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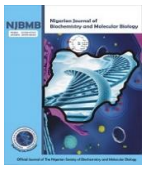

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Research Article

Bioassay Guided Evaluation and Characterization of *Solanum macrocarpon* Fruits and Leaf Fractions via *In Vitro* Antiglycation, Radical Scavenging and Molecular Docking Analyses

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ABSTRACT

Plants, both in their natural state and as extracts/fractions, have provided significant benefits to human health for centuries. The pharmacological benefits of these plants is attributed to the diversity of primary and secondary metabolites. This study evaluated the *in vitro* DPPH scavenging capacity, antiglycation activity and bioactive constituents of *Solanum macrocarpon* fruit and leaf fractions using Chromatographic, Spectrophotometric, Spectro-fluorescence, Spectrometry assays and Molecular docking methods. Results revealed that ethylacetate fruit fraction exhibited the highest activity overall, regarding both radical scavenging and antiglycation activities. Such potent dual bioactivity can be ascribed to the presence of some prominent compounds identified from GC-MS analysis, which includes tetradecanoic acid, n-hexadecanoic acid and cycloeicosane. Molecular docking studies highlighted five compounds (methoxyacetic acid, 3-pentadecyl ester; tetradecanoic acid, n-hexadecanoic acid, cycloeicosane- and 3-eicosene, (E)-) having a pronounced affinity with bovine serum albumin (BSA), indicating their potential to impede binding of AGEs to BSA. Findings from this study encourages further investigations on other biological activities of this plant besides its antioxidant and antiglycation activity.

Keywords: *Antiglycation, Radical Scavenging, GC-MS, Solanum macrocarpon, Molecular Docking*

INTRODUCTION

Protein glycation and oxidative stress are among the dual theories that explain aging due to their capacity to permanently alter the body's metabolic pathways. They have a substantial correlation with high levels of harmful free radicals (Premaranthne *et al*., 2021). On the other hand, oxidative stress and a mismatch between ROS generation and antioxidant defense can result in degenerative diseases such cancer, Alzheimer's, cardiovascular, aging and neurological problems (Kregel and Zhang, 2006). Through autoxidation of glucose and amadori products, protein glycation results in production of early (amadori) and late advanced glycation end products (AGEs) in conjunction with free radicals (Elosta *et al*., 2017). Increased protein glycation; a non-enzymatic interaction between protein amino groups and carbonyl groups from reducing sugars, results in an unstable schiff base that reorganizes into a more stable amadori product. Upon formation, the amadori products go through further reactions with dicarbonyl intermediates, including 3-deoxyglucosones, to generate advanced glycation end products (AGEs), which are mostly cross-linked and fluorescent structures (Ahmed, 2005; Elosta *et al*., 2012).

The continuous reoccurrence of inflation and fluctuation in economic stability in Nigeria and the world at large calls for serious concern. A remarkable coping strategy must include utilization of affordable as well as readily available nutraceuticals. One of such is *Solanum macrocarpon*; a

commonly known fruit-producing vegetable underutilized especially in Northern Nigeria. It is usually retailed by few vendors compared to other commonly known fruits like orange and banana. Hence, the need for an urgent approach on exploring the medicinal significance of this plant.

Solanum macrocarpon (Solanaceae) is a plant with approximately 1000 species globally, encompassing about 100 native species in Africa and nearby islands (Osei *et al*., 2010). Since the leaves are seen as having a high nutritional value and are so utilized to make soups and stews, they play a significant role in the local cuisine. In addition to having significant levels of sulfur-containing amino acid methionine (Messiaen, 1992; Komlaga *et al*., 2014), the leaves are high in protein, fat, crude fiber, calcium, and zinc (Oboh *et al*., 2005). In indigenous medicine, *S. macrocarpon* is used for a variety of purposes, such as weight loss and treatment of rheumatic disease, swollen joint pains, asthma, allergic rhinitis, nasal catarrh, skin infections, gastroesophageal reflux disease, constipation, and dyspepsia (Bello *et al*., 2005).The fruits are been consumed as a snack along with peanut paste.

Bioassay guided fractionation entails extraction of plant material; which is proceeded by testing for biological activity. Once the tested extract is found to be biologically active, the next step is fractionation. Subsequently, various fractions obtained are tested for biological activity and the most potent is then subjected to characterization (Ingle *et al*., 2017; Sasidharan *et al*., 2011). However, there is paucity of reports in literature on antiglycation activity of *Solanum macrocarpon* fruits and leaf fractions, including bioassay guided approach and *In silico* studies. Hence the novelty of this research.

MATERIALS AND METHODS

Chemicals and reagents

D-glucose, Bovine serum albumin (BSA), aminoguanidine and sodium azide were obtained 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

Solanum macrocarpon leaves and fruits were collected from natural habitats of Zaria, Kaduna State, Nigeria. Plant samples were authenticated at Department of Botany, Faculty of Life Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria; where a voucher number (ABU0560) was deposited.

Preparation of plant crude extracts

Using a pestle and mortar, air-dried plant samples (fruits and leaves of *Solanum macrocarpon*) were mashed into a fine powder. Twenty grams (20g) of finely ground plant material were steeped in 100 milliliters of methanol for a whole night before being filtered using Whatman No. 1 filter paper into glass vials that had been previously weighed, the supernatants were dried in a water bath at 45°C after being concentrated at 60°C with a rotary evaporator. Prior to partition chromatography, the dried extracts were kept at 4°C and labeled as crude extracts. The plant extract was kept in dark, airtight glass vials until when needed.

Liquid-liquid partition chromatography protocol

Dried methanolic extract (fruit 7.2 g and leaves 11.6g) was subjected to liquid-liquid partition chromatography. Two solvents (chloroform, ethyl acetate) with different polarities were used for the experiment. Briefly, methanol extract of plant materials (*S. macrocarpon* fruit and leaves) obtained by maceration was fractionated using chloroform and ethyl acetate. The supernatant was filtered using Whatman No. 1 sheet, pooled and concentrated using vacuum rotary evaporator. The eluents were then evaporated using a water bath at 45°C to obtain the dry form of the respective fractions. Each partition was conducted three times as described by Rajbir kaur *et al*., (2008) with slight modification.

Percentage yield

The percentage yield was obtained using dry weight, from the formula below (Adam *et al*., 2019).

%Yield of extract (g/100 g) = $(W_1 \times 100) / W_2$

Where:

W_1 is the weight of the plant extract residue after solvent removal

W_2 is the weight of dried plant powder.

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 (Othman *et al*., 2015).

Antioxidant effect of plant fractions

The antioxidant power of plant fractions was determined using DPPH free radical scavenging assay as described by Shah *et al*. (2013). The procedure was carried out in a clean and sterile environment. Briefly, 0.1 ml each of methanol, 1 mg/ml ascorbic acid and 1 mg/ml plant fraction was added, in triplicates; into control, standard and fraction test 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 or free radical scavenging activity of plant fractions and ascorbic acid was determined using the formula below:

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

Antiglycation effect of plant fractions

The antiglycation activity of plant fractions was estimated based on the method of Matsuura *et al.* (2002) and Kaewnarin *et al*. (2014). The experiment was carried out in a clean and

sterile environment. 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 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 using a spectrofluorometer. The percentage antiglycation activity of plant fractions and aminoguanidine was calculated using the following formula below:

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

Gas chromatography-mass spectrometry (GC-MS)

Ethyl acetate partition fractions of *Solanum macrocarpon* fruit samples was analyzed for bioactive compounds using GC-MS Agilent series 6890 with Hewlett Packard detector 5973. Separations were attained by column HP-5MS column (length30 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 fraction, 1.0µ L diluted with methanol as a solvent, was injected at 250°C in a split-less 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 (Mocan *et al*., 2016). 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.

Ligand preparation for molecular docking

The chemical structures of 10 compounds detected in *S. macrocarpon* fruits by GC-MS analysis (Undecane, 4,7-dimethyl- , Undecane, 4,7-dimethyl- , Methoxyacetic acid, 3-pentadecyl ester, Tetradecanoic acid, Undec-10-ynoic acid, dodecyl ester, 2-Piperidinone, N-[4-bromo-n-butyl]- , 1,6-Octadiene, 3-ethoxy-3,7-dimethyl-, n-Hexadecanoic acid, 3-Eicosene, (E)- and Cycloeicosane) 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) structures of the target protein, 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). 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, adding polar hydrogen atoms.

Molecular docking of ligand and protein

The graphical interface of PyRx 0.8 program was used to execute molecular docking study. The first step was to import and prepare protein and compounds (ligands) in the interface and then perform 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 ± 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 mean of various test solutions. P value less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Liquid-liquid partition chromatographic set up is shown in Plate 1. About 7.2 g and 11.6g of extract was obtained from 20 g of *S. macrocarpon* fruit. Liquid-liquid partition extract yielded fractions of chloroform and ethyl acetate at 2.6 g and 4.7 g respectively. The percentage yield obtained for chloroform and ethyl acetate fruit fractions was 13% and 23.5% respectively.

Plate 1. A Laboratory set up of Partition Chromatography

Solanum macrocarpon fruit and leaf fractions were developed as thin-layer chromatograms, depicted in plate 2. The thin layer chromatographic profiles of methanol extract, partitioned in ethyl acetate: chloroform solvent was developed using n-hexane:ethylacetate (7:3) ;these showed 10 spots respectively as shown. TLC Spot labels SMA, SMB, SMC and SMD denotes spots from fruit chloroform fraction, leaf ethylacetate fraction, fruit ethylacetate fraction and leaf chloroform fraction respectively.

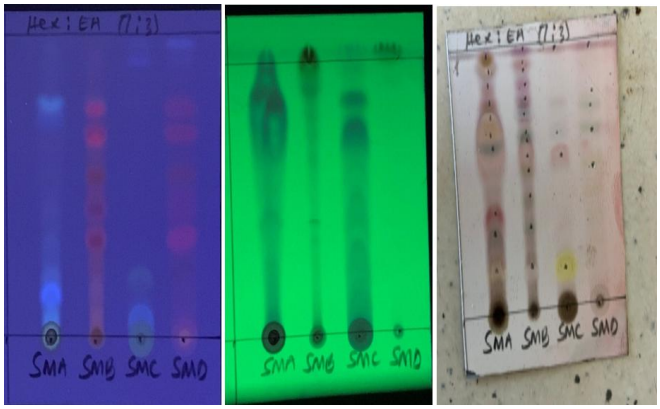

Plate 2. TLC Chromatogram of *Solanum macrocarpon* Fruit and Leaf Fractions on Precoated Silica gel Plate Observed Under UV Light at 254nm (Left) 365nm (Middle) and Daylight (Right) after Partition Chromatography

Antioxidant activity of *S. macrocarpon* fruits and leaf fractions (Figure 1) was analyzed using DPPH assay method. Results showed that *S. macrocarpon* fruit fraction had the highest antioxidant activity ($p < 0.05$) with a significant percentage outcome of 78% compared to the control (66%). However, there was a significant difference between all the fractions tested, with exception of ethylacetate and chloroform leaf fractions.

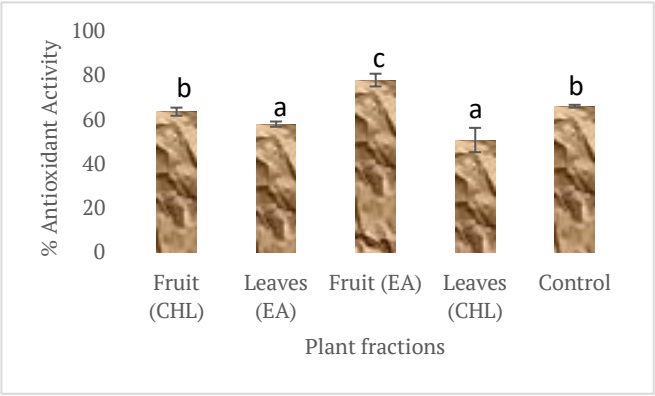

Figure 1. Antioxidant Activity of S. *macrocarpon* Fruit and Leaf Fractions
All data were represented as percentage activity

The antiglycation capacity of *S. macrocarpon* fruits and leaf fractions was assessed using BSA-Glucose model and the results are shown in Fig. 2. All samples presented statistically significant ($P < 0.05$) percentage antiglycation activity (77, 81,89 and 76%); when compared to the control (66%). However, the highest activity was recorded by ethylacetate fruit fraction (89%); which was able to inhibit glycation at 0.125mg/mL concentration (Figure 2).

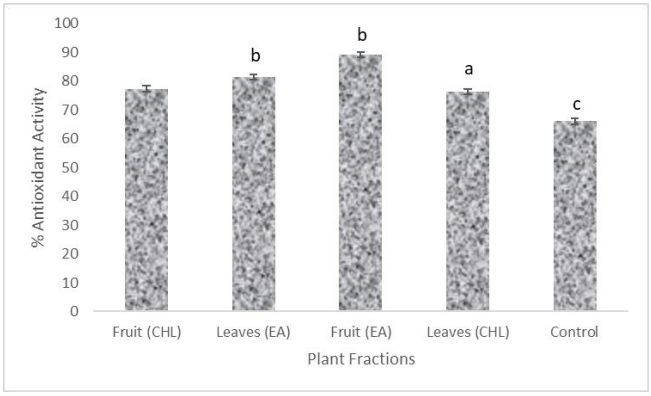

Figure 2. Antiglycation Activity of S. *macrocarpon* Fruit and Leaf Fractions
All data were represented as percentage activity

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of bioactive compounds identified in *S. macrocarpon* is shown in Table 1. Compounds present in the most active fraction with highest activity overall (ethylacetate fruit fractions) was analyzed using GC-MS and identified based on the database in the GC-MS library. The major groups of these spots were monoterpenoids and fatty acid-based compounds. The active fraction showed three major peaks (4, 8 and 10) at retention times of 36.2705, 37.8244 and 37.8244 minutes; identified as Tetradecanoic acid, n-Hexadecanoic acid and Cycloeicosane, respectively.

Molecular docking study of identified compounds was conducted to highlight the possible mechanism of antiglycation activity of *S. macrocarpon* fruit fraction. The docking process is based on the prediction of the position, orientation, and binding affinity of the ligand in the active sites of the targets. Docking results are presented in Table 2, while the interactions between the most active compound and the target is illustrated in Figures 3. The results showed that among the Nine (9) compounds determined by GC-MS, five compounds (Methoxyacetic acid, 3-pentadecyl ester; Tetradecanoic acid, n-Hexadecanoic acid, Cycloeicosane- and 3-Eicosene, (E)-), inserted perfectly into the binding site of BSA, with the lowest binding energy of −5.6, −5.8, −5.2, −8.3 and −7.0 kcal/mol respectively (Table 2). Methoxyacetic acid, 3-pentadecyl ester is linked to a Histidine residue by conventional hydrogen bond and arginine residue by a carbon-hydrogen bond. Tetradecanoic acid is linked to a Serine residue by conventional hydrogen bond and to Valine by carbon-hydrogen bond. Moreover, n-Hexadecanoic acid is linked to an Asparagine residue by carbon-hydrogen bond; however, the bond established with lysine was via an Alkyl/Pi-Alkyl interaction. 3-Eicosene, (E)- is linked to numerous amino acids residues (Ile7, Val23, Ala26, Phe27, Leu46, Leu66, His67, Leu69, Leu249, Leu250; Phe49) via Alkyl/Pi-Alkyl and Pi-Sigma interactions while for Cycloeicosane, the bond established with lysine was via an Alkyl interaction.

Table 1. Major Compounds Detected in *S. macrocarpon* Ethylacetate Fruit Fraction by GC-MS Analysis

Peak	Retention Time	Compounds	Similarity Index %	Common Names
1	12.9158	Undecane, 4,7-dimethyl-	83	
2	34.8295	Methoxyacetic acid, 3-pentadecyl ester	58	
3	36.2705	Tetradecanoic acid	90	Myristic acid
4	36.9485	Undec-10-ynoic acid, dodecyl ester	43	
5	37.1476	2-Piperidinone, N-[4-bromo-n-butyl]-	46	
6	37.2815	1,6-Octadiene, 3-ethoxy-3,7-dimethyl-	46	Ethyl linalool
7	37.8244	n-Hexadecanoic acid	95	Palmitic acid
8	38.1596	3-Eicosene, (E)-	50	Tetracosane
9	38.425	Cycloeicosane	90	

Table 2. Molecular Docking Score Values and Interactions of Ligands Present in *S. macrocarpon* Fruit Ethylacetate Fraction with Bovine Serum Albumin (BSA)

S/No	Ligands	PubChem ID	Binding energy (kcal/mol)	Interaction
1	Undecane, 4,7-dimethyl-	519389	-5.7	Alkyl/Pi-Alkyl: Phe506, Ala527, Leu574; Pi-Sigma: Phe508, Phe567
2	Methoxyacetic acid, 3-pentadecyl ester	542295	-5.6	Conventional Hydrogen Bond: His145; Carbon Hydrogen Bond: Arg144 ; Alkyl/Pi-Alkyl: Leu115, Pro117, Tyr137, Ile141, Phe164, Ile181, Val188
3	Tetradecanoic acid	11005	-5.8	Conventional Hydrogen Bond: Ser453; Carbon Hydrogen Bond: Val481 ; Alkyl/Pi-Alkyl: Phe205, Ala209, Leu346, Leu480; Unfavorable Acceptor-Acceptor: Ser343
4	Undec-10-ynoic acid, dodecyl ester_	91692432	-5.7	Alkyl/Pi-Alkyl: Leu505, Phe508, Lys524. Ala527, Leu528, Leu531, Val546, Met547, Leu574, Val575
5	2-Piperidinone, N-[4-bromo-n-butyl]-	536377	-5.1	Leu237, Arg256, Leu259, Ile289, Ala290
6	1,6-Octadiene, 3-ethoxy-3,7-dimethyl-_	175211	-5.6	Alkyl/Pi-Alkyl: Val554, Leu574; Pi-Sigma: Phe508
7	n-Hexadecanoic acid_	985	-5.2	Carbon Hydrogen Bond: Asn44; Alkyl/Pi-Alkyl: Lys20, Leu24, Phe36, Val40, Val43, Lys131, Lys132
8	3-Eicosene, (E)-	5365051	-7.0	Alkyl/Pi-Alkyl: Ile7, Val23, Ala26, Phe27, Leu46, Leu66, His67, Leu69, Leu249, Leu250 ; Pi-Sigma: Phe49
9	Cycloeicosane	520444	-8.3	Alkyl: Lys524; Vander Waals: Asn404, Ile512, Lys520, Gln521, Gln525, Leu528, Phe550, Val554
10	Calcitriol (control ligand)	5280453	-8.1	Alkyl/Pi-Alkyl: Ala193, Tyr451, Ile455. Vanderwaals: His145, Glu186, Leu189, Ala190, Glu424, Ser428, Lys431, Val432, Arg435, Leu454, Arg458.

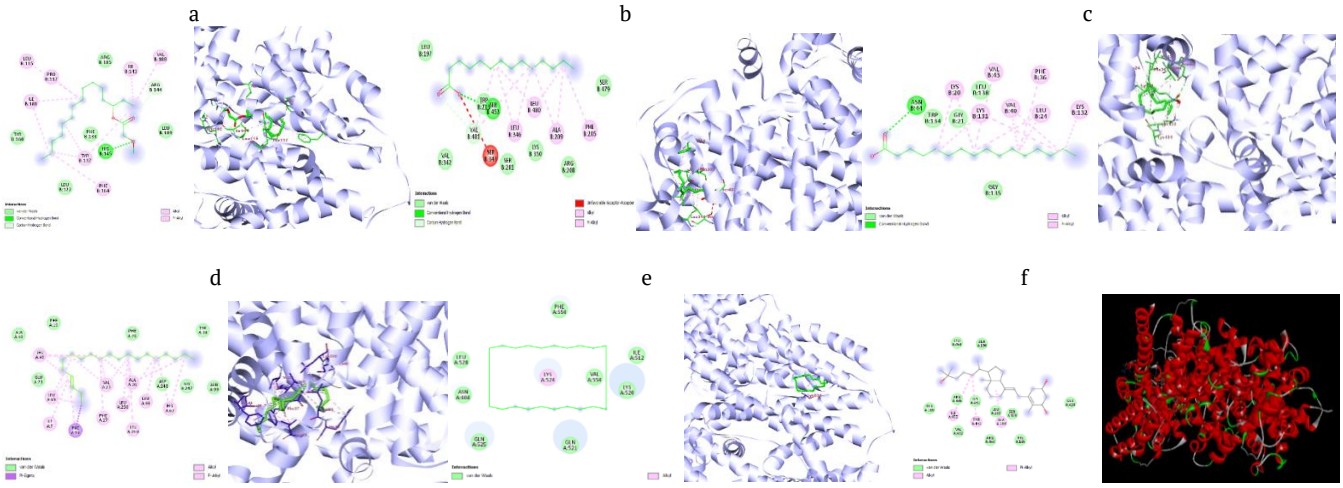

Figure 3. Two Dimensional (2D) and Three Dimensional (3D) View Showing Interactions Between (a) Methoxyacetic acid, 3-pentadecyl ester (b) Tetradecanoic acid (c) n-Hexadecanoic acid_ (d) 3-Eicosene, (E)- (e) Cycloeicosane, and (f) Calcitriol with neighboring residues of BSA.

DISCUSSION

In vitro antioxidant activity assay was carried out to assess the capacity of plant fractions to scavenge DPPH free radical. DPPH free radical scavenging is one of the accurate and most frequently employed assays for evaluating antioxidant activity. The ability of plant extractives to donate hydrogen atoms was tested by decolorizing a methanol solution of 2,2-diphenyl-1- picrylhydrazyl (DPPH). In the presence of antioxidants, DPPH generates a violet/purple color in methanol solution and diminishes to shades of yellow (Rahman *et al*., 2015). All fractions displayed high antioxidant capacities. However, ethylacetate fraction of *Solanum macrocarpon* fruits had the highest activity (77%) among other tested fractions.

To the best of our knowledge, this is the first-time research on antiglycation of *S. macrocarpon* fruits and leaf fractions was reported. Findings from our research revealed antiglycation activity of *S. macrocarpon* followed the same trend as the DPPH scavenging outcome, this was evident with ethylacetate fraction of the fruits having the highest activity (89%) among other tested fractions. Ethyl acetate fractions were more potent compared to chloroform fractions probably because the polarity of ethylacetate is moderate, so, ethylacetate been a moderately polar solvent must have extracted diverse phytocompounds that may have been both polar and non-polar, hence increasing its potency. Our findings are similar to some studies previously reported by Al-Musayeib *et al*., (2011) who reported anti-glycation, anti-inflammatory and antioxidant activities of ethyl acetate-soluble fraction of *Cordia sinensis* (Boraginaceae). Similarly, another research reported the efficacy of ethyl acetate fraction from *Rhus verniciflua* (lacquer tree) to inhibit recombinant human aldose reductase and accumulation of AGEs in BSA-glucose model system (Lee *et al*., 2008). Hence further study was embarked upon to elucidate the key principles in this fraction.

The chemical profiling of *Solanum macrocarpon* ethylacetate fruit fraction using GC-MS analysis showed presence of some compounds namely; Undecane, Methoxyacetic acid, Undec-10-ynoic acid, tetradecanoic acid, 2-piperidinone, 1,6-octadiene (monoterpenoid), n-hexadecanoic acid, 3-eicosene and cycloeicosane. interestingly, four of the compounds detected (cycloeicosane, tetradecanoic acid, undec-10-ynoic acid, dodecyl ester and n-hexadecanoic acid) in the present study were previously reported to have antioxidant activity (Dehpour *et al*., 2012).

Previous studies have confirmed that key amino acid residues involved in glycation process are lysine and arginine (Anguizola *et al*., 2013; Purnamasari *et al*., 2021), this commemorates most of the result findings in the present study. Molecular docking studies between identified GC-MS compounds and bovine serum albumin (BSA) revealed strong binding affinities ranging from -5.1 and -8.3 kcal/mol. Interestingly, methoxyacetic acid, tetradecanoic acid and n-hexadecanoic acid exhibited one polar contact each, involving residues Arg144, Ser453 and Asn44 respectively. However, n-hexadecanoic acid exhibited 3 non-polar contact with lysine residue; Lys20, Lys131 and Lys132. Additionally, both Undec-10-ynoic acid and 2-piperidinone established non-polar contacts with Lys524 and Arg256 respectively. Moreover, 3-eicosene and cycloeicosane did not establish any polar-contact with lysine or arginine residues.

Specifically, cycloeicosane- and 3-Eicosene, (E)- demonstrated a pronounced affinity for BSA with a binding score of -8.1 and -7.1 kcal/mol, respectively, indicating their potential to impede the binding of AGEs to BSA protein; however, for cycloeicosane, the bond established with lysine was via an Alkyl and Vanderwaal interactions (Figure 3). This is not in line with previous researches that reported bonding of antiglycating compounds with Lysine residue via carbon-hydrogen interactions (Anguizola *et al*., 2013; Purnamasari *et al*., 2021; Ouamnina *et al*., 2024).

To validate our docking protocol, redocking was carried out using a control ligand (calcitriol). Redocking results confirmed this compound's strong binding affinity to the BSA protein (8.1) which was similar to a study by Pawlukianiec *et al.*, (2025) whose docking score was -8. This proved the suitability of the docking protocol for the current study.

Findings from the present study highlighted an unconventional outcome in terms of hydrogen bonding of ligands and binding affinity. Three compounds (methoxyacetic acid, 3-pentadecyl ester; tetradecanoic acid and n-hexadecanoic acid) established hydrogen bond formation with BSA, while recording the least binding energy scores of −5.6, −5.8 and −5.2 kcal/mol respectively.

CONCLUSION

In conclusion, ethylacetate fraction of *Solanum macrocarpon* fruits had the highest activity for both DPPH scavenging and antiglycation assay. Compounds detected using GC-MS analysis have previously been reported to have antioxidant activity and hence it can be linked to the remarkable antiglycation activity observed in this study. However, an *in vivo* study is necessary to further elucidate these findings. Comparatively, two compounds (cycloeicosane- and 3-cicosene, (E)-) having higher binding energy scores of -8.1 and -7.1 established bonds via alkyl/Pi-Alkyl and Pi-Sigma bonds respectively.

AUTHORS' CONTRIBUTIONS

Conceptualization: HSU, ABS, MAS; Laboratory experiments: HSU, AY, MAS; Data Analysis: HSU, AY, MAS; Writing-original draft preparation: HSU, AY; Writing-review and editing: HSU, AY, FA, MAS, UAU, ABS; Resources: HSU, AY, FA, MAS, UAU, ABS; Supervision: HSU, ABS. All authors approved the final version of the manuscript.

FUNDING STATEMENT

No funding was received for the research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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