Effect of *Syzygium guineense* and *Borassus aethiopum* Leaves on Protein Glycation and Oxidative Stress Suppression

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**ABSTRACT**

The quest for discovery of a new antiglycation and antioxidant drug still remains a major priority in medicine and related clinical sectors. Against this backdrop, the antioxidant and antiglycation activities of ethylacetate, chloroform, methanol and aqueous extracts of *Syzygium guineense* (SG) and *Borassus aethiopum* (BA) leaves including their phytochemical compositions were evaluated in an in vitro trial. DPPH free radical scavenging capacity, antiglycation activity and qualitative phytochemical screening in vitro assay were employed respectively. Our result revealed that triterpenes, cardiac glycosides, tannins and flavonoids were detected in the plants leaves extracts. The results demonstrated a significantly ($p < 0.05$) low antioxidant and antiglycation activities except the aqueous extract of BA leaves, which displayed a significantly ($p < 0.05$) high antiglycation ability. Overall, data from the current study showed that ethylacetate, chloroform, methanol and aqueous extracts of the plants leaves have potential effect towards lowering oxidative stress and protein glycation and thus should be exploited for further research in the area of drug discovery.

**KEYWORDS:** *Syzygium guineense; Borassus aethiopum; Antioxidant; Antiglycation; Phytochemicals*

**INTRODUCTION**

Protein glycation and oxidative stress are physiological phenomena associated with a number of human ailments, such as cancer, neurological disorders and cardiovascular diseases (Jha *et al.*, 2017; Pizzino *et al.*, 2017). Protein glycation is a non-enzymatic reaction between carbonyl groups of reducing sugars and free amino groups of macromolecules like proteins, lipids and nucleic acid (Younus and Anwar, 2016; Froldi *et al.*, 2019). This process is a cascade reaction that promotes the generation of reactive oxygen species, auto-oxidation reactions and production of other reactive intermediates (Hellwig and Henle, 2014; Moldogazieva *et al.*, 2019). The association between them promotes oxidative damage to DNA, increases inflammatory response and decreases action of the endogenous antioxidant system (Hwang *et al.*, 2018; Froldi *et al.*, 2019). Unfortunately, the currently available treatment option for protein glycation has been compromised by toxic and severe side effects (Thomalley, 2003). Thus, the need to discover and develop a novel antiglycation agent cannot be overemphasized. Fortunately, there are a number of medicinal plants that have shown therapeutic effects against physiological conditions linked to development of diseases. This could serve as the foundation for isolation of bioactive compounds from plants in order to produce drugs to treat ailments.

Medicinal plants still play an important role in human and animal healthcare. About 60% of the world’s population and 80% of Africa’s population depend on herbal medicine for their primary healthcare (Opande *et al.*, 2022). One of such plants is *Syzygium guineense* (SG), which belongs to the family Myrtaceae. In Africa, the plant is widely distributed in Nigeria, Senegal, Cameroon and South Africa (Nvau *et al.*, 2011). Traditionally, the plant parts have been used for the treatment of menstrual cycle disorder (Nigatu, 2004), constipation, diarrhea, dysentery (Kisangau *et al.*, 2007), arthritis, rheumatism, venereal diseases, malaria (Kasali *et al.*, 2014), sleep disorder, anaemia (Nguyen *et al.*, 2016), diabetes mellitus, microbial and fungi infections (Ezenyi *et al.*, 2016). Additionally, scientific investigations have demonstrated that the methanol and aqueous extracts of the plant leaves possess antioxidant effect (Pieme *et al.*, 2014; Edewor *et al.*, 2021). However, the antioxidant activity of the ethylacetate and chloroform extracts of the plant leaves together with the antiglycation potential of these extracts remain a knowledge lacuna that needs to be bridged.

On the other hand, *Borassus aethiopum* (BA) Mart (Arecaceae) is a tropical plant species that grows widely across Africa (Ahmed *et al.*, 2010). Studies have indicated that the male inflorescences of the plant exhibited anti-inflammatory, antipyretic, pro-apoptotic, antifungal and antibacterial properties (Sakande *et al.*, 2004a, 2004b, 2011, 2012). Nonetheless, the antioxidant and antiglycation activities of extracts of the plant leaves are yet to be assessed in spite of all these preliminary scientific evidences. Herein, we conducted the present study in order to evaluate the in vitro antioxidant and antiglycation potentials of SG and BA leaves including their phytochemical constituents.

**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.
Plant Material
According to literature search, the SG and BA leaves were collected with the help of a traditional healer in May 2018 from local communities in Samaru and Zaria, Nigeria, respectively. The plants were identified at the herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria (ABUZ), Nigeria by matching them to voucher specimen deposited in the unit with the voucher number ABU0186 and ABU016312 for SG and BA leaves, respectively. The leaves of the plants were respectively cleaned, dried in open air in the laboratory for 10 days, pounded into a fine powder with a mortar and pestle and stored in airtight container until further usage.

Preparation of Plant Extract
Two hundred grams (200 g) of the fine powdered plants (SG and BA leaves) were soaked overnight in 500 ml each of ethylacetate, chloroform, methanol and water and filtered through 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. The extracts were stored at 4°C until required.

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

\[
\text{%Yield of extract (g/100 g) = (W_1 \times 100) / W_2}
\]

Where:
- \( W_1 \) is the weight of the plant extract residue after solvent removal
- \( W_2 \) is the weight of dried plant powder.

Phytochemical Analysis of Plant Extract
The extracts of SG and BA leaves were subjected to qualitative tests for anthraquinones, steroids, triterpenes, cardiac glycosides, saponins, tannins, flavonoids and alkaloids according to the method described by Evans (2009).

Antioxidant Effect of Plant Extract
The antioxidant power of the extracts, which is the ability of a given substance to scavenge DPPH free radical, was determined using DPPH free radical scavenging assay as described by Sirajuddin et al. (2012) and Shah et al. (2013). Briefly, 0.1 ml each of methanol, 1 mg/ml ascorbic acid and 1 mg/ml plant extract was added, in triplicate, into control, standard and extract 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 the extracts and ascorbic acid was determined using the formula below:

\[
\text{Antioxidant activity (\%) = (Absorbance of control - Absorbance of test) / Absorbance of control } \times 100
\]

Antiglycation Effect of Plant Extract
The antiglycation activity of the extracts was estimated based on the method of Matsuura et al. (2002) and 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, chloroform and methanol extracts). Following that, 20 µl each of 50 mM phosphate buffer (pH 7.4) containing 0.2 g/l sodium azide was added to testubes 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. 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 extracts and aminoguanidine was calculated using the following below:

\[
\text{Antiglycation activity (\%) = (Fluorescence intensity of control - Fluorescence intensity of test) / (Fluorescence intensity of control)} \times 100
\]

Data Analysis
Data were presented as mean ± standard deviation (SD) and analyzed using one way analysis of variance (ANOVA) with the help of Statistical Package for Social Science (SPSS) version 20 for windows. Duncan post-hoc test was conducted to detect differences amongst the mean of the various test solutions. P value less than 0.05 (p < 0.05) was considered statistically significant.

RESULTS
The yield of ethylacetate extract of SG leaves was higher (12.05 g) compared to the chloroform, methanol and aqueous extracts. Similarly, the highest yield was obtained when the leaves of BA were extracted with ethylacetate. However, the aqueous extract of SG and BA leaves recorded the lowest yield of 7.5 g and 5.5 g respectively (Table 1).

The phytochemical evaluation of the ethylacetate, chloroform, methanol and aqueous extracts of SG and BA leaves revealed the presence of phytoconstituents like triterpenes, cardiac glycosides, tannins and flavonoids. Furthermore, saponins were identified in all the extracts of the plants leaves with the exception of methanol and aqueous extracts of SG leaves (Table 2).
Table 1: Yield of Syzygium guineense and Borassus aethiopum leaves extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>Syzygium guineense</th>
<th>Borassus aethiopum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylacetate</td>
<td>6.025</td>
<td>4.05</td>
</tr>
<tr>
<td>Chloroform</td>
<td>4.75</td>
<td>3.65</td>
</tr>
<tr>
<td>Methanol</td>
<td>4.2</td>
<td>3.35</td>
</tr>
<tr>
<td>Aqueous</td>
<td>3.75</td>
<td>2.75</td>
</tr>
</tbody>
</table>

Table 2: Preliminary phytochemical profile of Syzygium guineense and Borassus aethiopum leaf extract

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>PLANT EXTRACTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Syzygium guineense leaves</td>
</tr>
<tr>
<td></td>
<td>EA</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: + = detected, - = not detected; EA= ethylacetate; CHL= chloroform; MEOH= methanol; AQ= aqueous

Compared to the ascorbic acid, the antioxidant activity of the ethylacetate and chloroform extracts of SG leaves was significantly ($p < 0.05$) low. Nonetheless, the ethylacetate extract displayed a higher antioxidant activity (53.67%) compared to the chloroform extract (45.33%) (Figure 1). Similarly, the antioxidant activity of BA leaves extracts was significantly ($p < 0.05$) low compared to the ascorbic acid. Nevertheless, the chloroform extract recorded the least activity of 3.3% (Figure 2).

Figure 1: Antioxidant activity of Syzygium guineense leaf extract.
Data are presented as the mean ± SD of triplicate values. *values with different alphabets over the bars are significantly ($p < 0.05$) different from each other.

Figure 2: Antioxidant activity of Borassus aethiopum leaf extract.
Data are presented as the mean ± SD of triplicate values. *values with different alphabets over the bars are significantly ($p < 0.05$) different from each other.

The antiglycation activity of SG leaves extracts was significantly ($p < 0.05$) low compared to the aminoguanidine. However, the ethylacetate extract had the least antiglycation activity (32.33%) (Figure 3). In addition, the antiglycation activity of the aqueous extract of BA
leaves was significantly ($p < 0.05$) increased compared to the aminoguanidine and other extracts. Nevertheless, the least antiglycation activity was observed with methanol extract of BA leaves (43.33%) (Figure 4).

To the best of our knowledge, studies on the antioxidant activity of chloroform and ethylacetate extracts of SG are scarce in literature; however, studies on aqueous and methanol extracts of the leaves of SA have been reported previously. Also, there is no existing literature on the antioxidant/antiglycation properties of BA leaf extracts. Previous studies have been geared towards determining antioxidant properties of BA fruit flour or fruit extracts (Abe-Inge et al., 2018), in lieu of this, the present research was embarked upon.

Our present findings showed that ethylacetate extract of SG and BA gave the highest percentage DPPH radical scavenging capacity in vitro (53% and 48% respectively), although this activity was lower compared to the standard (ascorbic acid-61%) used in the study; however, this is in line with the findings of Ibrahim et al., (2020) who reported DPPH radical scavenging capacity of Mangifera indica ethylacetate leaf extract to be 79%. DPPH scavenging activity of aqueous and ethanol extract of SG stem bark in vitro has also been reported previously (Pieme et al., 2014; Tankeu et al., 2016). Likewise, DPPH scavenging activity of BA flour was reported by previous in vitro studies (Amoateng et al., 2010). DPPH assay is conventionally considered as an indication of a plant extract’s ability to quench free radicals, as well as their hydrogen atom or electron donation ability, in the absence of any enzymatic action (Mileva et al., 2014). However, the antioxidant activities exhibited by plant extracts via DPPH scavenging capacity may be due to their hydrogen atom or electron donation ability. The hydrogen-donating ability, on the other hand, may be traceable to the presence of phenolic compounds in the extracts; as these secondary metabolites have been reported to possess antioxidant activities (Gruz et al., 2011).

DISCUSSION
The need to discover new therapeutic arsenal towards oxidative stress and protein glycation is on the rise due to the alarming increase in the incidences of diseases associated with these physiological phenomena. Sequel to this, the current study evaluated the phytochemical constituents as well as the in vitro antioxidant and antiglycation activities of the ethylacetate, chloroform, methanol and aqueous extracts of SG and BA leaves. The research revealed that the extracts of SG and BA leaves contain vast array of phytochemicals, namely flavonoids, steroids, triterpenes, cardiac glycosides, tannins, saponins and alkaloids. This is supported by previous studies on evaluation of phytoconstituents of plant extracts (Usman et al., 2018; Hassan et al 2020; Nazneen et al., 2016). These phytochemicals have been reported to exhibit potency in some physiological imbalance; for example, flavonoids play a role as antioxidant agents (Savithramma et al., 2011); alkaloids are important in antimicrobial, analgesic, and other antispasmodic actions (Savithramma et al., 2011; Chatoui et al., 2016; El Hattabi et al., 2016) also steroids have been found to possess anti-inflammatory potency (Chatoui et al., 2016).

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Glycation is a process of non-enzymatic reaction between reducing sugars (fructose or glucose) and amino groups of protein to form a Schiff base complex. The Schiff base formation is not stable; it’s rearranged to produce irreversible Amadori products before being involved in further reactions to produce highly reactive carbonyl compounds. The dicarbonyls intermediates can react with amino, sulfhydryl, and guanidine functional groups resulting in browning, denaturation, and cross-linking of the targeted proteins (Frye et al., 1998).

Our present findings have reported for the first time the antiglycation activity of BA and SG leaf extracts respectively. This study has shown that aqueous and methanol extract of BA and SG can lower formation of AGEs in vitro, with highest antiglycation activity of 96% and 64% for BA and SG respectively. However, BA’s ability to inhibit AGE formation was more than that of the standard aminguanidine (87%). Also, antiglycation activity varied according to the solvent of extraction been used for the experiment (Figures 3 and 4). The observed results for AGES inhibition by BA and SG are supported by other recent studies (Nampoothiri et al., 2011; Tupe et al., 2015).

Inhibitors of AGE products may act not only as quenchers of dicarbonyl intermediates, but also as antioxidants or metal ion chelators. Therefore, compounds with antioxidant activity could also inhibit the formation of AGE. Nakagawa et al., (2002) reported that green tea demonstrates strong antiglycation activity in addition to its known antioxidant potential. However, Chen et al. (2011) describe plant extracts that possess strong antiglycation, but low antioxidant activity (Astragalus membranaceus), or strong antioxidant, but low antiglycation potential (Periploca sepium). Our research findings thus showed that aqueous extract of BA leaves had a strong antiglycation potential (96%) but possess a lower antioxidant activity (44%) in vitro.

CONCLUSION

Our study shows for the first time extracts of Borassus aethiopum (BA) and Syzygium guineense (SG) leaves possess antiglycation and antioxidant activity in vitro. Aqueous extract of BA has the highest inhibitory effect on AGE formation. In order to validate these findings, in vivo research, which is typically the following step in the drug discovery and development pipeline, would be required. Moreover, due to vast array of phytochemicals identified, these plants, might serve potential role as antioxidant and antiglycation agents in modulating the progression of pathogenesis associated with diabetes, cancer, aging and Alzheimers disease.

ACKNOWLEDGEMENT

The management of Ahmadu Bello University, Zaria, Nigeria, provided the study facilities, for which the authors are grateful.

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