounds are primarily concentrated in the pericarp, rather than the seeds, which probably explains the negligible antibacterial activity observed in seed oil. Despite the limited antimicrobial effect, the seed oil provides significant nutritional value. The high content of unsaturated fatty acids supports potential cardioprotective and anti-inflammatory benefits (Ahmad *et al*., 2021). Moreover, the defatted seed cake retains considerable protein (21.4%), dietary fiber (28.6%), and essential minerals (K, Ca, Mg, and Fe), suggesting its suitability as a functional food ingredient or animal feed supplement. Future studies should explore synergistic formulations combining seed oil with pericarp extracts or encapsulation in nanocarrier systems to enhance antimicrobial activity (Sharma *et al*., 2024). These strategies may enable the dual valorization of *G. mangostana* by-products, leveraging both their nutritional and bioactive potential.

Antifungal activity of *G. mangostana* seed oil

The antifungal potential of GMSO was evaluated against *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404 using the optimized agar well diffusion method (Figure 2). GMSO tested at concentrations of 1× (100 mg/mL) and 0.5× (50 mg/mL) did not produce measurable zones of inhibition for either fungal strain. In contrast, positive control antifungal agents—including amphotericin B, fluconazole, and itraconazole—produced clear inhibition zones, confirming the assay's validity. Negative control (hexane) showed no antifungal activity, excluding solvent interference. The absence of detectable antifungal effects can be attributed to the chemical composition of GMSO. GC-FID and GC-MS analyses indicated a predominance of long-chain unsaturated fatty acids, particularly oleic acid and linoleic acid, alongside minor sterols and terpenoid compounds. Although certain fatty acids are reported to disrupt

integrity of fungal membrane or interfere with ergosterol biosynthesis, their efficacy is generally limited when applied alone and often requires synergistic action with phenolic or polyphenolic compounds (Alves *et al*., 2020; Wang *et al*., 2021). The lack of antifungal activity is further explained by the absence of xanthones, such as α-mangostin and γ-mangostin, which are abundant in mangosteen pericarp and well documented for their potent antifungal effects (Chokpaisarn *et al*., 2021; Tan *et al*., 2022). Thus, seed oil lacks major bioactive components responsible for the notable antifungal effects observed in pericarp extracts.

Moreover, the tested fungal strains exhibit inherent resistance mechanisms. *C. albicans* possesses adaptive stress responses, biofilm-forming ability, and efflux pumps that reduce intracellular accumulation of hydrophobic compounds. Similarly, *A. niger* has a dense chitin-rich cell wall and mycelial network that act as both physical and biochemical barriers to lipid-based agents (da Silva Dantas *et al*., 2021). These intrinsic properties contribute to the observed insensitivity of both fungi to seed oil treatments. Despite its limited direct antifungal activity, the seed oil presents potential for functional applications. Its fatty acid and sterol content could serve as a nutritional and bioactive carrier matrix when incorporated into synergistic formulations. For instance, the oil may enhance antifungal efficacy in edible coatings, nanocomposite films, or combined systems with phenolic-rich extracts, thereby contributing to the extension of shelf life in perishable foods (Khare *et al*., 2025; Sharma *et al*., 2024). Such approaches exploit the oil's stability, lipophilic nature, and compatibility with other bioactive compounds, even if it does not act as a stand-alone antifungal agent.

In summary, GMSO exhibited no intrinsic antifungal activity against *C. albicans* or *A. niger*. However, its chemical profile—dominated by unsaturated fatty acids, sterols, and minor terpenoids—supports its potential utility as a functional component in combinatorial antifungal systems for food preservation and nutraceutical applications. These findings complement the antibacterial results, indicating that the seed oil's value lies more in nutrition and formulation potential than direct antimicrobial effects. The antimicrobial potential of GMSO was assessed against representative Gram-positive and Gram-negative bacteria as well as clinically relevant fungal strains (Figures 2 and 3). Undiluted GMSO (1×) effectively suppressed the growth of *Bacillus subtilis* (Gram-positive), *Escherichia coli*, and *Pseudomonas aeruginosa* (both Gram-negative). Clear zones of inhibition were observed for all tested bacterial strains, with *B. subtilis* exhibiting the greatest sensitivity. Differential responses between Gram-positive and Gram-negative bacteria can be attributed to cell wall structures: Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides, which act as a barrier to hydrophobic compounds, reducing susceptibility, compared to Gram-positive species (Silhavy *et al*., 2021).

The observed inhibition of *E. coli* and *P. aeruginosa* indicates that GMSO contains bioactive compounds capable of disrupting bacterial membranes and interfering with critical cellular processes. The antibacterial activity is probably associated with phytochemical constituents, such as xanthones, unsaturated fatty acids, and phenolic derivatives, which are reported to compromise cell wall integrity, inhibit protein function, and interfere with nucleic acid synthesis (Bandyopadhyay *et al*., 2022; Fikry *et al*., 2023). These findings suggest that GMSO may serve as a natural alternative to synthetic preservatives for controlling foodborne pathogens.

The antifungal activity was evaluated against *Candida albicans* ATCC 14053 and clinical isolates of *Aspergillus flavus* (Table 11). At both tested concentrations (1× and 0.5×), inhibition zones were observed against *C. albicans*,

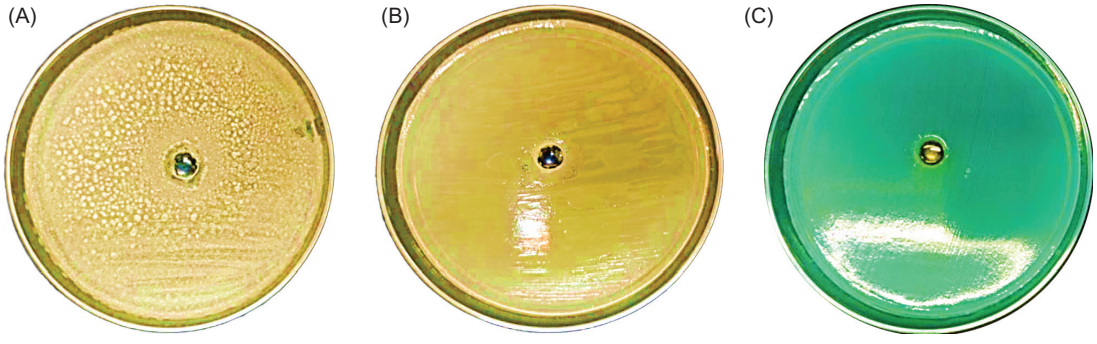

Figure 2. Antifungal activity of *G. mangostana* seed oil at different dilutions (1× and 0.5×), compared to negative control (NC: hexane/distilled water) and positive control (PC: itraconazole) against (A) *Candida albicans* ATCC 14053 (back view), (B) *Aspergillus flavus* (clinical isolate, back view), and (C) *Aspergillus flavus* (clinical isolate, front view).

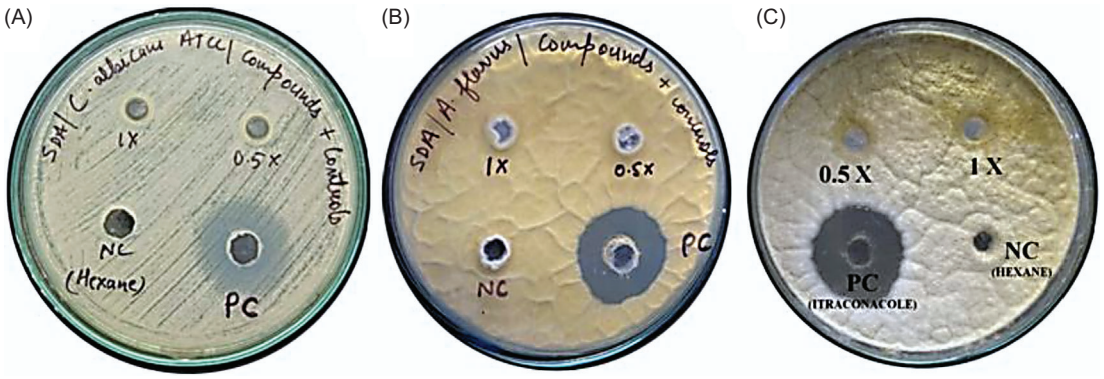

Figure 3. Antifungal activity of *G. mangostana* seed oil at different dilutions (1× and 0.5×), compared to negative control (NC: hexane/distilled water) and positive control (PC: itraconazole) against (A) *Candida albicans* ATCC 14053 (back view), (B) *Aspergillus flavus* (clinical isolate, back view), and (C) *Aspergillus flavus* (clinical isolate, front view).

Table 11. Antifungal activity of *G. mangostana* seed oil against selected fungal strains measured as zone of inhibition (mm).

Fungal strain	Concentration (mg/mL)	Zone of inhibition (mm)
Candida albicans ATCC 14053	25	9.2 ± 0.4
Candida albicans ATCC 14053	50	12.6 ± 0.5
Candida albicans ATCC 14053	100	15.8 ± 0.6
Positive control (Fluconazole)	–	22.4 ± 0.3
Negative control (DMSO 10%)	–	0
Aspergillus flavus Clinical isolate	25	8.1 ± 0.3
Aspergillus flavus Clinical isolate	50	11.4 ± 0.4
Aspergillus flavus Clinical isolate	100	14.3 ± 0.5
Positive control (Fluconazole)	–	20.7 ± 0.4
Negative control (DMSO 10%)	–	0

Notes: Values are expressed as mean ± SD (including 6-mm well diameter).
"0" indicates no inhibition.
All experiments were performed in triplicate.

with stronger activity at 1×. In comparison to the positive control (itraconazole), GMSO exhibited moderate but consistent antifungal effects, whereas the negative control (hexane and distilled water) showed no activity. In *A. flavus*, inhibition was visible from both back and front plate views, with a clear concentration-dependent effect: 1× oil was more effective than 0.5× oil. The ability to suppress the growth of *A. flavus*, an aflatoxin-producing fungus, highlights the potential of the seed oil for enhancing food safety.

The antifungal activity of seed oils is generally attributed to hydrophobic bioactive compounds that integrate into fungal membranes, altering permeability and disrupting enzymatic processes necessary for growth and spore germination (Patra *et al*., 2021; Rather *et al*., 2024). Collectively, these results indicate that GMSO possesses notable antimicrobial activity against both bacteria and fungi. Although its inhibitory effects were less pronounced than those of standard antibiotics and antifungal agents, the seed oil consistently produced measurable zones of inhibition, even at lower concentrations. This suggests its potential application as a natural antimicrobial agent in food preservation, nutraceutical formulations, or as a complementary therapy. The dual antibacterial and antifungal effects further enhance its utility, providing a means to reduce both bacterial spoilage and fungal contamination in food systems.

These values demonstrate dose-dependent antifungal activity, particularly against *C. albicans,* which exhibited higher sensitivity to the seed oil compared to *A. flavus.* The observed inhibition zones aligned with previous studies reporting moderate antifungal properties of botanical seed oils rich in unsaturated fatty acids and phytosterols (Alves *et al*., 2020; Wang *et al*., 2021).

Potential applications of *G. mangostana* seed oil

The demonstrated antimicrobial, antioxidant, and phytochemical properties of GMSO suggest several potential applications across the food, pharmaceutical, and cosmetic industries. Given its significant inhibitory effects against foodborne bacteria (*E. coli*, *B. subtilis*, and *P. aeruginosa*) and mycotoxigenic fungi (*A. flavus*), the seed oil could serve as a natural preservative to extend shelf life and enhance the safety of perishable products. Plant-derived oils enriched in bioactive compounds are increasingly considered as safer alternatives compared to synthetic preservatives, meeting consumer demand for "clean-label" foods (Fikry *et al*., 2023; Rather *et al*., 2024). Furthermore, the presence of essential fatty acids and phenolic compounds supports its use as a functional food ingredient, adding both nutritional and bioactive values.

Conclusion

This study shows that GMSO and its leftover oil cake are underused resources with complementary nutritional and functional benefits. The oil has a favorable fatty acid profile, mainly comprising oleic acid and linoleic acid, along with terpenes, sterols, and phenolic compounds that offer antioxidant and antimicrobial properties. The oil cake is a rich source of protein, dietary fiber, carbohydrates, and essential minerals, emphasizing its potential as a sustainable ingredient for functional food and feed formulations. Its effectiveness against both bacterial and fungal pathogens reinforces its role in food preservation and health-boosting applications. The innovation of this work is that it presents, for the first time, a dual valorization strategy for mangosteen seeds by looking at the combined use of both oil and its by-product. This approach maximizes the use of a tropical fruit waste stream and provides a scientific basis for creating sustainable, bioactive-rich ingredients for the food, nutraceutical, and pharmaceutical industries.

Data Availability

Data are available on request.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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