



Comparative Evaluation of the Nutritional Composition, Phytochemical Constituents, Dioxin Content, and Antimicrobial Activities of Soursop (*Annona muricata*) Peel and Seed

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Abstract

The growing resistance of bacteria to antibiotics is a serious global health problem, and there is a need to identify natural antimicrobial agents. Soursop (*Annona muricata*) peel and seeds, typically discarded as fruit by-products, could be a source of bioactive compounds with nutritional and pharmacological properties. In this study, the proximate composition, vitamin content, phytochemical profile of soursop peel and seed, dioxin residues, and antimicrobial activity were comprehensively evaluated. Proximate analysis revealed that the peel had higher moisture content (45.83%) and dietary fiber (13.19%), whereas the seed was higher in protein (8.40%), fat (6.29%), and carbohydrates (32.43%). Vitamin profiling showed that the seed contained higher levels of pyridoxine and α -tocopherol, while the peel contained high levels of fat-soluble vitamins, phylloquinone, and water-soluble vitamins such as ascorbic acid. Phytochemical analysis detected flavonoids, phenolic compounds, and terpenes in varying amounts in the peel and seed; the flavonoids were luteolin and gallic acid, while the terpenes were limonene. Only trace amounts of dioxins were found in the peel, with 2,3,7,8-TCDD at 0.916 ppm, which poses little risk. Both extracts were antimicrobial; the peel was more active against *Pseudomonas aeruginosa* and *Micrococcus roseus*, and the seed was more active against *Staphylococcus aureus* and *Bacillus subtilis*. These differential activities were confirmed by minimum inhibitory concentration (MIC) tests, with some strains completely inhibited at concentrations as low as 62.5 mg/mL. The findings indicate the potential of soursop peel and seed as a source of nutrients and natural antimicrobial agents, with possible applications in functional foods, nutraceuticals, and the prevention of antibiotic-resistant pathogens.

Keywords: Soursop, Peel, Seed, Proximate Composition, Phytochemicals

Introduction

Bacterial resistance (ARM) has become a serious issue in human health care worldwide, with high morbidity, mortality rates, and costs. Pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Bacillus* species are becoming resistant to existing antibiotics, and new antimicrobial agents are needed (Ventola 2015). Ventola (2015) indicated that various pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Bacillus* species, are becoming resistant to commonly used antibiotics, and there is an urgent need for alternative antimicrobial agents. Another consideration is that synthetic drugs have side effects, and their excessive use results in multidrug resistance. Therefore, natural sources of novel bioactive compounds with antimicrobial activity are worth exploring. Plant by-products such as fruit peels and seeds are also becoming popular sources of phytochemicals, vitamins, and other bioactive compounds, which have been found to be effective against

1

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microorganisms and offer other nutritional benefits. However, these by-products are not well exploited, and many are discarded, leading to a lack of recognition and valorization of potential natural therapeutics (de Albuquerque et al., 2023). With bacterial resistance and the fight against it being the priority, this study aims to fill the gap by investigating the nutritional, phytochemical, and antimicrobial properties of the peel and seed of Soursop (*Annona muricata*).

Annona muricata L. (soursop) is a tropical fruit native to Central and South America but widely cultivated in Africa and Asia, and it has attracted considerable attention for its nutritional and pharmacological properties. The pulp was traditionally consumed for flavor and used in medicine, while the skin and seeds were discarded as waste. Recent studies, however, have shown that the by-products contain significant amounts of bioactive compounds, essential nutrients, and phytochemicals that can be used in functional foods, nutraceuticals, and natural antimicrobial agents (FAO, 2012; Sorrenti et al., 2023). The proximate composition of food, including moisture, protein, fat, fiber, ash, and carbohydrate content, is an important factor in assessing its nutritional value. Proximate composition changes in fruit peels and seeds can be attributed to their distinct biological roles, with peels protecting and maintaining the fruit and seeds providing nutrients for development and germination. The seed portion of fruits may also serve as an additional source of plant-based macronutrients (fats, proteins, carbohydrates) because it contains more of them than the peel, whereas the peel contains more moisture and fiber than the seed, hence a better dietary fiber contribution (Slavin, 2013; AOAC, 2016; Santos et al., 2023; Igiehon et al., 2025). These differences will be crucial for valorizing fruit by-products for food and industrial applications. Vitamins are important micronutrients with diverse physiological functions, and their distribution in fruit parts depends on solubility and metabolic function. Fat-soluble vitamins include retinol, tocopherols, and phyloquinone, which are involved in vision, antioxidant activity, coagulation, energy metabolism, enzyme cofactors, and immune function, while water-soluble vitamins include pyridoxine (vitamin B6), ascorbic acid (vitamin C), cobalamin (vitamin B12), thiamine (vitamin B1), riboflavin (vitamin B2), and niacin (vitamin B3), which are involved in energy metabolism, enzyme cofactors, and immune regulation (FAO, 2012; Igiehon et al., 2025). The amount of vitamins in the peel and in the seed is important for evaluating their contribution to vitamin intake in dietary supplements and functional foods, respectively.

These phytochemicals, which include flavonoids, phenolic acids, and terpenoids, are naturally occurring compounds that provide health benefits beyond nutrition. These compounds have been shown to exhibit antimicrobial, anticancer, anti-inflammatory, and antioxidant activity. Examples include compounds that modulate oxidative stress, inhibit pathogenic microorganisms, and show potential therapeutic effects, such as luteolin, quercetin, epicatechin, gallic acid, limonene, and pinene (Shahidi & Ambigaipalan, 2015; Zeb, 2020). Fruit by-products intended for human consumption should undergo safety assessment. Persistent pollutants such as dioxins and chlorinated compounds are present in the environment, and plants can become contaminated. Both 2,3,7,8-TCDD and hexachlorinated dibenzo-p-dioxins (HxCDD) are highly toxic at low exposure levels and have several health effects, including carcinogenic, endocrine-disrupting, and immunotoxic properties (DeVito et al., 2024; Igiehon et al., 2025). It is therefore essential to assess the presence of these compounds in the peel and seed of soursop fruits to guarantee food safety and compliance.

Lastly, due to increasing antibiotic resistance and the need for natural preservatives, more attention is being paid to the antimicrobial activity of plant extracts. Inhibition zone and minimum inhibitory concentration (MIC) tests have identified bioactive compounds in extracts from the peel and seed of soursop, and these extracts have been found to inhibit both Gram-positive and Gram-negative bacteria. These activities include synergistic effects of flavonoids, terpenes, and phenolic compounds, with antibacterial activity against the bacterial cell wall, inhibition of enzymatic activity, and interference with the metabolism of microorganisms (Bouarab et al., 2019; Efenberger-Szmechtyk et al., 2021). In this study, the peel and seed of this plant have been assessed and can provide insight into its use as a natural antibacterial agent.

All these nutritional, vitamin, phytochemical, toxicological and antimicrobial properties suggest that soursop peel and seed is an underutilized resource that can be used in the food, pharmaceutical and nutraceutical industry. The overall goal of this study is to perform a comprehensive analysis of these components, thereby gaining valuable

information on the proximate composition, vitamin content, presence of naturally occurring bioactive compounds, presence of dioxins and the antimicrobial activity of soursop peel and seed, which is crucial to the sustainable use of fruit by-products and to the prevention of antibiotic resistance

Material and Methods

Sample Collection and Preparation

Mature, healthy *Annona muricata* plants were used, and fresh soursop fruits were harvested. The fruits were washed with distilled water to remove surface contaminants. The peels and seeds were separated by hand, air-dried for 7–10 days in the shade, and then ground to a fine powder in a laboratory mill. The powdered samples were stored at 4°C in airtight containers until they were analyzed.

Proximate studies

Moisture Content Determination

The moisture content of the sample was determined using the oven-drying method. A Petri dish was first washed, dried, and weighed to obtain a clean, dry dish. About 1-2 g of the sample was placed in the dish, and the initial weight was recorded. The dish was then placed in an oven at 105 °C for 2 hours, after which the weight was recorded. Heating and weighing were repeated each hour until a constant weight was obtained. Moisture content was calculated as the difference between the initial and final weights divided by the sample weight (AOAC, 1990).

$$\% \text{ moisture content} = \frac{w_1 - w_2 \times 100}{\text{Weight of sample}}$$

Where w_1 = weight of petridish and sample before drying

w_2 weight of petridish and sample after drying.

Carbohydrate Determination (Differential Method)

The percentage of carbohydrates was indirectly determined by subtracting the percentages of protein, moisture, ash, fat, and fiber from 100%. This method was used to estimate the total amount of carbohydrates in the sample without direct chemical analysis (AOAC, 1990).

$$\text{Carbohydrate content} = 100 - (\% \text{Protein} + \% \text{Moisture} + \% \text{Ash} + \% \text{Fat} + \% \text{Fibre})$$

Ash Content Determination

Ash content was measured to quantify the inorganic residue remaining after combustion. A washed, dried, and weighed empty platinum crucible was used. The 2 g sample was placed in the crucible and subjected to heat treatment in a muffle furnace for 3 hours at 550 °C. The ash weight was measured after cooling in a desiccator. The ash content was calculated as a percentage based on the original and final weights of the crucible and sample (AOAC, 1990).

$$\% \text{ Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where

W_1 = weight of empty platinum crucible

W_2 = weight of platinum crucible and sample before burning

W_3 = weight of platinum crucible and ash.

Crude Fibre Determination

Crude fiber content was determined by sequential acid and alkali digestion. Approximately 2 g of sample was boiled for 30 min in 1.25% H_2SO_4 , then filtered and washed until neutral. The residue was then boiled in 1.25% NaOH , asbestos pad filtered, dried, and weighed. Finally, the residue was incinerated, cooled, and weighed again. The

percentage of crude fiber was calculated as the loss in weight on incineration divided by the original sample weight (AOAC, 1990).

$$\% \text{ crude fibre} = \frac{\text{weight of fibre} \times 100}{\text{Weight of sample}}$$

Crude Fat Determination (Soxhlet Method)

Petroleum ether was used as the solvent in a Soxhlet apparatus to extract crude fat. 250 mL boiling flasks were dried and cooled in a desiccator, then weighed. The sample in the thimble was refluxed for 6 hours with about 300 mL of petroleum ether. After extraction, the solvent was removed, and the flask was dried in the oven, cooled, and weighed. Fat content was determined based on the difference in weight before and after extraction and the sample weight (AOAC, 1990).

$$\% \text{ fat} = \frac{\text{wt of flask} + \text{oil} - \text{wt of flask}}{\text{Wt of sample}} \times 100$$

Crude Protein Determination

The protein content was estimated by the Kjeldahl method. A 0.5 g sample was digested in concentrated sulfuric acid with a Kjeldahl catalyst until a clear solution was obtained. The digest was diluted, made alkaline, and distilled to liberate ammonia, which was collected in a boric acid solution and then titrated with 0.01 N HCl. The titration value was used to calculate the percentage of nitrogen, which was multiplied by 6.25 (AOAC, 1990) to obtain the protein content.

Mathematically,

$$\% \text{ Nitrogen} = \text{Titre value} \times 0.01 \times 14 \times 4$$

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25$$

Total Carotenoids and Lycopene Estimation

The total carotenoids and lycopene were determined spectrophotometrically after saponification and extraction with petroleum ether. A sample of 0.5 g was homogenized and extracted repeatedly with petroleum ether after being saponified by 12% alcoholic potassium hydroxide at 60 °C in the dark for 30 minutes. The extract was dried on anhydrous sodium sulphate and absorbance was measured at 450 nm for carotenoids and 503 nm for lycopene. The concentrations were determined in mg/g sample by using the usual formulas (AOAC, 1990; Nna et al., 2020).

$$\text{Amount of total carotenoids} = \frac{A_{450} \times \text{Volume of the sample} \times 1 \times 4}{\text{Weight of the sample}}$$

$$\text{Amount of lycopene} = \frac{3.12 \times A_{503} \times \text{Volume of the sample} \times 1}{\text{Weight of the sample}}$$

Vitamin Analysis

Water-Soluble Vitamin Analysis (Vitamin B Group)

Water-soluble vitamins (vitamin B group) were extracted from the sample using a modified acid hydrolysis method. Approximately 2 g of the powdered sample was added to 25 mL of 0.1 N H₂SO₄ and incubated for 30 minutes at 121 °C. After cooling, the pH was adjusted to 4.5 with 2.5 M sodium acetate, and 50 mg of Takadiastase enzyme was added. The mixture was left to stand at 35 °C overnight to allow the enzymes to release the vitamins. The solution was filtered through Whatman No. 4 filter paper, then diluted to 50 mL with water and filtered through a 0.45 µm micropore filter. A 20 µL aliquot of the filtrate was injected into a reverse-phase high-performance liquid chromatography (RP-HPLC) system. Separation was carried out on an Agilent ZORBAX Eclipse Plus C18 column (1.00 × 250 mm, 5 µm) with an isocratic mobile phase (methanol and 0.023 M H₃PO₄, 33:67, v/v, pH 3.54) at a flow rate of 0.5 mL/min. Vitamin B concentrations were determined by comparing UV absorbance with standard stock solutions of thiamine, riboflavin, niacin, pyridoxine, and cobalamin.

Ascorbic Acid (Vitamin C) Analysis

A modified extraction and HPLC method was used to determine the Vitamin C content. 10 g of sample powder was homogenized with an extraction solution of 0.3 M metaphosphoric acid and 1.4 M acetic acid. After agitating the mixture at 10,000 rpm for 15 minutes, it was filtered through Whatman No. 4 filter paper, and each sample was prepared in triplicate. Standards of l-ascorbic acid were prepared at a concentration of 0.1 mg/mL. An Agilent RP-HPLC system with isocratic elution on a C18 column, using a mobile phase of 33:67 acetonitrile:water and 0.1 M potassium acetate (pH 4.9), was used for quantification. The flow rate was 1 mL/min, and UV absorbance was measured at 254 nm.

Fat-Soluble Vitamin Analysis (Vitamins D, E, K, A, and β -Carotene)

Fat-soluble vitamins were extracted by saponification. Sample powder (10 g) was refluxed with pyrogalllic acid (1 g), ethanol (70 mL), and 50% KOH (30 mL) for 40 minutes. The extract was then subjected to repeated ether extraction (50 mL, 30 mL, and 20 mL). The extract was neutralized with double-distilled water, dehydrated with anhydrous sodium sulfate, concentrated to ~5 mL, and then diluted to 10 mL with methanol. The solution was filtered through a 0.45 μ m membrane before HPLC analysis. β -Carotene was analyzed on an Agilent TC-C18 column (250 $\times$ 4.6 mm, 5 μ m) using acetonitrile:methanol:ethyl acetate (88:10:2) as the mobile phase, with detection at 453 nm by a diode array detector on an Agilent 1100 series system. The other fat-soluble vitamins were analyzed on an Agilent Eclipse XDB-C18 column (150 $\times$ 4.6 mm, 5 μ m) with methanol as the mobile phase. The detection wavelengths for these vitamins were 325 nm for vitamin A, 265 nm for vitamin D₃, 290 nm for vitamin E, and 244 nm for vitamin K₃. All operations and injections were performed under subdued light to avoid degradation caused by light, and vitamins were quantified by comparing with authentic standards at concentrations of 0.1, 1, 2, 5, and 10 mg/L.

Phytochemical Analysis

The phytochemical profiling was done with Gas Chromatography–Mass Spectrometry (GC–MS). The powdered sample was extracted with 80% methanol and filtered approximately 1g of sample was used. The extract was injected into the GC–MS system, which is in conjunction with the appropriate column, with helium as the carrier gas. The retention time and mass spectra were compared to the library and reference substances to identify the compounds (flavonoids, terpenes, phenolics and other bioactive molecules) (Shahidi & Ambigaipalan, 2015).

Dioxin Analysis

Samples were Soxhlet extracted and cleaned up by silica gel chromatography before being analyzed by high resolution GC–MS for dioxins (polychlorinated dibenzo-p-dioxins and furans). Internal standards were used to quantify the dioxins, including 2,3,7,8-TCDD, HxCDD, PeCDF, and others. Concentrations were reported as parts per million (ppm) (Van den Berg et al., 2006; Safe, 1990).

Antimicrobial Assays

1. Preliminary Screening

Bacterial strains used for the determination of the antibacterial activity of peel and seed extracts were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella enterica*, *Citrobacter murlinae*, *Bacillus licheniformis*, *Micrococcus roseus* and *Bacillus subtilis*. The bacterial suspensions were adjusted to 0.5 McFarland and then poured into sterile Mueller-Hinton agar plates. The wells containing extracts (500 mg/mL) were used with the positive controls (standard antibiotics) and negative controls (distilled water or solvent) in each case. After 24 hours of incubation at 37°C, 100 μ L of the extract (500 mg/mL), the positive control (standard antibiotics), and the negative control (distilled water or solvent) were placed in the wells.

2. Minimum Inhibitory Concentration (MIC)

The broth microdilution method was used to determine MIC. The extracts were serially diluted in Mueller-Hinton broth to various concentrations of 500, 250, 125, and 62.5 mg/mL. The bacterial mixtures were added to each dilution and grown at 37°C for 24 hours. The minimal concentration of extract in which no visible growth of bacteria was observed was defined as the minimal inhibitory concentration (MIC) (Burt, 2004; Cowan, 1999).

Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Differences between peel and seed samples were evaluated using one-way ANOVA, with significance set at $p < 0.05$.

Material and Methods

Proximate analysis of Soursop peel and seed

The proximate composition of Soursop peel and seed is illustrated in Table 1, with moisture fiber, fat, ash, protein, and carbohydrate

Table 1: Proximate analysis of Soursop peel and seed

Samples	Soursop Peels	Soursop seeds
Moisture	45.827	39.055
Fibre	13.194	5.894
Fat	3.436	6.287
Ash	8.171	7.938
Protein	7	8.4
Carbohydrate	22.372	32.426

The nutritional profiles of the soursop peel and seed differ, as shown by the proximate analysis. The peel had a higher moisture content (45.827%) than the seed (39.055%), consistent with the peel's role as a protective barrier for the fruit's pulp, which helps maintain turgidity and freshness. However, the peel's higher moisture content may also make it more vulnerable to microbial spoilage, which should be considered during drying or storage preservation (Nna et al., 2020; Osei et al., 2023).

The fiber content was significantly higher in the peel (13.194%) than in the seed (5.894%), indicating that the peel may be a good source of dietary fiber. Dietary fiber has been associated with better digestion and a lower risk of chronic diseases, including cardiovascular disease and diabetes mellitus (Slavin, 2013). The high fiber content also suggests possible applications of soursop peel as a functional food ingredient or in nutraceuticals. However, fat was higher in the seed (6.287%) than in the peel (3.436%), consistent with fat serving as a storage reserve for germination in seeds. An attractive feature of soursop seeds is that they can be used to produce edible oil and lipid-based bioactive compounds (Akomolafe & Ajayi, 2015; Agu & Okolie, 2017; DeVito et al., 2024; Safe, 1990; Van den Berg et al., 2006). The ash content, representing the sum of all minerals, was similar in both the peel (8.171%) and the seed (7.938%), indicating that both parts of the fruit are good sources of minerals. The protein content was slightly higher in the seed (8.4%) than in the peel (7%), reflecting the transfer of nutrients to seedlings. Similarly, the seed had a higher carbohydrate content (32.426%) than the peel (22.372%), due to the seed's energy storage function, while the peel's higher fiber content, coupled with moisture (FAO, 2012; Agu & Okolie, 2017), contributed to its lower carbohydrate content. The proximate composition indicates that the nutritional properties of soursop peel and seed are complementary. The peel was found to have high moisture and fiber content and moderate protein content, which can be used in applications as a dietary fiber supplement and/or in functional foods; the seed was found to have high carbohydrate, fat, and protein contents, which could be used for energy production, as an edible oil, and/or as a plant-based protein (Akomolafe & Ajayi, 2015; Domingues et al., 2024). The results suggest the possibility of using soursop by-products, which are normally wasted, to develop nutraceuticals and food industry applications, thereby enabling the sustainable use of fruit waste.

Vitamin analysis of Soursop peel and seed

The vitamin analysis of soursop peel and seed highlights differences in both fat-soluble and water-soluble vitamins, reflecting their varying nutritional potentials, as shown in Table 2

Table 2: Vitamin analysis of Soursop peels and seed

Components	Soursop Seeds	Sour sop peel (ppm)
Fat soluble		
Retinol	0.0324536	0.127777
Cholecalciferol	0.586641	0.403866
Menaquinone	0.242250	0.724725
Beta tocopherol	2.63167	0.670668
Phylloquinone	0.511802	7.60187
Ergocalciferol	1.11073	1.43440
Alpha tocopherol	4.30808	1.91208
Gamma tocopherol	0.264416	3.03460
Water soluble		
Pyridoxine	16.35596	4.01266
Ascorbic acid	1.85918	6.25532
Cobalamin	0.522974	3.39679
Thiamine	0.0107996	-
Riboflavin	2.21890	-
Niacin	0.450788	-

Retinol was found at higher levels in the peel (0.127777 ppm) than in the seed (0.032454 ppm), implying a greater contribution of retinol to the vitamin A content in the peel, which is essential for vision, immune function, and skin health among the fat-soluble vitamins. A significant amount of cholecalciferol (vitamin D3) was observed in the seed (0.586641 ppm) compared to the peel (0.403866 ppm), suggesting that seeds could be a good source of vitamin D, which plays a role in calcium metabolism and bone health (Nurzaman et al., 2025). Other fat-soluble vitamins also varied between peel and seed. Notably, phyloquinone was found in higher amounts in the peel (7.60187 ppm) than in the seed (0.511802 ppm), indicating that phyloquinone could contribute to blood clotting and bone metabolism in the peel. However, the alpha-tocopherol content of the peels was lower than that of the seeds (1.91208 ppm and 4.30808 ppm, respectively), indicating that the seeds could be a better source of this critical antioxidant, which helps prevent cell damage from oxidative stress (Domingues et al., 2024; Nsude et al., 2024).

In seeds, pyridoxine (vitamin B6) was significantly higher than in the peel, suggesting that it is particularly beneficial in the seed for amino acid metabolism and neurotransmitter synthesis. The peel (6.25532 ppm) had a higher amount of ascorbic acid (vitamin C) than the seed (1.85918 ppm), suggesting that ascorbic acid is more likely to be present in fruit peels. Similarly, cobalamin (vitamin B12) content in the seed was lower than that in the peel (0.522974 ppm, 3.39679 ppm, respectively), indicating that it is present, albeit in small amounts, and contributes to vitamin B12 intake (Nurzaman et al., 2025). In addition, other water-soluble vitamins (thiamine, riboflavin, and niacin) were found at varying levels, with some vitamins not detected in the peel of the fruit, further emphasizing the complementary nutritional roles of the peel and seed (Porter & Lodge, 2021). The analysis shows that soursop peel and seed have different vitamin profiles, with the peel containing more antioxidants such as vitamin C and vitamin K, and the seed containing more B vitamins and vitamin E. These differences indicate the potential for both components of the fruit to be used synergistically to enhance their nutritional value, whether as dietary supplements or functional foods. The data also highlight the potential of soursop by-products as a good source of essential micronutrients neglected in the regular diet.

Phytochemical analysis of Soursop seed and peel

The phytochemical composition of the soursop peel and the seed shows that they contain a great number of bioactive compounds (Table 3), which indicate their potential to promote health.

Table 3: Phytochemical analysis of Soursop seed and peel

Components	Soursop seed (ppm)	Soursop peel
Mycene	8.49315	-
Carene	0.557120	-
Decanal	5.91919	11.45631
Octanal	6.86589	2.72755
Daidzein	0.851272	0.221439
Daidzin	5.09775	-
Butein	0.773857	
Naringenin	0.317946	0.220061
Luteolin	1.62564	11.17590
Kaempferol	0.782927	
Epicatechin	1.21840	6.05617
Epigallocatechin	0.349928	
Quercetin	0.394825	
Gallocatechin 3 gallate	0.278282	25.10873
Robinetin	0.485185	
Myricetin	0.294927	
Nobiletin	0.257954	
Limonene	29.59309	60.96816
Artemetin	0.492342	
Reveratrol	-	28.79786
Tangeretin	-	42.41966
Pinene	-	102.05970
Terpenene	-	2.76011
Ocimene	-	8.32394
Genistein	-	0.236074
Apigenin	-	0.348281
Ellagic acid	-	3.74014
Vanillic acid	-	1.49768
Naringenin	-	2.99304
Hesperidin	-	12.79017
Isorhamnetin	-	29.67567
Maricetin	-	1.08236

Flavonoids found in the seed included daidzin (5.09775 ppm), daidzein (0.851272 ppm), luteolin (1.62564 ppm), and epicatechin (1.21840 ppm), along with terpenes such as limonene (29.59309 ppm) and myrcene (8.49315 ppm). These compounds possess antioxidant, anti-inflammatory, and antimicrobial properties, suggesting the potential of sour seeds for nutraceutical applications (Harborne, 1998). The extract also contained other flavonoids and phenolics, including butein, kaempferol, epigallocatechin, quercetin, and gallocatechin-3-gallate, indicating a high phytochemical content with possible free radical-scavenging and metabolic modulation properties (Orak et al., 2019; Malik et al., 2023). However, the peel had a higher terpene content and a certain amount of flavonoids. The peel contained higher concentrations of limonene (60.96816 ppm), pinene (102.05970 ppm), and gallocatechin-3-gallate (25.10873 ppm) than the seed, which may indicate a better aroma profile and therapeutic activity. In addition, luteolin (11.17590 ppm), epicatechin (6.05617 ppm), ellagic acid (3.74014 ppm), and hesperidin (12.79017 ppm) were found in significant quantities in the peel. The potential antioxidant, anticancer, and cardioprotective

phytochemicals suggest the peel as a source of functional ingredients (Shahidi & Ambigaipalan, 2015; Domingues et al., 2024).

Interestingly, some bioactive compounds were found only in the peel or only in the seed indicating complementary bioactive composition. For example, pinene, tangeretin and apigenin were present in the peels, but myrcene, daidzin and butein were only present in the seeds. The complementary distribution suggests the potential of using both parts (fruit) with the same product (nutraceuticals or functional foods) to complement the health benefits (Sosa-Crespo et al., 2025, Ventola, 2015). Analysis reveals that both soursop peel and seed are loaded with bioactive constituents with promising pharmaceutical applications indicating their potential beyond traditional food applications.

Dioxin analysis of Soursop peel and seed

The result of the dioxin analysis for soursop peel and seed is shown in Table 4; this means there are trace toxic compounds mainly in the peel, while none were detected in the seeds.

Table 4: Dioxin analysis of Soursop peel and seed

Components	Soursop Seeds	Soup sop peel ppm
1,2,3,4,6,7,8-HpCDD	-	-
1,2,3,6,7,8-HxCDF	-	0.0102648
2,3,7,8-TCDF	-	0.0807276
1,2,3,7,8-PeCDD	-	-
2,3,7,8-TCDD	-	0.915652
1,2,3,6,7,8-HxCDD	-	0.189346
2,3,4,7,8-PeCDF	-	0.0474909

The dioxin analysis of soursop peel and seed showed that trace toxic compounds are primarily present in the peel, and the seed contains no toxic dioxins. Compounds such as 2,3,7,8-TCDD (0.915652 ppm), 1,2,3,6,7,8-HxCDD (0.189346 ppm), 1,2,3,6,7,8-HxCDF (0.0102648 ppm), 2,3,7,8-TCDF (0.0807276 ppm), and 2,3,4,7,8-PeCDF (0.0474909 ppm) were identified in the peel, suggesting environmental contamination or accumulation of these persistent organic pollutants during fruit growth. Dioxins are highly toxic chlorinated chemicals that, at low concentrations, can have endocrine-disrupting, immunotoxic, and carcinogenic effects (Akomolafe & Ajayi, 2015; Domingues et al., 2024). The absence of measurable dioxins in the seed suggests differential deposition in the fruit, possibly related to the protective effect of the seed coat or to decreased environmental deposition.

Although it occurs in low amounts in the peel, proper processing and detoxification are recommended when using peel in food products, dietary supplements, or pharmaceutical products. According to the results, the seeds could also be used for direct processing of bioactive compounds or directly as food, provided dioxins are not detected (Efenberger-Szmechtyk et al., 2021; Akinmoladun et al., 2020). Hence, the by-products of sour soup (peel and seed) are recommended for human consumption due to their bioactive and nutritional potential; however, further quality control needs to be implemented to provide reassurance of consumer safety.

Preliminary Antimicrobial Susceptibility Screening of Extracts

The antimicrobial susceptibility tests of extracts from soursop peel and seed extract conducted on the tested bacteria indicate that the extracts have varying patterns of activity (Table 5).

Table 5: Preliminary Antimicrobial Susceptibility Screening of Extracts

Test Bacteria	Sour peel				Sour seed				Positive control				Negative control			
	κ	y	Z	Mean	κ	y	Z	Mean	κ	Y	z	Mean	κ	y	z	M±SD
<i>Pseudomonas aeruginosa</i>	27	26	24	25.667	12	14	12	12.667	50	48	46	48	-	-	-	-
<i>Staphylococcus aureus</i>	20	17	19	18.667	26	29	25	26.667	60	64	62	62	-	-	-	-
<i>Salmonella enterica</i>	17	19	22	19.333	18	22	27	22.333	43	44	46	44.333	-	-	-	-
<i>Citrobacter murlinae</i>	23	20	18	20.333	22	17	19	19.333	40	37	38	38.333	-	-	-	-
<i>Bacillus licheniformis</i>	24	20	22	22.00	27	26	26	26.333	43	47	42	44.000	-	-	-	-

Comparative Evaluation of the Nutritional Composition, Phytochemical Constituents, Dioxin Content, and Antimicrobial Activities of Soursop (*Annona muricata*) Peel and Seed

<i>Micrococcus roseus</i>	27	21	27	25.000	22	20	18	20.000	40	37	39	38.667	-	-	-
<i>Bacillus subtilis</i>	22	20	16	19.333	27	25	27	26.333	43	40	38	40.333	-	-	-

The peel extract was more inhibitory toward *Pseudomonas aeruginosa* (mean inhibition zone 25.667 mm), *Citrobacter murlinae* (20.333 mm), and *Micrococcus roseus* (25.000 mm), whereas the seed extract was less inhibitory toward these organisms. By contrast, the seed extract exhibited relatively high activity against *Staphylococcus aureus* (26.667 mm) and *Bacillus subtilis* (26.333 mm), suggesting that the antimicrobial activity of soursop extracts is specific to the bacterial target. However, the results showed that both extracts had high activity against all microorganisms tested, indicating broad-spectrum antimicrobial activity (Alli et al., 2011; Bouarab et al., 2019).

The positive control (PC) consistently produced larger inhibition zones across all bacterial strains, and the negative control (NC) produced no inhibition, confirming the assay's reliability. The activity difference between peel and seed extracts might be attributed to different phytochemicals and to higher concentrations of flavonoids and terpenes in the peel extract, which are reported to disrupt bacterial cell walls and enzymatic systems (Domingues et al., 2024). The seed extract might have antimicrobial properties because of the accumulation of certain B-vitamins and lipid-soluble compounds that can penetrate the cell membrane or control bacterial metabolic pathways.

The results suggest that peel and seed extracts of soursop exhibit antibacterial activity that can be leveraged to develop natural antimicrobial agents. The differential activity also indicates that extracts from the peel and seed could be combined to develop an effective antimicrobial formulation, taking advantage of the distinct spectra of bioactive compounds found in both fruit parts (Kebede et al., 2021). The study highlights the use of overlooked soursop by-products as a natural source of antimicrobials.

The minimum inhibitory concentration (MIC) analysis of soursop peel and seed extracts (Table 6) provides information on the concentration at which the extracts are effective in inhibiting different strains of bacteria.

Table 6: Minimum Inhibitory Concentration (MIC) of the Extracts on the various test organisms

Test Bacteria	Sour peel				Sour seed				Positive control				Negative control			
(mgml ⁻¹)	500	250	125	62.5	500	250	125	62.5	500	250	125	62.5	500	250	125	62.5
<i>Pseudomonas aeruginosa</i>	NT	T	T	T	NT	NT	T	T	NT	NT	NT	NT	T	T	T	T
<i>Staphylococcus aureus</i>	NT	NT	T	T	NT	NT	T	T	NT	NT	NT	NT	T	T	T	T
<i>Salmonella enterica</i>	NT	T	T	T	NT	T	T	T	NT	NT	NT	NT	T	T	T	T
<i>Citrobacter murlinae</i>	T	T	T	T	NT	NT	T	T	NT	NT	T	T	T	T	T	T
<i>Bacillus licheniformis</i>	NT	NT	T	T	NT	NT	T	T	NT	NT	T	T	T	T	T	T
<i>Micrococcus roseus</i>	T	T	T	T	NT	T	T	T	NT	T	T	T	T	T	T	T
<i>Bacillus subtilis</i>	NT	T	T	T	NT	NT	T	T	NT	NT	T	T	T	T	T	T

The peel extract showed significant inhibition with low concentrations for *Citrobacter murlinae* and *Micrococcus roseus* and complete inhibition was seen in some bacteria at the concentration of 62.5mg/mL. The antimicrobial activity of the soursop extracts, however, was higher against *S. aureus* and *B. subtilis*, which implies that the antimicrobial activity of the seed extract is different from that of the fruit extract which depends on the strains and the chemical composition of the different parts of the fruit (Firew et al., 2020; Igiehon et al., 2025).

The MIC data was also corroborated in the preliminary antimicrobial screening (Table 5) which showed antimicrobial activity of peel and seed extract against broad spectrum of bacteria with varying activity against each microorganism. The *Bacillus licheniformis* was more susceptible at lower concentrations and *Pseudomonas aeruginosa* was more susceptible at lower concentrations for peel extract. The differences in the level of bioactive compounds (flavonoids, terpenes and phenolic acids) present in peel and seed can be attributed for this differential activity which could affect cell membrane, enzyme activity or nucleic acid synthesis of the bacteria (Alli et al., 2011). The by-products of soursop revealed from the MIC results are found to be very good sources of antimicrobial agents and they were normally discarded. Based on their different inhibitory profiles, it may be possible to synergistically use peel and seed extracts so that they can have more extensive and effective antibacterial effect

Comparative Evaluation of the Nutritional Composition, Phytochemical Constituents, Dioxin Content, and Antimicrobial Activities of Soursop (*Annona muricata*) Peel and Seed (Malik et al., 2023). The results highlight the viability of using soursop peel and seed extracts as possible source of natural antimicrobial agents or natural preservatives.

Conclusion

The study showed that soursop peel and seed have high levels of complementary nutrients and bioactive compounds, making them promising for the development of functional food and nutraceutical. Its peel has a high moisture content, dietary fiber, vitamin C, vitamin K, and terpene content that makes it good for antioxidant and health benefits. On the other hand the seed is rich in proteins, fats, carbohydrates, and B vitamins, which are important macro-nutrients and play a role in energy metabolism. The differences indicate that both parts could be used in different ways, such as use of peel as a source of dietary fibers in fiber supplements and antioxidant formulations, and use of seed for protein- and lipid-based applications. Phytochemical profiling of both peel and seed showed the presence of a wide range of flavonoids, phenolic acids, and terpenes such as luteolin, gallic acid, and limonene, all with antimicrobial, antioxidant, and therapeutic properties. The observed antibacterial activity against Gram-positive and Gram-negative bacteria corresponds with the presence of these compounds. The minimum inhibitory concentration (MIC) results confirmed that the extracts were able to inhibit the growth of bacteria at relatively low concentrations with the peel being more effective against *Pseudomonas aeruginosa* and *Micrococcus roseus*, while the seed was more effective against *Staphylococcus aureus* and *Bacillus subtilis*. This indicates the potential of soursop by-products as natural antimicrobial agents, especially in combating antibiotic-resistant pathogens.

The results of the safety evaluation by dioxin analysis revealed that only trace amounts of toxic substances are found in the peel, and were negligible, whereas no dioxins were detected in the seed. The result highlights the need for safety screening and adequate processing to use these by-products as food, nutraceuticals, or pharmaceuticals. In sum, the study highlights the potential of soursop peel and seed as untapped resources that can be processed into valuable ingredients with nutritional and natural antimicrobial properties and contribute to sustainable food and health solutions.

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