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Inhibitory effects of *Blackstonia grandiflora* extracts on the formation of advanced glycation end products (AGEs)

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ABSTRACT

Glycation, the nonenzymatic reaction of reducing sugars with amino groups, is increased in hyperglycemic physiological environments, leading to an acceleration of the formation of advanced glycation end products (AGEs). The AGEs are associated with many pathogenic disorders such as Alzheimer's disease, pathogenesis of diabetes, and atherosclerosis. They are also responsible for aging and tissue damage. In this study, the antiglycation activity of the extracts Blackstonia grandiflora (Viv.) was evaluated in the bovine serum albumin (BSA)/ribose system and the potency of these extracts was compared with the standard antiglycation agent aminoguanidine. The results showed that EtOAc extract had a significant inhibitory effect *on the formation of advanced glycation end products (AGEs).*

Keywords: Advanced glycation end-products (AGEs); *Blackstonia grandiflora*

INTRODUCTION

Advanced glycation end products (AGEs) are a heterogeneous group of molecules that are formed during the so-called Maillard reaction. This non-enzymatic reaction involves a reducing sugar such as glucose or ribose and the primary amino groups of a protein (lysine, arginine) **[1, 2].** It is initiated by the reversible formation of a Schiff base which undergoes a rearrangement to form a relatively stable Amadori product. After oxidation, the later gives a dicarbonyl intermediate which can react with an other primary amino groups of a protein to form heterocyclic AGEs **[3]** (**Figure 1**). Well-known AGEs are carboxymethyl-lysine (CML), pentosidine and pyrraline. Together with methylglyoxal, they have been used as biomarkers of glycoxidative damages **[4-8]**. Advanced glycation end-products (AGEs) are associated with many pathogenic disorders such as pathogenesis of diabetes, Alzheimer's disease **[9-11]** or atherosclerosis. More generally, they are responsible for aging and tissue damage **[12, 13].**

Molecules able to break AGEs or inhibit their formation may thus be considered as potential drugs, dietary supplements or other bioactive ingredients **[8, 14-15]**. These considerations have thus prompted the scientific community to identify advanced glycation end-products inhibitors or breakers. Recently, several studies were conducted to explore the anti-AGE activities of medicinal plant extracts. The potential of plant extracts to limit AGE formation correlates significantly with the polyphenolic content **[16, 17].**

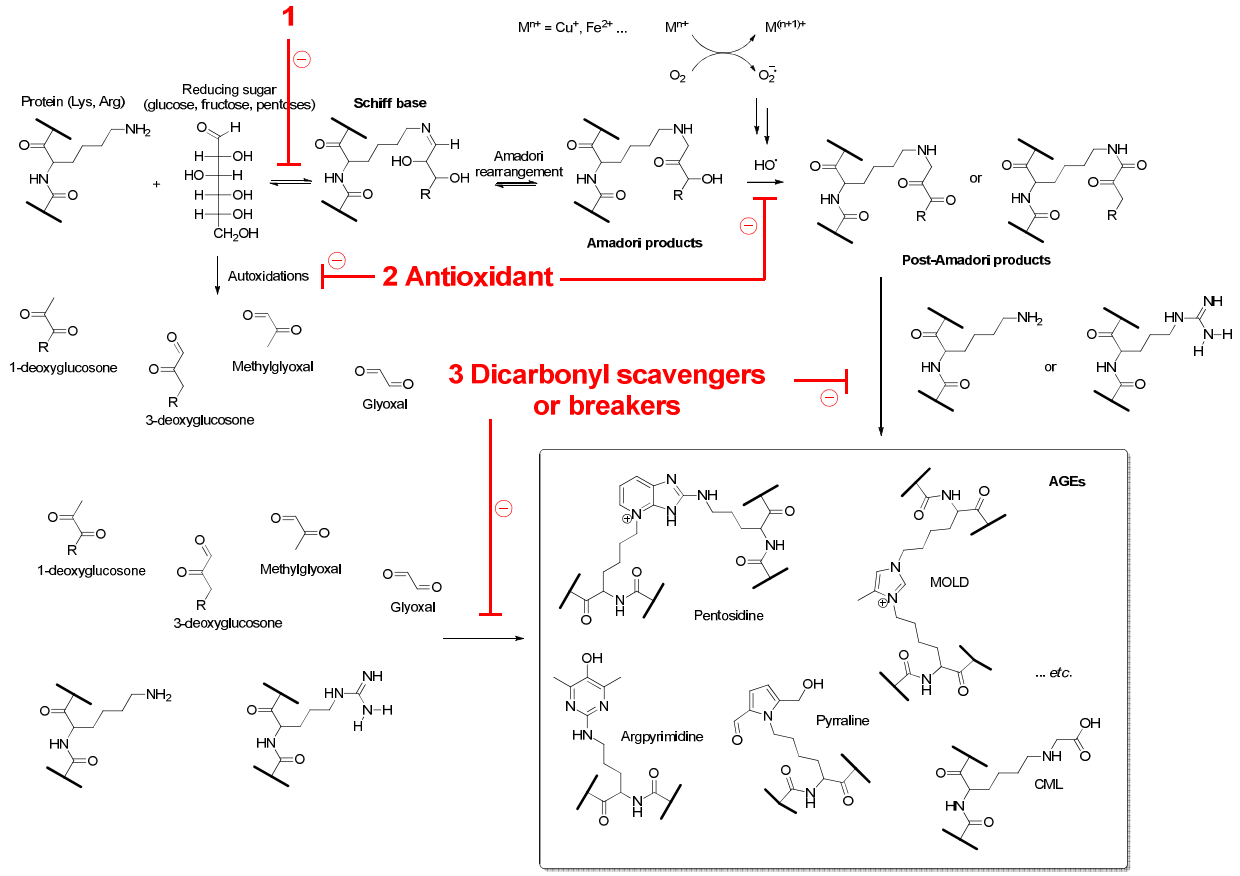

Figure 1: Schematic illustration of AGE formation and mean to inhibit it using plant secondary metabolites

The Gentianaceae, or Gentian family, is distributed worldwide with approximately 100 genera and about 1,800 species. It includes monocarpic and perennial herbs, shrubs, trees, and lianes, with terrestrial and epiphytic representatives **[18]**. The etymology of the name Gentian comes from Gentius, the second-century BC king of Illyria who has discovered the antipyretic properties of Gentians **[19]**. The Gentianceae have been widely used in traditional medicine and also as constituents in bitters and similar concoctions **[20, 21].** In this family, the genus *Blackstonia* is represented by four species. They are widely distributed in the Mediterranean, western and central European **[22]**.The genus has not been deeply studied from a pharmacological and phytochimical point of view; reports regarding its chemical composition are very few. The biologically active flavonoid glycosides **[23, 24],** secoiridoids and xanthones **[25]** were isolated from entire plants of *B. perfoliata*

Blackstonia grandiflora (Viv.) Pau is annual plant, 20-40cm high, stems single, erect, simple or ramified. In Algeria, this plant is growing in the forests, damp areas and in the high plateaus. It is widespread in northern Algeria **[26].** The aerial parts of *B. grandiflora* used in Algeria as a traditional remedy for fever and it is also known as a tonic **[27].** However, No phytochemical or biological sudies were carried out in this regard. To specify the benefits of this plant as functional food, we have investigated the potential of aerial parts extracts in preventing AGE formation.

MATERIALS AND METHODS

Plant material

Aerial parts of *Blackstonia grandiflora* (Gentianaceae) were collected during the flowering phase in may 2010 from El-kala, in the extreme north-east of the Algeria. Dr Gerard De Belair (Departement of biologiy, Annaba University, Algeria) ascertained botanical identity of the plant and voucher specimen was deposited in the Herbarium of our laboratory.

Plant extracts preparation

The aerial parts were dried and pulverized to a coarse powder. The powdered plant material (500 mg) was extracted three times with 70 % (V/V) aqueous methanol at room temperature overnight. The extracts were combined and concentrated under reduced pressure on a rotary evaporator and dissolved in distilled water (500 ml). The resulting solution was extracted successively with (3 × 300 ml) dichloromethane (CH_2Cl_2), ethyl acetate (3 × 300 ml) .and *n*-

butanol (3 × 300 ml). The organic phases were dried with Na_2SO_4, filtered and concentrated in vacuum at room temperature to obtain dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), *n*-butanol (BuOH) and the remaining hydromethanolic (MeOH) extracts.

Inhibition of AGE formation

The AGEs inhibition assay was carried out according the method by Derbre et al **[28]**, the assay involved incubating BSA (10 mg/mL) with D-ribose (0.5 M) and the tested extract (1 µg to 1 mg) in 50 mM phosphate buffer at pH 7.4 (NaN_3 0.02%). Solutions were incubated in 96-well microtiter plates at 37 °C for 24 h in a closed system before AGE fluorescence measurement. Fluorescence resulting from the incubation, under the same BSA (10 mg/mL) and tested extract (1 µg to 1 mg) conditions, was subtracted for each measurement. A control, *i.e.*, no inhibition of AGE formation, consisted of wells with BSA (10 mg/mL) and D-ribose (0.5 M). A blank of control, *i.e.*, 100% inhibition of AGE formation, consisted of wells with only BSA. The final assay volume was 100 µL. Both vesperlysines-like (λ_{exc} 370 nm; λ_{em} 440 nm) and pentosidine-like (λ_{exc} 335 nm; λ_{em} 385 nm) AGE fluorescence were measured using a microplate spectrofluorometer. The percentage of AGE formation was calculated as follows for each compound/extract concentration:

$$AGEs\ (\%) = \frac{[fluorescence\ intensity\ (sample) - fluorescence\ intensity\ (blank\ of\ sample)]}{[fluorescence\ intensity\ (control) - fluorescence\ intensity\ (blank\ of\ control)]} \times 100$$

Dose-effect curves were best fit with a sigmoidal dose-response equation using Sigma Plot 12.0 software, which enabled calculation of the IC50 values. Aminoguanidine and quercetin were used as references.

RESULTS AND DISCUSSION

The presence of advanced glycation end products (AGEs) in the body is correlated to many complications associated with diabetes because hyperglycaemia enhances protein glycation. The (AGEs) are also involved in the pathophysiology of Alzheimer´s disease, arthritis and ageing. Due to the importance of AGEs-related disorders issue, the investigation of AGEs formation inhibitors has received much attention.

In this study, bovine serum albumin (BSA)/ribose system was used for the evaluation of the inhibitory effects of *B. grandiflora* extracts against pentosidine and vesperlysines formation. The inhibitory activity is presented in **Table 1**.

Table 1: Anti-AGEs activities (IC_{50}) of the CH_2Cl_2, EtOAc, BuOH and MeOH extracts obtained from aerial parts of *Blackstonia grandiflora* and the reference compound aminoguanidine

Extracts	Effect on pentosidine-like AGE formation (IC50, mg/ml)	Effect on vesperlysines-like AGE formation (IC50, mg/ml)
CH_2Cl_2 extract	0,35	0,2
EtOAc extract	0,2	0,2
BuOH extract	0,6	0,6
MeOH	0,6	0,7
Aminoguanidine	0,15	1,0

AGEs; advanced glycation end products.
IC_{50}; 50% inhibitory concentration.

Our result indicate that the EtOAc extract exhibited a strong inhibitory activity against pentosidine and vesperlysine formation with the IC_{50} values of 0,2 mg/ml and 0,2 mg/ml respectively compared with the positive control aminoguanidine (IC_{50} = 0.15 mg/ml toward pentosidine, and IC_{50} = 1mg/ml toward vesperlysine). The CH_2Cl_2, BuOH and MeOH extracts also exhibited a potent inhibitory activity with the IC50 value 0, 2 mg/ml, 0,6 mg/ml and 0,7 mg/ml respectively against the formation of vesperlysines, whereas the glycation inhibitory activity of those extracts showed a lower activity against pentosidine formation than the aminoguanidine

Several studies have demonstrated that the anti-glycation activity correlates significantly with the phenolic content of the tested plant extracts **[29, 30]**. On the basis of a literature search, many purified phenolic compounds (involving chalcones, Flavones, Flavonols, F**l**avanones, Flavanonoles, isoflavones, anthocyanidins, and other phenolics) and phenolic-rich plant extracts have been found to have strong inhibitory activity in this bioassay **[31]**. Based in our results, the EtOAC extract has a high antiglycation potency which makes it worthy of further investigation on their purification and determination structural of the compound(s) responsible for inhibiting activity on the formation of advanced glycation end products (AGEs).

CONCLUSION

In this study, we evaluated the inhibition of pentosidine and vesperlysines formation as advanced glycation end products (AGEs) using *in vitro* glycation of in extracting aerial parts of *Blackstonia grandiflora*. The results indicated that EtOAc extract has a high inhibitory effect on formation of AGEs. The purification and characterization of the compound(s) from *Blackstonia grandiflora* responsible for inhibiting activity are under progress.

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