



Bioactivity-guided Evaluation of Alligator pepper (*Aframomum melegueta*): Phytochemicals, Antioxidant, Anti-inflammatory, Anti-diabetic Potentials and Anti-obesity

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ABSTRACT

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This study presents a bioactivity-guided evaluation of *Aframomum melegueta* (Alligator pepper) aimed at elucidating its nutraceutical potential through comprehensive phytochemical profiling and in vitro assessment of antioxidant, anti-inflammatory, anti-diabetic, and anti-obesity properties. The alligator pepper extract was prepared using water as the sole extraction solvent. Quantitative phytochemical analysis revealed the presence of diverse bioactive constituents, with alkaloids (62.05 mg/g) being the most abundant, followed by phenolics (25.56 mg GAE/g), saponins (15.58 mg/g), flavonoids (9.29 mg QE/g), tannins (1.35 mg/g), and steroids (0.06 mg/g). Antioxidant activity was confirmed through multiple assays: DPPH radical scavenging (83.4%), lipid peroxidation inhibition (45.07%), and ferric reducing antioxidant power (5.90 mg vitamin C equivalents/g). The seed powder also contained 1.22 mg/g of vitamin C. The anti-diabetic evaluation demonstrated moderate inhibition of key carbohydrate-hydrolysing enzymes, with 44.27% and 41.03% inhibition of α -glucosidase and α -amylase, respectively. Anti-inflammatory activity was evidenced by 85.56% antiproteinase inhibition and 50.31% inhibition of protein denaturation. In addition, the seed extract displayed appreciable anti-obesity potential, as shown by 40.18% inhibition of pancreatic lipase activity. Overall, *A. melegueta* demonstrates multifunctional bioactivities that support its potential application in nutraceutical formulations and complementary therapies in human and veterinary health. In conclusion, *Aframomum melegueta* exhibits significant antioxidant, anti-inflammatory, anti-diabetic, and anti-obesity activities, confirming its potential as a functional nutraceutical. Further in vivo investigations and mechanistic studies are recommended to substantiate these bioactivities and assess the safety and efficacy of its phytoconstituents in clinical and animal models.

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Introduction

The increasing prevalence of chronic non-communicable diseases (NCDs) such as type 2 diabetes mellitus, cardiovascular disorders, and inflammatory syndromes has created a significant global public health challenge (Gowshall and Taylor-Robinson, 2018). According to the World Health Organisation (WHO), metabolic and inflammatory diseases account for a substantial proportion of global morbidity and mortality, particularly in low- and middle-income countries where

healthcare infrastructure may be inadequate (Hossain et al., 2024). Central to the pathogenesis of these disorders is the overproduction of reactive oxygen species (ROS), leading to oxidative stress and the dysregulation of key metabolic and immune pathways (Afzal et al., 2023). This has spurred an urgent scientific search for safe, effective, and affordable alternatives to conventional synthetic drugs, many of which are associated with side effects, high cost, and limited accessibility.

One emerging area of therapeutic innovation is the use of nutraceuticals bioactive compounds derived from food sources, especially plants, which provide health benefits beyond basic nutrition (Oloruntola, 2024). Nutraceuticals offer a promising multi-targeted approach to disease prevention and management, particularly for complex disorders where oxidative stress, chronic inflammation, and metabolic dysregulation are interlinked (Dama et al., 2024; Oloruntola, 2022). Among the diverse array of botanicals with potential nutraceutical value, Alligator pepper, a spice from the *Aframomum* genus in the Zingiberaceae family, is of particular interest due to its rich ethnopharmacological history and underexplored biochemical properties (Adefegha et al., 2016).

Known locally in southwestern Nigeria as “Ataire” (Yoruba), Alligator pepper is traditionally used in both culinary and medicinal practices. The seeds and seed pods are consumed or applied in the management of ailments such as stomach pain, infectious diseases, infertility, and general inflammation (Abubakar et al., 2022). Despite these traditional applications, scientific validation of these claims remains limited. Several *Aframomum* species particularly *A. melegueta* have been reported to contain a variety of phytochemicals, including flavonoids, phenolic acids, alkaloids, terpenoids, and tannins, which may contribute to their pharmacological effects (Akinsanya et al., 2016). However, there is a noticeable gap in the literature regarding comparative evaluations of this phytogen with respect to its phytochemical profiles and biological activities, especially those relevant to the management of oxidative and metabolic disorders (Zhu et al., 2021).

Furthermore, while isolated studies have examined aspects of Alligator pepper’s antioxidant or anti-inflammatory effects, there is a lack of integrated bioactivity-guided assessments that align phytochemical composition with functional outcomes across multiple health endpoints. A comprehensive investigation that combines phytochemical profiling with robust in vitro assays for antioxidant capacity, anti-inflammatory potential, and glucose regulatory (anti-diabetic) effects is essential for substantiating its nutraceutical claims and supporting the development of evidence-based botanical therapeutics.

In addition, given the growing international interest in plant-based therapies, especially those rooted in African ethnobotanical knowledge, such a study also has implications for biodiversity conservation, local economic development, and the global recognition of indigenous knowledge systems (Mokonnen et al., 2022). Establishing a strong scientific basis for the use of Alligator pepper can stimulate its commercial valorization, promote sustainable agricultural practices, and contribute to health innovation in both local and global contexts.

Although *Aframomum melegueta* has been evaluated for certain biological properties, existing studies remain limited often focusing on single activities such as antioxidant, antimicrobial, or isolated anti-inflammatory effects (Adefegha et al., 2016). Multi-assay investigations are uncommon; most antioxidant studies use only one model (e.g., 2,2-diphenyl-1-picrylhydrazyl hydrate) without complementary assays like ferric reducing power or lipid peroxidation, and anti-inflammatory work rarely includes protein denaturation or anti-proteinase tests. Additionally, key metabolic assays such as α -amylase, α -glucosidase, and

pancreatic lipase inhibition have not been examined together within a unified system. No previous research has integrated the phytochemical profile of *A. melegueta* with a comprehensive evaluation of antioxidant, anti-inflammatory, anti-diabetic, and anti-obesity activities, underscoring the novelty and need for the present study.

The objective of this study is to provide a comprehensive, bioactivity-guided evaluation of *Aframomum melegueta* by integrating its phytochemical composition with functional in vitro assays. Specifically, the study aims to identify and quantify key phytoconstituents in the seed powder, and assess its antioxidant, anti-inflammatory, anti-diabetic, and anti-obesity activities using standardized in vitro models. This integrated approach is intended to generate robust evidence supporting the nutraceutical potential of *A. melegueta* and to fill critical gaps in the current literature.

Materials and Methods

Plant Sample Collection and Authentication

Fresh fruits of Alligator pepper (*Aframomum melegueta*) were collected from authenticated wild sources in Owo forest reserve, southwestern Nigeria. The plant species was taxonomically identified and authenticated at the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria.

Preparation of Aframomum melegueta seed Powder and Extract

The seeds were separated from the pods, air-dried at room temperature (25–28°C) for 21 days, and pulverised into fine powder using a mechanical grinder. The powdered sample was stored in airtight containers until analysis.

A measured quantity of *Aframomum melegueta* seed powder (50 g) was extracted using water (aqueous extraction). The powdered sample was soaked in 500 mL of distilled water and allowed to stand for 72 hours at room temperature with intermittent agitation to enhance solute–solvent interaction. The mixture was then filtered using Whatman No. 1 filter paper. The filtrate was transferred into evaporation flasks and concentrated under reduced pressure using a Rotary evaporator equipped with a digital water bath maintained at 40°C and a vacuum pump. Rotary evaporation was continued until the extract volume was reduced to approximately 10–20% of its original volume. The semi-concentrated extract was subsequently frozen at –20°C for 12–24 hours to ensure complete solidification. The frozen material was then subjected to lyophilization using a Freeze Dryer operating at a condenser temperature of –50°C to –80°C and a vacuum pressure of ≤ 0.1 mbar. Freeze-drying was continued until all moisture was removed and a dry, powdery extract was obtained. All reagents employed for the chemical analyses were of analytical reagent grade and were procured from Sigma-Aldrich (St. Louis, MO, USA).

Phytochemical Quantification

The procedures for determining total flavonoids, phenols, steroids, tannins, saponins, and alkaloids in *Aframomum melegueta* seed followed the methods described by Oloruntola and Ayodele (2022), with references to previously established protocols.

Total flavonoids were quantified using the aluminium chloride colourimetric method. The reaction mixture included methanol, aluminium chloride, potassium acetate, and distilled water added to the extract. Absorbance was read at 510 nm with i3 UV-Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China), and flavonoid content was expressed as mg rutin equivalents per gram (Surana et al., 2016).

Total phenols were measured by the Folin-Ciocalteu method. Extracts were reacted with Folin reagent and Na_2CO_3 , and absorbance was recorded at 760 nm after incubation in the dark. Results were expressed in mg gallic acid equivalents per gram (Ogles and Yalcin, 2012).

Steroids were determined following the method of Madhu et al. (2016). The extract was reacted with FeCl_3 , H_2SO_4 , and potassium hexacyanoferrate, heated in a water bath, and the absorbance was measured at 780 nm against a blank with i3 UV-Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China).

In the determination of the Tannins, the ethanolic extracts were prepared and quantified using the Folin-Ciocalteu method. Absorbance was read at 680 nm with i3 UV-Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China), and tannin content was expressed as mg tannic acid equivalent per gram dry weight (Biswas et al., 2020).

Total saponins were estimated using the vanillin-sulfuric acid colorimetric method. The extract was reacted with sulfuric acid, ethanol, and vanillin, incubated at 60°C, and absorbance measured at 545 nm with i3 UV-Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China). Results were expressed as mg tea saponin equivalents per gram dry weight (He et al., 2014).

Alkaloids were determined by the gravimetric method (Adeniyi et al., 2009). The sample was extracted with acetic acid in ethanol, concentrated, and precipitated with ammonium hydroxide. The precipitate was filtered, washed, oven-dried, and weighed. Alkaloid content was expressed as a percentage of the sample weight.

Antioxidant Assays

Ferric Reducing Antioxidant Power (FRAP)

The procedure for assessing the ferric reducing antioxidant capacity of *Aframomum melegueta* seed powder followed the method described by Oloruntola (2022), based on the protocol established by Pulido et al. (2000). To prepare the FRAP working reagent, a mixture was made by combining 300 mM acetate buffer, 10 mL of TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) in a ratio of 10:1:1, and pre-warmed to 37°C. A volume of 3.995 mL of the freshly prepared FRAP reagent was mixed with 5 μL of appropriately diluted *Aframomum melegueta* seed powder extract using a micropipette. The reaction, which involves the reduction of the ferric-TPTZ complex (Fe^{3+} -TPTZ) to its ferrous form (Fe^{2+} -TPTZ), results in the formation of an intense blue-colored complex. After incubating the mixture at 37°C for 30 minutes, the absorbance was measured at 593 nm using with i3 UV-Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China). A reagent blank (3.995 mL FRAP reagent plus 5 μL distilled water) served as the reference. A standard calibration curve was prepared using serial

dilutions of ascorbic acid (1–100 $\mu\text{g}/\text{mL}$), and the FRAP values of the samples were calculated from the linear equation of the standard curve. The antioxidant capacity of the sample was then expressed as milligrams of ascorbic acid equivalent per gram of sample (mg Vit C/g).

The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) Radical Scavenging Activity

The DPPH radical scavenging activity of *Aframomum melegueta* seed powder was evaluated following the analytical procedure previously established by Oloruntola (2022). Antioxidant potential was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method. A methanolic DPPH solution (6×10^{-5} M) was added to the sample extract or gallic acid standard, and the mixture was incubated in the dark for 20 minutes. Absorbance was measured at 515 nm against a methanol blank with i3 UV-Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China). Antioxidant capacity was quantified using a gallic acid standard curve (10–100 ppm).

Vitamin C Content

The quantification of vitamin C in *Aframomum melegueta* seed powder was conducted based on the protocol outlined by Oloruntola (2022), employing a DNPH (2,4-dinitrophenylhydrazine)-based colourimetric technique for analysis. A mixture of the extract, trichloroacetic acid, and water was reacted with DNPH solution, followed by incubation at 37°C for 3 hours. Sulfuric acid was then added, and absorbance was read at 520 nm with i3 UV-Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China). Ascorbic acid served as the calibration standard.

Lipid Peroxidation Inhibition Assay

The lipid peroxidation inhibitory potential of *Aframomum melegueta* seed powder was evaluated using a modified method adapted from Bajpai et al. (2015), following prior extraction of the bioactive compounds. The assay was performed using a reaction system containing 1 mM ferric chloride (FeCl_3), 1 mM ascorbic acid, and 50 μL of bovine brain phospholipids (5 mg/L) in 20 mM phosphate buffer (pH 7.4). Various concentrations of AMSP extract (50–250 $\mu\text{g}/\text{mL}$) or a standard antioxidant compound were added to the reaction mixtures. A control group was prepared without the extract. All reaction mixtures were incubated at 37°C for 60 minutes to induce lipid peroxidation through hydroxyl radical generation. The extent of lipid peroxidation was determined by measuring malondialdehyde (MDA) formation via the thiobarbituric acid (TBA) reaction. Briefly, TBA reacts with MDA to form a pink chromogen, whose absorbance was read at 532 nm using with i3 UV-Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China). The percentage inhibition (PI) of lipid peroxidation was computed using the following formula:

$$\text{PI (\%)} = \frac{(\text{A control} - \text{A test})}{(\text{A control})} \times 100$$

A control: Absorption of the controlling reaction

A test: Test reaction absorbency.

Enzyme-Based Antidiabetic Activity *Aframomum melegueta* seed Powder

The α -Amylase Inhibitory Activity

The inhibitory effect of *Aframomum melegueta* seed powder on α -amylase activity was assessed using the 3,5-dinitrosalicylic acid (DNSA) method, as adapted from Wickramaratne et al. (2016). *Aframomum melegueta* seed powder extract was initially dissolved in at least 10% dimethyl sulfoxide (DMSO) and further diluted with phosphate buffer (0.02 M $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, pH 6.9, containing 0.006 M NaCl) to obtain a concentration range of 10–1000 $\mu\text{g/mL}$. For the assay, 200 μL of each extract concentration was mixed with 2 mL of α -amylase solution and incubated at 30°C for 10 minutes. Subsequently, 200 μL of 1% (w/v) soluble starch solution was added, and the mixture was further incubated for 3 minutes. The reaction was terminated by adding 200 μL of DNSA reagent, prepared by dissolving 12 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 20 mL of 96 mM DNSA. The mixture was then heated in a water bath at 85–90°C for 10 minutes. After cooling to room temperature, the reaction mixture was diluted with 5 mL of distilled water, and absorbance was measured at 540 nm using i3 UV-Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China). A negative control (without extract) represented 100% enzyme activity, while blanks (without enzyme) were prepared for each concentration to correct for background absorbance. Acarbose (100–200 $\mu\text{g/mL}$) served as the positive control. The percentage inhibition of α -amylase activity (PAI) was calculated using the equation:

$$\text{PAI} = 100 \times \frac{\text{Abs 100\% Control} - \text{Abs Sample}}{\text{Abs 100\% Control}}$$

The concentration of extract required to inhibit 50% of enzyme activity (IC_{50}) was obtained by plotting the inhibition percentages against extract concentrations.

The α -Glucosidase Inhibitory Activity

The ability of the sample to inhibit α -glucosidase was evaluated following the procedure of Dej-adisai and Pitakbut (2015), using p-nitrophenyl- α -D-glucopyranoside (pNPG) as the substrate. The assay was conducted in 96-well microplates. Each reaction well contained 50 μL of phosphate buffer (10 mM, pH 7.0) supplemented with 0.2 mg/mL sodium azide and 2 mg/mL bovine serum albumin. To this, 50 μL of *Saccharomyces cerevisiae* α -glucosidase enzyme solution (1 U/mL) and 50 μL of the test sample (8 mg/mL in 5% DMSO) were added. The mixture was incubated at 37°C for 2 minutes. Then, 50 μL of 4 mM pNPG was added to initiate the enzymatic reaction. The reaction was allowed to proceed for 5 minutes at the same temperature, during which the release of p-nitrophenol (pNP), a yellow product, was monitored every 30 seconds at 405 nm using i3 UV-Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China). Acarbose (8 mg/mL) served as the positive control, while 5% DMSO served as the negative (solvent) control. Reaction velocity (RV) was determined using the linear portion of the absorbance-time curve:

$$\text{RV} = \frac{\Delta \text{Absorbance at 405 nm}}{\Delta \text{Time}}$$

The percentage of enzyme inhibition (PEI) was calculated using:

$$\text{PEI} = \frac{V \text{ control} - V \text{ sample}}{V \text{ control}} \times 100$$

Where:

V control = Reaction velocity without extract

V Sample = Reaction velocity with the extract

In Vitro Anti-Inflammatory Properties *Aframomum melegueta* seed Powder

Albumin Denaturation Inhibition Assay

The anti-denaturation effect of *Aframomum melegueta* seed powder was assessed using a modified method based on Osman et al. (2016). Standard non-steroidal anti-inflammatory drugs (NSAIDs), ibuprofen and diclofenac (each at 0.1% or 1.0 mg/mL), served as reference controls. The reaction setup consisted of 200 μL of fresh egg albumin, 1.4 mL of phosphate-buffered saline (PBS, pH 6.4), and 1.0 mL of AMSP extract at the desired test concentration. A negative control was prepared by replacing the extract with distilled water. The reaction mixtures were incubated at 37°C for 15 minutes, followed by heating at 70°C for 5 minutes to induce protein denaturation. After cooling to room temperature, absorbance was measured at 660 nm using a i3 UV-Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China).

The percentage inhibition of protein denaturation (PDI) was calculated as:

$$\text{PDI\%} = \frac{1 - (\text{ARTS})}{\text{ARWTS}(-\text{ve control})} \times 100$$

ARTS: Absorbance reading of the test sample

ARWTS: Absorbance reading without test sample

Anti-proteinase Activity Assay

The ability of *Aframomum melegueta* seed powder to inhibit proteinase activity was evaluated following the method of Rajesh et al. (2019), with slight modifications. A reaction mixture of 2.0 mL was prepared, containing 1.0 mL of *Aframomum melegueta* seed powder extract at various concentrations (100–500 $\mu\text{g/mL}$), 0.06 mg of trypsin enzyme, and 20 mM Tris-HCl buffer (pH 7.4). The mixture was pre-incubated at 37°C for 5 minutes to allow enzyme-extract interaction. Following incubation, 1.0 mL of 0.8% (w/v) casein solution was added as the substrate, and the reaction was continued at 37°C for an additional 20 minutes. The enzymatic reaction was terminated by adding 2.0 mL of chilled 70% perchloric acid, which precipitated undigested proteins. The reaction mixtures were then centrifuged or allowed to stand for sedimentation, and the absorbance of the supernatant was measured at an appropriate wavelength (e.g., 280 nm) i3 UV-Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China). The percentage of protease inhibition (PRI) was calculated using the formula:

$$\text{PRI} = \frac{(\text{Abs control} - \text{Abs sample}) \times 100}{\text{Abs control}}$$

In vitro Anti-Obesity Property

In Vitro Lipase Inhibitory Assay

The pancreatic lipase inhibitory activity of *Aframomum melegueta* seed powder was evaluated using a colourimetric method adapted from previously validated protocols, with minor modifications to suit the test matrix (Bandara et al., 2023). The assay measured the ability of *Aframomum melegueta* seed powder extract to inhibit the enzymatic hydrolysis of p-nitrophenyl palmitate (pNPP), a synthetic substrate for lipase. The concentrated extract was reconstituted in 10% DMSO to prepare test concentrations ranging from 100 to 1000 µg/mL. The lipase activity assay was performed in 96-well microplates. Each well received 100 µL of pancreatic lipase enzyme solution (1 mg/mL in 100 mM Tris-HCl buffer, pH 7.4) and 50 µL of the *Aframomum melegueta* seed powder extract at various concentrations. The enzyme–extract mixtures were preincubated at 37°C for 15 minutes. Thereafter, 50 µL of pNPP substrate solution (prepared in isopropanol and emulsified with gum arabic or Triton X-100 if required) was added to initiate the enzymatic reaction. The plates were further incubated at 37°C for 30 minutes. Following incubation, the release of p-nitrophenol (pNP) a yellow chromophore was monitored by measuring absorbance at 405 nm using i3 UV-Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China).

Negative control was enzyme and substrate without extract, the blank was extract without enzyme to correct for background absorbance, while the positive control was Orlistat (100–500 µg/mL), a known lipase inhibitor

The percentage inhibition of lipase activity (ILA) was calculated using the following expression:

$$ILA (\%) = ((A \text{ control} - A \text{ sample}) / (A \text{ control})) \times 100$$

Where:

A control = Absorbance of the control (enzyme + substrate only)

A sample = Absorbance in the presence of extract

The extract concentration required to inhibit 50% of lipase activity (IC₅₀) was determined from the dose-response curve using non-linear regression analysis.

Determination of IC₅₀ Values

For antioxidant, anti-inflammatory, anti-diabetic, and anti-obesity assays, dose-response curves were generated from five concentrations. IC₅₀ values were determined using nonlinear regression (sigmoidal curve-fitting) performed.

Statistical Analysis

All assays were performed in triplicate (n = 3). Data were expressed as mean ± standard deviation (SD). Although raw data were initially organized using Microsoft Excel, statistical analysis including IC₅₀ determination was conducted using GraphPad Prism 9.5.1.

Results

Phytochemical Composition of Aframomum melegueta Seed Powder

The quantitative phytochemical analysis of *Aframomum melegueta* seed powder revealed the presence of various bioactive constituents in differing concentrations (Figure 1). Alkaloids were the most abundant phytochemicals, with a concentration of 62.05 mg/g. Phenolic compounds followed, recorded at 25.56 mg GAE/g. Saponins were also present in appreciable quantity (15.58 mg/g). Flavonoid content was 9.29 mg QE/g. Tannins were detected at 1.35 mg/g, while steroids were found in trace amounts, with a concentration of 0.06 mg/g.

Antioxidant Properties of Aframomum melegueta Seed Powder

The antioxidant properties of *Aframomum melegueta* seed powder are presented in Table 1. The ferric reducing antioxidant power was 5.90 ± 0.07 mg vitamin C equivalent/g, reflecting the capacity of the extract to donate electrons and reduce oxidants. The extract demonstrated a strong free-radical scavenging response in the DPPH assay, producing 83.40 ± 1.17% inhibition with an IC₅₀ of 0.02 mg/g, indicative of high radical-quenching potency.

The extract also inhibited lipid peroxidation, achieving 45.07 ± 8.12% inhibition with an IC₅₀ of 0.04 mg/g, suggesting effective suppression of oxidative degradation of lipids. The vitamin C content of the seed powder was 1.22 ± 0.02 mg/g, confirming the presence of endogenous ascorbic acid that may contribute to the overall antioxidant activity.

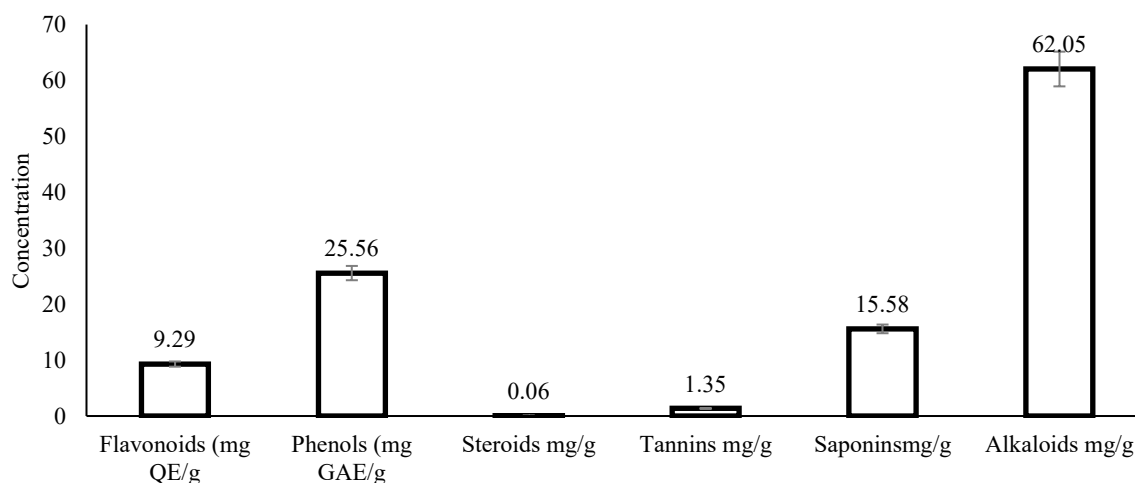


Figure 1. Phytochemical composition of *Aframomum melegueta* seed powder

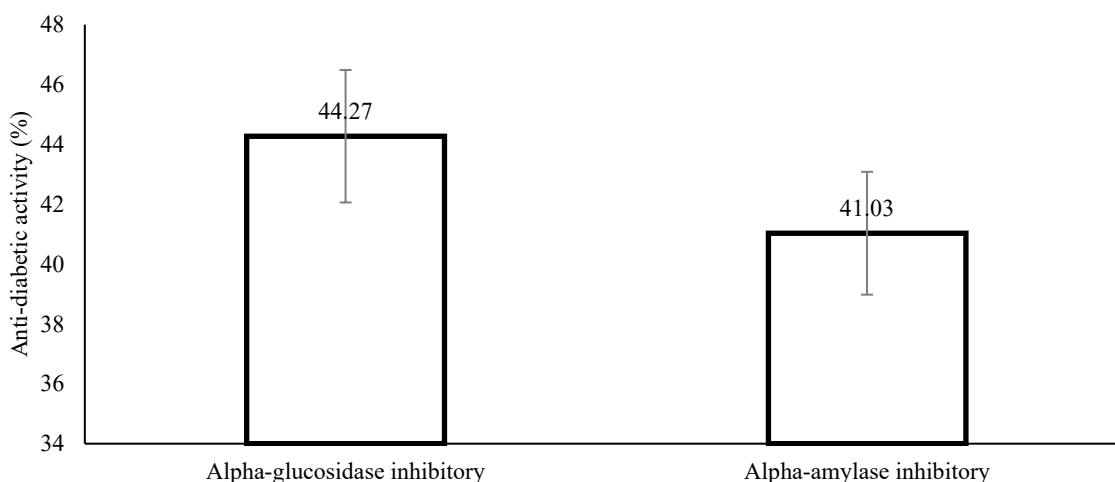


Figure 2. Anti-diabetic activities of Aframomum melegueta seed powder

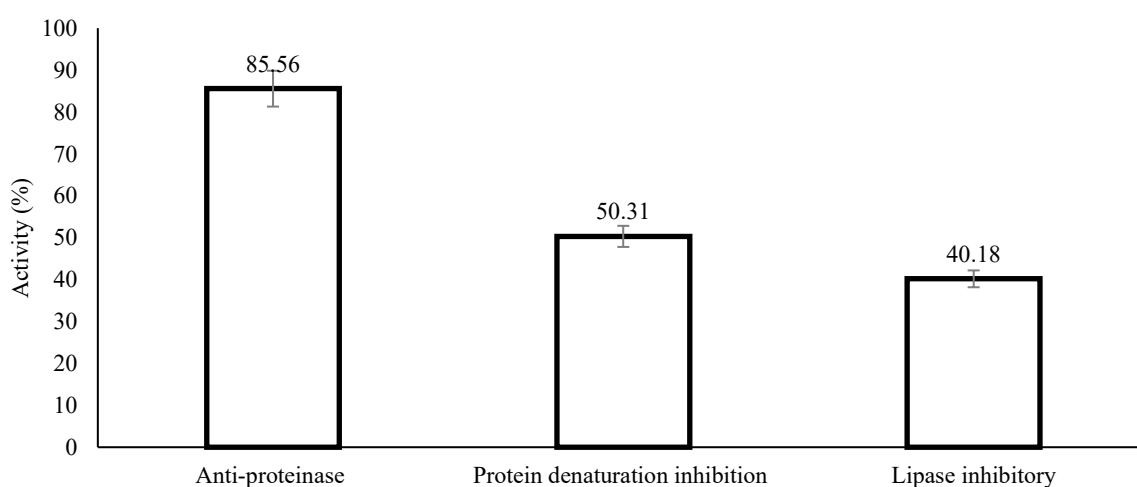


Figure 3. Anti-inflammatory and Anti-obesity Activities of Aframomom melegueta seed powder

Table 1. Antioxidant properties and vitamin C content of *Aframomum melegueta* seed powder

Assay	Inhibition or Quantity	IC ₅₀ (mg/g)
Ferric Reducing Antioxidant Power (mg (Vit. C)/mg)	5.90 ± 0.07	Not applicable
2,2-diphenyl-1-picrylhydrazyl hydrate (%)	83.40 ± 1.17	0.02
Lipid Peroxidation Inhibition (%)	45.07 ± 8.12	0.04
Vitamin C (mg/g)	1.22 ± 0.02	Not applicable

Anti-diabetic, Anti-inflammatory and Anti-obesity of Aframomum melegueta seed powder

Figure 2 presents the anti-diabetic potential of *Aframomum melegueta* seed powder. The extract exhibited moderate inhibitory effects on key carbohydrate-digesting enzymes, with 44.27 ± 0.47% inhibition with an IC₅₀ of 0.04 mg/g of α-glucosidase and 41.03 ± 0.58% inhibition with an IC₅₀ of 0.04 mg/g of α-amylase.

Figure 3 illustrates the anti-inflammatory and anti-obesity potential of *Aframomum melegueta* seed powder. The seed powder demonstrated pronounced anti-inflammatory activity, as evidenced by a high antiproteinase inhibition of 85.56 ± 0.31% with an IC₅₀ of 0.02 mg/g and a notable inhibition of protein denaturation at 50.31 ± 0.69% with an IC₅₀ of 0.05 mg/g. Furthermore, the seed extract exhibited appreciable anti-obesity activity, reflected in its 40.18 ± 2.33% inhibition with an IC₅₀ of 0.04 mg/g of pancreatic lipase activity.

Discussion

The present study elucidates the bioactivity profile of *Aframomum melegueta* seed powder, establishing its potential as a functional food ingredient and natural therapeutic agent. The quantitative phytochemical composition revealed a rich profile of bioactive constituents, among which alkaloids were the most abundant, followed by phenolics, saponins, flavonoids, tannins, and trace levels of steroids. These results corroborate earlier findings by Onoja et al. (2014) and Ogwu et al. (2024), who reported high alkaloid and polyphenol contents in *A. melegueta*, highlighting the species' consistent phytochemical richness across diverse geographical ecotypes.

Alkaloids, being the predominant phytoconstituents, are known for their diverse pharmacological functions, including analgesic, antimicrobial, and metabolic

modulatory effects (Dey et al., 2020). Their high concentration in the current study suggests a strong contribution to the observed bioactivities, particularly the enzyme inhibitory and anti-inflammatory responses. Phenolic compounds, detected at appreciable levels, are well-established antioxidants that modulate oxidative stress and inflammation by scavenging free radicals and modulating inflammatory pathways (Dey et al., 2020). The quantified flavonoid content also affirms the antioxidant, anti-diabetic, and anti-obesity potential, as flavonoids such as quercetin and catechins are recognised inhibitors of α -glucosidase and pancreatic lipase (Lam et al., 2024).

The present study provides a bioactivity-guided evaluation of *Aframomum melegueta* seed powder, demonstrating that the plant possesses significant antioxidant, anti-inflammatory, anti-diabetic, and anti-obesity potentials. The findings build on traditional knowledge of the spice and highlight its relevance as a functional botanical with promising therapeutic implications (Niu et al., 2022; Sokamte et al., 2018).

The antioxidant assays provide the foundation for interpreting the plant's wider bioactivities. The strong DPPH radical scavenging activity ($83.40 \pm 1.17\%$; $IC_{50} = 0.02$ mg/g) suggests the presence of highly reactive electron- and hydrogen-donating phytochemicals capable of neutralizing free radicals (Ponnampalam et al., 2022). Such activity is typically associated with phenolic acids, flavonoids, and volatile constituents reported in *A. melegueta*. These compounds act as primary antioxidants by terminating free-radical chain reactions, thereby preventing cellular oxidative damage (Onoja et al., 2014). The moderate ferric reducing antioxidant power (5.90 ± 0.07 mg vitamin C eq/g) further supports the ability of the extract to participate in electron transfer processes essential for redox balance. Together, these results confirm that *Aframomum melegueta* has a robust antioxidant profile that can modulate oxidative stress pathways (Onoja et al., 2014).

The observed inhibition of lipid peroxidation ($45.07 \pm 8.12\%$; $IC_{50} = 0.04$ mg/g) provides additional evidence that the seed powder effectively protects membrane lipids from oxidative degradation. Lipid peroxidation is a central mechanism linking oxidative stress to inflammation, mitochondrial dysfunction, impaired insulin signaling, and adipocyte dysregulation (Masenga et al., 2023). Therefore, the capacity of *Aframomum melegueta* to suppress this process supports its potential to modulate the biochemical pathways associated with chronic inflammatory and metabolic diseases. The measurable vitamin C content of the seed powder (1.22 ± 0.02 mg/g) was relatively low compared with values typically reported for vitamin C-rich guava leaves (142.55 mg/100g) (Kumar et al., 2021). However, despite its low concentration, the presence of vitamin C may still contribute functionally by supporting radical scavenging and regenerating oxidized phenolic antioxidants, thereby enhancing overall antioxidant synergy within the extract (Alberts et al., 2025).

In this study, the anti-diabetic potential of the seed powder was evidenced by moderate inhibition of α -glucosidase and α -amylase, which are critical enzymes involved in carbohydrate digestion (Alqahtani et al., 2019). Inhibiting these enzymes slows glucose absorption and reduces postprandial blood glucose spikes, a mechanism comparable to the action of synthetic drugs like acarbose

(Gong et al., 2020). Mechanistically, phytochemicals, particularly phenolics, flavonoids, and alkaloids may interact with the active sites of these enzymes through hydrogen bonding, hydrophobic interactions, or π - π stacking, thereby reducing substrate accessibility and catalytic turnover (Ding et al., 2023). Polyphenols are also known to alter the conformational structure of amylolytic enzymes, leading to partial denaturation or allosteric modification that decreases enzymatic efficiency (Martinez-Gonzalez et al., 2017). Collectively, these mechanisms suggest that *Aframomum melegueta* may modulate glucose metabolism through both direct enzyme inhibition and ancillary redox-mediated pathways, enhancing its relevance as a natural anti-diabetic agent. These results align with the earlier findings of researchers, who demonstrated significant α -amylase and α -glucosidase inhibition by phenolic-rich spices (Kashtoh and Baek, 2015; Tiji et al., 2021). In animal production, such enzyme inhibitory activity could be exploited to modulate glycemic control and improve feed efficiency, especially in animals under high-energy diets or metabolic stress conditions (Wang et al., 2024).

The anti-inflammatory efficacy of *Aframomum melegueta* seed powder was remarkable, with antiproteinase inhibition and protein denaturation inhibition. Protein denaturation is a hallmark of inflammatory processes, and compounds that inhibit this pathway are considered potent anti-inflammatory agents (Hasan et al., 2023). The bioactive constituents of *A. melegueta*, including phenolic compounds, may contribute to these effects through several mechanisms. First, these phytochemicals have been reported to interfere with key inflammatory enzymes such as cyclooxygenase (COX), lipoxygenase (LOX), and phospholipase A₂, thereby reducing the synthesis of prostaglandins and leukotrienes (Metkin et al., 2025). Second, the observed antiproteinase activity suggests a possible inhibitory effect on neutrophil-derived proteases (e.g., elastase), which helps limit tissue damage during inflammation (Assiry et al., 2022). Additionally, inhibition of protein denaturation indicates potential membrane-stabilizing properties, which may prevent lysosomal enzyme leakage and attenuate downstream activation of NF- κ B-regulated cytokines (Hasan et al., 2023). The known antioxidant properties of *A. melegueta* may further complement these pathways by limiting ROS-driven inflammatory signaling.

These values are comparable to those obtained for *Ficus racemosa*, a phytochemical anti-inflammatory agent (Mohan et al., 2021). The rich alkaloid and phenolic content likely underpins these effects through the suppression of pro-inflammatory mediators such as NF- κ B and TNF- α (Rahman et al., 2021). In animal production systems, especially under tropical climates where subclinical inflammation is common due to environmental stressors and feed toxins, the inclusion of natural anti-inflammatory agents in feed could enhance gut health, improve nutrient absorption, and reduce antibiotic reliance (Obianwuna et al., 2024).

The *Aframomum melegueta* seed powder also exhibited notable anti-obesity potential, with a 40.18% inhibition of pancreatic lipase a key enzyme in dietary fat digestion. This level of inhibition of *Aframomum melegueta* is consistent with earlier reports on lipase-inhibiting activity

of phenolic-rich spices (Fernando et al., 2019). This activity is nutritionally relevant, as reducing fat absorption can help manage obesity and related disorders (Subramanian and Hanim, 2025). Moreover, in livestock nutrition, especially in monogastric species like poultry and swine, strategic modulation of fat digestion using natural inhibitors could regulate lipid metabolism, reduce fat deposition, and improve carcass quality (Obianwuna et al., 2024; Subramanian and Hanim, 2025).

While *A. melegueta* exhibits multiple beneficial bioactivities, potential toxicity and safety considerations should be acknowledged. Alkaloids, phenolics, and other bioactive constituents may exert dose-dependent effects, with excessive intake potentially leading to gastrointestinal disturbances or metabolic imbalances (Kmail, 2025). Previous studies on *A. melegueta* and related Zingiberaceae species indicate that moderate consumption is generally safe, with no significant cytotoxic or organ-specific adverse effects reported at physiologically relevant doses (Alolga et al., 2022; Nwarienne et al., 2023). Nevertheless, bioactivity-guided applications in humans or animals warrant careful dosage optimization and monitoring to balance efficacy with safety. Future investigations including acute and sub-chronic toxicity assessments are recommended to fully establish the safety profile of this nutraceutical candidate.

Collectively, the integration of these phytochemicals and bioactivities highlights *Aframomum melegueta* as a multi-functional nutraceutical candidate. The *Aframomum melegueta* seed's antioxidant, anti-inflammatory, anti-diabetic and anti-obesity properties confer potential for dietary inclusion in human and animal health formulations aimed at disease prevention, metabolic regulation, and performance enhancement (Almoraie and Shatwan, 2024; Mohammed et al., 2017; Mayele et al., 2025).

Conclusion

Aframomum melegueta seed powder exhibits notable antioxidant, anti-inflammatory, anti-diabetic, and anti-obesity activities, supporting its potential as a functional food ingredient or nutraceutical. Further in vivo studies are needed to confirm its efficacy and safety.

Declarations

The authors declare that there are no competing interests.

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