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Research Article

Evaluation of Advanced Glycation End Products and DPPH (1-DIPHENYL-2-PICRYL-HYDRAZYL) Inhibitory Potential of *Solanum macrocarpon* Fruits and Leaves: An *In vitro* Study

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ABSTRACT

Advanced Glycation End Products (AGEs), are produced when sugars and free amino groups in proteins undergo a non-enzymatic event known as glycation. A key therapeutic strategy against the advancement of glycation and its associated health issues, such as hyperglycemia and atherosclerosis, is the inhibition of AGE generation. The present study was carried out to evaluate the qualitative phytochemical constituent as well as in vitro antioxidant and antiglycation potential of extracts from fruit and leaves of *Solanum macrocarpon*. AGEs derived from incubation of bovine serum albumin (BSA) and glucose was characterized by spectrofluorescence. Fruit and leaf samples were extracted with chloroform, ethyl acetate and methanol, followed by phytochemical constituent evaluation, antioxidant and antiglycation determination. Our results indicated the presence of alkaloids, saponins, tannins and flavonoids in all the plant extracts. Results obtained from the antioxidant assay showed highest percentage antioxidant activity for both the fruit and leaf extracts of *S. macrocarpon* were found in the methanolic extract (98.95%) and chloroform extract (92.09%) respectively. Similarly, the highest percentage antiglycation activity for both fruit and leaf extracts were found in methanolic extract (71.84%) and ethyl acetate extract (62.91%) respectively. The plant thus, exhibit the ability to significantly decrease 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical levels, alongside advanced glycation end products (AGES), implying its potential as an ameliorating agent.

Keywords: *Antiglycation, Free radical, Phytochemicals, Solanum macrocarpon*

INTRODUCTION

Glycation is a non-enzymatic process that results in the production of advanced glycation end products (AGEs) when proteins react with reducing sugars. Endothelial dysfunction, lipid peroxidation, protein structural alterations, and promotion of inappropriate cellular activity are the causes of AGE accumulation, which damages cells and tissues (Singh et al., 2017; Galiniak et al., 2017). Previous research indicates that a higher prevalence of myasthenia gravis (Adamsczyk-Sowa et al., 2017), diabetes and its effects (Adamska et al., 2018; Rhee and Kim, 2018), and neurodegenerative disorders (Pinkas and Aschner, 2016) is linked to increased glycation.

The well-known antiglycating drug; aminoguanidine (AG) stops the development of diabetic complications by blocking the production of AGEs. However, major side effects such as myocardial infarction, congestive heart failure, atrial fibrillation, anemia, and gastrointestinal disruption have led to the discontinuation of aminoguanidine (Thornalley, 2003; Friedman, 2010). Anti-glycation medicines generated from plants have recently been seen as viable instruments to deal with human aging and the emergence of many disorders. There is mounting evidence that natural substances provide anti-glycation efficacy (Intagliata et al., 2021).

An imbalance between the production of reactive oxygen species (ROS) and the body's natural antioxidants causes oxidative stress in humans, which can harm proteins, lipids, and DNA through a series of reactions (Rudrapal et al., 2022). Because it can neutralize and stop free radicals, such as reactive oxygen species (ROS) and their derivatives, from harming cells, antioxidant activity is crucial to cellular physiology. By scavenging reactive free radicals and preventing lipid peroxidation and other associated processes, antioxidants shield the organism against the diseases that can arise from them (Pande and Srinivasan, 2013).

Solanum macrocarpon is a common plant genus in the Solanaceae family; with approximately 1000 species globally including at least 100 native species in Africa and the nearby islands (Osei et al., 2010). In indigenous medicine, *S. macrocarpon* is used for a variety of purposes, such as weight loss and the treatment of rheumatic disease, swollen joint pains, asthma, allergic rhinitis, nasal catarrh, skin infections, gastroesophageal reflux disease, constipation, and dyspepsia (Bello et al., 2005). 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 the sulfur-containing amino acid methionine (Messiaen, 1992; Komlaga et al., 2014), the leaves are high in calcium and potassium (Ojo et al., 2015; Sereno et al., 2018). Scientific studies opined that *S. macrocarpon* constitutes valuable nutrients and bioactive compounds. While its widely used in cooking and traditional medicine, its medicinal potential extends to pharmaceuticals and health products, utilizing not only the fruit and leaves but also other parts like flowers and roots (Ahn et al., 2025).

Previous studies reported antioxidant activities of aqueous and ethanol extracts of Solanum macrocarpon leaves (Okesola et al., 2020; Osei-Owusu et al., 2023). However, there is paucity of data on bioassay guided assessment of the aforementioned plant using chloroform, ethyl acetate and methanol solvent systems with regards to in vitro radical scavenging and antiglycation approach, hence the need to carry out this research. In this context, we made an attempt to determine the DPPH radical scavenging capacity and antiglycation activity of Solanum macrocarpon fruits and leaf extracts using different solvent system.

MATERIALS AND METHODS

Chemicals and reagents

Methanol, Ethyl-acetate, Chloroform, 1,1-diphenyl-2-picrylhydrazyl (DPPH). Phosphate buffer, Ascorbic acid, Bovine serum albumin (BSA), Glucose, Phosphate buffered saline (PBS), Trichloroacetic acid, Aminoguanidine,

Plant material

Solanum macrocarpon leaves and fruits were collected in August of 2019 from a natural habitat in Zaria, Kaduna state. Plant samples were authenticated at the Department of Botany, Faculty of Life Science, Ahmadu Bello University, Zaria, Kaduna state, Nigeria; where a voucher number (019380) was deposited.

Plant extract preparation

Using a pestle and mortar, air-dried plant samples were mashed into a fine powder. Then 150 ml of methanol, ethyl-acetate, and chloroform were used to extract 10 g of powdered plant material separately. After being filtered through a Whatman No. 1 filter paper into glass vials that had been previously weighed, the supernatants were 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.

DPPH radical scavenging activity

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay was used to quantitatively measure each extract's antioxidant activity spectrophotometrically (Shah *et al*., 2013). A solution of DPPH (0.135 mM) in methanol was made, and 1 ml of it was combined with 1 ml of extracts at various concentrations (1000 µg/l, 500 µg/l, 250 µg/L, 125 µg/l, and 62.5 µg/L). For half an hour, the mixture was incubated. Vitamin C, or L-ascorbic acid, served as a control. A spectrophotometer was used to detect absorbance at 517 nm. The extent of discolouration reveals the extracts' capacity for hydrogen donation and scavenging. The following formula was used to convert the acquired absorbance values to % scavenging activity:

$$\% \text{ inhibition} = \frac{(A517\text{nm of control} - A517\text{nm of sample}) \times 100)}{(A517\text{nm of blank})}$$

Where; Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract or standard.

Antiglycation assay

Protein glycation inhibition was carried out in accordance with Matsuura et al. (2002) and Kaewnarin et al. (2014). 800 µg/mL bovine serum albumin (BSA), 200 mM D-glucose with or without the extract (1 mg/mL) in phosphate buffer (50 mM, pH 7.4), and 0.2 g/L sodium azide (NaN_3) all constitutes the reaction mixture (2 mL). For seven days, the reaction mixture was incubated at 37°C. The intensity of the fluorescence was measured using a Cary Eclipse fluorescence spectrophotometer at 370 nm for excitation and 440 nm for emission. As a positive control, 1 mg/mL of aminoguanidine was utilized. The following formula was used to determine the results, which were expressed as the percentage of AGE inhibition:

$$\text{Inhibition (\%)} = [(F0 - F1)/F0] \times 100$$

Where F1 and F0 represent the fluorescence intensity of the sample and the control mixtures, respectively.

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

Table 1 shows various phytoconstituents identified in ethylacetate, chloroform and methanol extracts of *Solanum macrocarpon* leaves and fruits, revealing the presence of saponins, triterpenes, cardiac glycosides, tannins and flavonoids. Moreover, alkaloids were identified in all the plant extracts evaluated.

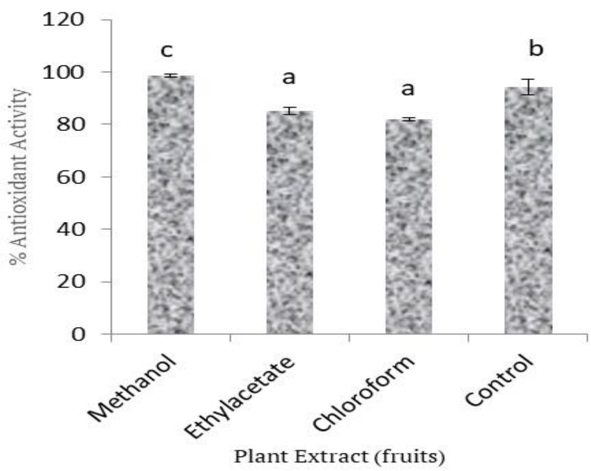
Table 1. Preliminary Phytochemical Profile of Fruits and Leaf Extracts of *Solanum macrocarpon*

Phytochemicals	SMMF	SMCF	SMEF	SMML	SMCL	SMEL
Saponins	-	-	-	+	-	-
Cardiac Glycosides	-	-	+	-	-	+
Steroids And Triterpenes	+	-	-	+	+	+
Flavonoids	-	-	+	-	+	+
Tannins	-	-	-	+	+	+
Anthraquinones	+	-	-	-	-	+
Alkaloids	++	++	++	++	++	++

Key: (+) = present, (−) = absent, (++) = highly present

SMMF: *S. macrocarpon* methanol fruit extract, SMCF: *S. macrocarpon* chloroform fruit extract, SMEF: *S. macrocarpon* ethyl acetate fruit extract, SMML: *S. macrocarpon* methanol leaf extract, SMCL: *S. macrocarpon* chloroform leaf extract, SMEL: *S. macrocarpon* ethyl acetate leaf extract.

Figure 1 shows antioxidant activity of *Solanum macrocarpon* fruit (left) and leaf (right) extracts. Compared to ascorbic acid standard, antioxidant activity of methanol extract of S. macrocarpon fruit was significantly ($p < 0.05$) high (98.52%). Comparatively, leaf chloroform extract displayed a higher antioxidant activity (92.71%) compared to methanol (83.25%) and ethyl acetate extract (84.25%). The DPPH radical scavenging capacity between chloroform leaf extract and ascorbic acid was significantly low ($p < 0.05$).



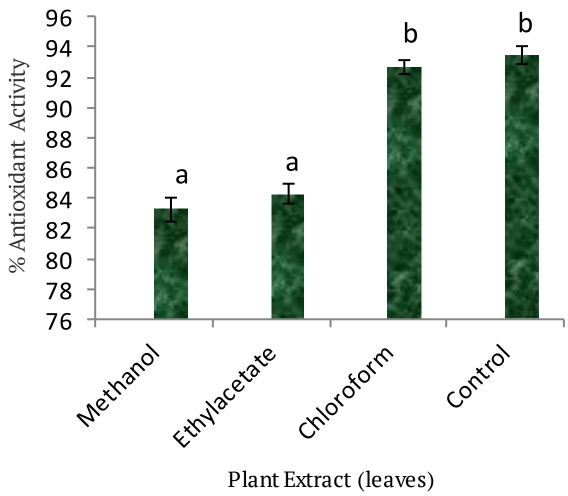
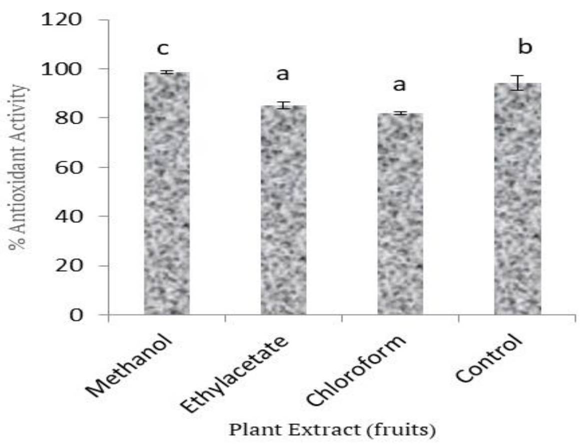


Figure 1: Antioxidant Activity of *Solanum macrocarpon* fruit (left) and leaf (right) extracts: data are shown as percentage antioxidant activity.

Figure 2 shows antiglycation activity of *Solanum macrocarpon* fruit (left) and leaf (right) extracts. *S. macrocarpon* fruit extract had the highest antiglycation activity (71.84%), however this was significantly ($p < 0.05$) low compared to the control (aminoguanidine). Overall, methanol extract had the least antiglycation activity (38.38%).



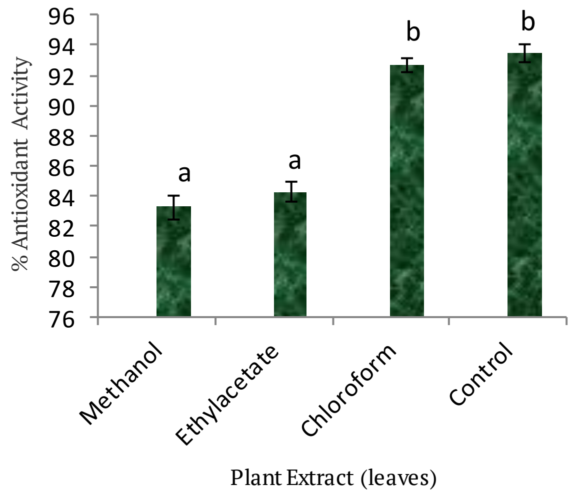


Figure 2: Antiglycation Activity of *Solanum macrocarpon* fruit (left) and leaf (right) extracts: data are shown as percentage antiglycation activity.

DISCUSSION

According to World Health Organization (WHO) reports, around 80% of people worldwide rely on plant-based medications, due to favorable impacts on health and reduced risk of side effects (WHO, 2024). In Nigeria, Solanum macrocarpon is a green leafy vegetable customarily eaten as an additive in soups. The fruits are often eaten as a snack or dessert, with a peanut-paste dip. Findings from this research revealed the presence of the following phytochemicals; alkaloids, anthraquinones, flavonoids, glycosides, saponins, tannins and terpenes in the leaf and fruit extracts of S. macrocarpon; which appear to be the commonly reported phytochemicals (Saxena et al., 2013; Usman et al., 2018; van Wyk et al., 2017; Usman et al., 2023a). Laboratory-based in vitro studies have associated the aforementioned compounds with anti-inflammatory, antidiabetic, anticancer, antiglycation and antioxidant properties (Usman et al., 2023b; Proshkina et al., 2020; Maisetta et al., 2019).

DPPH free-radical scavenging activity of S. macrocarpon fruit and leaf extracts was evaluated at 517 nm. Decrease in absorbance with an increase in concentration of the extracts was an indication of the extracts donating protons to DPPH. However, with respect to percentage inhibitory activity of the extracts, methanol fruit extract exhibited highest DPPH inhibitory activity of 98% overall. This was followed by ascorbic acid (94%) standard, and chloroform leaf extract (92%). Inferentially, DPPH scavenging activity of S. macrocarpon fruit extract tend to increase with increase in polarity of the solvent used for the extraction increases, implying the more polar methanol extract in this study had the highest DPPH free-radical activity compared to ethylacetate and chloroform extracts. This finding is intriguing considering the fact that the methanol extract was more active than the standard antioxidant compound used. A similar trend was reported by previous research (Mapfumari et al., 2022) in which the antioxidant activity of methanolic extract of a South African Mistletoe tends to increase as the polarity of the extraction solvent increases. Moreover, a reverse trend was observed in the leaf extract of S. macrocarpon; which showed increase in DPPH scavenging activity increases with decrease in polarity of the solvent used for the extraction. A rational explanation to this observation could be that the scavenging of free radicals from DPPH species by Solanum macrocarpon extracts is not a function of solvent polarity.

Inferentially, a major phytochemical found in this study—the flavonoid—may be responsible for the DPPH scavenging ability. Because of their antioxidant qualities, flavonoids help shield plants from a variety of biotic and abiotic stressors. Plants' secondary metabolic pathways are essential for producing flavonoids in response to oxidative stress. Furthermore, leafy plants' flavonoids are important because they serve as a barrier against strong sunlight exposure (Behn et al., 2011).

Studies have demonstrated that AGEs produced during glycation processes can raise oxidative stress; thus, an increase in free radicals causes sugars in various biomolecules to glycate more readily. Because AGEs regulate oxidative stress and protein glycation, antioxidant and anti-glycation properties are therefore connected (Dil et al., 2019; Neha et al., 2019). In the present study, the antiglycation activity of the extracts (S. macrocarpon) was assessed in vitro. Both fruit and leaf extracts showed antiglycation activity against glycation, demonstrated in the Bovine Serum Albumin (BSA)-glucose model. However, methanolic extract from fruit samples had the highest antiglycation activity overall. Similar studies demonstrated antiglycation activity of plant compounds on glycating agents (Gilabert-Oriol et al., 2015; Prasad et al., 2019; Usman et al., 2025).

AGEs mainly affect long-lived proteins, like hemoglobin, collagen, or elastin (Nadjib et al., 2018; Bansode and Gacche, 2019). Effects of glycation and AGEs generation on short-lived proteins, including plasma albumin, are also of interest because they cause several structural and functional modifications (Zendjabil, 2020). For this reason, study of the impacts of glycation in albumin, is an emerging trend. In vitro model systems of protein glycation using BSA (BSA has approximately 76% sequence homology to human serum albumin) incubated with sugars, have appeared as an interesting option to investigate deleterious consequences of protein glycation (Rabbani and Ahn, 2019) as well as the protective effects of natural compounds (Khan et al., 2020).

CONCLUSION

Conclusively, our preliminary findings opined that extracts of *S. macrocarpon* fruits and leaves present antiglycation and antioxidant activity in BSA-glucose model *In vitro*. However, methanolic fruit extract showed a greater activity in the evaluations performed, demonstrating that the polar bioactive compounds present in the extract may be directly associated with the activities investigated. Further studies will focus on bioassay guided fractionation of the most potent extract to elucidate and characterize the active principles present in the plant.

AUTHORS' CONTRIBUTIONS

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

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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