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Bioactive Composition and Antioxidant Characteristics of *Calophyllum inophyllum* Seed Flour: Potential Relevance for Functional Food Development

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ABSTRACT

Metabolic diseases such as obesity, type 2 diabetes mellitus, and dyslipidemia are health challenges, with over one billion people affected by obesity and 589 million adults living with diabetes. This study evaluated the antioxidant activity, anthocyanin content, amino acid profile, proximate composition, and fatty acid (FFA) profile of *Calophyllum inophyllum* seed flour as a functional food ingredient. Seeds were collected from the Batam Botanical Garden, Indonesia, and processed by freeze-drying. Antioxidant activity was determined using the DPPH assay (IC_{50}), anthocyanin content was measured spectrophotometrically, and proximate composition, amino acid profile, and FFA composition were analyzed using standardized methods, including GC-FID. All analyses were performed in triplicate ($n = 3$), and results were expressed as mean ± standard deviation. The flour was predominantly composed of lipids (65.26 ± 0.13%), followed by carbohydrates (21.92 ± 0.10%) and proteins (8.39 ± 0.02%), with low moisture content (2.64 ± 0.03%). The DPPH assay yielded an IC_{50} value of 13,362.04 ± 1.70 mg/L, indicating limited free radical scavenging activity, while anthocyanins were not detected. The amino acid profile was characterized by high glutamic acid content and the presence of essential amino acids, including leucine, valine, and lysine. The fatty acid profile was dominated by unsaturated fatty acids, particularly oleic and linoleic acids. These findings indicate that *Calophyllum inophyllum* seed flour contains nutritionally relevant amino acids and unsaturated fatty acids, supporting its potential relevance for functional food development. Further studies are required to characterize additional bioactive compounds and evaluate their biological activities in vivo.

Keywords: *Calophyllum inophyllum* seed flour; fatty acid; amino acids; functional food; metabolic diseases

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INTRODUCTION

Metabolic diseases, particularly obesity, type 2 diabetes mellitus (T2DM), and dyslipidemia, represent major global public health challenges. According to the International Diabetes Federation, approximately 589 million adults were living with diabetes worldwide in 2024, and this number is projected to rise substantially in the coming decades (1). In parallel, the prevalence of obesity continues to increase globally, affecting more than one billion individuals and contributing significantly to cardiometabolic morbidity and mortality (2,3). These disorders are characterized by impaired energy metabolism, chronic low-grade inflammation, and excessive oxidative stress, which promote lipid peroxidation, protein modification, and DNA damage (4,5). Although dietary interventions rich in fruits, vegetables, and antioxidant-containing foods have shown beneficial effects on metabolic health, their long-term effectiveness is often limited by variability in bioactive compound content, accessibility, cost, and consumer adherence (6,7). Consequently, there is growing interest in identifying novel, sustainable, and nutritionally valuable functional food sources that may complement existing dietary strategies. In this context, food ingredients containing bioactive compounds with antioxidant and metabolic regulatory properties have attracted considerable attention because of their potential to support health and reduce oxidative stress-related metabolic disturbances (8).

Previous studies have demonstrated that *Calophyllum inophyllum* contains a variety of bioactive compounds, including phenolic compounds, flavonoids, and lipid-soluble antioxidants with potential biological activities (9–11). Extracts and seed oil derived from this species have been reported to exhibit antioxidant properties, suggesting that *C. inophyllum* may represent a valuable source of bioactive constituents for functional food applications (11,12). However, existing research has primarily focused on leaves, extracts, and seed oil, while information regarding the antioxidant characteristics and nutritional composition of *C. inophyllum* seed flour remains limited. Therefore, investigating the antioxidant activity and compositional profile of the seed flour is important to evaluate its potential as a novel food ingredient and to provide scientific evidence supporting its future utilization in functional food development (13–15).

Beyond phytochemical constituents, the nutritional value of a potential functional food is strongly influenced by its macronutrient composition and lipid profile. In particular, the quality and composition of dietary lipids play a crucial role in metabolic health, as different fatty acids exert distinct physiological effects on lipid metabolism, insulin sensitivity, inflammation, and cardiovascular risk (16,17). Diets rich in unsaturated fatty acids, especially oleic acid and linoleic acid, have been associated with improved plasma lipid profiles and reduced cardiometabolic risk factors, whereas excessive consumption of certain saturated fatty acids may contribute to metabolic dysfunction (16). Therefore, characterization of the lipid fraction is essential when evaluating novel food materials intended for functional food applications. In addition to total fat content, free fatty acid (FFA) profiling provides important information regarding lipid quality, stability, and potential biological functionality. FFAs are generated through triglyceride hydrolysis and are widely used as indicators of lipid degradation and oxidative stability during food processing and storage (18,19). Furthermore, the relative abundance of individual fatty acids can provide insight into the nutritional and physiological relevance of a food matrix, particularly in relation to metabolic health and functional food development (20,21). Consequently, comprehensive analysis of proximate composition and fatty acid profiles is necessary to assess both the nutritional quality and functional food potential of *Calophyllum inophyllum* seed flour (17).

In addition to lipid composition, the protein quality of a potential functional food can be evaluated through its amino acid profile. Essential and non-essential amino acids contribute to various physiological functions, including protein synthesis, metabolic regulation, and tissue maintenance (22–26). The composition and balance of amino acids are therefore important indicators of nutritional quality and potential functional value in food materials (27). Despite growing interest in *Calophyllum inophyllum* as a source of bioactive compounds, information regarding the amino acid composition of its seed flour remains limited. Characterizing its amino acid profile may provide valuable insight into its nutritional potential and suitability for future functional food applications.

Calophyllum inophyllum is a tropical species widely distributed throughout coastal regions of Asia and the Pacific, including Indonesia (28). Previous studies have primarily focused on its seed oil, leaf extracts, and pharmacological properties, reporting the presence of bioactive compounds with antioxidant, anti-inflammatory, and other biological activities (11,29). In addition, the seed oil has attracted considerable attention for applications in the pharmaceutical, cosmetic, and biodiesel industries (11). However, despite the growing interest in *C. inophyllum*, limited information is available regarding the nutritional composition, amino acid profile, antioxidant characteristics, and fatty acid composition of its seed flour. This knowledge gap is important because seed flour may retain valuable nutrients and bioactive constituents after processing, making it a potentially useful ingredient for functional food development. Therefore, this study comprehensively evaluates the proximate composition, amino acid profile, free fatty acid (FFA) composition, anthocyanin content, and in vitro antioxidant activity of *C. inophyllum* seed flour. To the best of our knowledge, this study is among the first to provide an integrated characterization of the nutritional and antioxidant properties of *C. inophyllum* seed flour, thereby providing a scientific basis for its future utilization as a novel functional food ingredient.

METHOD

Plant samples of *Calophyllum inophyllum* were collected from the Batam Botanical Garden, Batam City, Riau Islands Province, Indonesia (Location 1: 104°05′06.729″ E, 1°10′22.825″ N; Location 2: 104°05′07.387″ E, 1°10′22.825″ N; Location 3: 104°05′07.826″ E, 1°10′23.002″ N) (Figure 1). This study employed a descriptive analytical design to characterize the physicochemical properties, nutritional composition, amino acid profile, fatty acid composition, anthocyanin content, and antioxidant activity of *C. inophyllum* seed flour. Seeds were collected from three different mature and healthy trees to improve biological representativeness, and the independently collected samples served as biological replicates (n = 3). Sampling was conducted at approximately 09:00 a.m. under ambient temperature conditions of approximately 31 °C. The seeds were washed under running water for 1–2 min to remove impurities and residual fruit tissues before further processing. Subsequently, the seeds were cut into small pieces and pre-frozen at −40 °C for 24 h using a deep freezer. Freeze-drying was then performed under vacuum pressure (0.01–0.05 mbar) at a condenser temperature of −50 °C for 24–48 h until complete drying was achieved (30). This process was applied to minimize thermal degradation and preserve the stability of bioactive compounds during storage and subsequent analyses.

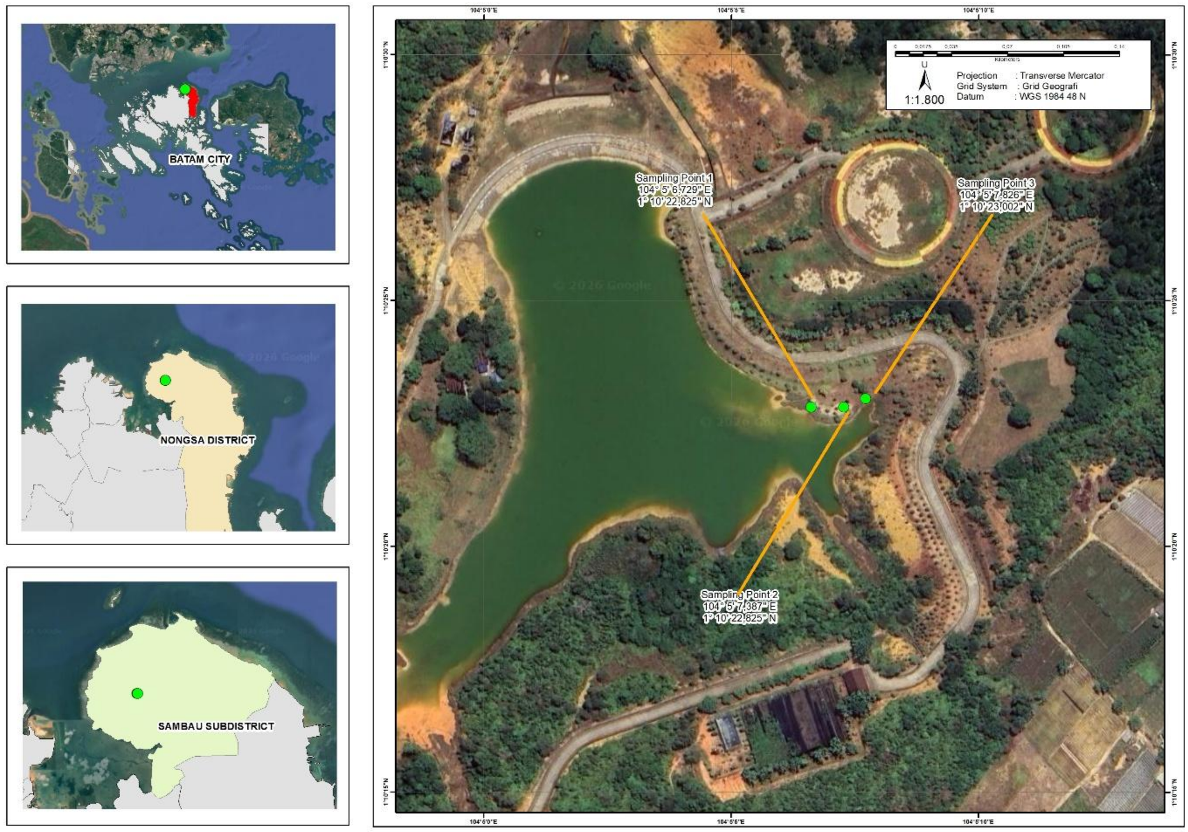

Figure 1. Location of the Batam Botanical Garden area in Batam City, Riau Islands Province, Indonesia.

The pH of the flour was determined by dispersing 5 g of sample in 45 mL distilled water (1:10, w/v), homogenizing for approximately 5 min, and measuring with a calibrated pH meter at room temperature. Antioxidant activity was assessed using the DPPH radical scavenging assay according to AOAC 2012.04. Briefly, 5 g of sample was extracted using a methanol water mixture (1:1, v/v), sonicated for 10 min, reacted with DPPH solution (50 mg/L), incubated in the dark for 30 min, and measured at 519 nm. Antioxidant activity was expressed as the IC_{50} value (11,31). Total anthocyanin content, amino acid profile, proximate composition, and free fatty acid (FFA) content were analyzed using standardized protocols at PT. Saraswati Indo Genetech (SIG), Bogor, Indonesia. Total anthocyanin content was determined using the pH differential method according to AOAC 2005.02, with absorbance measured at 520 and 700 nm and results expressed as cyanidin-3-glucoside equivalents. Amino acid profiling was performed using chromatographic methods, including UPLC for most amino acids, LC-MS/MS for cystine and methionine, and HPLC for L-tryptophan. Proximate composition analysis included determination of moisture, ash, protein, fat, and carbohydrate contents using standardized analytical procedures. Fatty acid composition was analyzed using gas chromatography coupled with a flame ionization detector (GC-FID) following conversion to fatty acid methyl esters (FAMEs), with compound identification based on retention times and quantification by peak area normalization. Results were expressed as percentages of total detected fatty acids (16,17,19,32).

All analyses were performed using three independent biological replicates obtained from seeds collected from three different mature trees. Data are presented as mean ± standard deviation (SD). Because the study was designed as a descriptive characterization of a single plant material without treatment groups or comparative interventions, inferential statistical analyses were not considered appropriate. Therefore, results were summarized using descriptive statistics and reported as mean ± SD from three biological replicates (33). Ethical approval was not required because this study did not involve human participants, animals, or clinical specimens and was conducted exclusively using plant materials collected for analytical purposes.

RESULTS

Amino acid profiles

The amino acid profile of *Calophyllum inophyllum* seed flour is presented in Table 1. The analytical results demonstrated the presence of a diverse range of both indispensable amino acids (IAA) and dispensable amino acids (DAA) in varying concentrations. Among the indispensable amino acids, L-leucine exhibited the highest concentration (5099.02 ± 1.16 mg/kg), followed by L-valine (2914.12 ± 0.48 mg/kg), L-phenylalanine (2862.62 ± 0.18 mg/kg), and L-lysine (2656.50 ± 1.63 mg/kg). In contrast, L-methionine was present at the lowest concentration (26.63 ± 0.00 mg/kg). Other essential amino acids, including L-isoleucine, L-threonine, L-histidine, L-tryptophan, and L-tyrosine, were detected at intermediate levels.

Among the indispensable amino acids, L-glutamic acid was the predominant component (13,556.13 ± 3.44 mg/kg), followed by L-arginine (10,338.90 ± 2.26 mg/kg) and L-aspartic acid (5777.93 ± 1.26 mg/kg). Moderate concentrations were observed for L-serine, glycine, L-alanine, and L-proline. Overall, dispensable amino acids were present at higher concentrations than indispensable amino acids, with glutamic acid and arginine representing the dominant amino acid constituents of the seed flour.

The predominance of glutamic acid and arginine observed in *C. inophyllum* seed flour is consistent with amino acid patterns reported in several seed-derived functional food ingredients, including pumpkin seed flour and pumpkin seed protein isolates, where glutamic acid and arginine are among the most abundant amino acids (34,35). Similarly, soybean flour, which is widely recognized as a high-quality plant protein source, contains substantial levels of essential amino acids such as leucine, valine, lysine, and phenylalanine (36). Although the total protein content and amino acid balance of *C. inophyllum* seed flour differ from those reported for soybean-based ingredients, the presence of a broad spectrum of indispensable amino acids together with high concentrations of glutamic acid and arginine indicates a nutritionally relevant amino acid composition. These findings suggest that *C. inophyllum* seed flour may represent a complementary source of plant-derived amino acids and support further investigation of its nutritional quality and protein bioavailability.

Table 1. Amino Acid Composition of *Calophyllum inophyllum* Seed Flour (mg/kg sample).

Amino Acids	Mean ± SD
IAA*	
L-Leucine	5099.02 ± 1.16
L-Isoleucine	2442.38 ± 0.58
L-Methionine	26.63 ± 0.00
L-Phenylalanine	2862.62 ± 0.18
L-Lysine	2656.50 ± 1.63
L-Tryptophan	406.98 + 0.03
L-Histidine	1305.61 ± 0.04
L-Threonine	1752.09 ± 0.32
L-Valine	2914.12 ± 0.48
DAA**	
L-Aspartic Acid	5777.93 ± 1.26
L-Tyrosine	843.13 ± 0.39
L-Glutamic Acid	13556.13 ± 3.44
L-Serine	3396.53 ± 0.89
L-Proline	2785.82 ± 0.58
Glycine	3268.55 ± 0.79
L-Alanine	2812.17 ± 0.48
L-Arginine	10338.90 ± 2.26

IAA*: indispensable amino acids
DAA**: dispensable amino acids

Proximate and Antioxidant Properties

The proximate composition and antioxidant properties of *Calophyllum inophyllum* seed flour are presented in Table 2. The analysis revealed that lipids were the predominant macronutrient (65.26%), followed by carbohydrates and protein. Conversely, moisture and ash contents were relatively low, indicating reduced water availability and limited inorganic mineral content within the flour. The high energy value was consistent with the elevated lipid concentration, as fats contribute substantially to the caloric content of food materials. The flour also exhibited a slightly acidic pH, which may influence the stability and interactions of food constituents during storage.

The exceptionally high lipid content distinguishes *C. inophyllum* seed flour from many conventional cereal- and legume-based flours and may influence its potential applications in food systems. High-fat seed flours can serve as concentrated sources of dietary energy and lipid-soluble bioactive compounds, but their incorporation into conventional flour-based products may require formulation adjustments to maintain desirable technological and sensory properties (37,38). In addition, materials with elevated lipid concentrations are generally more susceptible to oxidative deterioration during storage, particularly when unsaturated fatty acids are abundant (39). Nevertheless, the low moisture content observed in the present study may contribute to improved storage stability by reducing hydrolytic reactions, microbial growth, and moisture-related deterioration processes (40). Therefore, while the high lipid content may present formulation and storage challenges, it may also provide opportunities for the development of specialized functional food products with enhanced energy density and lipid-derived nutritional components.

Table 2. Proximate Analysis and Antioxidant Properties of *Calophyllum inophyllum* Seed Flour.

Proximate Analysis	Mean ± SD
Ash Content (%)	1.66 ± 0.03
Total Fat (%)	65.26 ± 0.13
Moisture Content (%)	2.64 ± 0.03
Carbohydrate (%)	21.92 ± 0.10
Protein Content (%)	8.39 ± 0.02
Calorie From Fat (Kcal/100 g)	587.30 ± 1.21
Total Calories (Kcal/100 g)	709.75 ± 0.69
pH	5.52 ± 0.00
Antioxidant Properties	
Antioxidant Activity [IC50] (mg/L)	13,362.04 ± 1.70
Anthocyanins (mg/kg)	ND

ND: Not Detected

Regarding the antioxidant properties, the DPPH assay yielded an IC_{50} value of 13,362.04 mg/L, indicating very weak free radical scavenging activity under the assay conditions. Since antioxidant activity is inversely related to IC_{50} values, the high IC_{50} observed in the present study suggests limited antioxidant effectiveness compared with extracts commonly regarded as potent antioxidants (39,40 NEW). The absence of detectable anthocyanins further indicates that these pigments do not contribute to the observed antioxidant response. Therefore, the nutritional relevance of *Calophyllum inophyllum* seed flour appears to be more strongly associated with its macronutrient composition and fatty acid profile than with antioxidant activity. Further studies are required to identify other phytochemical constituents and evaluate their biological activities using complementary analytical approaches.

Free Fatty Acid Composition

The fatty acid profile of *Calophyllum inophyllum* seed flour is summarized in Table 3. The analysis showed a predominance of unsaturated fatty acids over saturated fatty acids. Among the unsaturated fractions, monounsaturated fatty acids (MUFA) represented the major group, followed by polyunsaturated fatty acids (PUFA). Oleic acid was the dominant individual fatty acid, while linoleic acid, palmitic acid, and stearic acid were also identified as major components.

Minor fatty acids, including capric, lauric, palmitoleic, heptadecanoic, and heptadecenoic acids, were detected at low concentrations. Several long-chain fatty acids, such as arachidic, behenic, lignoceric, and nervonic acids, were also present in small amounts. In contrast, a number of fatty acids, including undecanoic, tridecanoic, myristic, arachidonic, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), were not detected in the sample. Overall, the fatty acid profile of *C. inophyllum* seed flour was characterized by high proportions of oleic and linoleic acids, indicating that unsaturated fatty acids constitute the predominant lipid fraction of the seed flour.

Table 3. Free fatty acids composition in *Calophyllum inophyllum* Seed Flour.

Compound	Chemical formula	Mean ± SD (%)
Omega 3 Fatty Acids	$C_{18}H_{30}O_2$	0.187 ± 0.00
Omega 6 Fatty Acids	$C_{18}H_{32}O_2$	19.019 ± 0.07
Omega 9 Fatty Acids	$C_{18}H_{34}O_2$	26.291 ± 0.06
Linoleic Acid	$C_{18}H_{32}O_2$	18.949 ± 0.07
Linolenic Acid	$C_{18}H_{30}O_2$	0.257 ± 0.00
Oleic Acid	$C_{18}H_{34}O_2$	26.284 ± 0.06
Capric Acid	$C_{10}H_{20}O_2$	0.005 ± 0.00
Lauric Acid	$C_{12}H_{24}O_2$	0.015 ± 0.00
Palmitic Acid	$C_{16}H_{32}O_2$	9.443 ± 0.08
Palmitoleic Acid	$C_{16}H_{30}O_2$	0.157 ± 0.00
Heptadecanoic Acid	$C_{17}H_{34}O_2$	0.065 ±0.00
Heptadecenoic Acid	$C_{17}H_{32}O_2$	0.027 ±0.00
Stearic Acid	$C_{18}H_{36}O_2$	9.426 ± 0.05
Arachidic Acid	$C_{20}H_{38}O_2$	0.395 ± 0.00
Eicosyanoic Acid	$C_{22}H_{44}O_2$	0.097 ± 0.00
Behenic Acid	$C_{24}H_{48}O_2$	0.106 ± 0.00
Lignoceric Acid	$C_{24}H_{46}O_2$	0.024 ± 0.00
Nervonic Acid	$C_8H_{16}O_2$	0.007 ± 0.00
Caprylic Acid	$C_{18}H_{30}O_2$	0.003 ± 0.00
Saturated Fat	$C_{18}H_{32}O_2$	19.483 ± 0.13
Unsaturated Fat	$C_{18}H_{34}O_2$	45.777 ± 0.13
Polyunsaturated Fat	$C_{18}H_{32}O_2$	19.206 ± 0.07
Monounsaturated Fat	$C_{18}H_{30}O_2$	26.571 ± 0.06
Free Fatty Acid	$C_{18}H_{34}O_2$	9.935 ± 0.05
Undecanoic Acid	$C_{11}H_{22}O_2$	0.000 ± 0.00
Tridecanoic Acid	$C_{13}H_{26}O_2$	0.000 ± 0.00
Myristic Acid	$C_{14}H_{28}O_2$	0.000 ± 0.00
Miristoleic Acid	$C_{14}H_{26}O_2$	0.000 ± 0.00
Pentadecanoic Acid	$C_{15}H_{30}O_2$	0.000 ± 0.00
Pentadecenoic Acid	$C_{15}H_{28}O_2$	0.000 ± 0.00
T-Oleic Acid	$C_{18}H_{34}O_2$	0.000 ± 0.00
T-Linoleic Acid	$C_{18}H_{32}O_2$	0.000 ± 0.00
Eicosadienoic Acid	$C_{20}H_{36}O_2$	0.000 ± 0.00
Eicosatrienoic Acid	$C_{20}H_{34}O_2$	0.000 ± 0.00
Arachidonic Acid	$C_{20}H_{32}O_2$	0.000 ± 0.00
Heneicosanoic Acid	$C_{21}H_{42}O_2$	0.000 ± 0.00
Erucic Acid	$C_{22}H_{42}O_2$	0.000 ± 0.00
Docosadienoic Acid	$C_{22}H_{40}O_2$	0.000 ± 0.00
Trichosanoic Acid	$C_{23}H_{46}O_2$	0.000 ± 0.00
Butyric Acid	$C_4H_8O_2$	0.000 ± 0.00
Caproic acid	$C_6H_{12}O_2$	0.000 ± 0.00
Eicosapentaenoic Acid (EPA)	$C_{20}H_{30}O_2$	0.000 ± 0.00
Docosahexanoic Acid (DHA)	$C_{22}H_{32}O_2$	0.000 ± 0.00

DISCUSSION

This study provides important information regarding the nutritional composition, fatty acid profile, amino acid composition, anthocyanin content, and antioxidant activity of *Calophyllum inophyllum* seed flour. The findings demonstrate that this underutilized plant material possesses a distinctive compositional profile characterized by high lipid content, a predominance of unsaturated fatty acids, and the presence of both indispensable and dispensable amino acids. Although antioxidant activity was detected using the DPPH assay, the observed IC_{50} value indicated limited free radical scavenging activity under the assay conditions. Furthermore, anthocyanins were not detected in the samples analyzed. These findings suggest that the antioxidant activity of *C. inophyllum* seed flour is not associated with anthocyanin content; however, the specific compounds responsible for the observed activity cannot be determined from the present study because no comprehensive phytochemical characterization was performed. Therefore, further studies are required to identify and characterize the bioactive constituents present in the seed flour and to better understand their potential biological activities (41,42).

The antioxidant activity evaluated using the DPPH assay yielded an IC_{50} value of 13,362.04 mg/L, indicating very weak free radical scavenging activity under the experimental conditions. This finding suggests that *Calophyllum inophyllum* seed flour possesses limited antioxidant effectiveness when assessed using the DPPH method. In addition, anthocyanins were not detected in the analyzed samples. Because no comprehensive phytochemical characterization was performed in the present study, the compounds potentially contributing to the observed antioxidant response cannot be identified. Therefore, the current results should be interpreted as evidence of limited in vitro antioxidant activity rather than strong antioxidant potential. Further phytochemical investigations are required to determine the presence of other bioactive constituents and to better understand their contribution to the overall biological properties of the seed flour(43).

Oxidative stress has been widely recognized as an important factor associated with the development and progression of various metabolic disorders, including type 2 diabetes mellitus, obesity, and dyslipidemia(21). Antioxidant compounds may contribute to the neutralization of reactive oxygen species and help reduce oxidative damage to biomolecules (31). However, the present study evaluated only the in vitro antioxidant activity of *Calophyllum inophyllum* seed flour and did not directly assess biological effects related to metabolic diseases. Therefore, while the observed antioxidant activity provides information regarding one aspect of the functional characteristics of the seed flour, the findings should not be interpreted as evidence of efficacy for the prevention or management of metabolic disorders. Further studies using appropriate cellular, animal, or clinical models are required to evaluate the biological relevance of these observations.

The DPPH method applied in this study is generally more sensitive to hydrophilic antioxidant compounds and may underestimate the contribution of lipophilic constituents present in complex food matrices (44). Therefore, the measured antioxidant activity should be interpreted with caution, particularly for materials characterized by a high lipid content. In the present study, *Calophyllum inophyllum* seed flour contained 65.26% lipids and was dominated by unsaturated fatty acids, especially oleic and linoleic acids. These characteristics suggest that antioxidant mechanisms relevant to this material may be associated not only with direct free radical scavenging in solution but also with the protection of lipid fractions against oxidative deterioration. Consequently, the relatively weak DPPH activity observed may not fully represent the oxidative stability or antioxidant behavior of the lipid-rich matrix. Limitations of the DPPH assay for evaluating lipid-dominated food systems have been recognized in previous studies, which recommend the use of complementary methods capable of assessing antioxidant activity in lipid environments or biological systems (40,44). Assays such as β-carotene bleaching, oxygen radical absorbance capacity (ORAC), lipid peroxidation inhibition, or thiobarbituric acid reactive substances (TBARS) measurements may provide additional information regarding the ability of bioactive compounds to protect lipid fractions from oxidative damage. Therefore, future studies employing multiple antioxidant assessment approaches would provide a more comprehensive evaluation of the functional properties of *C. inophyllum* seed flour.

In addition to its antioxidant characteristics, *Calophyllum inophyllum* seed flour exhibited a distinctive nutritional composition characterized by a high lipid content and a predominance of unsaturated fatty acids. Oleic acid and linoleic acid were identified as the major fatty acids, which is consistent with previous studies reporting that *C. inophyllum* seed oil is predominantly composed of oleic acid (30–50%) and linoleic acid (20–40%) as its principal unsaturated fatty acids (45,46). The similarity between the fatty acid profile observed in the present study and those reported for *C. inophyllum* oil suggests that nutritionally relevant lipid components remain associated with the seed flour following processing. The predominance of oleic and linoleic acids is noteworthy because these fatty acids are widely recognized as important constituents of plant-derived oils. Oleic acid has been associated with favorable lipid profiles and cardiovascular health, whereas linoleic acid is an essential fatty acid involved in maintaining cell membrane integrity and normal physiological functions (47,48). Nevertheless, the present study evaluated only the compositional characteristics of *C. inophyllum* seed flour and did not assess biological or metabolic outcomes. Therefore, although the fatty acid profile supports the nutritional relevance of the seed flour, further studies are required to evaluate the bioavailability and physiological significance of these lipid constituents in food applications.

The amino acid profile provides an additional perspective on the nutritional characteristics of *Calophyllum inophyllum* seed flour. The presence of essential amino acids such as L-leucine, L-valine, and L-lysine indicates that the flour contains nutritionally relevant protein components. Previous studies have reported that these amino acids are involved in physiological processes related to protein metabolism and energy regulation (49). Similarly, L-arginine has been recognized as a precursor of nitric oxide and has been associated with endothelial function and vascular homeostasis (50), whereas glutamic acid contributes to nitrogen metabolism and various cellular functions (51). In the present study, glutamic acid was identified as the predominant amino acid, followed by arginine and aspartic acid, highlighting the distinctive amino acid composition of the seed flour. In addition to its nutritional relevance, the high glutamic acid content may influence the sensory properties of food products because glutamic acid is known to contribute to umami taste perception. However, the present study evaluated only the amino acid composition of *C. inophyllum* seed flour and did not assess protein digestibility, amino acid bioavailability, or physiological effects. Therefore, the biological significance of the observed amino acid profile cannot be directly inferred from the current findings and requires further investigation through appropriate nutritional and functional studies (52).

The proximate composition further contributes to the characterization of *Calophyllum inophyllum* seed flour. The low moisture content observed in the present study suggests reduced water availability, which is generally associated with improved storage stability, reduced microbial growth, and slower deterioration reactions in dried food materials (40,53). In addition, the slightly acidic pH may influence the physicochemical properties of the flour and contribute to product stability during storage and processing (54). These characteristics are important considerations for the development of shelf-stable food ingredients.The presence of free fatty acids (FFA) reflects the lipid-rich nature of the seed flour and provides additional information regarding its lipid composition and quality. Together with the predominance of unsaturated fatty acids, these findings highlight the distinctive compositional characteristics of *C. inophyllum* seed flour. However, the effects of these properties on long-term storage stability and preservation of bioactive compounds were not directly evaluated in the present study and therefore require further investigation.

Methodologically, the use of freeze-drying in this study provided advantages for sample preservation because it removes water under low-temperature conditions, thereby minimizing thermal degradation of heat-sensitive constituents compared with conventional drying methods such as hot-air or oven drying (55,56). Previous studies have reported that freeze-drying is generally more effective in retaining nutritional and bioactive components in plant-derived materials, making it particularly suitable for compositional and bioactivity-related analyses (56). Consequently, the analytical results obtained in the present study are expected to more closely reflect the intrinsic characteristics of *Calophyllum inophyllum* seed flour. Overall, the findings demonstrate that *C. inophyllum* seed flour possesses a distinctive compositional profile characterized by high lipid content, a predominance of unsaturated fatty acids, the presence of essential and non-essential amino acids, and limited in vitro antioxidant activity. These characteristics highlight the potential relevance of this underutilized plant material for functional food development. However, the present study was limited to compositional characterization and in vitro analyses; therefore, no conclusions can be drawn regarding biological efficacy, physiological mechanisms, or disease-related outcomes. Further studies are required to evaluate the bioavailability of key nutrients and bioactive compounds, assess their biological activities in appropriate experimental models, and determine their suitability for food product development.

CONCLUSIONS AND RECOMMENDATIONS

Calophyllum inophyllum seed flour exhibits distinctive nutritional and compositional characteristics, including a high lipid content, a predominance of unsaturated fatty acids, and the presence of both essential and non-essential amino acids. The DPPH assay indicated limited in vitro antioxidant activity, while anthocyanins were not detected in the analyzed samples. These findings suggest that the observed antioxidant activity is not associated with anthocyanin content, although the specific compounds contributing to this activity remain to be identified. Overall, *C. inophyllum* seed flour demonstrates potential as a functional food ingredient warranting further biological investigation. Future studies should evaluate the bioavailability and biological relevance of its nutritional and bioactive components, as well as its suitability, safety, and stability for food product development.

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