



## Research Article

## The Potential of *Diospyros mespiliformis* and *Carissa edulis* Leaves Towards Inhibition of Protein Glycation and Oxidative Stress

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## ABSTRACT

Glycation is the spontaneous reaction between structural or functional proteins and reactive sugar moieties which results in the formation of advanced glycation end-products (AGEs). AGEs play a crucial role in the pathogenesis of diabetic complications, including natural aging, oxidative stress, and chronic inflammation. As a result, the phytochemical profile, antioxidant and antiglycation activities of ethylacetate and chloroform extracts of *Diospyros mespiliformis* (DM) and *Carissa edulis* (CE) leaves were investigated, *in vitro*. *In vitro* DPPH free radical scavenging assay of the extracts was assessed while BSA-glucose glycation model was utilized to assess the *in-vitro* inhibition of protein glycation, using spectrofluorescent assay. The result showed that alkaloids, phenols, flavonoids, steroids, triterpenes, cardiac glycosides, saponins and anthraquinones were present in the extracts of the plants. Furthermore, the extracts displayed a significantly ( $p < 0.05$ ) low antioxidant activity (43%) compared with ascorbic acid (61%). Moreover, ethylacetate extract of DM leaves, exhibited a significantly ( $p < 0.05$ ) high antiglycation effect (98%) in comparison with aminoguanidine (87%). The current study showed that ethylacetate and chloroform extracts of the plant leaves have potential effect towards lowering oxidative stress and protein glycation and should be further explored for drug discovery.

**Keywords:** Phytochemicals, Antioxidant, Antiglycation, *Diospyros mespiliformis*, *Carissa edulis*

## INTRODUCTION

Since ancient times, the use of medicinal plants to manage and cure various diseases has been of utmost importance (Cai *et al.*, 2004). In affluent nations, there has been an upsurge in the acceptance of crude herbal products (Street and Prinsloo, 2013). Many cultures continue to rely on information that has been passed down from earlier generations about conventional medical practices. In nations like China and India, this information has evolved into a sophisticated system of diagnosis, medicinal preparations and treatment (Houghton, 2010). Natural products are a significant source of unique compounds that may aid innovative approaches in the development of drugs. In the past, the rising interest in the usage of medicinal herbs has been driven by the pursuit of

innovative substances for the treatment of ailments. The World Health Organization (WHO) has advised that medicinal plants be really identified, empirically, used, and developed for their safety usage and efficacy in medical treatment. This is an acknowledgment of the significant relevance of herbal medicine to the delivery of primary health care (Newman and Cragg, 2007).

*Diospyros mespiliformis* (DM), also known as Jackal Berry or African Ebony, is a member of the Ebenaceae plant family. In Nigeria, the Hausa and Yoruba names for the plant are *Kanya* and *Igidudu*, respectively. The leaves are simple, alternately arranged, and dark green in color. The plant, which

has enormous yellow berries when fully grown, is dioecious and flowers in April and May (Dangoggo *et al.*, 2012). Traditionally, a decoction of the leaves is utilized to cure wounds, treat whooping cough, and reduce fever (Adzu *et al.*, 2002a; Abubakar *et al.*, 2007). In addition, scientific studies have shown that the aqueous, methanol, ethanol, petroleum ether, and N-butanol leaf extracts of the plant have antioxidant, antimicrobial (Shagal *et al.*, 2011; Dangoggo *et al.*, 2016), antiplasmodial (Oguche and Nzeliibe, 2016) and analgesic (Adzu *et al.*, 2002b) activities. However, despite all these preliminary scientific pieces of evidence, the antioxidant and antiglycation effects of the ethylacetate and chloroform leaf extracts of the plant are yet to be evaluated.

*Carissa edulis* (CE) Valh (Apocynaceae) is a plant widely distributed in Africa (Bentley *et al.*, 1984). It is used traditionally to manage chest complaints (Bentley *et al.*, 1984), rheumatism (Giday *et al.*, 2003), gonorrhoea, syphilis, rabies, diuretics, headache (Addis *et al.*, 2001) and hypertension (Houngue *et al.*, 2022). Furthermore, a number of studies conducted on the aqueous, methanol, and hydroethanol leaf extracts of the plant have reported its antioxidant (Fanta Yadang *et al.*, 2019), antimicrobial (Ibrahim *et al.*, 2010), antidiabetic (El-Fiky *et al.*, 1996), antiplasmodial (Kirira *et al.*, 2006) and neuroprotective (Fanta Yadang *et al.*, 2020) activities. Notwithstanding, the activities of the ethylacetate and chloroform leaf extracts of the plant toward DPPH radical scavenging and protein glycation are yet to be assessed.

Considering the reported pharmacological activities of *D. mespiliformis* and *C. edulis* leaf extracts, we evaluated the potential *in vitro* phytochemical constituents, antioxidant and antiglycation activities of ethylacetate and chloroform leaf extracts of the plants.

## MATERIALS AND METHODS

### Chemicals and Reagents

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

### Plant Material

The DM and CE leaves were collected with the assistance of a traditional healer in May 2018 from local communities in Azare and Zaria, Nigeria, respectively. The plants were identified at the herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria (ABUZ), and Nigeria and were assigned voucher numbers ABU0938 and ABU900182

for DM and CE leaves, respectively. The leaves of the plants were respectively cleaned, dried in the open air in the herbarium unit of the laboratory for 10 days, pounded into a fine powder with a mortar and pestle, and stored in an airtight container until needed.

### Preparation of Plant Extract

Two hundred grams of the finely powdered plants (DM and CE leaves) were soaked overnight in 500 mL of each ethyl acetate and chloroform 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 yields were 10.79 g and 2.1 g of crude DM and CE leaves ethylacetate extract, respectively. However, for crude DM and CE leaves chloroform extract, the yields were 9.7 g and 8.5 g, respectively. The extracts were stored at 4°C until required.

### Phytochemical Screening of Plant Extract

The extracts of DM and CE leaves were subjected to qualitative tests for carbohydrates, saponins, anthraquinones, cardiac glycosides, alkaloids, flavonoids, triterpenes, and steroids based on the method described by Evans (2009).

### DPPH radical Scavenging Activity of Plant Extract

The antioxidant power of the extracts was estimated using DPPH free radical scavenging assay as described by Brand-Williams *et al.* (1995) with slight modifications by 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 tubes labeled control, standard, and extract, respectively. Following that, 3 mL of 0.24 mg/mL DPPH (prepared in methanol) was added into the test tubes. The aliquot 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 DPPH radical scavenging activity of the extracts and ascorbic acid was computed using the formula hereunder:

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

### Antiglycation Activity of Plant Extract

The assessment of the antiglycation potential of the leaf extracts was conducted according to the method of Matsuura *et al.* (2002) with certain modifications by Kaewnarin *et al.* (2014). In brief, 20 µL each of 800 µg/mL BSA and 200 mM D-glucose were added, in triplicate, into tubes labeled control, standard, and extract. Afterward, 20 µL each of 50 mM phosphate buffer (pH 7.4) containing 0.2 g/L sodium azide, 1 mg/mL aminoguanidine and 1 mg/mL plant extract (prepared in phosphate buffer containing sodium azide) was added into

the test tubes, respectively. Next, 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 (Cary series). The percentage antiglycation activity of the extracts and aminoguanidine was calculated using the formula below:

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

### Data Analysis

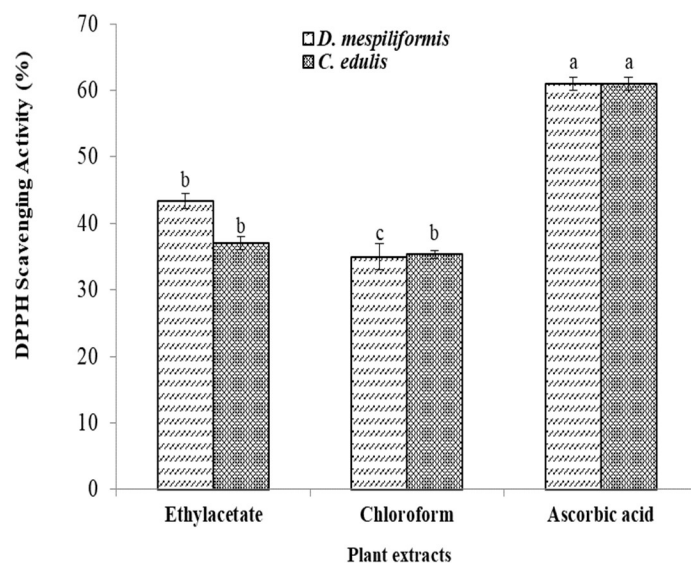
Data are presented as mean  $\pm$  standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) with the aid of Statistical Package for the Social Science (SPSS) version 20 for Windows. Duncan post hoc test was done to detect differences amongst the mean of the various treatment groups. P value less than 0.05 ( $p < 0.05$ ) was considered statistically significant.

## RESULTS

The phytochemical studies on the ethylacetate and chloroform extracts of DM and CE leaves revealed the presence of both cardiac glycosides and triterpenes. Additionally, alkaloids were detected in both extracts of the plant leaves except in the chloroform extract of the CE leaves (Table 1).

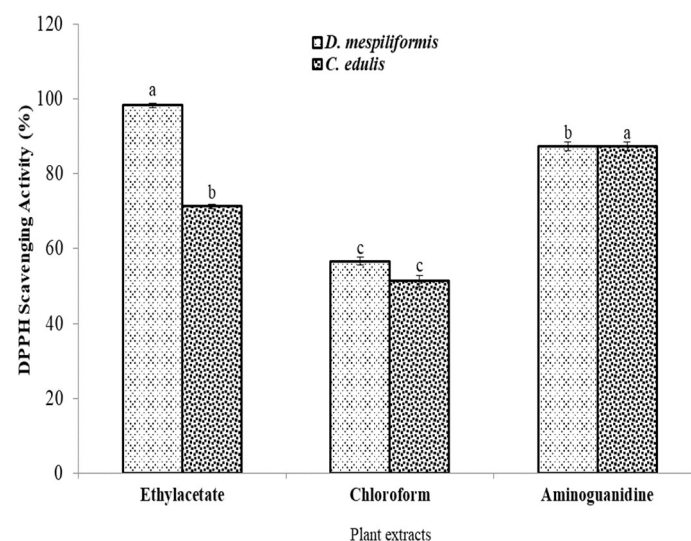
DPPH radical Scavenging Activity of the leaf extracts of DM was significantly ( $p < 0.05$ ) lower compared to ascorbic acid. Notwithstanding, ethyl acetate extract displayed a higher activity (43.33%) compared to the chloroform extract (35.00%) (Figure 1). Similarly, compared to the ascorbic acid, the DPPH radical scavenging activity of CE leaf extract was significantly ( $p < 0.05$ ) lower. Although insignificant, the ethylacetate extract had a higher activity (37.00%) compared to the chloroform extract (35.33%) as seen in Figure 1.

The antiglycation activity of DM leaves ethylacetate extract was significantly ( $p < 0.05$ ) higher compared to the aminoguanidine and chloroform extract. However, chloroform extract of *D. mespiliformis* had the least antiglycation activity (56.67%) (Figure 2). Moreover, the antiglycation activity of the chloroform extracts of CE leaves was significantly ( $p < 0.05$ ) lower compared to aminoguanidine Figure 2.



**Figure 1.** DPPH Scavenging Activity of *Diospyros mespiliformis* and *Carissa edulis* Leaf Extracts.

Data are presented as mean  $\pm$  SD of triplicate values. <sup>a-c</sup> different alphabets over the bars are significantly ( $p < 0.05$ ) different from each other.



**Figure 2.** Antiglycation Activity of *Diospyros mespiliformis* and *Carissa edulis* Leaf Extracts.

Data are presented as mean  $\pm$  SD of triplicate values. <sup>a-c</sup> different alphabets over the bars are significantly ( $p < 0.05$ ) different from each other.

**Table 1.** Preliminary Phytochemical Profile of *Diospyros mespiliformis* and *Carissa edulis* Leaf Extracts

Phytochemicals	Ethylacetate extract		Chloroform extract	
	<i>D. mespiliformis</i>	<i>C. edulis</i>	<i>D. mespiliformis</i>	<i>C. edulis</i>
Saponins	-	-	-	+
Anthraquinones	+	-	-	-
Cardiac glycosides	+	+	+	+
Alkaloids	+	+	+	-
Flavonoids	-	-	-	+
Triterpenes	+	+	+	+
Steroids	+	-	-	+

Key: + = present, - = absent

## DISCUSSION

The hunt for brand-new antioxidant and antiglycation agents has been intensified. This is largely due to the problems of toxicity and severe side effects associated with the currently available inhibitors of AGEs (Peng *et al.*, 2008a,b). Excessive accumulation of these AGEs in living organisms leads to cellular dysfunction by inhibiting cell signaling, and alterations of structural and functional modifications of tissue proteins (Barlovic *et al.*, 2010). The current research demonstrated that the extracts of DM and CE leaves contain numerous phytochemicals, namely; flavonoids, steroids, triterpenes, cardiac glycosides, tannins, saponins, and alkaloids. This is supported by previous studies on the evaluation of phytoconstituents of plant extracts (Usman *et al.*, 2018; Nazneen *et al.*, 2016). These phytochemicals have been reported to exhibit potency in some physiological imbalances; for example, flavonoids play a role as antioxidant agents (Savithamma *et al.*, 2011); also steroids have been found to possess anti-inflammatory potency (Chatoui *et al.*, 2016).

Several studies have demonstrated that oxidative stress can greatly contribute to the degenerative processes linked to aging and diseases (Lemberkovics *et al.*, 2002; Shon *et al.*, 2003). Scientific information on the antioxidant ability of a number of medicinal plants has been documented. Nevertheless, non-scientific pieces of evidence have shown that the plant leaves were used to treat fever and headache (Addis *et al.*, 2001; Abubakar *et al.*, 2007), which are important features of oxidative stress. This may indicate the capability of plant leaves to combat disorders induced by oxidant damage. Results obtained from this study showed that the leaf extracts have a considerable antioxidant effect. This therapeutic activity might primarily be attributed to the phytochemical components of the plants, which are generally known to possess free radical scavenging capacity. Overall, an *in vivo* study, which is normally the next step in the drug

discovery and development pipeline, would be needed to confirm or refute these findings. Protein glycation is another vital factor that can speed up physiological processes associated with aging and diseases. The control of this phenomenon is a good measure of the management of diseases. In view of this, the antiglycation activity of the ethylacetate and chloroform extracts of DM and CE leaves were investigated. However, ethylacetate extract of the DM leaves was able to significantly inhibit the formation of advanced glycation end products by 98.00% compared to aminoguanidine (87.33%). This medicinal effect may partly be linked to the flavonoids contents of the plant extract because studies have suggested that flavonoids are responsible for the antiglycation potential of plant extracts (Kim and Kim, 2003; Matsuda *et al.*, 2003; Wu and Yen, 2005; Ardestani and Yazdanparast, 2007; Peng *et al.*, 2008a; Wang *et al.*, 2011). Findings from this research revealed that the plant extracts showed a low DPPH radical scavenging activity as compared with the high antiglycation activity observed. A similar trend was observed from previous studies on antidiabetic Chinese herbal medicine, including leaf extracts of *Syzygium guineense* and *Borassus aethiopicum* (Chen *et al.*, 2011; Usman *et al.*, 2023).

In conclusion, this study shows for the first time extracts of *Diospyros mespiliformis* (DM) and *Carissa edulis* (CE) leaves possess antiglycation and low antioxidant activity *in vitro*. Ethylacetate extract of DM showed the highest AGE inhibitory effect. Moreover, due to the presence of a vast array of phytochemicals, these plant extracts, might serve the potential role as antiglycation agents in modulating the progression of related diseases.

## AUTHORS' CONTRIBUTIONS

Conceptualization: HSU, ABS, MAU, FEA, SMH; Laboratory experiments: IU, HSU; Data Analysis: MAU, HSU, IU; Writing- original draft preparation: HSU, MAU; Writing-review and editing: IU, FEA, SMH, ABS; Resources: IU, HSU, MAU, FEA, SMH, ABS; Supervision: HSU, ABS.

All authors approved the final version of the manuscript

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## CONFLICT OF INTEREST

The authors declare no conflict of interest

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