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ARTICLE

Solanum melongena Fruits and Leaf Extracts can Inhibit Advanced Glycation End Products (AGEs) and 1-diphenyl-2-Picryl-hydrazyl (DPPH) Radical *In vitro*: A Preliminary Study

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Abstracts

Background: Oxidative stress is a deleterious effect of several diseases and is linked to production of advanced glycation end products (AGEs). Nigeria and other countries use medicinal herbs, such *Solanum melongena* L. (Solanaceae), extensively for the traditional treatment of illnesses. Aim: The current study aimed at assessing phytochemical components, *in vitro* antioxidant and antiglycation properties of extracts from *S. melongena* fruit and leaves. Methods: AGEs derived from incubating bovine serum albumin (BSA) and glucose was characterized by Spectro-fluorescence. Fruit and leaf samples were extracted with chloroform, ethyl acetate and methanol and tested for antioxidant and antiglycation activities following phytochemical constituents evaluation. Results: Our result indicated the presence of alkaloids, saponins and flavonoids in all the plant extracts. Furthermore, the chloroform extract of the fruit displayed a significant ($p < 0.05$) activity on 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging (97.21%) and antiglycation assay (79.14%), compared to other extracts. Additionally, the ethyl acetate extract of the leaves exhibited significantly more potent DPPH radical scavenging (86%) and antiglycation (79%) activities compared to other extracts, respectively. Conclusion: Evidence from this study demonstrated that fruit and leaves of *S. melongena*, particularly the chloroform and ethyl acetate extract, has potent antioxidant and antiglycation potentials that necessitate further research studies to identify the active key principles.

Keywords: Antiglycation, Antioxidant, Phytochemicals, Fruit, Leaves, *Solanum melongena*

1.0 Introduction

There is growing evidence that pathogenesis of numerous human illnesses, such as cancer, heart disease, inflammatory conditions, and neurological problems, are significantly influenced by Free radical-induced oxidative stress (Schieber and Chandel, 2014). Furthermore, glycation and oxidative stress are closely related events that are commonly referred to as "glycoxidation" (Chaouch et al., 2016). An early glycation product is formed when reducing sugars and proteins undergo an impromptu, non-enzymatic amino–carbonyl reaction. This product then goes through cyclization, dehydration, and rearrangement to produce Schiff base and Amadori products, which in turn forms advanced glycation end products (AGEs) (Zhu et al., 2019a; Wu et al., 2011). Numerous chronic illnesses, including aging, arteriosclerosis, and complications from diabetes, can be caused by AGEs (Zhang et al., 2011; Lee et al., 2007). Studies have shown that the mechanism of antiglycation may be linked to its antioxidant activity (Zhu et al., 2019b).

The therapeutic qualities of plants have garnered more interest recently, especially their capacity to treat a range of illnesses linked to inflammation and oxidative stress. *Solanum melongena* commonly known as Eggplant, is a popular vegetable that is prized for its varied phytochemical content and high nutrient profile. Although it has long been utilized in culinary traditions across many cultures, its medicinal uses are starting to get scientific attention, especially in relation to its antioxidant and anti-hyperglycemic qualities (Gürbüz et al., 2018; Silva et al., 2020).

Plate 1: Fruits and Leaves of *Solanum melongena*(https://harvesttotable.com/howtogroweggplant/)

Solanum melongena L. (Solanaceae) is commonly known as aubergine and 'yalon turawa' in Hausa language; it is grown all over the world, primarily in tropical and subtropical areas, but thrives in temperate temperatures as well. Furthermore, because it grows in warm, humid areas, it is regarded as a valuable commercial item. These and other qualities allow for a global production of 55,197,878 tons, with China, India, Egypt, and Turkey being the primary producers (Lim, 2012; Sultana et al., 2013; Karimi et al., 2021). In some previous studies, antidiabetic (Nwanna et al., 2013), anti-inflammatory (Umamageswari et al., 2015; Im et al., 2016), antiproliferative (Milner et al., 2011), analgesic (Mutalik et al., 2003), antimicrobial (dos Santos Oliveira and Furlong, 2008; Salamatullah et al., 2021) and anticancer properties (Afshari et al., 2017; Fekry et al., 2019; Shabana et al., 2013) are mainly related to its secondary metabolite contents.

This study aims to present the potential of *S. melongena* fruits and leaf extracts in inhibition of Advanced Glycation End Products (AGEs) and DPPH radical *in vitro*. Investigating *Solanum melongena* may open up new possibilities for natural treatment approaches to oxidative stress and glycation-related conditions. The preliminary findings reported here will also serve as a basis for additional studies that could clarify the mechanisms and possible therapeutic uses of *S. melongena* extracts in the field of drug development and discovery.

Only few studies exist in literature, regarding antiglycation activity of *S. melongena* fruits and leaves; which were based on aqueous extracts (Nisha et al., 2009; Sathishkumar et al., 2015), there remains a conspicuous research gap regarding their antiglycation activity using different solvent systems, especially chloroform and ethylacetate, hence the need for this research.

2.0 EXPERIMENTAL

2.1 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.

2.2 Sample Collection

Solanum melongena leaves and fruits were collected from a farm in Katsina State, Nigeria. Plant samples were authenticated by a taxonomist at Ahmadu Bello University's Department of Botany, Faculty of Life Sciences, Zaria, Kaduna State, Nigeria; where a voucher number of (ABU01054) was deposited.

2.3 Preparation of Plant Crude Extracts

Using a pestle and mortar, air-dried whole plant materials were mashed into a fine powder. Ten grams of powdered plant material were extracted individually using 150 milliliters of methanol, ethyl-acetate, and chloroform. The supernatants were filtered through a Whatman No. 1 filter paper into glass vials that had been previously weighed and allowed to air dry. The amount of plant material that was extracted was measured and kept in dark, airtight glass vials until it was needed (Oloya et al., 2022).

2.4 Phytochemical Evaluation of Plant Extracts

Phytochemicals in fruit and leaf extracts of *S. melongena* was evaluated through colour change or precipitate formation assays such as flavonoids (Shinoda Method), phenolic compounds (Ferric chloride), alkaloids (Dragendorff, Wagner and Mayer Methods), triterpenes and steroids (Liebermann–Buchard Method). Considering the following symbols: presence [+] and absence [-], the results were expressed as the relative presence of the phytochemicals (Harborne, 1998; Evans, 2002).

2.5 Antioxidant Activity of Plant Extracts

The plant extracts' capacity to function as antioxidants was assessed using the DPPH free radical scavenging assay reported by Shah et al. (2013). In brief, 0.1 ml of methanol, 1 mg/ml ascorbic acid, and 1 mg/ml plant extract were applied in triplicate to the control, standard, and extract tubes, respectively. Then, 3 ml of 0.24 mg/ml DPPH (prepared in methanol) was added to the test tubes. The mixture was then mixed for 5 minutes before incubating in the dark at 25 °C for 30 minutes. The absorbance was measured at 517 nm. The percentage antioxidant or free radical scavenging activity of extract and ascorbic acid was calculated using the formula below (Shah et al. 2013):

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

2.6 Antiglycation Activity of Plant Extracts

The antiglycation activity of the extracts was estimated based on the method of Kaewnarin et al., (2014). In brief, 20 µl each of 800 µg/ml BSA and 200 mM D-glucose were added, in triplicate, into test tubes labeled; standard and plant extracts (ethyl acetate and chloroform). Thereafter, 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 extracts as mentioned above, 1 mg/ml of both aminoguanidine and 1 mg/ml plant extract (prepared in phosphate buffer containing sodium azide) was added into test tubes labeled standard and plant extracts respectively. Afterwards, the mixture was incubated at 37 °C for 7 days. Fluorescence intensity was measured at 370 nm excitation and 440 nm emission. Percentage antiglycation activity of the extracts and aminoguanidine was estimated using the following formula (Kaewnarin et al., 2014):

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

2.7 Data Analysis

Data was analyzed by one-way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) version 20 for Windows, with all trials conducted in triplicate. The Duncan post-hoc test was used to find discrepancies between the means of several test solutions. A P value of less than 0.05 ($p < 0.05$) indicated statistical significance.

3.0 RESULTS AND DISCUSSION

Saponins, triterpenes, tannins, and flavonoids were found to be the phytoconstituents in ethylacetate, chloroform, and methanolic extracts of *Solanum melongena* leaves and fruits. Additionally, all plants contained flavonoids, saponins, and alkaloids extracts (Table 1). According to Osei Akoto et al., (2020), the phytochemical makeup of plants dictates their pharmacological and biochemical action. Our findings showed that all of the plant extracts of *S. melongena* included alkaloids, saponins, and flavonoids; a similar pattern was noted in a prior study (Usman et al., 2018). Flavonoids have been implicated in the prevention of cancer, heart disease, asthma, and stroke, while saponins have been shown to possess anti-inflammatory activity (Ravikumar et al., 2016). Alkaloids have been reported to have pharmacological activities which include wound-healing, antimicrobial, antioxidant and antiplasmodial (Vigbedor et al., 2022).

Table 1: Qualitative Phytochemical constituents of *Solanum melongena* fruit and leaf extracts

Phytochemicals	FEM	FEC	FEEA	LEM	LEC	LEEA
Alkaloids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Steroids	+	ND	+	+	ND	ND
Anthracenes	ND	ND	+	+	ND	ND
Triterpenes	ND	ND	ND	+	+	ND
Flavonoids	+	+	+	+	+	+
Tannins	ND	ND	ND	+	+	ND

KEY

ND signify not detected while **+** signifies the presence of a phytochemical

FEM = Fruit Extract Methanol **FEC** = Fruit Extract Chloroform

FEAE = Fruit Extract Ethyl Acetate **LEM** = Leaf Extract Methanol

LEC = Leaf Extract Chloroform **LEAE**= Leaf Ethyl Acetate Extract

Figure 1 illustrates chloroform extract's antioxidant activity of *S. melongena* fruit, this was significantly ($p < 0.05$) high (97.21 %) compared to ascorbic acid (control). Comparatively, leaf ethylacetate extract displayed a higher antioxidant activity (86.29 %) compared to methanol (82.66 %) and chloroform extract (51.13 %) (Figure 2). The DPPH radical scavenging capacity between ethylacetate leaf extract and ascorbic acid was significantly low ($p < 0.05$). All data were presented as mean ± SD of triplicate values. a-c values with different alphabets over the bars are significantly ($p < 0.05$) different from each other.

By comparing the ability of various samples to scavenge free radicals, the DPPH radical is commonly used as a substrate to evaluate the efficacy of antioxidants (Amarowicz et al., 2004). A primary finding from this study is the substantial DPPH radical scavenging activity exhibited by *S. melongena* fruit chloroform extract (97 %). This is consistent with a previous study that have demonstrated the antioxidant potential of various parts of eggplant (Jung et al., 2011). However, our findings differ from previous research (Nisha et al., 2009) in which the fruit methanol extract of the big purple eggplant variety, gave the lowest DPPH scavenging score as compared to the small and moderate variety; this observation may be due to the influence of geographical location on plants and their variations on constituents. Also, the solvent used for the extraction may account for the differences. Furthermore, a study by Gan et al., (2017) reported a positive correlation between alkaloids and DPPH scavenging ability, which correlates with the phytochemical profiling of our findings. Previous research also indicates a positive correlation between antioxidant activity and antiglycation activity (Ramkissoon et al., 2013; Usman et al., 2023a).

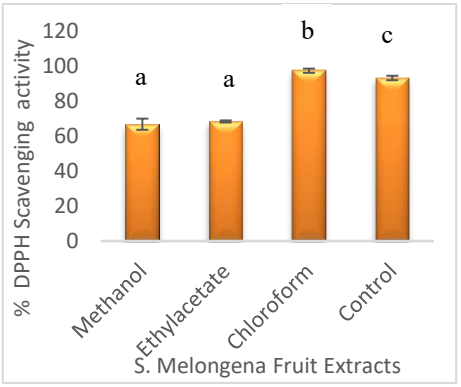

Figure 1: *In vitro* DPPH Radical Scavenging Activity of *Solanum melongena* Fruit Extracts: data are shown as percentage DPPH scavenging activity.

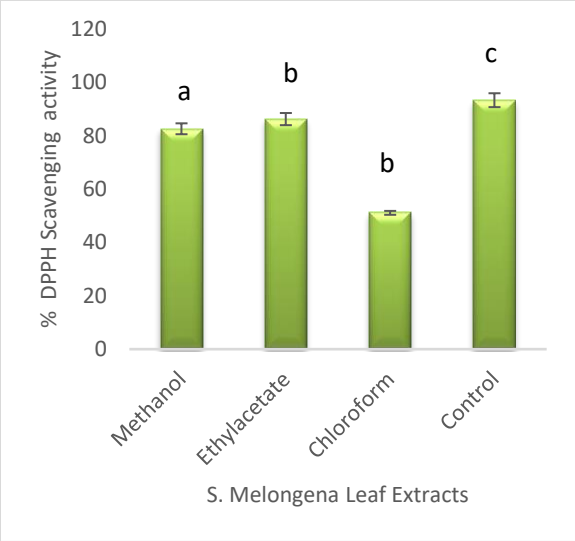

Figure 2: *In vitro* DPPH Radical Scavenging Activity of *Solanum melongena* Leaf Extracts: data are shown as percentage DPPH scavenging activity.

The results from antiglycation activity of *S. melongena* fruits is depicted in Figure 3. *S. melongena* chloroform fruit extract had the highest antiglycation activity (79.14 %) compared to methanol (49.17 %) and ethylacetate (21.29 %), however this was significantly (p < 0.05) low compared to the control (aminoguanidine). Overall, ethylacetate fruit extract had the least antiglycation activity (21.29 %) as shown below (Figure 3) while ethylacetate leaf extract had the most antiglycation activity (79.26 %) as shown in (Figure 4). All data were presented as mean ± SD of triplicate values. a-d values with different alphabets over the bars are significantly (p < 0.05) different from each other.

The inhibition of AGEs by *S. melongena* extracts represents an important aspect of this research. Advanced Glycated End-Products (AGEs) are connected to several long-term conditions, including diabetes and heart disease, and their formation is often influenced by oxidative stress (Giacco and Brownlee, 2010). In the present study, *S. melongena* fruit and leaf extracts demonstrated a notable capacity (79 %) to inhibit the formation of AGEs. This finding slightly differs from a previous study on *S. melongena* fruit, which revealed 80 % and 58 % antiglycation activity for acetone and hot water extracts respectively (Sathishkumar et al., 2015). The rich phytochemical profile of *Solanum melongena* extracts, especially flavonoids, which have been demonstrated to disrupt Maillard reaction pathway and hence stop the formation of AGEs, may be responsible for the extracts' anti-glycation efficacy. Phytochemicals and polyphenolic substances from different plant sources have been shown to have similar anti-glycation properties (Usman et al., 2023b; Afzan et al., 2024). In order to support the development of functional foods that specifically target glycation-related disorders, future research should concentrate on finding particular bioactive components in the extracts that inhibit AGEs.

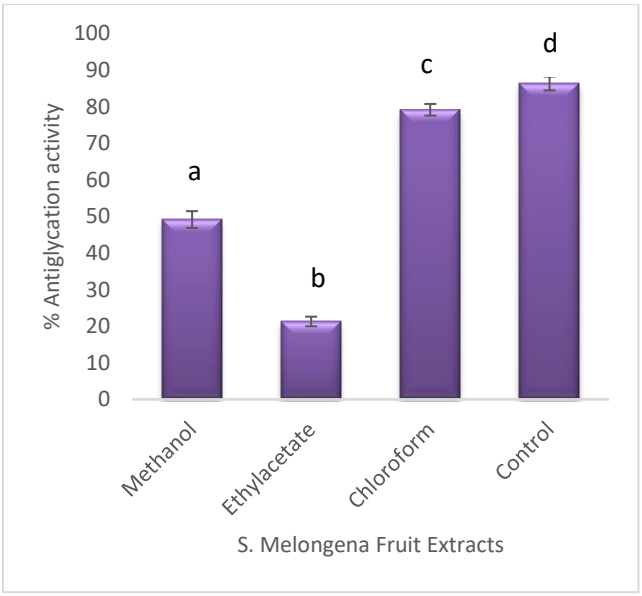

Figure 3: *In vitro* Antiglycation Activity of *Solanum melongena* Fruit Extracts: data are shown as percentage antiglycation activity

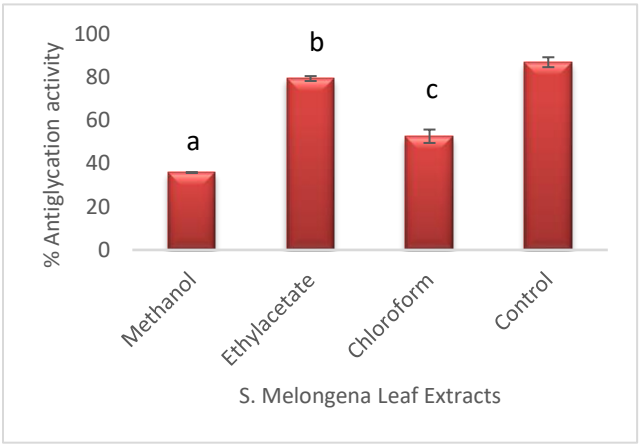

Figure 4: *In vitro* Antiglycation Activity of *Solanum melongena* Leaf Extracts: data are shown as percentage antiglycation activity.

4.0 Conclusion

The significant findings obtained from this study indicate that *S. melongena* fruits and leaves extracts possess notable antioxidant and anti-glycation properties, aligning with previous research and reinforcing its therapeutic potential in managing oxidative stress and related pathologies. These results would pave way for additional research on eggplant's potential as a candidate in drug design and discovery that guards against chronic illnesses and oxidative stress. Foods with natural antioxidant and anti-glycation capacity, such as *S. melongena*, may be beneficial dietary treatments as the prevalence of oxidative stress and glycation-related disorders (diabetes, Alzheimer's disease, and other age-related conditions) keeps increasing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

INDIVIDUAL'S AUTHOR CONTRIBUTION

Conception: HSU, ABS
Design: HSU, IG, ABS
Execution: HSU, IG, FA, UAU
Interpretation: HSU, IG, FA, UAU, ABS
Writing the paper: HSU, IG; FA, UAU, ABS

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