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EVALUATION OF THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF THE DRIED SEED AND FRUIT COAT OF *DIALIUM GUINEENSE* WILD (FABACEAE)

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ABSTRACT

The study evaluated the phytochemical constituents, proximate composition, antimicrobial, and antioxidant activities of the dried pulverised seed and fruit coat of *Dialium guineense* wild (fabaceae). It addresses the need for alternative antimicrobial agents, cheaper phytogenic feed additives, and antioxidants due to rising health concerns. The study's significance lies in exploring underutilized parts of *Dialium guineense* and contributing to potential applications in medicine and animal nutrition. The ripe fruits were harvested, identified, and authenticated before undergoing extraction, partitioning into polar and non-polar fractions, and subsequent phytochemical screening. Proximate analysis, including moisture, lipid, ash, carbohydrate, crude fiber, and protein content, was determined using AOAC guidelines. Agar diffusion method was utilized to measure the antimicrobial activities of the fractions against selected microorganisms *(E. coli, Klebsiella spp, S. aureus, Aspergilus niger, Penicillium spp, Fusarium spp, Candida spp.).* Antioxidant capacities were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. GC-MS analysis was used for compound identification, and statistical analyses were performed using Graph Pad Prism version 8.0 software. The results of the Phytochemical screening showed that the fruit coat and seed had relevant Phytochemicals present in them. The GCMS analysis results of the non-polar fractions of the seed and fruit coat was found to contain 10 and 13 bioactive compounds respectively. The seed exhibited significant ($p < 0.001$) antioxidant activity compared to the fruit coat with an IC_{50} of 11.49 µg/ml. The antimicrobial susceptibility test showed that the seed and fruit coat had activity against *S. aureus, Klebsiella spp* and *Pseudomonas spp*. The MIC values of the total crude extract of the seed against *S. aureus, Pseudomonas app*, and *Klebsiella spp* was 25%, 50%, and 12.5% respectively. Therefore, the seeds have better Antioxidant and antimicrobial activity compared to the fruit coat.

KEYWORDS: *Dialium guineense* wild, antimicrobial activity, antioxidant activity.

1. INTRODUCTION

According to WHO 2023 statistics, chronic diseases account for 41 million deaths annually, or 74% of all deaths worldwide.[1] Oxidative stress is the common denominator among these diseases, and it describes an imbalance between the body's production of oxidants and antioxidant defenses, which can lead to biological system damage.

Furthermore, a growing number of farmers involved in poultry, especially in developing countries, are worried about the exorbitant cost of purchasing conventional feeds and in-feed additives mostly antibiotics. Many countries have banned the sale of poultry raised with antibiotics because of health concerns. Nonetheless, bacterial resistance to several antibiotics has been connected to the ongoing use of in-feed antibiotics.[2] Due to widespread public health concerns about antibiotic resistance and the likely negative effects of increased residue in animal products, the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) banned the use of antibiotics from livestock feed as growth promoters in a number of European countries.[3] There has been a lot of research interest in the search for alternative phytogenic-based feed additives to replace conventional antibiotic growth promoters due to the demand to stop using them in livestock diets.

Since oxidative stress is strongly linked to several diseases and newly emerging health issues, there is a growing need for antioxidants to help reduce the causes of oxidative stress. According to a few reports, the upsurge in the cost of conventional antibiotic growth

promoters and their negative health effects necessitates the discovery of alternative phytogenic feed additives that are high in nutrients and relevant phytochemicals.[2]

Plants are increasingly being recognized for their role in health care on a global scale and there is a growing focus on the utilization of plant-based natural products in pharmaceutical care to treat a range of illnesses.[4] Natural products of plant origin are now seen as essential sources of medicinal agents, and traditional medicine has made use of their products. Although there are medicinal plants all over the world, most of them are thought to be found in tropical regions.

One of such is "Black Velvet Tamarind", or *Dialium guineense* wild- a tropical tree; indigenous to West and Central Africa. Approximately 20 metres high and 0.8 metres in diameter, it is a fruit tree of the Fabaceae family that grows in dense savannahs, dark canyons, thick forests, and gallery forests. The tree is low-branching, has a compact, but densely leafy crown and is usually branched. They bear a profusion of round, flattened fruits, sometimes to the point where a brittle shell encloses the seed, which is enclosed in a dry, brownish, very sweet edible pulp which is acidic.[5]

Dialium guineense wild has numerous biological activities, of which two are its antimicrobial and antioxidant qualities. Antioxidants are substances that lessen the dangerous effects of reactive oxygen species (ROS) and free radicals in the body. This lowers the risk of disease and minimizes cellular damage.

The bulk of the world's health issues are brought on by metabolic and chronic illnesses, such as atherosclerosis, diabetes, cancer, chronic obstructive pulmonary disease, cardiovascular disease, and stroke.[6] Free radicals are the main cause of these illnesses.

Dialium guineense wild is an abundant reservoir of various antioxidants, which includes triterpenoids, carotenoids, flavonoids, and phenolic acids, among other phytochemicals. These substances have extraordinary scavenging properties that allow them to capture free radicals and halt the cascades that result in oxidative damage. In addition to being essential for its survival against environmental stressors, the complex antioxidant defense system may also have health benefits for people. Apart from its antioxidant properties, *Dialium guineense* is also well known for its antimicrobial properties.

Antimicrobials are an important class of agents utilized in modern medicine, agriculture, and many other industries. They are vital in the fight against microbial infections. These additives or materials are made to stop the proliferation of microorganisms like fungi, viruses, bacteria, and parasites, or to eradicate them entirely. Antimicrobials also play a significant role in agriculture, protecting crops and maintaining animal health.

Dialium guineense has a broad range of phytochemicals, which highlights its potential as a functional food and medicinal resource. To fully comprehend the phytochemical profile and its individual or combined effects on human and animal health, more scientific research and studies are necessary. The potential health-enhancing effects of these compounds have been highlighted by ongoing research. The fruit possess antioxidant, antimicrobial, and anti-inflammatory qualities. Research findings indicate that these phytochemicals may be have contributed to these activities.[7]

These various compounds found in *Dialium guineense* wild are consistent with the fruit's historical applications in West African indigenous medicine. For a variety of medical uses, local communities have used the plant's fruit, bark, and leaves, among other parts.

Even though it is high in phytochemicals, it has also been shown to have nutritional value. Its dietary significance in regional cuisines and customs is attributed to the essential nutrients it contains, including minerals and vitamins like vitamin C, B_1, B_2, and B_3.

The investigation of the phytochemical components of *Dialium guineense* shows promise for future studies into its possible uses in nutraceuticals, functional food development, and medicine. Comprehensive analyses are necessary to determine the precise compounds present, their concentrations, bioavailability, and potential synergistic effects. Only then can the full range of health benefits linked to this tropical fruit be unlocked. Gas chromatography-mass spectroscopy (GC-MS) is one of the analytical assay techniques used to effectively carry out identification and characterization of the various phytochemicals present in *Dialium guineense* wild. Prior studies have been conducted on the pulp, bark, leaves, flowers, and stem sections of *Dialium guineense* wild. However, no research has been done on the seed, and scanty work on the fruit coat to ascertain its pharmacological activity.

2. METHODS AND MATERIALS

Collection of Plant Materials

2.1 COLLECTION OF SAMPLES

The ripe fruits of *Dialium guineense* wild were harvested from Owerri west local government area (LGA) of Imo State. Prior to inclusion in the study, the sample was identified and authenticated with Voucher Specimen Number UPHF0600 by a Taxonomist, Dr. Suleiman Mikailu, of the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences, University of Port-Harcourt.

2.2 MATERIALS/REAGENTS

2.2.1 MATERIALS

Gas-Chromatography Mass Spectrophotometer model QP-2010 (Shimadza), UV Spectrophotometer, rotary evaporator, retort stand, separating funnel, Erlenmeyer

flask, desiccator, hot air oven, water bath, Whatman filter paper no. 1, sterile pipette, analytical weighing balance , sterile cork borer, test tubes, volumetric flasks, glass funnel (Pyrex), beakers, porcelain crucibles, petri dishes, sterile Pasteur's pipette, incubator, meter rule, universal bottles, stop watch, masking tapes, cotton wool, aluminium foil, maceration jars.

2.2.2 REAGENTS

Dichloromethane, methanol, n-hexane, distilled water, dimethyl sulfoxide, 2,2'-dipheny-1-picrylhydrazyl, sulphuric acid.

2.3 PREPARATION

The collected fruits were carefully screened to ensure total removal of bruised and spoilt fruits. The screened fruits were subjected to air drying; the pulps were peeled off the seeds and fruit coats. The pulps were properly discarded afterwards and the fruit coats including seeds were adequately air dried to ensure complete absence of moisture. Upon achieving the desired dryness, the seeds and fruit coats were pulverized into coarse and fine powder forms respectively using a grinder. The powdered samples were then stored carefully in airtight glass containers for subsequent utilization, ensuring protection against external contaminants.

2.4 EXTRACTION

After pulverization, 400 g of powdered seeds, and 500 g of the finely powdered fruit coat were accurately weighed and subsequently transferred into an amber-colored macerating glass jar. The maceration process commenced by immersing the powdered fruit coats, and seeds in 1 litre and 820 ml of dichloromethane-methanol respectively, maintaining a precise ratio of 1:1. This maceration was done at room temperature within the maceration jar, utilizing intermittent agitation for a duration of 72 hours. Following the maceration process, the resulting dichloromethane-methanol extract of seeds and fruit coats were filtered utilizing Whatman NO. 1 filter paper. Both filtrates obtained were concentrated using a rotary evaporator. The weight of the extracts was recorded including the percentage yield. The Total extracts were afterwards, transferred carefully into appropriately labelled glass containers and stored within an activated desiccator until further analysis necessitated its use.

2.5 PARTITIONING

The total crude extract obtained previously was used for the partitioning process. 25 grams of the total fruit coat extract was dissolved in 100 ml of 90%v/v aqueous methanol and afterwards partitioned using 200 ml n-hexane in a separating funnel. Upon vigorous shaking, thorough mixing and partitioning of the components, the formation of two distinct layers: an aqueous methanol layer and a nonpolar n-hexane layer was obtained from the settled mixture. The n-hexane layer was successfully separated from the aqueous methanol layer and concentrated on a rotary evaporator. These partitioning steps were repeated by adding an equal volume of n-Hexane to the aqueous methanol obtained from the first partitioning step was in a separating funnel. Again, vigorous shaking and subsequent settling led to the separation into two layers. This partitioning effectively separated constituents into fractions based on varying polarities. Each collected layer was concentrated using a rotary evaporator to obtain the desired compounds. The same procedure was repeated for the total seed extract by dissolving 7 g of extract in 250 ml of 90%v/v aqueous methanol and partitioning with 120ml n-hexane to obtain the non-polar fraction.

2.6 PHYTOCHEMICAL SCREENING

The phytochemical analysis of the extracts was carried out according to Trease and Evans.[8] to identify any phyto-constituents that may have been present, including glycosides, terpenoids, alkaloids, tannins, and flavonoids.

2.7. PROXIMATE ANALYSIS

2.7.1 Moisture Content (AOAC 925.10 Method - Air Oven)

1g of the sample was weighed into a dried porcelain evaporating dish. The dish, containing the sample, was then placed in an oven set precisely at 105°C for a duration of 6 hours. The evaporating dish, now holding the dried sample, was allowed to cool in a desiccator at room temperature. Subsequently, the dish, along with the dried sample, was re-weighed, and this new weight was recorded accurately. The moisture content was determined using the formula:

% Moisture = [(Weight of fresh sample - Weight of dried sample) / Weight of sample used] × 100.

2.7.2 Lipid Content (AOAC 963.15 Method - Soxhlet Extraction)

2g of the powdered sample were carefully placed into a filter paper, which was then inserted into a Soxhlet extractor. This extractor was positioned within a pre-weighed, dried distillation flask. Acetone was introduced into the distillation flask through the condenser attached to the Soxhlet extractor. A cooled water jet was directed into the condenser, allowing the heated solvent to reflux continuously. This refluxing action resulted in the extraction of lipids from the sample, accumulating in the solvent chamber. Upon complete extraction of lipids from the sample, the condenser and extractor were disconnected, and the solvent was evaporated to concentrate the lipid extract. The concentrated lipid extract in the flask was then dried in an air oven until a constant weight was achieved. Subsequently, the flask containing the dried lipid extract was re-weighed to accurately measure the weight of the lipid.

The calculation of % Lipid was performed using the formula

% Lipid = [(Weight of flask and extract - Weight of empty flask) / Weight of sample extracted] × 100.

2.7.3 Ash Content (AOAC 942.05 Method - Furnace)
1g of the dried sample was weighed into a preheated and pre-weighed porcelain crucible and was carefully placed into a muffle furnace, where it was exposed to a regulated temperature of 630°C for a period of 3 hours. Subsequently, the crucible was allowed to cool down to room temperature before being re-weighed.

The calculation for % Ash was performed using the formula
% ASH = [(Weight of crucible + Ash sample - Weight of crucible and sample after ash) / Weight of sample] × 100.

2.7.4 Carbohydrate Content (AOAC 920.39 Method - Clegg Anthrone)
0.1 g of the sample was precisely weighed and placed into a 25 ml volumetric flask. To this, 1 ml of distilled water and 1.3 ml of 62% perchloric acid were added. The mixture was vigorously shaken for 20 minutes to ensure complete homogenization. The volumetric flask was filled up to the 25 ml mark with distilled water and securely stoppered. The resultant solution was then allowed to settle for decanting purposes. From the filtrate, 1 ml was transferred into a 10 ml test tube and diluted to volume with distilled water. Subsequently, 1 ml of the working solution was pipetted into a clean test tube, to which 5 ml of Anthrone reagent was added. A mixture of 1 ml distilled water and 5 ml Anthrone reagent was also prepared. Both mixtures were read at a wavelength of 630 nm using the prepared blank (1ml distilled water and 5ml Anthrone reagent) for calibration purposes. Additionally, a solution of glucose (0.1ml) was treated as the sample with Anthrone reagent, and its absorbance was measured.

The % Carbohydrate as glucose was calculated using the formula
% CHO as glucose = (25 x absorbance of sample) / Absorbance of standard glucose.

2.7.5 Crude Fibre Determination (AOAC 962.09 Method)
2g of the sample were initially extracted with petroleum ether. Subsequently, the sample was boiled under reflux for 30 minutes in the presence of 200 ml of dilute hydrochloric acid. After this step, the mixture underwent filtration. The resulting residue was thoroughly washed with water until it reached an acid-free state. This residue was then transferred into a beaker and boiled for an additional 30 minutes, this time with 200 ml of dilute sodium hydroxide solution. Following this, the mixture was filtered once more, and the residue was collected into an ignited crucible. The residue collected in the crucible underwent a washing process: three washes with 20 ml ethanol and two washes with 10 ml ether. After the washing steps, the residue was dried in an oven until constant weight was achieved. This dried residue was then cooled and weighed. Subsequently, the dried residue was placed in a furnace and subjected to ignition. After the ignition process, the residue was cooled once more and weighed.

The calculation for Crude Fiber content involves the following formula
% Crude Fiber = [(Weight of dried residue - Weight of ignited crucible with residue) \Weight of sample used] × 100

2.7.6 Protein Content (AOAC 984.13 Method - Kjeldahl)
0.1 g of the sample was precisely weighed into a clean 250 ml conical flask. To this, 3 grams of digestion catalyst and 20 ml of concentrated sulphuric acid were added. The mixture was heated to initiate digestion, resulting in a colour change from black to sky-blue. After cooling the digest to room temperature, it was diluted to a final volume of 100 ml using distilled water. From this diluted digest, 20 ml was decanted into a distillation flask. The distillation flask was secured on an electrothermal heater or hot plate and attached to a Liebig condenser, leading to a receiver containing 10 ml of 2% boric acid indicator. Through a syringe attached to the mono-arm steelhead, 40 ml of Sodium hydroxide was incrementally introduced into the digest until it became strongly alkaline. The mixture was then heated to boiling, and the resultant distilled ammonia gas passed through the condenser into the boric acid indicator, causing the colour to change from purple to greenish. The distillate in the receiver was titrated back to a purple colour from the greenish hue using standard 0.1N Hydrochloric acid solution. The volume of hydrochloric acid required for this colour change was noted as the titre value.

The calculation for % Organic Nitrogen (used to estimate protein content) is:
% Organic Nitrogen = Titre value × Factor × 100 × 100) / (V × W)
Where:
- Titre value is the volume of standard acid (e.g., 0.1N Hydrochloric acid) used in titration.
- Factor is a conversion factor, often 1.4, representing the conversion of nitrogen to protein.
- V is the volume of the sample used in the digestion step (in liters).
- W is the weight of the sample used in grams.

This formula allows the determination of the organic nitrogen content, which is then used to estimate the protein content in the sample.

2.8. ANTIMICROBIAL SUSCEPTIBILITY TESTING: AGAR WELL DIFFUSION METHOD
2.8.1 BACTERIA
The bacterial strains used were *Escherichia coli., Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumonia.* Sterile petri dishes were labelled in duplicate for each bacterial strain. Mueller-Hinton agar pour was uniformly inoculated with 0.1ml of

standardized bacterial cultures and thoroughly mixed. After pouring the inoculated agar into the labelled petri dishes, it was allowed to cool and solidify on the workbench. Then, a sterile cork borer was used to aseptically create wells in the solidified agar. Preparation of 100%w/v solutions of total crude extract of the seeds and fruit coats was carried out. A few drops of each extract solutions were added into the corresponding wells on the agar plates before being incubated at 37°C for 24 hours under optimal conditions. After incubation, observation for zones of inhibition around each well was conducted, with Gentamicin serving as the positive control and Dimethyl sulfoxide (DMSO) as the negative control. Accurate measurements of the diameter of these zones were taken using a caliper.

2.8.2 FUNGI

The chosen fungal strains were *Candida albicans*, *Fusarium oxysporum*, *Aspergillus niger*, and *Penicillium chrysogenum.*

Sterile petri dishes were labelled in duplicate for each fungal strain. Potato Dextrose agar pour was uniformly inoculated with 0.1ml of standardized fungal cultures and thoroughly mixed. After pouring the inoculated agar into the labelled petri dishes, it was allowed to cool and solidify on the workbench. Then, wells were aseptically created in the solidified agar using a sterile cork borer. A 100%w/v solution of the total extracts for both seeds and fruit coats was prepared. The respective extract solutions were added into the wells on the agar plates. The plates were incubated at 25°C for 72 hours under optimum conditions. Following incubation, observation for zones of inhibition around each well was carried out, using Fluconazole as the positive control and Dimethyl sulfoxide (DMSO) as the negative control. Accurate measurements of the diameter of these zones were taken with a caliper.

2.8.3 MINIMUM INHIBITORY CONCENTRATION (MIC) DETERMINATION

Sterile petri dishes were labelled in duplicates for each bacterial strain. Mueller-Hinton agar pours were inoculated with 0.1ml of standardized bacterial cultures, ensuring thorough mixing. The inoculated agar was carefully poured into the labelled petri dishes and left to solidify on the workbench. After solidification of the agar, five (5) discs were carefully extracted from the agar layer using a sterile cork borer. This process created five (5) wells in each agar plate. These wells were labelled to accommodate five (5) concentrations of each extract identified with activity during the preliminary study. Concentrations included 50%, 25%, 12.5%, 6.25%, and 3.125%. With a separate sterile Pasteur's pipette, 0.1ml of each extract concentration was meticulously added tio the corresponding wells. The plates were left on the workbench for 15 minutes to ensure proper diffusion of the extracts. Subsequently, all plates were incubated at 37°C for 24 hours. Post-incubation, the diameter of resulting zones of inhibition was measured in millimeters (mm) using a caliper, directly through the base of the plates.

2.9. ANTIOXIDANT ASSAY

A weighing balance was used to weigh 50 mg of the polar and non-polar extracts of the fruit coat and seeds, which were then divided into separate 50 ml beakers. The extracts were dissolved in 5 ml of methanol, reaching the 50 ml threshold in the measuring cylinders in the process. The glass rod stirrer was used to stir until a clear, homogenous solution was achieved. After which, the mixture was transferred to a 10 ml volumetric flask and further diluted to produce six different concentrations, which ranged from 500 µg/ml to 15.625 µ/ml. The next step involved the dissolution of 2.4 mg of DPPH in 100 ml of methanol to create a solution of the DPPH radical, which was allowed to dissolve completely until a homogenous solution was obtained. Subsequent procedures entailed supplementing each of the diluted concentration samples with 2.5 ml of the methanolic DPPH solution. The mixtures were gently incubated at room temperature for 30 minutes in the dark after a thorough shaking. The absorbance values of the reaction mixtures were then obtained using spectrophotometric measurement at a wavelength of 517 nanometers. After that, the DPPH scavenging capacity was computed. A plot was created, with the logarithm of each individual concentration (x-axis) and the average percentage inhibition (y-axis) correlated. Based on the graph, the IC_{50} value was calculated to determine the level of antioxidant activity that the plant sample displayed.

2.10. GAS CHROMATOGRAPHY MASS-SPECTROSCOPY (GC-MS) CHARACTERIZATION

The compounds were identified using GC-MS model QP-2010 (Shimadza), which was fitted with a 30-meter-long Restek column with an internal diameter of 0.25 mm and a thickness of 0.25 µm. The carrier gas used was Helium as the GC was operated in the splitless mode and flow rate was set at 1 ml/min. The 8 µl of the sample was injected into the injection port and the injection temperature was maintained at 250°C. The set temperatures for the columns were 60°C for 1.50 minutes, 260°C for 1.50 minutes, and 300°C for 3.3 minutes at 14^{o}C min-1. At an interface temperature of 250 °C and an ion source temperature of 230°C, samples were automatically injected into the mass spectrometer. Election impact ionisation (EI) was conducted at 70 eV. The identification criteria were the retention time and the abundance of the confirmation ions in relation to the quantification ions. The National Institute of Standards and Technology (NIST) data was compared with the fragmentation pattern.

2.11. STATISTICAL ANALYSIS

Every test was run three times (triplicates). Standard error of mean (SEM) ± mean was used to express the values. The significant differences between all columns and the control were found using the Student's T-test. A

P value of < 0.05 was deemed significant. Graph Pad Prism version 8.0 was used for all statistical analysis.

3. RESULTS

3.1 Proximate Analysis

Table 1: Comparing the proximate compositions of the seeds and fruit coat.

Nutrients	Seed %	Fruit coat %
Carbohydrate	26.38	11.68
Protein	10.43	7.80
Lipid	2.80	4.15
Moisture	21.89	16.14
Fibre	35.79	56.83
Ash	2.71	4.30

Table 1. compares the Proximate compositions of the fruit coat and the seeds. The percentage abundance of carbohydrates in the fruit coat was found to be 11.88%, thus lower than the seeds which had a percentage abundance of 26.38%. The protein composition in the seeds was found to be higher than the fruit coat; 10.43% in seeds and 7.80% in fruit coat. Interestingly, the percentage abundance of lipids in the fruit coat was found to be higher than in the seeds which gave a value of 2.80%. The fruit coat of *Dialium guineense* showed a higher fibre content of 56.83% compared to 35.00% abundance of fibre in the seeds.

Also, as shown in Figure 1, the seeds had a greater abundance value of 21.89% with respect to moisture content whereas the percentage abundance of moisture in fruit coat was 16.14%. The fruit coat was also discovered to have an ash value of 4.30%, which was higher than 2.71% ash value of the seeds.

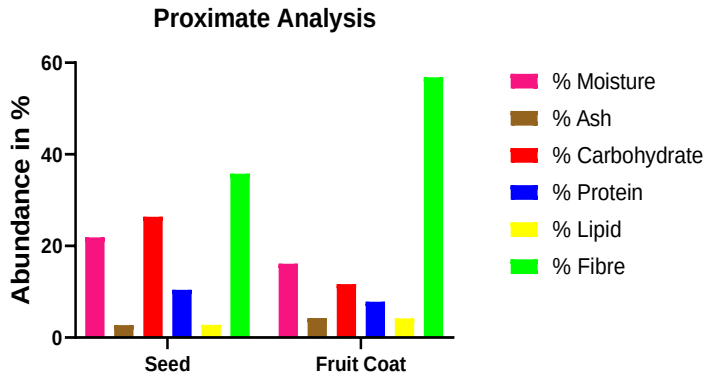

Figure 1: A chart comparing the different proximate compositions of the seeds and fruit coats of *Dialium guineense*.

3.2 Phytochemical screening of the total crude extract of the dried seeds and fruit coats of *Dialium guineense* wild (fabaceae)

Table 2: Screened phytochemicals in seeds and fruit coats *of Dialium guineense*

Phytochemicals	Test	Seed extract	fruit coat extract
Carbohydrates	Molisch test	+	+
	Fehling's test	+	+
Phenolics	Ferric chloride test	+	+
Saponins	Frothing test	+	+
	Emulsion test	+	+
Alkaloids	Wagner's test	+	+
Tannins	Ferric chloride test	+	+
Flavonoid	Shinoda test	+	+
Cardiac glycosides	Keller-Kiliani	+	_
	Kedde test	+	+
Triterpenoid	Salkowski's test	+	+
	Liebermann-Burchard test	+	+
Proteins	Million's test	+	+

	Picric acid test	+	+
Anthraquinones		+	+

Key: (+) - Present; (-) – Absent

The qualitative phytochemical screening carried out showed that both fruit coats and seeds *Dialium guineense* had an array of relevant phytochemicals which not only contribute to plant survival but maybe responsible for its bioactive activities.

The total crude extract of the fruit coat revealed presence of bioactive compounds comprising of phenolics, flavonoids, alkaloids, tannins, anthraquinones, triterpenoids, proteins, carbohydrate, and saponins. Phytochemicals identified in the seed extract includes phenolics saponins, flavonoids, cardiac glycosides, alkaloids, tannins, anthraquinones, triterpenoids and proteins (Table 2). The seed extract showed presence of cardiac glycosides, but the fruit coat extract was devoid of it.

3.3. ANTIMICROBIAL ASSAY

Table 3: Antimicrobial assay of the total crude extracts of the seeds and fruit coats showing the inhibition zone diameters in mm.

Test organisms	Fruit coat Extract	Seed extract	Positive control	Negative control
Escherichia coli.	-	-	-	-
Staphylococcus aureus	6.7± 0.3	10.0 ± 0.0	18.5± 0.3	-
Klebsiella pneumoniae	5.7 ± 0.3	10.6 ± 0.3	13.0 ± 0.6	-
Pseudomonas aeruginosa	4.7 ± 0.3	9. 0 ± 0.0	23.5± 0.3	-
Candida albicans	-	-	-	-
Aspergillus niger	-	-	1.3±0.3	-
Penicillium chrysogenum	-	-	7.3±0.3	-
Fusarium oxysporum	-	-	-	-

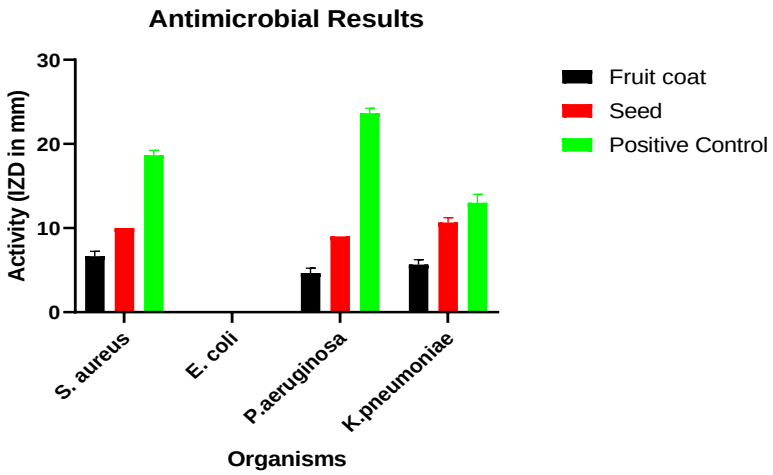

Figure 2: A chart showing the zones of inhibition of the samples and control against the test organisms.

The various fractions showed different levels of antibacterial activity against various bacteria strains and no antifungal action.

Methanol-dichloromethane extract of seeds and fruit coats showed inhibitory activity against staphylococcus aureus with the inhibition zone diameter of 10.0 mm and 6.7 mm respectively with the seed extract having the highest inhibition. The extracts of seeds and fruit coats also demonstrated activity against *Klebsiella pneumoniae* with the corresponding diameter zones of 10.7 mm and 5.7 mm respectively.

Antibacterial activity of the extracts against *Pseudomonas aeruginosa* was also demonstrated. An inhibition zone diameter of 9.0 mm was obtained for the seed extract while the fruit coat extract yielded 4.7 mm for the inhibition zone diameter.

The negative control demonstrated no antimicrobial activity whereas the positive control (Gentamicin) had antibacterial activity against *Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae* with corresponding inhibition zone diameters of 18.7 mm, 13.0 mm, and 23.7 mm.

Furthermore, antimycotic activity was shown by the positive control (fluconazole) with inhibition zone diameters of 7.3 mm and 1.3 mm respectively.

Table 4: Showing inhibitory activity (MIC) of the crude total extract of the seeds of *Dialium guineense* wild at different concentrations.

Test organisms	Seed extract				
	3.125%	6.25%	12.5%	25%	50%
Staphylococcus aureus	-	-	-	4.7 ± 0.3	6.7±0.3
Pseudomonas aeruginosa	-	-	-	-	4.7±0.3
Klebsiella pneumoniae	-	-	4.3±0.3	5.7±0.3	6.5±0.3

Results were expressed as mean ± SEM (n = 3).

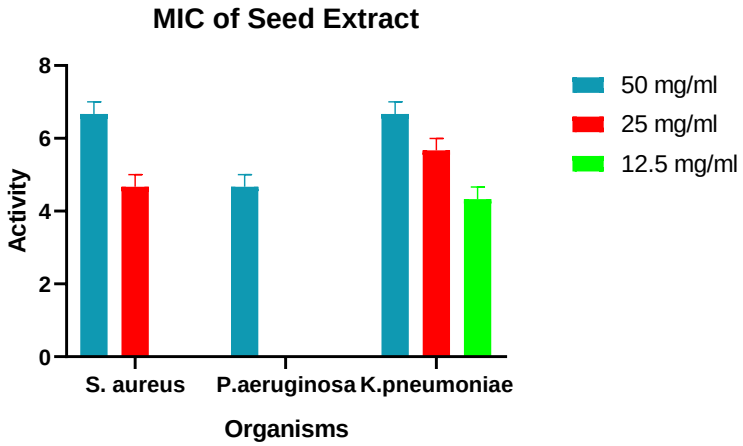

Figure 3: A chart showing the zones of inhibition of the seed extract against the test organisms at different concentrations.

The minimum inhibitory concentration (MIC), of the total crude extract of the seeds against Staphylococcus aureus was 25% and the inhibition zone diameter of 4.7 mm was obtained. Against Pseudomonas aeruginosa, the MIC was found to be 50% for which an inhibition zone diameter of 4.7 mm was shown. The seed extract also demonstrated activity against Klebsiella pneumoniae with IZD of 6.7 mm, 5.7 mm, and 4.3 mm at concentrations of 50 µg/ml, 25 µg/ml, and 12.5 µg/ml respectively. Hence, the MIC was found to be 12.5 µg/ml.

3.4. ANTIOXIDANT ASSAY

Table 5: Showing the antioxidant activity of the Polar and Non-polar fractions of the seeds and fruit coats of *Dialium guineense* wild.

	AVERAGE % INHIBITION OF FRACTIONS			
Conc. mcg/ml	FCME	FCNE	SME	SNE
500	83.625±0.216	78.597±0.008	77.304±0.304	84.378±0.464
250	81.878±0.070	77.131±0.002	76.211±0.190	71.748±0.473
125	79.913±0.177	23.708±0.578	65.379±0.290	47.149±0.237
62.5	74.329±0.921	12.624±0.143	64.949±0.236	26.729±0.618
31.25	24.084±0.921	4.400±0.365	45.520±0.142	14.590±0.141
15.625	7.168±0.942	0.305±0.312	28.438±0.473	9.098±0.413

Results were expressed as mean ± SEM (n = 3).

KEY
FCME = Fruit coat methanolic fraction**, FCNE** = Fruit coat n-hexane fraction**, SME** = Seed methanolic fraction**, SNE** = Seed n-hexane fraction.

Table 6: Showing the IC_{50} values for different fractions in µ/ml.

FCME	FCNE	SME	SNE
39.81	148.2	11.49	133.0

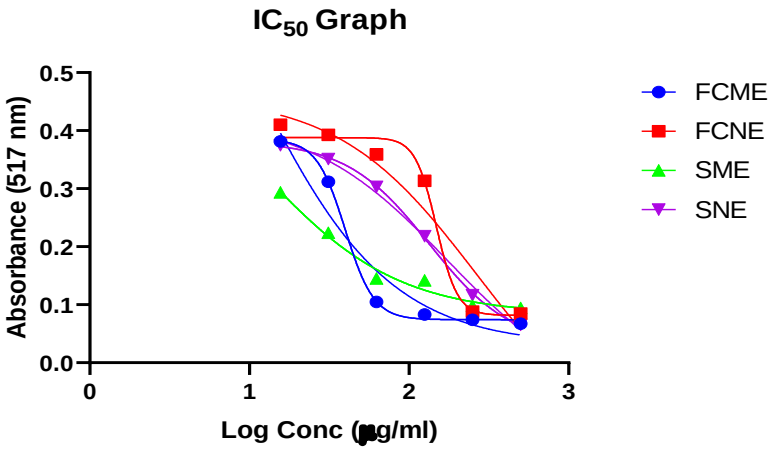

Figure 4: Showing the IC_{50} plot of absorbance against log concentration for polar and nonpolar fractions of the seeds and fruit coat of *Dialium guineense* wild.

Antioxidant assay carried out showed that the scavenging activity of DPPH free radical by the Polar (90% aqueous methanolic) and non-polar (n-hexane) fractions of the seeds and fruit coat was concentration dependent. The average percentage inhibition of various extracts increased with an increase in concentration (Table 5). Interestingly, the polar fraction of seed extract had the highest activity at 15 µg/ml and 31.25 µg/ml. At concentrations of 12.5 µg/ml, 250 µg/ml and 500 µg/ml, polar fraction of the fruit coat exhibited increased Antioxidant activity with inhibitory effects of 79.91%, 81.876% and 83.625% respectively.

Although, the n-hexane fraction of the fruit coat had least inhibitory effects of 0.305%, 4.400%, 12.624% and 23.708% at concentrations of 15.625 µg/ml, 31.25 µg/ml, 62.5 µg/ml, and 125 µg/ml respectively, it demonstrated an increased Antioxidant activity at 250 µg/ml and 500 µg/ml.

A comparison of the percentage radical scavenging activity of the four extracts showed that n-hexane fraction of the seeds demonstrated highest (84.378%) activity (Table 5).

IC_{50} values of 11.49 µg/ml, 39.81 µg/ml, 133.00 µg/ml, and 148.20 µg/ml were obtained for polar fractions of seed, fruit coat, non-polar fractions of the fruit coat and seeds respectively.

3.5. GAS CHROMATOGRAPHY-MASS SPECTROPHOTOMETRY (GCMS) RESULTS

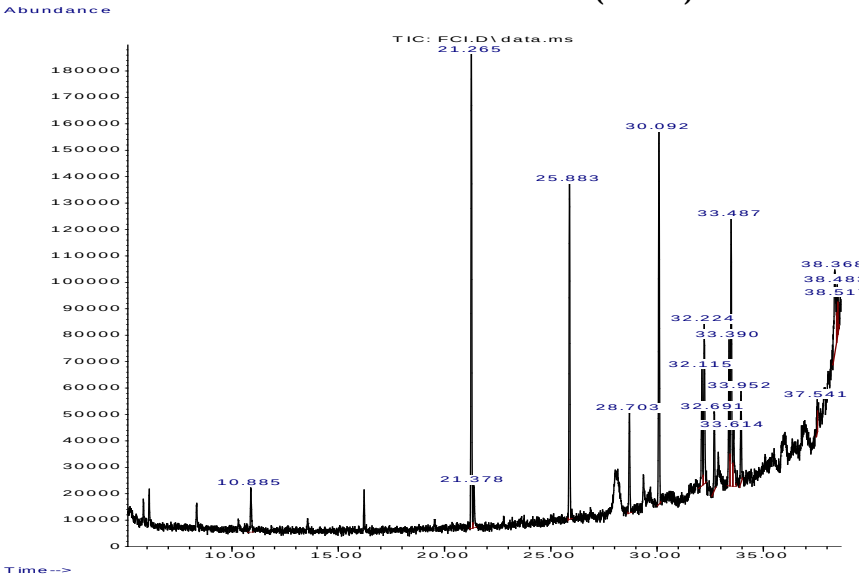

Figure 5: Chromatogram of the analysed n-hexane fraction of the fruit coat.

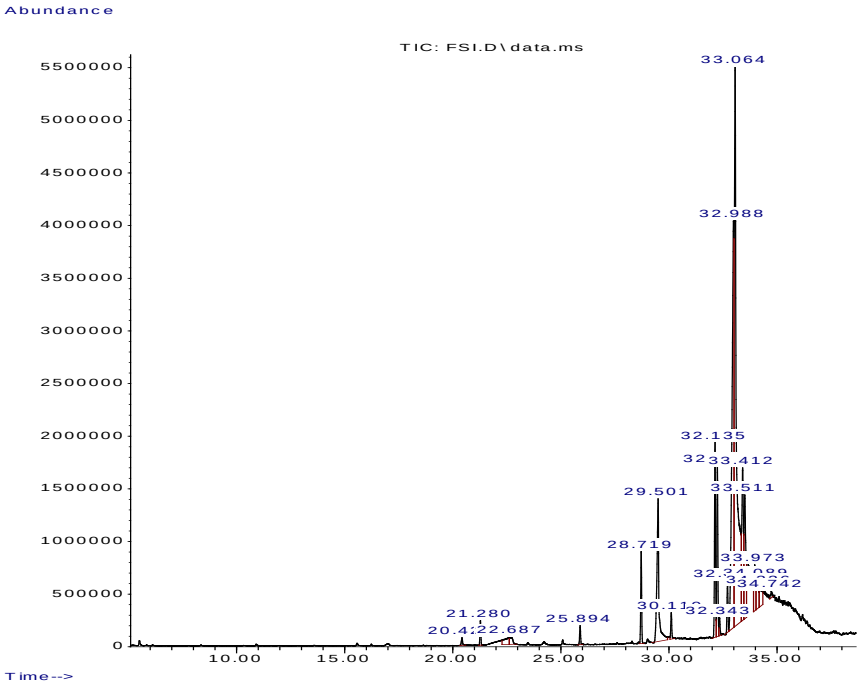

Figure 6: Chromatogram of analysed n-hexane fraction of the seed.

Table 7: GCMS Analysis of the Crude n-hexane extract of the dried fruit coats of *Dialium guineense*.

Peak	Retention Time (min)	Area %	Molecular Formular	Molecular Weight (g/mol)	Compound	Match Quality %
1	21.2655	17.2766	$C_{14}H_{28}O_2$	228.3709	Dodecanoic acid, ethyl ester	95
2	21.3783	2.032	$C_{12}H_{14}O_4$	222.24	Diethyl Phthalate	80
3	25.8826	12.2384	$C_{16}H_{32}O_2$	256.4241	Tetradecanoic acid, ethyl ester	95
4	28.7029	3.9737	$C_{17}H_{34}O_2$	270.4507	Pentadecanoic acid, 14-methyl-, methyl ester	94
5	30.0915	14.5536	$C_{18}H_{36}O_2$	284.4772	Hexadecanoic acid, ethyl ester	95
6	32.1148	4.6177	$C_{19}H_{34}O$	294.4721	9,12-Octadecadienoic acid, methyl ester	99
7	32.6906	3.0019	$C_{16}H_{32}O$	256.4241	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	83
8	33.3898	5.4856	$C_{20}H_{36}O$	308.4986	Linoleic acid ethyl ester	98
9	33.9524	3.7042	$C_{20}H_{40}O$	312.5304	Octadecanoic acid, 17-methyl-, methyl ester	90
10	37.5409	0.8257	$C_{19}H_{40}O$	284.5203	Hexadecyl propyl ether	72

Table 8: GCMS Analysis of the Crude n-hexane extract of the dried seeds of *Dialium guineense*.

Peak	Retention Time (mm)	Area %	Molecular Formular	Molecular Weight (g/mol)	Compound	Match quality %
1	20.4288	0.269	$C_{12}H_{24}O_2$	200.3178	Dodecanoic acid	95
2	21.2801	0.6066	$C_{14}H_{28}O_2$	228.37	Dodecanoic acid, ethyl ester	97
3	25.8935	0.5047	$C_{13}H_{26}O_2$	214.34	Undecanoic acid, ethyl ester	91
4	28.7189	2.3606	$C_{17}H_{34}O_2$	270.4507	Pentadecanoic acid, 14-methyl-, methyl ester	98
5	29.5013	7.7833	$C_6H_{32}O_2$	256.4241	n-Hexadecanoic acid	99
6	30.1104	0.7456	$C_{13}H_{26}O_2$	214.3443	Undecanoic acid, ethyl ester	86
7	32.1347	5.3846	$C_{19}H_{34}O$	294.4721	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	99
8	32.2431	4.6311	$C_{19}H_{36}O$	296.4879	9-Octadecenoic acid, methyl ester, (E)-	99
9	32.3434	0.4995	$C_{19}H_{36}O_2$	296.5	12-Octadecenoic acid, methyl ester	95
10	32.7108	1.2937	$C_{19}H_{38}O$	298.5038	Methyl stearate	97
11	32.9881	21.5391	$C_{13}H_{32}O$	280.4455	9,12-Octadecadienoic acid (Z, Z)-	99
12	34.0893	1.7459	$C_{19}H_{36}O$	280.5	2-Methyl-Z, Z-3,13-octadecadienol	89
13	34.7422	0.2534	$C_{17}H_{32}O$	252.4	8-Hexadecenal, 14-methyl-, (Z)-	72

4. DISCUSSION

After the extraction process, it was discovered that the fruit coat gave percentage yield of 7.52%w/w, while the pulverised seeds yielded 2.47%w/w. This difference could be because of difference in particle size as well as the surface area. The fruit coat was pulverised to fine powder with increased surface area, hence better contact of the solvent with the powder matrix. Due to the hard nature of the seeds of *Dialium guineense*, coarse powder was obtained upon grinding. Increasing the Surface area increases interaction between the solvent and the powder which, in turn, increases the rate at which the components of interest are brought into solution.[9]

Table 1 compares the percentage abundance of various nutrients found in the seeds and fruit coats of *Dialium guineense* wild. The percentage abundance of carbohydrates in the fruit coat was found to be 11.88%,

thus lower than the seeds which had a percentage abundance of 26.38%. This implies that the seeds would provide a better supply of carbohydrates in diets compared to the fruit coat. Interestingly, the fruit had the highest fibre composition of 56.83% which justifies its potential as a rich source of dietary fibre as stated by Osanyinlusi et al.[10] Also, the seeds had a greater abundance of moisture content (21.89%) whereas the percentage abundance of moisture in fruit coat was 16.14%.

According to Osanyinlusi et al.[10], the moisture content is a significant parameter as it has serious impacts on storage life and other processing characteristics. The lower moisture content of fruit coat is highly beneficial as it protects it from microbial infestation and makes it less susceptible to spoilage due to bacteria proliferation.

Table 2 revealed that *Dialium guineense* possessed a variety of relevant phytochemicals in both its fruit coats and seeds, which may be responsible for the bioactive properties in addition to aiding in plant survival. The total crude extract of the fruit coat revealed presence of bioactive compounds comprising of phenolics, flavonoids, alkaloids, tannins, anthraquinones, triterpenoids, proteins, carbohydrate, and saponins. Phenolics, saponins, flavonoids, cardiac glycosides, alkaloids, tannins, anthraquinones, triterpenoids, and proteins are among the phytochemicals found in the seed extract. While there were cardiac glycosides in the seed extract, none was present in the fruit coat extract. This confirms the findings of Besong et al.[5] Phenolic compounds have been discovered to have a role in the antimicrobial and antioxidant effects of *Dialium guineense* wild.[11]

The various fractions showed different levels of antibacterial activity against various bacteria strains and no antifungal action. Methanol-dichloromethane extract of seeds and fruit coats showed inhibitory activity against *Staphylococcus aureus* with the inhibition zone diameter of 10.0 mm and 6.7 mm respectively with the seed extract having the highest inhibition. The extracts of seeds and fruit coats also demonstrated activity against *Klebsiella pneumoniae* with the corresponding diameter zones of 10.7 mm and 5.7 mm respectively.

The antibacterial activity of the extracts against *Pseudomonas aeruginosa* was also demonstrated. An inhibition zone diameter of 9.0 mm was obtained for the seed extract while the fruit coat extract had an inhibition zone diameter of 4.7 mm. These results suggest that the seed extract had higher antibacterial activity compared to the fruit coat extracts. This activity could be linked to the presence of Phytochemicals such as phenolics, tannins, flavonoids, and alkaloids which confer antibacterial properties to the extracts.

For the Minimum Inhibitory Concentration (MIC) analysis, only the seed extract showed inhibitory effect against *Staphylococcus aureus, Klebsiella pneumoniae, and Pseudomonas aeruginosa* at concentrations of 25%, 12.5% and 50% respectively. Therefore, it can be said that the seeds have better antibacterial activity compared to the fruit coat.

DPPH radical scavenging assay was carried out to determine the hydrogen atom donating ability of the polar and non-polar fractions of the extracts through the decolourization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produced violet colour in methanol solution and this colour faded to various shades of yellow colour with different intensity corresponding to the various concentrations of the polar and non-polar fractions.

IC_{50} values of 11.49 µg/ml, 39.81 µg/ml, 133.00 µg/ml, and 148.20 µg/ml were obtained for polar fractions of seed, fruit coat, non-polar fractions of the fruit coat and seeds respectively (Table 6). The lower the value of IC_{50}, the higher the free radical scavenging activity of the fraction. Hence, the polar fraction of the seed extract has better free radical scavenging activity compared to the other fractions of the fruit coat.

Also, the methanol fraction of the fruit coat had significant ($p < 0.001$) percentage inhibition compared to the n-hexane fraction. And the methanolic fraction of the seeds showed significant ($p < 0.001$) percentage inhibition compared to the n-hexane fraction.

Hence the methanol fractions are better than the n-hexane fractions, and interestingly, the seed is better than the fruit coat.

From the GC-MS analysis, a total of 10 bioactive compounds were contained in the fruit coat as shown in Table 7 and 13 bioactive compounds were found in the seed as shown in Table 8. Among the detected bioactive components of n-hexane extract of the fruit coat of *Dialium guineense* included, Dodecanoic acid, ethyl ester ($C_{14}H_{28}O_2$), Diethyl Phthalate ($C_{12}H_{14}O_4$), Tetradecanoic acid, ethyl ester ($C_{16}H_{32}O_2$), Pentadecanoic acid, 14-methyl-, methyl ester ($C_{17}H_{34}O_2$), 9,12-Octadecadienoic acid, methyl ester ($C_{19}H_{34}O$), Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$), Tetradecanoic acid, 12-methyl-, methyl ester, (S)- ($C_{16}H_{32}O$), Linoleic acid ethyl ester ($C_{20}H_{36}O$), Octadecanoic acid, 17-methyl-, methyl ester ($C_{20}H_{40}O$), Hexadecyl propyl ether ($C_{19}H_{40}O$).

Table 8 provides comprehensive data on the 13 bioactive compounds found in the seed. These compounds are majorly, carboxylic acids, saturated fatty acids, isoprenoid lipid, saturated alkanes and are known to confer Antioxidant, anti-inflammatory, antimicrobial properties to *Dialium guineense*. Dodecanoic acid (Lauric acid) has antioxidant, and anti-microbiological activities against several bacteria, which might be useful in ensuring a balance of the distribution of bacteria

associated with gut microbiota and for protection against microbial infection.[12]

5. CONCLUSION

This study disclosed that seeds of *Dialium guineense* possess significant antioxidant and antimicrobial effects compared to the fruit coat. The antioxidant activities of the fractions studied were in the order; polar fraction of seed > polar fraction of fruit coat > non-polar fraction of seed > non-polar fraction of fruit coat.

The seeds and fruit coat are highly underutilized since they are usually discarded on the streets after consumption of the pulp. In addition to revealing the antioxidant and antimicrobial potential of the seed and fruit coat, this study has also shown that they can serve as a source of supplementary dietary nutrients for humans and can be utilized as phytogenic feed additives for animals.

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