

Partition Fractions of *Dialium guineense* Fruits and Leaves Inhibits AGEs and DPPH-radical formation: An *In vitro* - Computational Study.Hauwa S. Usman^{1*}, Auwal Adamu¹, Umar A. Umar¹, Funmilola Audu², Mukhtar A. Suleiman¹, Murja I. Danja³, Zakariyya A. Inuwa¹ and Abdullahi B. Sallau¹¹Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria²Department of Biochemistry, University of Abuja, FCT, Nigeria³Federal University of Education, Zaria, Nigeria**ABSTRACT**

Non-enzymatic reaction between proteins and reducing sugars (glycation) results in production of advanced glycation end products (AGEs), leading to build up and cellular injuries via lipid peroxidation, endothelial dysfunction and protein structural alterations. Anti-glycation phytoconstituents seems promising for addressing human aging and emergence of many disorders. This study aimed to explore antioxidative and antiglycation effects of *Dialium guineense* (DG), a plant commonly known for its medicinal properties. Partition fractions from both fruits and leaves of this plant were utilized, focusing on their ability to inhibit Advanced Glycation End-products (AGEs) formation and scavenge DPPH radicals, utilizing both *in vitro* experimental assays and *in silico* molecular docking techniques. Antioxidant activity revealed DPPH scavenging capacity of 94% for ethylacetate fruit fraction and 85% for ethylacetate leaf fraction. A similar trend was observed for antiglycation results. Ethylacetate fruit fraction showed significant efficacy (95%) against glycated bovine serum albumin (BSA) when compared to the control (aminoguanidine). The leaf chloroform fraction displayed a lower (73%) effectiveness overall. Both fractions inhibited AGE formation in BSA-Glucose model. Molecular docking analyses revealed strong binding affinities of -6.4 and -6.1 kcal/mol, involving a non-polar interaction with Arg185 residue respectively; which is highly susceptible to glycation. Carbonic acid and Oxalic acid exhibited one polar contact each, involving two residues (Arg-208 and Arg-144). Hence, compounds in fruit ethylacetate fraction had higher binding affinity for BSA, which correlated with superior anti-oxidant and anti-glycation activity. These findings suggest that the bioactive compounds from *D. guineense* have therapeutic potential in combating various diseases.

Keywords: Antiglycation, *Dialium guineense*, Molecular Docking, Chromatography, Fruits, Leaves

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Copyright: © 2026 Usman *et al.* This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.**Introduction**

Advanced Glycation End-Products (AGEs) are generated via the classical Maillard reaction, which involves reducing sugars reacting non-enzymatically with amino groups in proteins, lipids, and nucleic acids. This process involves formation of a Schiff base, an Amadori rearrangement, and further oxidative modifications.^{1,2} While AGEs are formed normally at moderate levels, continuous hyperglycemia causes increased glucose levels, which greatly elevates their formation.^{3,4}

The harmful effects of Advanced Glycation End-Products (AGEs) are primarily due to their ability to cause permanent damage to proteins by forming crosslinks, both intermolecular and intramolecular.⁵ These crosslinks alter the structure and functionality of proteins, rendering enzymes and proteins that are physiologically active inert,⁶ making them resistant to proteolytic digestion,⁷ and facilitating the production of ROS.⁸

This leads to pro-inflammatory responses,⁹ Several metabolic and biochemical abnormalities.^{4,10,11} When bound to their receptor, AGEs trigger several signaling pathways that increase oxidative stress and inflammation, which impacts cellular function and metabolism.¹²

AGEs can build up endogenously or exogenously.¹³ Highly reactive glycation products found in cigarette smoke also serve as a precursor to AGEs formation.^{13,14} High amount of exogenous AGEs can be found in the modern western diet. Significant amount of AGEs are formed when food is thermally processed, which often entails employing dry heat technology for cooking methods like frying, grilling, baking and barbecue.¹⁴ Food processing aimed at conservation, flavor and appearance also results in the formation of various food derived advanced glycation end products (AGEs) referred to as glycotoxins.^{4,15}

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is conventionally used to assess plants antioxidant potential. One of the most important ways that phytochemicals protect against oxidative stress is by neutralizing free radical's effect.¹⁶

The search for natural products with therapeutic potential has attracted a lot of interest from scientist and medical professionals because of the necessity to find new ways to treat diseases linked to oxidative stress and metabolic disorders.^{17,18,19} Among numerous candidates, *Dialium guineense* (DG) known by many as black velvet tamarind; is an important tropical fruit known for both its nutritional and therapeutic properties. Primarily found in west Africa, it has long been used in traditional medicine to treat a variety of conditions such as respiratory disorders, diabetes and diarrhea.^{20,21,22} A rich phytochemical composition made up of flavonoids, tannins and other phenolic compounds with strong antioxidant properties has been found through phytochemical profiling of *D. guineense*, especially its root and leaf extracts.^{23,24}

*Corresponding author. mail: ummisa71@gmail.com

Tel: +2348069676503

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Presently, an enormous research gap exists due to lack of reports on antiglycation-based *In vitro* and *In silico* studies on DG. By bridging this gap, our research seeks to elucidate the potentials of *D. guineense* as a natural therapeutic agent against oxidative stress-related complications. These findings will contribute to the expanding body of knowledge regarding usage of antioxidants and antiglycants derived from plants in disease prevention and management, as well as offer insightful information about the bioactive compounds present in these fractions.

Materials and Methods

Chemicals and Reagents

D-glucose, bovine serum albumin (BSA), aminoguanidine, sodium azide were purchased from Sigma Aldrich Company, USA. Methanol, ethylacetate, chloroform, ascorbic acid and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from British Drug House Chemical Limited, Poole, England.

Sample Collection

Dialium guineense leaves and fruits were collected from a local farm in (Saminaka) of Kaduna State, Nigeria on 16 May, 2023. Plant samples were authenticated at Department of Botany, Faculty of Life Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria; where a voucher number of (ABU07181) was deposited.

Preparation of Plant Crude Extracts

Based on a prior activity study on the crude extracts of DG,³³ the extracts with the highest activity were selected for the present study. Two hundred grams (20 g) of fine powdered sample of DG was soaked overnight in 100 mL each of methanol (fruit); leaf samples were extracted separately using, chloroform and ethylacetate, followed by filtration using a filter paper (Whatman No. 1). The extracts were concentrated at 60 °C using a rotary evaporator and dried in a water bath at 45 °C. Dried extracts were labeled as crude extracts and stored at 4 °C prior to partition chromatography.

Liquid-Liquid Partition Chromatography Protocol

Dried crude extracts of both fruits and leaves of DG (7.2 g) were subjected to liquid-liquid partition chromatography. Two solvents (chloroform, ethyl acetate) with different polarities were used as described by²⁵ with slight modification. Each partition was conducted three times and the eluent was pooled and dried in a water bath at 45 °C.

Thin Layer Chromatography

Thin layer chromatography was conducted using a plate precoated with silica gel 60 F245 (0.25 mm thick and 7.5 cm long). The developer solvent was hexane: ethylacetate with a ratio 7:3. The developed TLC plate was then viewed under UV at wavelength 254 nm and 365 nm.²⁶

Antioxidant Effect of Plant Fractions

The antioxidant power of the fractions was determined using DPPH free radical scavenging assay as described by.²⁷ Briefly, 0.1 mL each of analytical grade methanol, 1 mg/mL ascorbic acid and 1 mg/mL plant fraction was added, in triplicates, into control, standard and fraction tubes, respectively. Thereafter, 3 mL of 0.24 mg/mL DPPH (prepared in methanol) was added into the test tubes. The mixture was then stirred for 5 min and incubated in the dark at 25 °C for 30 min. The absorbance was read at 517 nm. The percentage antioxidant activity of the fractions and ascorbic acid was determined using the formula below:

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Antiglycation Effect of Plant Fractions

The antiglycation activity of the fractions was estimated based on standardized methods.^{28,29} In brief, 20 µL each of 800 µg/mL BSA and 200 mM D-glucose were added, in triplicates, into test tubes labeled; standard and plant fractions (ethyl acetate and chloroform). Following that, 20 µL each of 50 mM phosphate buffer (pH 7.4) containing 0.2 g/L sodium azide was added to test tubes labeled standard and the various plant fractions as mentioned above, 1 mg/mL of both aminoguanidine and 1 mg/mL plant fraction (prepared in phosphate buffer containing sodium azide) was added into test tubes labeled standard and plant fractions respectively. Afterwards, the mixture was incubated at 37 °C for 7 days. The fluorescence intensity was read at an excitation wavelength of 370 nm and an emission wavelength of 440 nm. The percentage antiglycation activity of the fractions and aminoguanidine was calculated using the formula :

$$\text{Antiglycation activity (\%)} = \frac{\text{Fluorescence intensity of control} - \text{Fluorescence intensity of test}}{\text{Fluorescence intensity of control}} \times 100$$

Gas Chromatography-Mass spectrometry (GC-MS)

The fraction with the highest activity overall (DG ethyl acetate fruit fraction) was analyzed for bioactive compounds using GC-MS Agilent series 6890 with Hewlett Packard detector 5973. Separations were attained by a HP-5MS column (length 30 m × diameter 250 µm × thickness of film 0.25 µm). An electron ionization system with high energy electrons (70 eV) was utilized for spectroscopic detection by GC-MS. The temperature of the injector was 220 ± 0.2 °C and the transfer line 240 °C. The temperature of the oven was programmed from 60 °C to 246 °C at 3 °C /min. Pure helium gas was passed as a carrier at 1.02 mL/min at 210 °C. Prepared fractions (1.0 µL) diluted with methanol as a solvent, were injected at 250 °C in a splitless method. The early temperature was positioned at 50–150 °C with a rising rate of 3 °C / min and held for 10 min. Finally, the temperature was amplified to 300 °C at a rate of 10 °C / min.³⁰ Detection was completed using a full scan mode between 35 to 600 m/z and with a gain factor of 5. All peak areas were compared with the database in the GC-MS library version NIST 08-S.

Molecular Docking Studies

Ligand Preparation

The chemical structures of 9 compounds detected in DG fruits by GC-MS analysis (Carbonic acid, prop-1-en-2-yl tridecyl ester, 17-Pentatriacontene, Oxalic acid, cyclobutyl hexadecyl ester, Undecane, Hexadecanoic acid, n-Hexadecanoic acid, 1,6-Octadiene, 5,7-dimethyl-, (R)-, 9,17-Octadecadienal, (Z)-, Thirane, hexyl-) were downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/> (accessed on 11th January, 2025) in SDF format.

Preparation of the Target Protein

The three-dimensional (3D) structure of the target protein, namely the crystalline structure of bovine serum albumin (BSA) (PDB ID: 4OR0) was obtained from the Protein Data Bank of the Research Collaboratory for Structural Bioinformatics (RCSB) (<https://www.rcsb.org/> (accessed on 15th January, 2025). The PyRx software (Version: PyRx. 0.8) was utilized to prepare the protein and perform molecular docking. The protein was prepared for docking by removing all heteroatoms and water molecules, thereby adding polar hydrogen atoms.

Molecular Docking of Ligand and Protein

The graphical interface of the PyRx 0.8 program was used to execute the molecular docking study. The first step was to import and prepare the protein and compounds (ligands) in the interface and then perform the molecular docking using a grid box dimension of x = 106.04, y = 65.72, and z = 115.14 with a grid center of x = -49.02, y = -3.92, and z = -27.96 for BSA with an exhaustiveness of 8. Discovery Studio Visualizer v21.1.0.20298 (BIOVIA, San Diego, CA, USA) was used to visualize the binding interactions of the protein-ligand complex.

Data Analysis

All experiments were carried out in triplicates with data presented as mean \pm standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) version 20 for windows. Duncan post hoc test was conducted to detect differences amongst the mean of various test solutions. P value less than 0.05 ($p < 0.05$) was considered statistically significant.

Results and Discussion

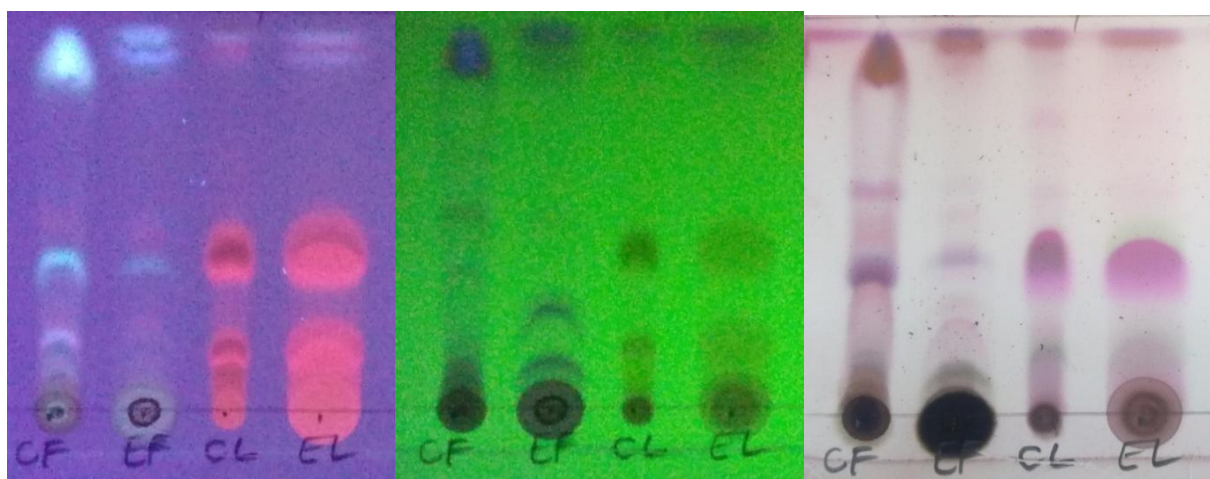


Plate 1: TLC chromatogram of *D. guineense* fruit and leaf fractions on precoated silica gel plate observed under UV light at 254nm (left) 365nm (middle) and daylight (right) after partition chromatography

The antiglycation capacity of DG fruits and leaf fractions was assessed using BSA-Glucose model and the results are shown in Fig. 2. The highest activity was seen in the ethylacetate fruit fraction (95 %); the result was significant ($p < 0.05$) when compared to the control-aminoguanidine (61 %). However, there was no significant ($p < 0.05$) difference between ethylacetate fruit and chloroform fruit fractions.

In particular, ethylacetate fruit fraction showed significant efficacy (95 %) against glycated bovine serum albumin (BSA) when compared to the control (aminoguanidine). The leaf chloroform fraction displayed a lower (73 %) effectiveness overall; this might be attributed to the higher polyphenolic content observed in the fruits as compared to the leaf fractions.^{21,24,32} Both fractions inhibited AGE formation in BSA-Glucose model. This complements previous preliminary findings on DG extracts, which revealed a significant antiglycation activity by both DG fruits and leaf extracts.³³ Our findings demonstrated that both DG fruits and leaf fractions significantly decreased the level of AGEs in bovine serum albumin compared to aminoguanidine.

Antioxidant activity of DG fruits and leaf fractions (Figure 1) was evaluated using DPPH assay. Results showed that chloroform fruit fraction had the highest antioxidant activity with a significant ($p < 0.05$) percentage outcome of 94% compared to the control (63 %). However, there was a significant ($p < 0.05$) difference between all the fractions tested, with the exception of ethylacetate and chloroform leaf fractions. Additional mechanisms of AGEs inhibition involve antioxidant activity of inhibitors by scavenging free radicals or chelating metal ions.³⁴ According to previous studies, antiglycation activity is positively correlated with antioxidant activity.^{35,36} Thus, DPPH radical scavenging activity was assessed in the present study and the results shows remarkable outcome similar to previous studies.³⁷

The DPPH scavenging activity was greater in ethylacetate fruit fraction (94 %) and leaf fraction (85 %) respectively.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of active compounds present in DG is shown in Table 1. Compounds present in the most active fraction (ethylacetate fruit fraction) was analyzed using GC-MS and identified based on the database (NIST) in the GC-

The antiglycation potential of *Dialium guineense* fruit ethylacetate fraction was substantiated through inhibition of Advanced Glycated End Products (AGEs) formation in glycated albumin. Glycation increases the level of amyloid cross β -structure, thus aggravating the cytotoxicities of protein aggregation and in general hyperglycaemia.³¹ *Dialium guineense* fruit and leaf fractions were developed as thin-layer chromatograms. The thin layer chromatographic profiles of crude extracts, partitioned in ethyl acetate: chloroform solvent was developed using n-hexane:ethylacetate (7:3); these showed 14 spots as shown below. TLC Spot labels CF, EF, CL and EL denotes spots from chloroform fruit, ethylacetate fruit, chloroform leaf, ethylacetate leaf, respectively as depicted in plate 1.

MS library. Chemical profiling of *D. guineense* ethylacetate fruit fraction showed presence of some compounds namely; Carbonic acid, Pentatriacontene, Oxalic acid- cyclobutyl hexadecyl ester, Undecane, Hexadecanoic acid- methyl ester, n-Hexadecanoic acid, 1,6-Octadiene - 5,7-dimethyl- (R)-, 9,17-Octadecadienal, (Z)- and Thiirane, hexyl-; these compounds have been previously investigated for their antioxidant and various biological activities.³⁸

n-hexadecanoic acid was reported as an inhibitor of phospholipase A2, it is also an anti-inflammatory compound and an antioxidant compound.^{39,40} Undecane, 4,7-dimethyl- was reported for treatment of skin inflammatory disorders, such as atopic dermatitis and allergy.⁴¹ 1,6-Octadiene was previously reported in ethylacetate fraction of *Pycnanthus angolensis* (Welw.) Warb. leaves, possessing strong bacteriostatic effect against *E. coli*, but no activity against *Staphylococcus aureus* and *Candida albicans*.⁴² Oxalic acid, was reported as one of the major constituents of *Ricinus communis* seed oil, exhibited anti-inflammatory, antibacterial, antioxidant, anthelmintic, antidiabetic, anticancer, mosquitocidal, and insecticidal activity.⁴³ 9,17 Octadecadienal, (Z)- was reported to possess antimicrobial activity.⁴⁴ Hexadecanoic acid, methyl ester was observed to exhibit antioxidant, antimicrobial, hypocholesterolemic, nematocidal, pesticide, antiandrogenic, insecticide properties.⁴⁵ 17-Pentatriacontene possess anti-inflammatory, anticancer, antibacterial and antiarthritic.⁴⁶

Thiirane (ethylene sulfide), a three membered sulphur-containing heterocycle is a highly reactive organic compound due to its ring strain.⁴⁷ Thiirane and its derivatives have shown many pharmacological activities including anticancer, antimicrobial, and antibacterial.⁴⁸ Steroidal compounds containing thiirane moiety also exhibit biological activities like peptidase inhibitors, carboxypeptidase A inhibitors, aromatase and metalloproteinases inhibitors.^{49,50,51,52} Various thiirane containing compounds include natural hydrocarbons, cyclic and acyclic alcohols, natural or semi-synthetic steroids, peptides and polyethers.^{53,54} Thus, various reports in literature suggest that thiirane serve as an important lead for the synthesis of many polymers,

pharmaceuticals, pesticides, herbicides, liquid crystals, adhesives and glycomimetics.^{55,56}

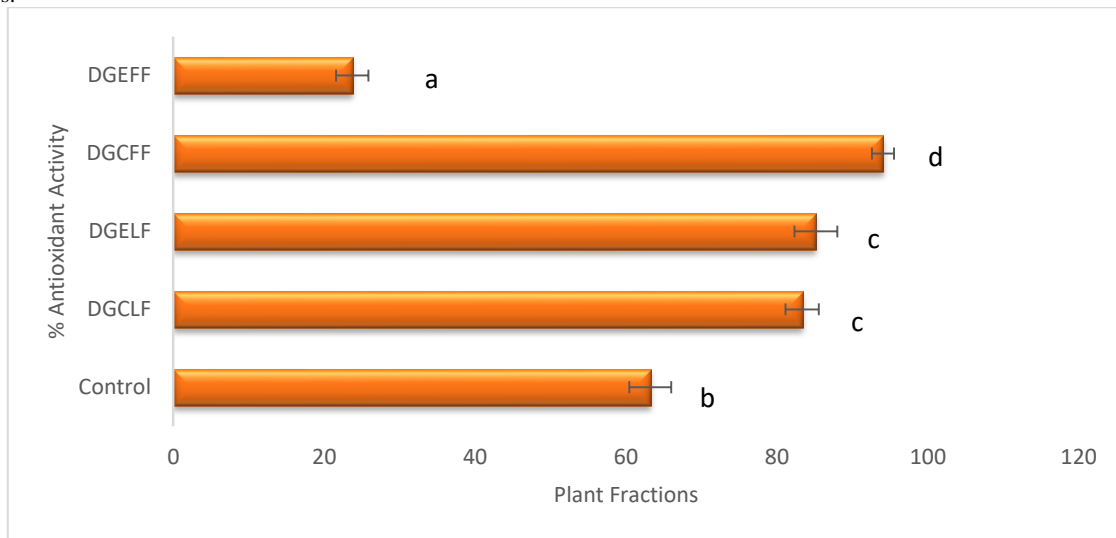


Figure 1: Antioxidant activity of *D. guineense* fruits and leaf fractions. DGEFF denote *D. guineense* ethylacetate fruit fraction, DGCF- denotes *D. guineense* chloroform fruit fraction, DGELF- *D. guineense* ethylacetate leaf fraction, DGCLF- *D. guineense* chloroform leaf fraction

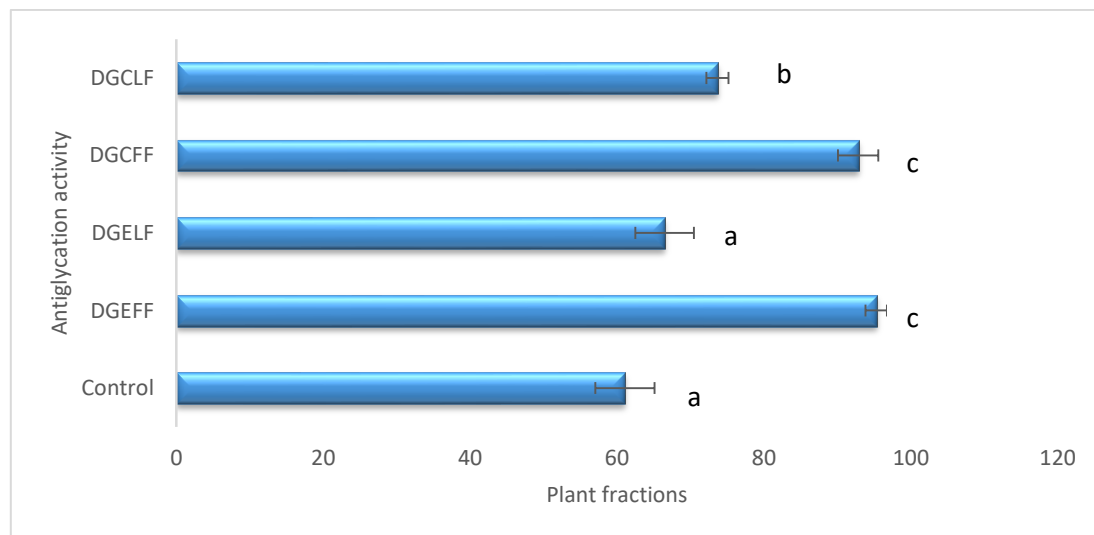


Figure 2: Antiglycation activity of *D. guineense* fruits and leaf fractions. DGEFF denote *D. guineense* ethylacetate fruit fraction, DGCF- denotes *D. guineense* chloroform fruit fraction, DGELF- *D. guineense* ethylacetate leaf fraction, DGCLF- *D. guineense* chloroform leaf fraction.

Table 1: Major compounds identified in *D. guineense* ethylacetate fruit fraction by GC-MS analysis

Peak	Retention Time	Compounds	Similarity %	Index	Common Names
1	32.1842	Carbonic acid, prop-1-en-2-yl tridecyl ester	86		
2	36.0704	17-Pentatriacontene	53		
3	36.319	Oxalic acid, cyclobutyl hexadecyl ester	30		
4	36.8806	Undecane	25		
5	37.2817	Hexadecanoic acid, methyl ester	89		Palmitic acid
6	37.8319	n-Hexadecanoic acid	99		
7	38.2322	1,6-Octadiene, 5,7-dimethyl-, (R)-	38		Isocitronellene
8	38.4184	9,17-Octadecadienal, (Z)-	97		
9	38.5604	Thiirane, hexyl-	68		Ethylene Sulphide

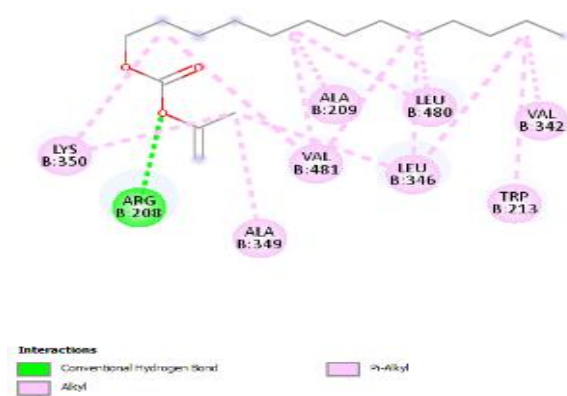
Molecular docking, a widely used *in-silico* technique in drug discovery, is used to predict optimal binding conformation of a ligand with a macromolecule such as a protein.⁵⁷ In the present study, nine compounds, namely- Carbonic acid, Pentatriacontene, Oxalic acid,

Undecane, Hexadecanoic acid, n-Hexadecanoic acid, Octadiene, Octadecadienal and Thiirane were docked with bovine (BSA).

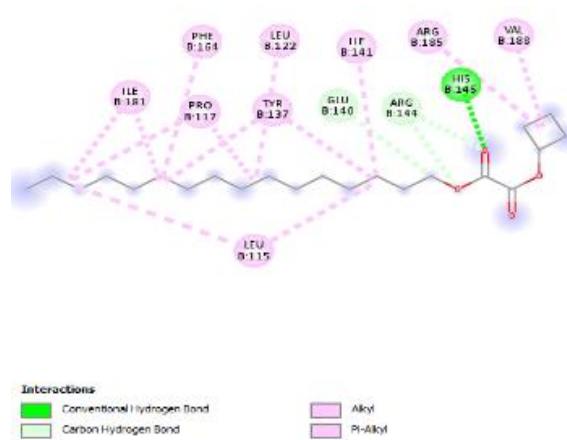
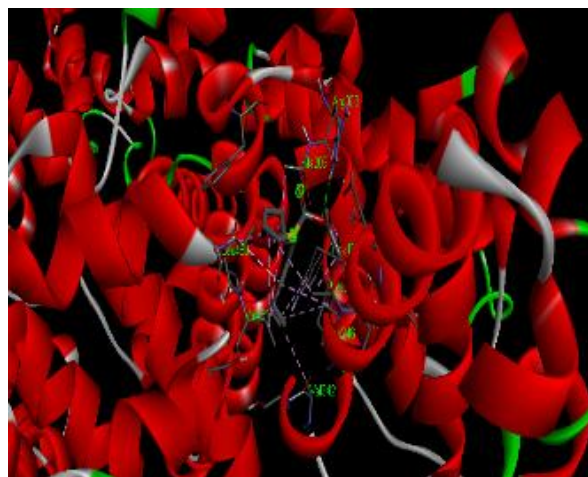
The results of virtual screening of active phytocompounds with BSA are presented in Table 2. The binding energies, estimated through

docking studies of compounds with target protein, revealed that Carbonic acid, Pentatriacontene, Oxalic acid, Undecane, Hexadecanoic acid, n-Hexadecanoic acid, Octadiene, Octadecadienal and Thiirane had docking scores of -5.7, -5.6, -5.8, and -5.4, -6.1, -5.6, -5.8, -6.4, -1.8 (kcal/mol), respectively, with BSA. However, Octadecadienal showed the highest affinity towards BSA (-6.4 kcal/mol). The interactions between the compounds and the protein is depicted in Figure 3. It was observed that all the compounds showed intersecting molecular interactions within the binding site of the protein. Previous studies have confirmed that the main amino acid residues involved in the glycation process are lysine and arginine,^{58,59}

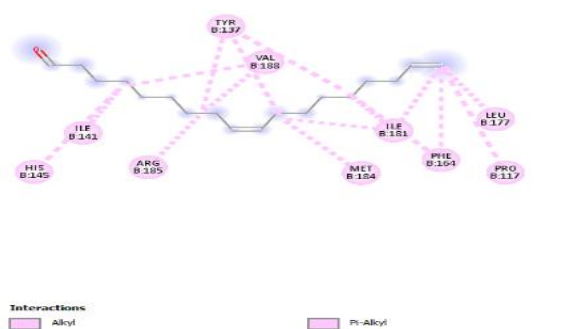
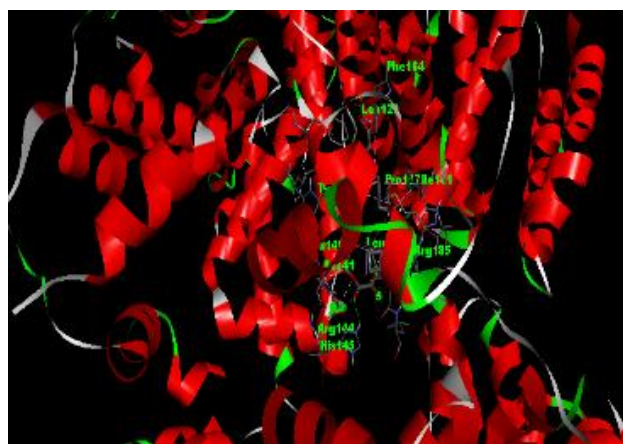
which is in line with some of the results obtained in the present study and a similar study on *Solanum macrocarpon*.⁶⁰ Molecular docking studies between Octadecadienal and n-Hexadecanoic acid with bovine serum albumin (BSA) revealed strong binding affinities of -6.4 and -6.1 kcal/mol, involving a non-polar interaction with Arg185 residue; for both compounds respectively. Interestingly, both Carbonic acid (prop-1-en-2-yl tridecyl ester) and Oxalic acid (cyclobutyl hexadecyl ester) exhibited one polar contact each, involving two residues (Arg-208 and Arg-144). An additional polar contact was also established by oxalic acid with His145 residue. However, the molecular docking assessment did not show any polar contacts for Pentatriacontene, Undecane, Hexadecanoic acid, n-Hexadecanoic acid, Octadiene, Octadecadienal and Thiirane.



(a) Carbonic acid, prop-1-en-2-yl tridecyl ester



(b) Oxalic acid, cyclobutyl hexadecyl ester



(c) 9,17-Octadecadienal, (Z)-

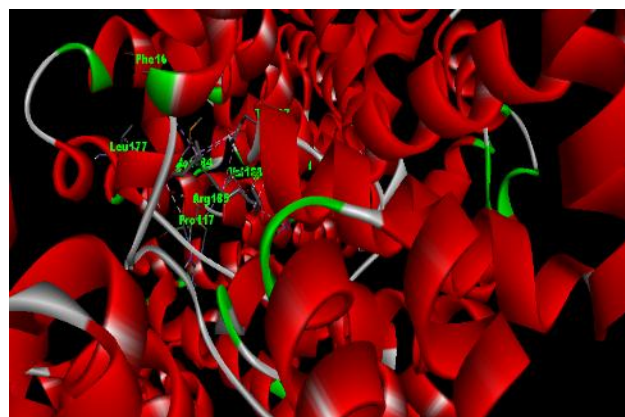


Figure 3: Molecular docking of identified compounds with BSA. (a) Carbonic acid, prop-1-en-2-yl tridecyl ester (b) Oxalic acid, cyclobutyl hexadecyl ester (c) 9,17-Octadecadienal**Table 2:** Outcome of binding affinities for docking positions of various compounds identified in DG ethylacetate fruit fraction to bovine serum albumin (BSA) in a molecular docking simulation.

Ligands	PubChem ID	Binding energy (kcal/mol)	Interaction
Carbonic acid, prop-1-en-2-yl tridecyl ester	91692935	-5.7	Conventional Hydrogen Bond: Arg208 Alkyl/Pi-Alkyl: Ala209, Trp213, Val342, Leu346, Ala349, Lys350, Leu480, Val481
17-Pentatriacontene	5365022	-5.6	Alkyl/Pi-Alkyl: Tyr149, Trp213, Arg 217, Leu218, Leu237, His241, Leu259, Ala260, Ile263, His287, Ile289, Ala290
Oxalic acid, cyclobutyl hexadecyl ester	6420625	-5.8	Conventional Hydrogen Bond: His145 Carbon Hydrogen Bond: Glu140, Arg144 Alkyl/Pi-Alkyl: Leu115, Pro117, Leu122, Tyr137, Ile141, Phe164, Ile181, Arg185, Val188
Undecane	14257	-5.4	Alkyl/Pi-Alkyl: Val23, Ala26, Leu46, Leu66, His67, Leu69, Leu250
Hexadecanoic acid	985	-6.1	Alkyl/Pi-Alkyl: Pro117, Pro119, Leu122, Tyr137, Phe164, Leu177, Leu178, Ile181, Met184, Arg185, Val188
n-Hexadecanoic acid	8181	-5.6	Alkyl: Ala209, Ala212, Leu326, Leu330, Leu346, Ala349, Lys350, Leu480, Val481
1,6-Octadiene, 5,7-dimethyl-, (R)-	10964588	-5.8	Alkyl/Pi-Alkyl: Pro117, Pro119, Leu122, Phe164, Leu177, Leu178, Ile181
9,17-Octadecadienal, (Z)-	5365667	-6.4	Alkyl/Pi-Alkyl: Pro117, Tyr137, Ile141, His145, Phe164, Leu177, Ile181, Met184, Arg185, Val188
Thiirane, hexyl-	9865	-1.8	Ala190, Tyr451, Leu454

Conclusion

This study provides compelling evidence that *Dialium guineense* possess antioxidant and antiglycation activities through inhibition of AGE formation and free radical scavenging. Nine (9) compounds were identified from the fruit ethyl acetate fraction by GC-MS analysis, with octadecadienal and hexadecanoic acid as major components. The *in vitro* results, supported by molecular docking data, highlights the potential therapeutic applications of this plant in managing oxidative stress and AGEs-related diseases. Further investigation into the mechanistic role of specific compounds responsible for these effects and their *in vivo* activity is warranted to fully elucidate their clinical relevance.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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