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Anti-glycation properties of 05 spices and culinary herbs used in Benin

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Abstract

Diabetes mellitus is a major public health problem characterized by chronic hyperglycemia leading to several other pathologies. It causes oxidative stress, resulting in the overproduction of superoxide radicals and protein glycation, ultimately leading to the formation of Advanced Glycation Endproducts (AGEs). It has been shown elsewhere that spices and herbs commonly used in the culinary arts inhibit glycation and AGE formation. The aim of this study is to evaluate the inhibitory effect of five aromatic herbs and spices commonly used in Benin on protein glycation and AGE formation.

Total flavonoids content and polyphenolic compounds were determined with respectively the aluminium chloride method and the Folin-Ciocalteu method. For glycation testing, serum pools from diabetics and non-diabetics were used. Serum fructosamines were measured using the nitro blue tetrazolium colorimetric test and AGEs were detected using a fluorescence method. All five herbs and spices had shown notable ability to inhibit fructosamine and AGE formation. *L. nobilis* was very rich in flavonoids and exhibited the strongest inhibitory activity against glycation and AGE formation. The results of this study suggested that the investigated five spices and culinary herbs have interesting properties to take in account in the therapy of diabetes and its related complications.

Keywords: Diabetes; Anti-glycation effect; AGE inhibition; Herbs and spices used in Benin

1. Introduction

Diabetes mellitus is a progressive metabolic disorder characterized by chronic hyperglycaemia. This is due to a defect in insulin secretion as a result of insufficient insulin production or cell insensitivity to insulin action, or both [1]. It is a major public health problem due to its significant and growing prevalence on the one hand, and its socio-economic impact on the other [2]. According to reports by the International Diabetes Federation, 415 million people suffered from diabetes worldwide in 2017 [3]. It is assumed that it will affect around 642 million people by 2040. Diabetes mellitus is assumed to be primarily caused by persistent hyperglycaemia, ultimately leading to numerous secondary complications such as neuropathy, nephropathy, retinopathy, stroke and peripheral arterial disease [4,5]. The chronic hyperglycaemic state of diabetes mellitus leads to oxidative stress through several mechanisms such as: glucose auto-oxidation, overactivity of polyol pathway, overproduction of superoxide radicals and increased protein glycation [6].

Glycation of tissue and serum proteins is a spontaneous chemical reaction known as the Maillard reaction between glucose or its intermediates and amino groups of proteins, leading to the formation of fructosamines or glycated serum proteins and after a cascade of further reactions to AGEs formation. Glycation affects the structure and function of the modified proteins and is implicated in various pathologies such as diabetes mellitus, atherosclerosis, Alzheimer's disease and amyloidosis. The common chronic diabetes complications have been related to glycated proteins and AGE accumulation in lens, kidney and nerve [7,8].

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Anti-glycation compounds can act at several levels to prevent the deleterious action of glycated proteins and AGEs. Ascorbic acid, aspirin and metformin are known for their ability to block the binding reaction of sugar residues to proteins [8,9]. Calcium antagonists, amlodipine, kinetin and quinine, can scavenge free radicals to slow or inhibit AGE formation [8]. Aminoguanidine, a hydrazine derivative, is the first antiglycative molecule to be used in human experiments *in vivo*. It is a scavenger of free radicals and reactive carbonyls that also possesses the ability to react with early glycosylation products to form compounds unsuitable for generating AGEs [10,11]. However, side effects such as liver disorders, digestive problems and anaemia led to the discontinuation of the trial [12]. This has prompted the search for new molecules with little or no side-effects.

Nowadays, herbal remedies for diabetes are gaining in popularity as they are inexpensive compared to synthetic hypoglycaemic drugs [13]. Plants contain a variety of nutritional and functional components such as vitamins, minerals, dietary fibers and phytochemicals, and also abound in useful components for health maintenance. Folk medicine has always attributed curative properties to certain foods. Plants, herbs and culinary spices have been used in traditional medicine for centuries to treat diabetes mellitus and its complications [14]. Ethnobotanical studies have identified over 1,200 species of plants, herbs and spices with hypoglycaemic activity [15]. In Mauritius, authors have demonstrated the efficacy of ten commonly used spices and aromatic herbs to inhibit the glycation of serum bovine albumin [16]. Similar works have been carried out by Dearlove on spices and herbs acquired from a supermarket in Athens in Greece, and by Starowicz on samples collected in Poland and Slovakia [17,18]. In Benin, herbs and spices that are commonly used in the culinary arts have not been investigated in such studies, mainly their effects on glycation with a view to understand their usefulness in the treatment of diabetes and its complications.

Thus, this study was initiated in the aim to evaluate the inhibitory effect of five aromatic herbs and spices commonly used in Benin on protein glycation and AGE formation. In the first part of the study, we have evaluated the inhibitory properties of 14 spices and aromatic herbs acquired in a market in Abomey-Calavi, in southern Benin (data not published). Basil (*Ocimum basilicum*), turmeric (*Curcuma longa*), coriander (*Coriandrum sativum*), laurel (*Laurus nobilis*) and ginger (*Zingiber officinale*), the five species with the highest inhibitory effect on glycation were selected for the present study.

2. Material and methods

2.1. Chemical and reagents

The chemical used were procured from either MolyCHEM (Badiapur, India) or Merk Sigma-Aldrich (Darmstadt, Germany). The serum glucose determination kit was obtained from ELITechgroup (Puteaux, France).

2.2. Vegetal and biological materials

The various herbs and spices were purchased in February 2021 at a market in Abomey-Calavi, southern Benin. The area is characterized by an equatorial climate with high humidity and alternating dry seasons (November to March and mid-July to mid-September) and rainy seasons (April to mid-July and mid-September to October). Annual rainfall is 1245 mm (https://presidence.bj/home/le-benin/geographie). The herbs and spices were identified by a botanist from the University of Abomey-Calavi.

The study required human serums from anticoagulant-free tubes. Confirmed diabetic and non-diabetic patients were selected on the basis of their fasting blood glucose levels at a health center in Abomey-Calavi, Benin.

2.3. Blood glucose assay

Venous blood samples were collected from patients after overnight fast. Blood glucose levels were determined using the glucose oxidase/peroxidase colorimetric method on the semi-automated biochemistry analyzer Genrui WP21B (Genrui-Biotech Co, China). Subjects with blood glucose level between 0.65 and 1.10 g/L were considered normoglycemic and those with a level higher than 1.25 g/L were declared diabetic. For the purpose of this study, the diabetic patients included have fasting blood glucose level above 1.5 g/L. Ten diabetic serums were combined into a single sample with an average blood sugar of 2.18 g/l whereas the pool of ten normoglycemic serums gave an average glucose value of 0.82 g/l. The obtained serums served for the antiglycative tests with following extracts: leaves of basil and laurel, rhizomes of ginger and turmeric and seeds of coriander.

2.4. Preparation of plants extracts

All spices and herbs were washed under running tap water to remove dirt and earth remains. Rhizomes were cut into fine slices. The plants parts were then dried at room temperature under shade in the laboratory for two weeks. All spices and herbs were ground to a fine powder using a suitable blender. The powders were used for phytochemical screening and preparation of decoctions. For the decoction 50 g of powdered herbs or spices was added to 1000 ml of distilled water. The mixture was boiled for 30 minutes and filtered. The filtrates were evaporated under vacuum until dryness. They are distributed in tubes, stored at -20° C and were further used to carry out glycation tests for the determination of fructosamines and AGEs.

2.5. Phytochemical screening

The major phytochemical groups were determined using various methods described by Houghton [19]. Mucilages were detected by the ethyl ether test and saponosides by the foam test. The presence of tannins was revealed by the $FeCL_3$ test. Anthocyanins were identified using HCL and KOH reagents. To detect the presence of leuco-anthocyanins, ethanol and HCl are used. The cyaniding test was carried out to detect flavonoids while the appearance of a clear fluorescence under UV rays indicated the presence of coumarins. The Fehling reaction was performed to detect reducing sugars.

2.6. Determination of total polyphenol content

The Folin-Ciocalteu method was used to measure the total polyphenol content of the aromatic herbs and spices. Briefly 0.4 ml of the plant extract is introduced into a test tube containing 2 ml of Folin-Ciocalteu reagent. After 5 min, 1.6 ml of a Na_2CO_3 solution (20%) is added. The mixture obtained is incubated at room temperature for 2 hours in the dark. The absorbance is then measured with a spectrophotometer at 760 nm. Gallic acid at different concentrations served for the calibration plot and an ethanol solution was used as the blank. The results obtained are expressed in milligram of gallic acid equivalent per gram dried weight (mg GAE/100 g DW).

2.7. Determination of total flavonoids

The flavonoids were determined by the aluminium chloride method using quercetin as standard. To 500 µl of the spice or herb extract, 2.5 ml of distilled water and 300 µl of a $NaNO_2$ solution (5%) were added. After 6 minutes, 300 µl of $AlCl_3$ (10%) are added to the mixture. We let the mixture at room temperature for further 5 minutes then added 1 ml of NaOH (1N) and 400 µl of distilled water. The absorbance of the mixture obtained was measured with a spectrophotometer at 415 nm. The blank contained the same reagent, but the extract was replaced by ethanol. The results are expressed in milligram quercetin equivalent per gram of dry weight (mg QE/ 100 g DW).

2.8. Evaluation of antioxidant activity

The chosen method for assessing the anti-radical activity of extracts is the 2.2-diphenyl-2-picrylhydrazyl hydrate (DPPH) method described by Novak with slight modifications [20]. The principle is based on the bleaching rate of the purple coloured DPPH radicals test solution. The addition of an antioxidant agent to the reaction medium provokes the reduction of DPPH- radicals and a decoloration. The reaction was monitored at 517 nm using a Genesys 50 spectrophotometer (Illkirch, France). In the reaction below, the DPPH- radical is represented by Z, the donor molecule by AH and ZH is the reduced form of the DPPH- radical.

$$Z\bullet + AH \rightarrow ZH + A\bullet \quad (1)$$

As the DPPH solution is relatively "unstable", the "real" concentration was determined by measuring the absorbance against a "blank" of 1/10 diluted ethanol. The results were expressed as percentages of inhibition calculated by comparison to the sample without any extract.

$$PI\ (\%) = [(Abl - Aext)/ Abl] \times 100$$

PI: percentage of inhibition
Abl: absorbance of negative control (containing no extracts)
Aext: absorbance of extracts.

2.9. Glycation of serums from non-diabetic patients and inhibition by the herbs and spices extracts

Serum from non-diabetic patients was subjected to glycation with a 2% glucose solution by the method described by Ardestani [21]. To evaluate the inhibition potential of basil, turmeric, laurel, coriander and ginger on glycation, the extract of each spice or aromatic herb was added to the glycation reaction medium. In the test tubes 1 ml of 2% glucose

solution, 1 ml of serum (the proteins concentration was adjusted to 50 g/l), 50 µl of a sodium azide solution at the concentration of 20 mg/L, 100 µl of 0.1 M phosphate buffer (pH = 7.4) and 100 µl of the extract at the concentration of 150 mg/ml are mixed. All the solutions were prepared in the phosphate buffer. The reaction mixtures were incubated at 37° C in the dark for 7 days. Glycated proteins or fructosamines were determined by the nitroblue tetrazolium method.

2.10. Determination of the antiglycation effect of the five herbal extracts and spices on the serum pool of the diabetic patients

The procedure is similar to that described above (Glycation of serums from non-diabetic patients and inhibition by the herbs and spices extracts), except for the addition of the 2% glucose solution. The diabetic serum pool was not subjected to glycation to assess the effect of spice and herb extracts at blood glucose concentrations corresponding to the physiological conditions of diabetics. Test tubes containing serum from diabetic patients, sodium azide solution, phosphate buffer and aqueous extract of each aromatic herb or spice were incubated at 37 °C protected from light for 7 days. Glycated protein or fructosamine levels were determined by the nitroblue tetrazolium method.

2.11. Determination of serum fructosamines by the nitroblue tetrazolium (NBT) method

The determination of serum glycated proteins or fructosamines was performed by the nitroblue tetrazolium method with minors modifications [22]. Serum fructosamines were measured using a colorimetric assay based on the ability of ketoamines to reduce NBT to formazan. Serum samples from non-diabetic patients and diabetic patients subjected to glycation inhibition by the spice and herb extracts were used. To 1 ml of the reaction medium the same quantity (1 ml) of carbonate buffer at pH 10.8 and then 0.5 ml NBT at 0.48 mmol/L was added. The mixture was incubated for 15 min at 37 °C. The spectrophotometric analysis was carried at 550 nm using a KENZA-Biolabo spectrophotometer (Maizy, France).

2.12. Determination of fluorescent AGEs

The reaction medium was the same as that used for glycation tests with normoglycemic serums to which the spice or aromatic herb extract was added. Test tubes contained serum, 15% glucose solution, sodium azide solution, phosphate buffer and the aqueous extract of each aromatic herb or spice at a concentration of 150 mg/ml. Two groups of reaction media were produced for this purpose. To evaluate the effect of the extracts on already formed AGE, one group of reaction media was incubated for three weeks. The spice or aromatic herb extracts were added the day before AGEs determination. Another group was treated as usual by incubating the reaction media for 4 weeks with the extracts added at the start of the experiment. Aminoguanidine was used as a reference molecule. The inhibition rate was determined by comparison with the reaction medium without extract. AGEs were determined by a "Turner Design" brand TD-700 type fluorimeter at an excitation wavelength of 365 nm and emission wavelength of 450 nm [16,23].

2.13. Data collection and analysis

Variance was calculated using ANOVA software to compare glycation inhibition percentages. The Shapiro-Wilk and Brown-Forsythe tests were used respectively to check normality and to calculate the variance of each series consisting of three measurements. Paired comparisons of values were made using Dunnett test and Kruskal-Wallis test where appropriate. The significance level was set at 5%. Graphs were produced using Excel and Sigmaplot V14.5.

3. Results and discussion

The first part of this study focused on 14 spices and aromatic herbs, all of which showed anti-glycative activity (not published data). The five extracts with the highest fructosamines inhibition rates were the subject of the work presented in this paper. These were ginger (*Zingiber officinale*) and turmeric (*Curcuma longa)* rhizomes, coriander (*Coriandrum sativum)* seeds and laurel (*Laurus nobilis*) and basil (*Ocimum basilicum*) leaves. Several studies have reported the potential of natural compounds with antiglycation activity [24]. It has been shown that plants extracts exert their effect at various stages by inhibiting the reaction between sugar and proteins, preventing the oxidation of glycated proteins or influencing the metal-catalysed oxidation of glucose that leads to AGE formation [25].

3.1. Phytochemical analysis

All five aromatic herbs and spices contain mucilages, condensed tannins, flavonoids and reducing sugars. The absence of leuco-anthocyanins in *C. savitum* extracts is noteworthy. Almost all the extracts have been found to contain tannins and flavonoids (Table 1). Phytochemical screening of the aqueous extract of *O. basilicum* showed the presence of mucilages, tannins, anthocyanins, flavonoids, leuco-anthocyanins, reducing sugars and the absence of coumarins (Table

1). These results are supported by findings obtained by N'guessan [26]. With regard to the aqueous extract of *C. longa*, the presence of alkaloids, tannins, flavonoids and saponins was noted. There is a notable difference between our results and those reported by Mahmood [27], who noted the absence of all chemical groups except tannins in aqueous extracts of *C. longa* [27]. Similar to the results of Lakhera et al, (2015), our study showed that *C. sativum* aqueous extracts are rich in flavonoids, alkaloids and polyphenols [28]. In addition to these three major groups, the presence of reducing sugars, coumarins and mucilages was observed. Polyphenols, flavonoids, tannins, alkaloids, anthocyanins and saponins were mainly detected in the aqueous extract of *L. nobilis* leaves. Screening of *Z. officinale* revealed the presence of flavonoids, alkaloids and tannins. These results are similar to those found by Jan [29]. The observed differences are common since it is known that geographic location, season, and other environmental factors can influence the phytochemical composition of plant species.

Table 1 Phytochemical composition of the 05 spices and herbs tested

Chemical compounds	***Ocimum basilicum***	***Curcuma longa***	***Coriandrum sativum***	***Laurus nobilis***	***Zingiber officinale***
Mucilages	++	++	+	+	++
Saponosides	++	++	-	+++	-
Condensed tannins	+	+	+	++	+
Gallic tannins	++	+	-	+	+
Anthocyanins	+	+	+	++	-
Flavonoids	+	+	+	+++	+
Leuco-anthocyanins	+	+	-	++	+
Reducing sugars	+	+	+	++	+
Coumarins	-	+	+	+	+

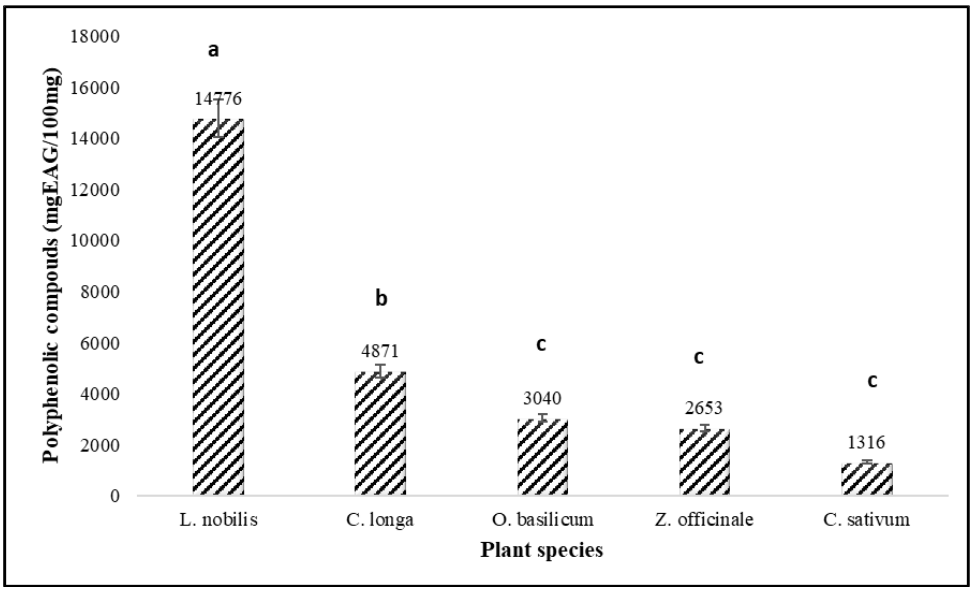
Legend: (-) absent; (+) present; (++) abundant; (+++) very abundant

3.2. Total polyphenols contents of the tested herbs and spices

All aromatic herbs and spices contained phenolic compounds at concentrations varying from 1316.22 for *Z. officinale* to 14776.00 mg EAG/100 g for *L. nobilis*. However, the highest level of phenolic compounds was found in the leaves of *L. nobilis* followed from rhizomes of *C. longa* (Fig. 1). The other three species showed lower and variable levels that were not statistically different.

Zheng and Wang working on 39 culinary herbs found total phenolic content between 0.26 and 17.51 mg EAG/g fresh weight. The phenolic compounds level obtained in our study for *L. nobilis* (147.76 mg GAE/g) and *O. basilicum* (39.96 mg GAE/g) were significantly higher than those of respectively 4.02 mg GAE/g and 2.3 GAE/mg reported by these authors. This difference may be partly due to the fact that we used dried spices and herbs while Zheng and Wang used fresh product [30]. Moreover, other authors who have also used dried aromatic herbs and spices have reported polyphenol contents similar to ours.

The value of 11.3 mg/g GAE found for ginger by Starowicz in their work on dried and homogenized spices is close to our total polyphenol content of 13.16 mg EAG/g while Dearlove reported a higher value of 17.7 mg GAE/g for the same spice [17]. However laurel, turmeric and basil plants studied in our work exhibited elevated phenolic content compared to the values found in the study of Dearlove. Some studies have shown that phenolic compound contents vary considerably from one species to another and within the same species, due to temperature, climate, [31] variety and species origin [32]. The distribution of secondary metabolites can change during plant development, which can be linked to harsh climatic conditions (temperature, drying, etc.) that stimulate the biosynthesis of secondary metabolites such as polyphenols [33].

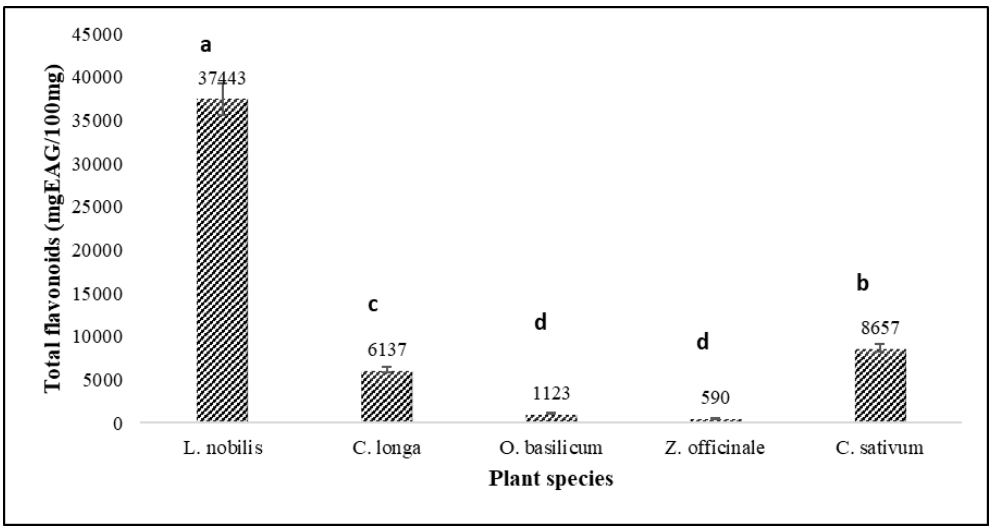


Data are expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of dry extract. Same letters indicated that the mean values are not significantly different. Different letters indicated mean values that are statistically different with P<0.05 (Dunnett test).

Figure 1 Average total polyphenolic contents in mg GAE/ 100 g extract

3.3. Total flavonoids contents of the extracts

The 05 aromatic herbs and spices contained flavonoids ranging from 590 to 37443.33 mg quercetin /100 g extract. *L. nobilis* had the highest total flavonoid content and the lowest value was found in *Z. officinale* (Fig. 2).



Significant means values are marked with different letters while values with the same letter are not significantly different. Significance level was set to 0.05 (Dunnett test)

Figure 2 Average total flavonoids contents in mg QE/100g extract.

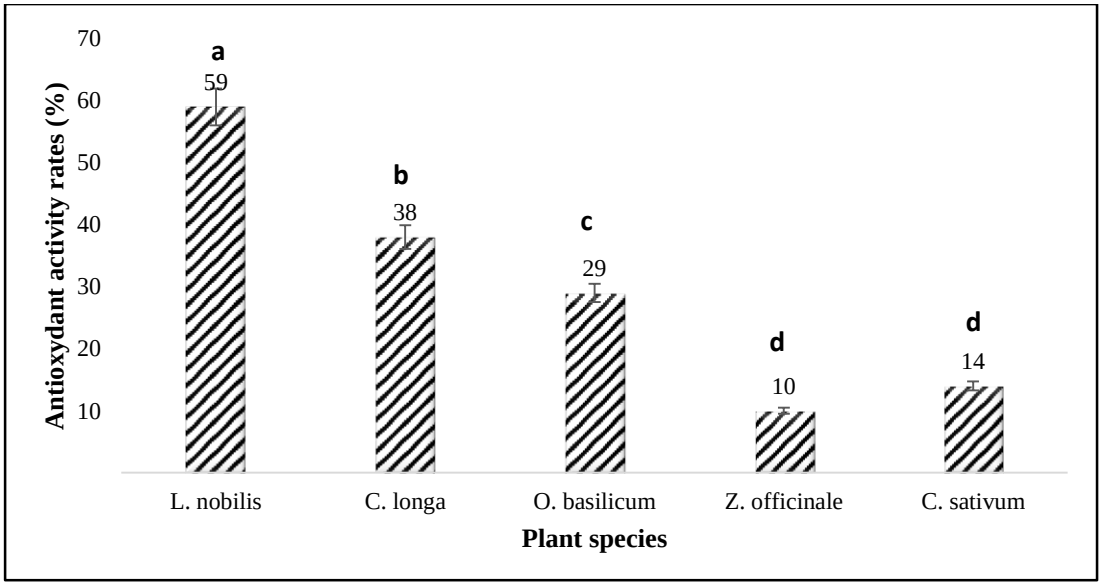
Using aqueous extraction, other authors have reported high flavonoids content in extracts of turmeric, followed by laurel, ginger and coriander respectively [34]. The study by Hayat and Sabri (2016) on *C. longa* in Pakistan reported flavonoid contents of 2.282 mg EQ/g of dry matter, which is lower than that found in our study [35]. In 2021, Muzolf-Panek recorded a lower level of flavonoids (3.59 mg EQ/g) compared with those found in our study [36]. The composition and quantity of secondary metabolites in an extract can be influenced by a number of factors, such as extraction mode and time, temperature, the nature of the solvent and its polarity, which enables compounds of similar polarity to the solvent [37].

3.4. Antioxidant activity of the five spices and herbs extracts

In this study, the highest DPPH radical scavenging activity was recorded in *L. nobilis* (58.8%), followed respectively by *C. longa* (38.2%), *O. basilicum* (29.4%), *C. savitum* (14.1%) and *Z. officinale* (10.1%). These values were as weak as those found by Kim who reported percentage of 39.63% for basil and 24.43% for turmeric aqueous extracts [38]. Except coriander, the polyphenols content of the tested herbs and spices followed the same order as the antioxidant activity (Table 2). Laurel exhibited the highest and ginger possessed the lowest polyphenol compounds value. Our results are similar to those found by Slowianek on polish spices in that ginger exhibited the lowest values for polyphenols content and antioxidant activities when compared to basil, laurel and turmeric. According to this author, laurel showed the greatest value of polyphenols content and the second highest antioxidant activity [39]. In agreement with these findings, Lu also highlighted a strong positive correlation between the total phenolic composition and antioxidant capacity of spices commonly consumed in China [40].

Similar to our study, Muñiz-Márquez et al. in their work on aqueous extracts of selected herbs and spices showed that *L. nobilis* had a higher antioxidant activity than *C. sativum* [41]. Authors have noticed that spices of the Umbelliferae family such as coriander among others displayed weaker antioxidant activities than those from the Lauraceae family like laurel and other species [34]. It is known that the antioxidant activities of plants extracts are generally related to their polyphenol content. However, due to the complexity in the composition of the compounds, the polyphenol content is not always correlated with the antioxidant capacity. Other authors have reported poor correlation between antioxidant activity and polyphenol content [42].

Among polyphenols, phenolic acids and flavonoids have the ability to scavenge free radicals and are known for their notable antioxidant properties. In our study, the flavonoids content of the extracts decreased in following order: laurel, coriander, turmeric, basil and ginger while the percentage of DPPH inhibition was laurel > turmeric > basil > coriander > ginger. Laurel showed the highest radical scavenging activity and the greatest flavonoid content. In contrary to these results, Hamrouni-Sellami who worked on extracts including laurel, turmeric, ginger and coriander, reported the lowest flavonoid content associated with the highest antioxidant activity in turmeric [34]. Some studies pointed out a correlation between flavonoids content of plants extracts and their antioxidant activity [43,44] while other authors reported a weak or no correlation [36].



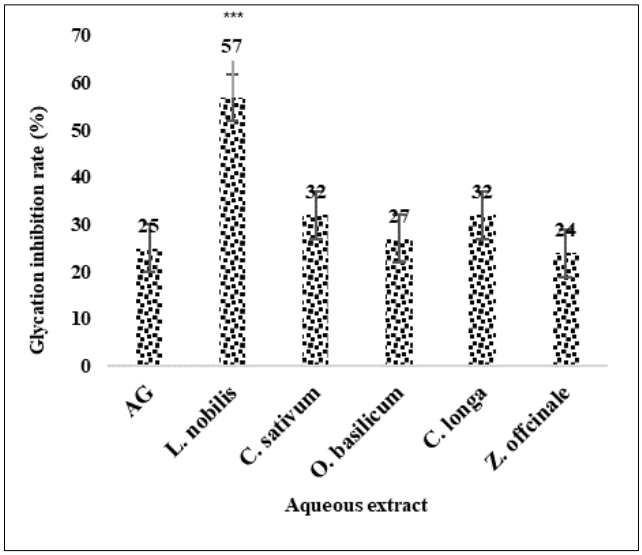
Antioxidant capacity was performed using DPPH radical scavenging ability. Results are expressed in percentage in comparison to a blank without extract. Same letters indicated that the mean values are not significantly different. Significance level was set to 0.05 (Dunnett test).

Figure 3 DPPH free radical scavenging activities of the spices and herbs extracts tested

3.5. Inhibition of serum glycated proteins

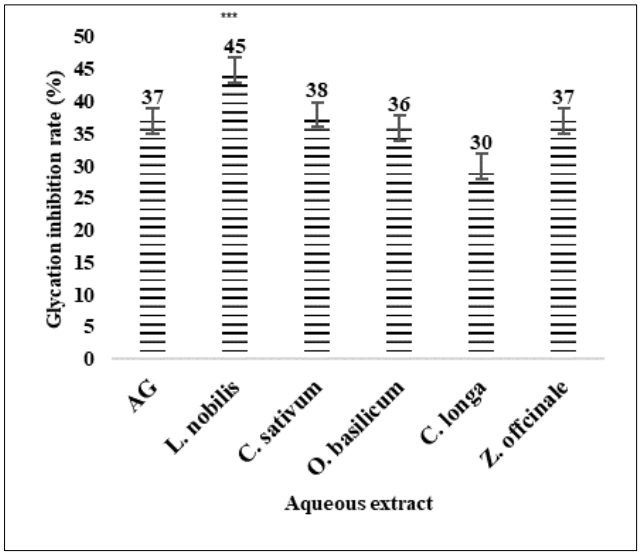
The five extracts tested in this study (rhizomes of *Z. officinale* and *C. longa*, seeds of *C. savitum*, leaves of *L. nobilis* and basil (*O. basilicum*) showed significant inhibitory potential against protein glycation *in vitro*. For the evaluation of the glycation inhibition, two types of samples were used. On the one hand, a pool of non-diabetic serum that has been subjected to glycation with 2% glucose and on the other hand a pool of diabetic serum that has been directly used for the inhibition tests.

Glycation inhibition percentages varied depending on extracts in the non-diabetic serums pool from 24% for ginger to 57% for laurel (Fig 4). The inhibition rates found for the serum of diabetic patients were all below 50%, ranging from 30% for turmeric to 45% for laurel (Fig. 5). Regarding the non-diabetic serum, the reference molecule aminoguanidine exhibited the lowest inhibition rate of 25%. Overall the inhibition rates of the glycated non diabetic serum were slightly lower than those of the diabetic serum pool. In both cases laurel displayed the highest inhibition rates in comparison to aminoguanidine and the other spices and herbs (Fig 4 and fig 5). This differences were statistically significant (*p=0.0001*). Although the values were different, the differences were not statistically different between aminoguanidine and the other 04 spices and herbs which have displayed a similar inhibitory potential as the reference molecule.



The glycation inhibition percentages were calculated using the values of the sample without extract considered as none inhibition. The inhibition percentages of extracts were compared to those of aminoguanidine. Only *L. nobilis* is significantly different with P=0.0001 and marked with the sign*** (Kruskal-Wallis test)

Figure 4 Glycation inhibition rates of aqueous extracts of aromatic herbs and spices in non-diabetic serum



The glycation inhibition percentages were calculated with the values of the sample without extract considered as none inhibition. The inhibition values of extracts were compared to those of aminoguanidine. *L. nobilis* exhibited the highest inhibition rate with P=0.0001 (Kruskal-Wallis test).

Figure 5 Glycation inhibition rates of aqueous extracts of aromatic herbs and spices in the diabetic serum pool

Extracts of aromatic herbs and spices conceal several mechanisms of action, some of which are very little studied in our country, notably the antiglycative property that has been demonstrated by numerous studies carried out elsewhere. A study has shown that aqueous extracts of cinnamon bark have an inhibition potential similar to that of aminoguanidine [45]. Another study in Pakistan revealed the inhibitory effect of ginger on proteins glycation. Moreover, this author showed an important reduction of glucose diffusion [46]. Naderi in Iran reported good inhibition capacity of wild caraway, turmeric, cardamom and black pepper on haemoglobin glycation [47]. These authors and numerous other studies have pointed out the inhibiting effects of culinary herbs and spices on proteins glycation.

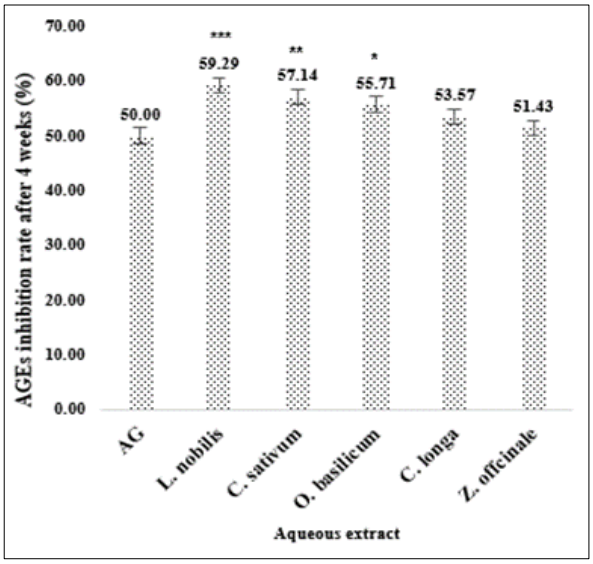
3.6. Determination of AGE inhibition

The formation of AGE occurs at the last stage of the Maillard reaction during the glycation process. Lasting hyperglycemia generates a high level of AGEs which accumulate in the organs and ultimately lead to tissue damages linked to diabetes complications. Numerous studies have shown that besides other biological properties, spices and culinary herbs displayed the ability to counteract the harmfulness of AGEs. According to literature, AGEs are generally determined by their fluorescence after an incubation time of two to five weeks [16,46,48]. We have chosen the incubation time of four weeks to assess the inhibition of AGE formation by the 05 extracts.

The extracts showed good inhibitory activity against AGE formation with percentages ranging from 50.00 to 59.29% with aminoguanidine having the lowest. Three extracts out of five namely laurel (*L. nobilis*) turmeric (*C. longa*) and basil (*O. basilicum*) showed statistically significant differences when compared aminoguanidine (Fig. 6). No significant differences were observed between the inhibition rates of coriander (*C. savitum*) and ginger (*Z. officinale*) in comparison to that of aminoguanidine. Studies by Perez Gutierrez (2012) in Mexico on methanolic extracts of marjoram revealed a

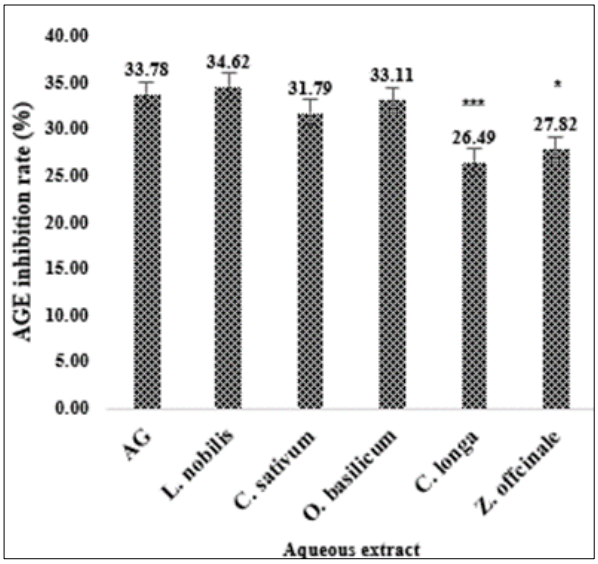
better *in vitro* inhibition of AGE formation than aminoguanidine with the experimental models BSA-glucose and BSA-methylglyoxal [48]. Similar to the findings of our study, a work of Ramkissoon et al, (2016) on various spices and herbs including ginger (*Z. officinale*) and turmeric (*C. longa*) using herbs and spices infusions, revealed glycation inhibition percentages ranged from 20.2% to 59.4%. These results are in agreement with those of Dearlove et al, (2008) and Starowicz et al, (2019) who worked respectively on twenty spices and aromatic herbs in Greece, and on fourteen samples of culinary species collected in Poland and Slovakia [17,18]. The AGEs inhibitory effect of various spices has been proved. Starowicz reported higher inhibition percentage of respectively 88%, 87% and 81% for clove, oregano and anise however the rate of inhibition found for ginger is completely comparable to ours and those of other authors. We note that this author has used ethanolic extracts. Another in vitro study showed that aqueous extracts of *Z. officinale* inhibited AGE formation in lens proteins. The authors demonstrated that administration of *Z. officinale* to diabetic rats significantly inhibited the formation of various AGEs in the ocular lens [49].

The inhibitory activity of the five herbs and spices on already formed AGEs was assessed by incubating the reaction medium for 3 weeks, and adding the extracts 24 hours before the determination of fluorescent AGEs. Notable AGE inhibition levels varying from 26.49 for turmeric to 34.62% for laurel were recorded (Fig. 7). Turmeric and ginger displayed statistically lower inhibition rates than the 3 other extracts which showed a similar activity as the reference molecule aminoguanidine. These results indicated the ability of the tested extracts to counteract already formed AGEs. These values were as expected lower than those depicted in figure 6 which were obtained after 4 weeks incubation time of the extracts with the reaction medium. Several mechanisms are involved in the inhibition of AGE formation such as chelating of metal ions, scavenging of free radicals, preventing of the conversion of Amadori products to AGE and the degradation of already formed AGEs [11,17].



The AGEs inhibition percentages were calculated with the value of the sample without extract considered as none inhibition. The inhibition values of extracts were compared to those of aminoguanidine. Significant values P<0,001 are marked ***, 0.001<P<0.01 are marked ** and 0.001<P<0.05 are marked * (Kruskal-Wallis test)

Figure 6 Inhibition rate of fluorescent AGEs after four weeks incubation of the reaction medium



The AGEs inhibition percentages were calculated with the value of the sample without extract considered as none inhibition. The inhibition values of extracts were compared to those of aminoguanidine. Significant values P<0,001 are marked ***, 0.001<P<0.01 are marked ** and 0.001<P<0.05 are marked * (Kruskal-Wallis test)

Figure 7 Fluorescent AGE inhibition rate after 3 weeks incubation with the addition of extracts 24 h before AGE determination

Out of the five tested herbs and spices, laurel leaves exhibited high polyphenols and flavonoids contents associated with the more potent antioxidant capacity and glycation inhibition. Ginger, which showed the lowest level of flavonoids, revealed an antioxidant activity close to that of coriander. The percentages of inhibition of glycated proteins and those of the reduction in the level of AGEs of ginger are higher or comparable to those of coriander. Polyphenolic and flavonoid contents varied from one extract to another, as varied also the antioxidant activity and the glycation inhibition rate. All plant species in this study are rich in phytochemicals such as coumarins, tannins and polyphenols including flavonoids that are reported to possess antiglycative and antioxidant properties [11,50]. Tannins are known to have high antioxidant activity and are very good scavengers of free radicals, thus inhibiting the formation of the superoxide radical [51]. Flavonoids were described to possess antioxidant and anti-inflammatory activities and are benefit in the treatment

of cardiovascular and neurodegenerative diseases [52]. It is well known that antioxidant properties of plants are linked to the presence of phenolic acids and flavonoids [53,54].

Since the aim of this study was to investigate the antiglycative properties of 05 herbs and spices widely used in the culinary art in South Benin, very promising results have been achieved. The results of our study on the antiglycative effects of herbs and spices are in agreement with the literature data. Ginger is widely used in different regions of the world in making drinks, candies and as a seasoning. Its antihyperglycemic properties have been reported and it has been the subject of *in vivo* experiments in humans. The rhizomes of ginger contain a notable amount of polysaccharides [55,56]. The most studied constituents are gingerols, which are responsible for its antioxidant and antiglycative properties. Akash demonstrated its capacity to prevent diabetes complications through several metabolic processes such as inhibition of lipid peroxidation, interaction with carbohydrate metabolism, the activation of antioxidant enzymes [55]. In turmeric, another rhizome of the Zingiberaceae family, the presence of several natural compounds has been described. The most important and best studied is curcumin to which the antidiabetic activities are due. Antioxidant and antiglycative activities of turmeric extracts have been reported [50,57]. Numerous studies have identified essential oils in basil and proved their antidiabetic effects. Eugenol, pinene, farnesol which are among the identified constituents [57] are antioxidants found also in other medicinal plant species [58]. In addition, a study showed the antiglycative activity of eugenol, which is also present in other plants of the genus Ocimum [59]. The antidiabetic activity of coriander has been reported [56] and its antioxidant and antiglycative activities have been proven [44]. Among its antidiabetic properties, its beneficial effects on lipid metabolism were pointed out. Linalool, γ-terpinene, terpinen-4-ol and ρ-cymene are the main components of coriander seeds [57]. The leaves of laurel showed in this study the highest contents of polyphenolic compounds and flavonoids. The extract of this culinary herb exhibited the greatest antioxidant activity, reduction of glycated serum proteins as well as inhibition of AGEs. Its effect on glycation was more effective than that of aminoguanidine. Some authors have reported a strong antiglycative activity of culinary herbs of the Lauraceae family in comparison with other spices. A good anti-radical capacity as well as the inhibition of glycation have been reported by several authors for laurel [60,61]. Among the reported components, quercetin, gallic acid, linalool and eugenol [61] could explain the observed effects. Among the antidiabetic plants, culinary herbs and spices have gained more interest because they are considered safe due to their long last consumption in human nutrition.

Table 2 Contents of polyphenolic compounds, antioxidants and flavonoids of herbs and spices and their inhibition rates of glycation and AGE formation

Herbs and spices	**Total polyphenolic compounds**	**Total flavonoids compounds**	**DPPH Antioxi-dant activity (%)**	**Glycation inhibition in non-diabetic sample (%)**	**Glycation inhibition in diabetic sample (%)**	**AGE inhibition with extracts added 24h before determination (%)**	**AGE inhibition with extracts during 4 weeks (%)**
L. nobilis	14775±1775.89	37443.33±3379.75	58.8	57	45	35	59
C. savitum	1316.22±200.01	8656.67±932.42	14.1	32	38	32	57
C. longa	4870.67±228.74	6136.67±121.72	38.2	27	30	26	56
O. basilicum	3039.56±413.68	1123.33±623.28	29.4	32	36	33	54
Z. officinale	2652.89±311.51	590±30	10.1	24	37	28	51
Aminogua-nidine	-	-	-	25	37	34	50

4. Conclusion

Our study showed that five herbs and spices (*O. basilicumum, C. longa, C. savitum, L. nobilis and Z. officinale*) possessed strong activity in inhibiting serum protein glycation and AGE formation. The polyphenolic and flavonoids content of the tested herbs and spices would confer the observed activities. These results show that these herbs and spices, commonly used in the culinary arts in Benin, have an interesting potential, as their anti-glycative properties suggest a notable role in slowing the development of diabetic complications. Further studies to determine the therapeutic doses of these herbs and spices will enable us to explore their potential as dietary supplements. As plant extracts are characterized by their richness in various phytochemical compounds, further *in vivo* and *in vitro* investigations are needed to probe other mechanisms involved in the monitoring of diabetes.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors have no conflict of interest to declare.

Statement of ethical approval

The present research work does not contain any studies performed on animals or humans subjects by any of the authors.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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