

PROXIMATE COMPOSITION, MINERAL ANALYSIS, PHYSICOCHEMICAL, ANTIBACTERIAL AND CYTOTOXIC PROPERTIES OF *PSIDIUM GUAJAVA L.* SEED

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Abstract

Guava (*Psidium guajava*) seeds are an underutilized by-product with potential nutritional and bioactive value. The present study evaluated the proximate composition and mineral profile of guava seed powder (GSP), together with the physicochemical characteristics, antibacterial activity, and preliminary toxicity of guava seed oil (GSO). Proximate analysis showed that crude fiber was the predominant component of GSP (45.20 ± 0.60 g/100 g), followed by total carbohydrate (31.15 ± 0.20 g/100 g) and crude fat (14.32 ± 0.50 g/100 g). The powder contained 8.10 ± 0.50 g/100 g protein, 8.03 ± 0.10 g/100 g moisture, and 1.23 ± 0.10 g/100 g ash, with a calorific value of 294.10 kcal/100 g. Mineral analysis revealed potassium as the most abundant mineral (540.3 ± 0.1 mg/100 g), followed by sodium (321.0 ± 0.5 mg/100 g), phosphorus (190.7 ± 0.5 mg/100 g), calcium (140.2 ± 0.5 mg/100 g), and magnesium (115.3 ± 0.3 mg/100 g). GSO exhibited an iodine value of 140 ± 0.5 g I₂/100 g, saponification value of 188 ± 0.8 mg KOH/g, acid value of 0.66 ± 0.1 mg KOH/g, peroxide value of 2.11 ± 0.1 meq/kg, and oxidative stability of 5.8 ± 0.1 h. The oil demonstrated antibacterial potential contrary to all tried bacterial species, with the greatest zone of inhibition observed contrary to *Escherichia coli* (17 ± 0.4 mm), which also showed the lowest MIC (150 mg/mL) and MBC (300 mg/mL). In the *Artemia salina* lethality assay, GSO produced 0% mortality at 10, 100, and 1000 µg/mL after 24 h, whereas the etoposide positive control produced 100% mortality. Overall, the findings demonstrate that guava seed powder is a fiber- and mineral-rich plant material, while its oil possesses favorable physicochemical characteristics and measurable antibacterial activity, supporting the potential utilization of guava seeds as a value-added nutritional and bioactive resource.

Introduction

The human diet is a complex mix of metabolites, and the composition of what we eat can affect our health and the development of diseases like diabetes, cardiovascular complications and cancer (Senyilmaz-Tiebe *et al.*, 2018). The family Myrtaceae is included in the genus guava (*Psidium guajava*), which consists of about 100 species of tropical and subtropical shrubs and small trees. It is inherent to subtropical and tropical areas of Mexico, the Caribbean, Central and northern South America and is planted around the world in subtropical and tropical areas of Africa, Asia, Pacific islands, and the USA (Malacrida and Jorge, 2013). As agro-industrial processing spreads around the globe, significant amounts of agricultural and food-processing residues have been produced. A share of these residues is used as animal feed, compost or soil amendments, but a significant fraction is dumped without proper treatment which may lead to environmental pollution. In addition, inappropriate disposal is an economic loss due to the fact that many plant residues contain biologically active constituents that have potential antioxidant and other efficient possessions, like fatty acids and phenolic compounds (Uchoa-Thomaz *et al.*, 2014). In guava processing, juices, purees and instant fruit powder are produced, and significant amounts of solid by-products are created. Around 30% of the processed fruit can be left as waste like seeds, peel and pulp leftover after processing (Swati *et al.*, 2020). Guava seeds are of special interest among these residues as they have significant lipid content and other bioactive constituents. The palmitic and stearic acids like fatty acids are responsible for the possible industrial applications of guava seed oil. The fat is ironic in fatty acids which can be used in the manufacture of soap, lubricants and softening agents. Furthermore, oil extracted from the guava seeds, which is an agricultural by-product, has the potential to be used in blends with the conventional edible oils to alter the physicochemical and nutritional properties. Thus, the recovery and exploitation of seed oil of guava can be a solution to use fruit-processing waste as a

valuable product, and, at the same time, minimize environmental impact of agro-industrial wastes (Uchoa-Thomaz *et al.*, 2014).

Amongst the generated waste, the seeds of fruits produces big waste portion that are castoff annually in dump outcomes in discarding issues and cargoes ecosystem weight. Fruit processing industry by-product has enormous efficiency for the production of seeds oils from the fruits, which is financially beneficial as it is rich in natural bio-components which can be used for edible and non-edible purposes (Swati *et al.*, 2020). Fruit processing wastes are considered as potential pollutant sources and reduction of the quantity of these wastes is important. Therefore, the valorisation of fruit-processing by-products and the recovery of bioactive compounds is a growing interest, as it is the future of sustainable use and the potential for waste valorisation. (Uchoa-Thomaz *et al.*, 2014).

The utilization of seeds in all their variants resolve facilitate the reduction of waste, the commercialization of the product, aversive material for technology and nutrition purposes. Therefore, attention needs to be given to using all the fruit seeds produced by the trees year after year to help solve environmental problems and find a new income source (Swati *et al.*, 2020).

Therefore, the current study aims was to add value to a waste product (guava seeds). This will be achieved through the evaluation of the proximate and minerals composition guava seed powder (GSP), physio-chemical composition of guava seed oil (GSO), and antibacterial and toxicity activities of GSO.

Materials and Methods

Collection of Guava Seeds

Guava seeds were collected from Food Pilot Plant, Pakistan Council of Scientific and Industrial Research (PCSIR), Peshawar, Khyber Pakhtunkhwa-Pakistan along with wet skin sticking to seed and very small quantities of flesh.

Guava Seed Powder (GSP) Preparation

The seeds were wash-down with ordinary clean H₂O to eliminate their seed skin and pulp residue. The seeds were then dried in a Hot-Air Tray Drier

(England) for 24 hours at 40°C. The dehydrated guava seeds were coarse ground using a Laboratory Mill (Standard Model No.3 Wiley Mill- USA). The resulting powder was then put in sealed polyethylene containers for storage before analysis.

Proximate Composition of GSP

Moisture, ash, fat, crude fibre and protein content was measured based on the method described by Ali *et al.* (2025). The content of moisture was done through Electric Oven (Mettler- Germany) drying the samples to constant weight at 100–110 °C, and the loss of weight was considered as quantity of moisture. The quantity of ash was measured through burning the GSP in an Electric Furnace (Vulcan D-550, S#. JCE 1350106V, USA) at a temperature of about 550 °C for 5-6 hours till a persistent mass was obtained. The crude fat was extracted by Soxhlet Apparatus (Quickfit- England) extraction with petroleum ether for 6-8 hours. Estimation of crude fibre was done by sequential alkali digestion (1.25% NaOH) and acid digestion (1.25% H₂SO₄). The basic protein content was determined by multiplying nitrogen (N) quantity by 6.25 (N × 6.25) using the Kjeldahl method using Micro Kjeldahl (Bloc Digest, Spain). While quantity of carbohydrate was measured through deducting the percentage of ash, fiber, fat and protein from 100% of dehydrated mass (Ali, 2011). The gross energy values were calculated using physiological fuel-value coefficient of 4.1, 9.3 and 4.1 kcal/g protein, fat and carbohydrate correspondingly (Ali, 2011).

Minerals Analysis

Take one gram of GSP and digested in twenty milliliter of a mixture (acids) i.e. nitric acid (80 mL), perchloric acid and sulfuric acid (20 mL each). The sample was then placed in a digestion flask with the acid mixture and heated to produce a clear digest, which was then diluted to a final capacity of five hundred milliliter with distilled H₂O. The subsequent digest was analyzed by atomic absorption spectrophotometry through the use of element-specific filters, and the concentration of minerals was determined from calibration curves made from standard solutions. The concentrations of zinc (Zn), iron (Fe), copper (Cu), manganese

(Mn) and magnesium (Mg) were measured in diluted ashed GSP solution through Atomic Absorption Spectrophotometer (Z-2000, Hitachi, Japan) according to the method of Ali *et al.* (2025). The levels of sodium, calcium and potassium were determined by Flame Photometer (Jenway PFP7, UK). The estimation of phosphorus content was carried out through Molybdate Colorimetric technique as reported by Ali *et al.* (2025). In brief, the mineral digest solution (0.5mL) was blended with trichloroacetic acid (TCA) 9.5 mL at approximately 10% and allowed to stir for five minutes and after that sieved by means of filter paper (Whatman® Grade 42). Take a five milliliter of the filter solution and placed in cuvette and the working standard and blank (5 mL of standard solution and 5 mL of TCA, respectively) were prepared in parallel cuvettes. The Molybdate reagent (0.5 mL) was added was shifted to every cuvette, and shaken well; H₂SO₄ reagent (0.2 mL) was mixed to each cuvette. Colour development proceeded for 10 minutes before optical density using Spectrophotometer (UV-1700- Shimadzu, Japan) at 660nm. The quantity of Phosphorus (P) was measured as:

$$P = (\text{Optical density of sample} / \text{Optical density of standard}) \times \text{Conc. of Standard (5 mg/dl)} \times 100$$

Extraction of Guava Seed Oil (GSO)

The GSP was used to extract the seed oil with petroleum ether for 6 hours in a Soxhlet Extractor (Quickfit- England) and then evaporated following vacuum evaporation till the petroleum ether was completely eliminated, in a Büchi Rotary Evaporator (Model R-200, Switzerland) at 40 °C. the oil recovered was stored in glass bottles (amber), purged with N₂, wrapped and deposited at –18 °C for further analysis.

Physio-chemical characterization of GSO

Refractive index (at 40 °C), peroxide value (meq/kg), acid value (mg KOH/g), saponification value (mg KOH/g) and iodine value (g I₂/100 g) were measured by the standard methods (A.O.C.S., 2004). The unsaponifiable matter was determined by the method used by Malacrida and Jorge (2013). The oxidative stability was measured using Rancimat (Metrohm Ltd., Herisau, Switzerland)

using the oxidative stability index (OSI) which is the number of hours (hr) taken until an oil is able to produce measurable rancidity at 100 °C with 20 L/h air flow.

Antibacterial Activities of GSO

The six bacterial strains tested were: *Klebsiella pneumoniae* (K. pneumoniae), *Pseudomonas aeruginosa* (P. aeruginosa), *Escherichia coli* (E. coli), *Salmonella typhi* (S. typhi), *Bacillus subtilis* (B. subtilis) and *Staphylococcus aureus* (S. aureus). All the strains were procured from the Microbiology Research Section of PCSIR Laboratories Complex, Peshawar, Pakistan and stock cultures were stored on Nutrient Agar (NA) at 4 °C in cooled incubator.

Antibacterial Activity by Disc Diffusion Technique

The GSO antibacterial potential was assessed applying agar disc diffusion technique. The microbial species were incubated overnight in Nutrient Broth (NB) and then re-calibrated to 0.5 McFarland ($\sim 1.5 \times 10^8$ CFU/mL). All the sterile NA plates were swabbed with the standardized inoculum. Hundred μ L of GSO solution (400 mg/mL) was loaded onto sterile Whatman 6 mm filter discs which were then dried in a sterile atmosphere and transferred to the inoculated plates. Streptomycin 20 μ L (10 mg/mL) served as a positive control, 5% DMSO was used as a blank control. The plates were kept in an incubator (Mettler-Germany) then incubated for twenty-four hours with 37 ± 2 °C. The inhibition zones diameters were calculated in millimetres (mm).

Minimum Inhibitory Concentration (MIC) Determination

The Tube Broth Dilution Method was used to determine the MIC. A dilution of the GSO were prepared in sterile Mueller–Hinton broth within a test tubes, yielding final concentrations from 500 to 0.195 mg/mL (500, 400, 300, 250, 200, 150, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391 and 0.195 mg/mL). A standardized bacterial inoculum ($\sim 5 \times 10^5$ CFU/mL) was added to all the tests tubes in equal amount. Tests tubes were incubated 18-24 hours at 37 ± 2 °C and the MIC was considered as the lowest concentration of GSO where no bacterial growth was observed.

Minimum Bactericidal Concentration (MBC) Determination

The MBC is the lowermost dosage at which the level of bacteria drops to zero. After the MIC assay, 10 μ L of the aliquots from the tests tubes that did not show turbidity were transferred to fresh NA and were incubated in an incubator (Mettler-Germany) for twenty-four hours at 37 ± 2 °C. The MBC was determined as the lowermost quantities of GSO where no colonies grew on subculture in the medium.

Cytotoxicity Assessment (*Artemia salina* Lethality Assay)

To determine the cytotoxic potential of GSO, a brine shrimp (*Artemia salina*) lethality bioassay was applied, as modified by Javid and Bashir (2015). A stock solution of GSO (10 mg/mL) was obtained through liquifying twenty milligram of GSO in two milliliter of dimethyl sulfoxide (DMSO). Aliquots of 500 μ L, 50 μ L and 5 μ L of this stock were transferred into sterile glass vials for closing quantities of 1000, 100 and 10 μ g/mL dosages, respectively. Vials were exposed to the air at room temperature overnight to allow DMSO to evaporate to create a thin film of the test sample on the inside of the vial.

The *Artemia salina* eggs were hatched in an non-natural salt-water (38 g sea salt per litre of distilled H₂O) for 24–48 hours under continuous aeration and light at 28 ± 2 °C and the swimming nauplii were collected for testing. Ten nauplii were added to each vial and the volume raised to 5 mL using artificial seawater for a total of 30 larvae being tested per concentration. The vials containing DMSO were used as vehicle controls, and the vials containing seawater were used as negative controls. No feeding was performed and ampoules were stored for twenty-four hours at room temperature, after which surviving and dead nauplii were counted and the ones that did not respond to light shaking were considered dead. The mortality was thus measured as a %.

$$\text{Mortality (\%)} = \frac{\text{No. of dead larvae}}{\text{Total No. of larvae}} \times 100$$

Statistical analysis

Every trail was repetitive 3 times (n=3) and outcomes were expressed as mean $\pm$ standard deviation (SD). A p value less than 0.05 were stared as statistically significant. The results were investigated applying SPSS software (Version 10).

Results

The proximate composition of GSP is shown in Table 1. The moistness quantity of the prepared GSP was 8.03 ± 0.10 g/100 g, indicating the presence of residual moisture in the dried powder. Among the analyzed components, crude fiber was

the predominant constituent (45.20 ± 0.60 g/100 g), followed by total carbohydrate (31.15 ± 0.20 g/100 g) and crude fat (14.32 ± 0.50 g/100 g). The quantity of crude protein was 8.10 ± 0.50 g/100 g, while the ash content was 1.23 ± 0.10 g/100 g. The calorific value of GSP was 294.10 kcal/100 g. These findings demonstrate that GSP is characterized by a high crude fiber content, together with appreciable levels of carbohydrates and fat.

Table 1. Proximate Composition of GSP (g/100 g dry weight basis)

Parameters	Results
Moisture	8.03 ± 0.1
Ash	1.23 ± 0.1
Crude Fat	14.32 ± 0.5
Crude Fiber	45.2 ± 0.6
Crude protein (Nx6.25)	8.1 ± 0.5
Total carbohydrate*	31.15 ± 0.2
Calorific value(kcal/100g)	294.101

Results are presented as mean (n=3) $\pm$ standard deviation (SD). *By difference.

The mineral composition of GSP is presented in Table 2. Among the analyzed minerals, potassium (K) was the utmost plentiful element, with a concentration of 540.3 ± 0.1 mg/100 g, tailed by Na (321.0 ± 0.5 mg/100 g), phosphorus (190.7 ± 0.5 mg/100 g), calcium (140.2 ± 0.5 mg/100 g), and magnesium (115.3 ± 0.3 mg/100 g). Iron was detected at 11.2 ± 0.5 mg/100 g, whereas comparatively lower concentrations were observed for zinc (3.1 ± 0.1 mg/100 g), copper (1.2 ± 0.1 mg/100 g), and manganese (Mn) was 1.1 ± 0.1 mg/100 g. Comparison with the recommended dietary allowances (RDAs) presented in Table 2 showed that the iron content of GSP was close to the recommended daily intake for children aged 7–

10 years and adult males (10 mg/day), while it was lower than the recommended intake for adult females (15 mg/day) and pregnant/lactating women (13 mg/day). The potassium content represented approximately 67.5% of the listed RDA for children and adults, while it accounted for approximately 45.0% of the RDA for pregnant and lactating women. The sodium content corresponded to approximately 80.3% of the listed RDA for children and adult females, 64.2% for adult males, and 64.2% for pregnant and lactating women. The calcium concentration was substantially lower than the listed daily recommendations. Overall, GSP contained appreciable levels of potassium, sodium, phosphorus, calcium, and magnesium, with iron being the most prominent trace mineral detected.

Table 2. Minerals Analysis of GSP (mg/100 g dry weight basis)

Minerals	Results	Recommended Dietary Allowances (mg/day)				RDA References
		Children 7-10 Years	Male Adult	Female Adult	Pregnant and Lactating mother	
Manganese	1.1±0.1	~	~	~	~	
Zinc	3.1±0.1	10	15	12	19	Ali et al., 2011
Iron	11.2±0.5	10	10	15	13	Ali et al., 2011
Magnesium	115.3±0.3	~	~	~	~	
Calcium	140.2±0.5	1600	2000	2000	2000	Ali et al., 2011
Copper	1.2±0.1	~	~	~	~	
Sodium	321.0±0.5	400	500	400	500	Ali et al., 2011
Potassium	540.3±0.1	800	800	800	1200	Ali et al., 2011
Phosphorus	190.7±0.5	~	~	~	~	

Results are expressed as means (n=3) ± standard deviation (SD).

The physio-chemical characteristics of GSO are depicted in Table 3. The refractive index of GSO at 40 °C was 1.40 ± 0.00. The oil showed an iodine value of 140 ± 0.5 g I₂/100 g and a saponification value of 188 ± 0.8 mg KOH/g. The percentage of

unsaponifiable matter was 1.11 ± 0.1%. The acid value of GSO was 0.66 ± 0.1 mg KOH/g, while the peroxide value was 2.11 ± 0.1 meq/kg. The oxidative stability was 5.8 ± 0.1 h. Overall, the obtained physicochemical parameters demonstrate that GSO possessed a high iodine value, moderate saponification value, and relatively low acid and peroxide values.

Table 3. Physico-chemical characteristics of GSO

Characteristics	Values
Peroxide value (meq/kg)	2.11±0.1
Refractive index (40 °C)	1.4±0.0
Saponification value (mg KOH/g)	188±0.8
Acid value (mg KOH/g)	0.66±0.1
Unsaponifiable matter (%)	1.11±0.1
Stability oxidative (h)	5.8±0.1
Iodine value (g I ₂ /100g)	140±0.5

Mean ±standard deviation (SD) with n=3

The antibacterial activities of GSO contrary to the study bacterial strains is presented in Table 4. GSO exhibited antibacterial activity against all six tested bacteria, as demonstrated by the formation of inhibition zones. The largest inhibition zone (IZ) was observed against *E. coli* (17 ± 0.4 mm),

followed by *B. subtilis* (12 ± 0.5 mm) and *K. pneumoniae* (11 ± 0.5 mm). GSO produced inhibition zones of 9 ± 0.2 mm against *S. typhi* and 9 ± 0.1 mm against *P. aeruginosa*, while the smallest inhibition zone was recorded against *S. aureus* (8 ± 0.1 mm). The MIC of GSO ranged from 150 to 250 mg/mL. The bottom MIC (150 mg/mL) was

observed against *E. coli*, whereas MIC values of 200 mg/mL were recorded against *B. subtilis* and *K. pneumoniae*. The remaining three bacterial strains, *S. aureus*, *S. typhi*, and *P. aeruginosa*, showed MIC values of 250 mg/mL.

Similarly, the MBC reached from 300 to 500 mg/mL. The lowest MBC (300 mg/mL) was observed against *E. coli*, followed by 400 mg/mL against *B. subtilis* and *K. pneumoniae*. The MBC for *P. aeruginosa*, *S. typhi* and *S. aureus* was 500 mg/mL.

Table 4. Antibacterial Activity of GSO

Bacteria	GSO			Streptomycin	5% DMSO
	IZ	MIC	MBC	ZI	ZI
<i>S. aureus</i>	8±0.1	250±00	500±00	16±0.1	0.0
<i>B. subtilis</i>	12±0.5	200±00	400±00	20±0.4	0.0
<i>S. typhi</i>	9±0.2	250±00	500±00	20±0.2	0.0
<i>E. coli</i>	17±0.4	150±00	300±00	14±0.1	0.0
<i>P. aeruginosa</i>	9±0.1	250±00	500±00	12±0.5	0.0
<i>K. pneumoniae</i>	11±0.5	200±00	400±00	19±0.2	0.0

Each value represents Mean ± standard deviation (SD) with n=3.

The cytotoxicity of GSO was evaluated using the *Artemia salina* lethality assay after 24 h of exposure, and the outcomes are shown in Table 5. No mortality was detected in any of the GSO-treated groups at 1000, 100 and 10 µg/mL doses. In each treatment, all 10 larvae remained alive, corresponding to 0% mortality. Similarly, no mortality was observed in either the blank control containing synthetic saline and DMSO or the

The reference antibiotic, streptomycin (10 mg/mL), produced inhibition zones reaching from 12 ± 0.5 to 20 ± 0.4 mm across the tested bacteria, with the largest zones observed against *B. subtilis* and *S. typhi* (20 ± 0.4 and 20 ± 0.2 mm, respectively). Overall, among the tested organisms, *E. coli* showed the greatest susceptibility to GSO, exhibiting the largest inhibition zone and the lowest MIC and MBC values, whereas *S. aureus* showed the lowest inhibition zone.

negative control containing synthetic saline alone, with all larvae remaining viable after 24 h. In contrast, the positive control (etoposide, 1000 µg/mL) produced 100% mortality, with all 10 larvae recorded as dead. These findings indicate that GSO did not exhibit detectable lethality against *A. salina* at the tested concentrations up to 1000 µg/mL under the experimental conditions used, whereas the positive control demonstrated the expected lethal response.

Table 5. *Artemia salina* lethality assay of GSO after 24 h of exposure

Treatment	Dose (µg/mL)	Live larvae (n = 10)	Dead larvae (n = 10)	Mortality (%)
GSO	1000	10	0	0
GSO	100	10	0	0
GSO	10	10	0	0
Blank (Synthetic saline + DMSO)	-	10	0	0
Negative control (Synthetic saline only)	-	10	0	0
Positive control (Etoposide)	1000	0	10	100

Each trial contained of 10 *Artemia salina* larvae (n = 10). Blank: synthetic saline comprising 0.5 mL DMSO; Negative control: artificial saline only.

Discussion

Proximate, physiochemical and minerals composition of Guava Seed

The ash values obtained here give an indication of the mineral content of a food sample, since it is the total inorganic matter that remains after combustion (Shovon *et al.*, 2013); the results are summarized in Table 1.

Dietary fibre has established physiological significance, in addition to bulk. Fibre enhances stool bulk and promote intestinal peristalsis which aids in maintaining regular bowel actions and preventing constipation (Ponnusamy and Vellaichamy, 2012). It's also been shown to slow the conversion of starch to sugar and to decrease cholesterol absorption in the gut, which could help keep blood glucose from spiking after eating. A higher fibre content is associated with a lower incidence of cardiovascular disease, colorectal cancer and obesity over the longer term (Shovon *et al.*, 2013), as a result of which the content of crude fibre is considered a significant indicator when assessing food materials or agricultural residues for their health-promoting potential.

The food energy value represents the amount of energy or metabolic energy available to the body to utilize, and is primarily based on the amount of carbohydrate, protein, fat and in some cases organic acids present in the food. This permits energy value calculation to be a regular method for measuring a material's general dietary value (Shovon *et al.*, 2013).

The value of iodine (I) of the GSO was 134 g I₂/100 g oil, the unsaponifiable matter 0.49% (w/w) and the saponification value was 196 mg KOH/g which are in accordance with previous studies (Prasad and Azeemoddin, 1994; Piombo *et al.*, 2006). The small acid value (0.40 ± 0.02 mg KOH/mg) indicated high oxidative stability followed by low peroxide value ($0.62 \pm 0.05\%$), corroborating the findings of Swati *et al.* (2020). The previous studies have indicated that the seed contains approximately 61.4% crude fibre, 7.6% protein and 16% oil (Noor *et al.*, 2015), which makes the seed a potential raw material for oil

extraction, formulation of food and nutritional supplementation.

Minerals Analysis

The mineral profile of GSP previously reported as follows: Ca was 0.05 mg/100 g, magnesium 0.13 mg/100 g, sulfur 0.09 mg/100 g, iron 13.8 mg/100 g, manganese 0.44 mg/100 g, zinc 3.31 mg/100 g, sodium 0.05 mg/100 g, potassium 0.20 mg/100 g, and phosphorus 0.30 mg/100 g (Uchoa-Thomaz *et al.* (2014). Manganese is a cofactor of several enzyme systems, and acts as an activator to enhance the catalytic activity of these enzymes (Ali *et al.*, 2024a). On the other hand, zinc supports brain health, behavioral development, normal insulin release, growth and wound healing, and has a general regulatory function in cell-shape maintenance and enzymatic catalysis throughout the body (Naseem *et al.*, 2012; Ali *et al.*, 2024a).

Iron is an essential portion of hemoglobin (Hb) and is also involved in the degradation of proteins, fats and carbohydrates; with iron shortage, anemia is a common occurrence (Naseem *et al.*, 2012). Meanwhile, magnesium regulates glucose, insulin, vitamin D and calcium levels and is also associated with healthier muscle tissues, stronger cardiac muscles and reduced anxiety (Ali *et al.*, 2024b).

Calcium is beneficial in milk production, blood clotting, skeletal and dental development, cardiac and nerve function (Ali *et al.*, 2024a). As the calcium within bone is lost over time (as in osteoporosis), there is an increased risk of fracture. Notably, when phosphorus intake exceeds calcium intake (either in the short term or over the long term), the body compensates for this imbalance by removing calcium from the bone stores, which over time or in the short term, can affect the integrity of the bones.

Some of the physiologically important enzymes are copper-dependent such as cytochrome c oxidase (in cellular energy production), lysyl oxidase (connective tissue formation and stabilization), and ceruloplasmin (iron oxidation from ferrous to ferric form) (Shovon *et al.*, 2013).

Potassium and sodium are the two most important electrolytes in the body, which regulate osmotic pressure, cellular fluid balance and acid base

homeostasis, all of which are vital to normal functions of body cells and organs (Ali *et al.*, 2024a).

Phosphorus is a macro-mineral, a component of cells and tissues, and is involved in many metabolic pathways, and acts as a buffer to bodily fluids. It is also a part of ATP, the main energy molecule in the cell, as well as a component of nucleic acids and other phosphorylated energy intermediates and is essential to energy metabolism, nucleic acid biosynthesis and general metabolism (Ali *et al.*, 2025).

Antibacterial Activities

Guava seed meal extracts also exhibited antibacterial activities contrary to *S. aureus*, *S. typhimurium*, *Listeria monocytogenes*, *Bacillus cereus* and *E. coli* O157:H7 with IZ array from 9.0 to 22.3 mm (Mohamed *et al.*, 2011). The other investigation, the guava seed extract at 5 mg/mL concentration showed moderate inhibition zone (6-20 mm) contrary to *P. aeruginosa*, *E. coli*, *B. subtilis* and *S. aureus*, but the extract was not effective against the fungi *Candida albicans* and *Aspergillus niger* (Subal *et al.*, 2011). In addition, a glycine-rich peptide originally believed to have only a storage role in the guava seed was shown to inhibit gram-negative bacteria that cause gastrointestinal illness in vitro, suggesting a potential future application against *Proteus* spp. and *Klebsiella* spp, and, perhaps, as an inhibitor of the development of resistance to existing antibiotics (Patricia *et al.*, 2011).

Cytotoxic Activity applying larvae of *Artemia salina*

In this study no extract was found to be toxic to *Artemia salina*. Meyer *et al.*, (1982), classified extracts according to the severity of toxicity based on median lethal concentration (LC_{50}); the value of 1,000 $\mu\text{g/mL}$ was used as the threshold for categorizing extracts as nontoxic.

The results of this are in accordance with Uchoa-Thomaz *et al.* (2014) who found that methanol, acetone, ethanol and hexane extract of GSP had no toxic effect. Avocado seed hexane extract has however, been reported to exhibit measurable toxicity, with an LC_{50} of 2.37 mg/mL (Leite *et*

al., 2009). The nontoxic profile established for guava seed material further bolster its potential use as an herbal or human product.

Conclusion

In conclusion, these findings support the nutritional and bioactive potential of guava seeds, highlighting them as a valuable plant resource. The seed powder was particularly high in crude fibre content and contained significant amounts of carbohydrate, fat, protein and minerals, including potassium, phosphorus, calcium and magnesium. Guava seed oil, on the other hand, exhibited promising physicochemical properties, with a moderate iodine value and low peroxide and acid values, which are indicators of its good oxidative properties. The oil was also shown to inhibit all the bacterial strains tested with the most sensitive being *Escherichia coli*. Critically, it showed no lethal effect on *Artemia salina* at concentrations up to 1000 $\mu\text{g/mL}$. Overall, the results place guava seed as an underutilized source of both nutritional compounds and bioactive components and indicate the need for a detailed characterization of its fatty acid and bioactive compound content, as well as an assessment of the safety, stability and applied potential of the oil for utilization in various food, pharmaceutical and other value-added product applications.

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