WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 11, 17-31. Research Article ISSN 2277–7105

INHIBITION OF SERUM PROTEINS GLYCATION AND ALPHA-AMYLASE ACTIVITY BY AQUEOUS EXTRACTS OF CULINARY HERBS AND SPICES

Guilphados Djogbede[1], Eugénie Anago[1,2*], Atchadé Pascal Tchogou[4,1], Maïmounatou Atindehou[1] and Lamine Saïd Baba-Moussa[3]

[1]Laboratory of Research in Applied Biology (LARBA), EPAC, University of Abomey-Calavi, B.P. 2009, Cotonou, Benin.
[2]Laboratory of Biochemistry and Molecular Biology (LBBM), FAST, University of Abomey-Calavi, 03 B.P. 0420, Cotonou, Benin.
[3]Laboratory of Biology and Molecular Typing in Microbiology, (LBTMM), FAST, University of Abomey-Calavi 01 BP 188 Cotonou, Benin.
[4]Experimental and Clinical Biology Unit (UBEC), Biotechnology Research Laboratory Medical and Pharmaceutical (LaRBiMeP), National School of Biosciences and Biotechnology of Dassa-Zoumé (ENSBBA), National University of Science, Technology and Engineering of Abomey (UNSTIM), BP: 14, Dassa-Zoumé, Benin.

Article Received on
05 April 2024,
Revised on 25 April 2024,
Accepted on 15 May 2024
DOI: 10.20959/wjpr202411-32527

***Corresponding Author**
Dr. Eugénie Anago
Laboratory of Research in Applied Biology (LARBA), EPAC, University of Abomey-Calavi, B.P. 2009, Cotonou, Benin.

ABSTRACT

Diabetes continues to be a public health problem worldwide. However, especially in low-income countries in Asia, Africa and Latin America, the search for treatments based on natural compounds is being considered following the serious side effects of certain synthetic molecules. In this study we explored the potential of 14 spices and herbs commonly used in southern Benin for seasoning to inhibit protein glycation as well as alpha-amylase. Glycation inhibition was performed using the method of thiobarbituric acid to determine the serum fructosamine level. The substrate chloronitro-3-phenol served to measure the inhibition of porcine alpha-amylase. The results showed that turmeric, coriander, ginger and laurel displayed the highest inhibition rates of glycation ranging from 71 to 79% while anise, mint, laurel and ginger revealed the highest inhibition percentages of alpha-amylase with values varying from 87 to 93%. The properties highlighted in these spices and culinary herbs are based on their chemical composition in polyphenolic compounds and especially specific flavonoids which have been reported in the

literature. This work contributes to the effort to identify herbal preparations which can be used in the prevention or alleviation of diabetes.

KEYWORDS: Diabetes, protein glycation, alpha-amylase, fructosamines, spices, Benin.

INTRODUCTION

Diabetes mellitus is a metabolic disease that continues to spread throughout the world. In 2019, diabetes was the direct cause of 1.5 million deaths, and 48% of all deaths due to diabetes occurred before the age of 70 years. Furthermore, elevated blood glucose causes around 20% of cardiovascular deaths.[1] The situation is particularly complicated in developing countries because of a weak health system and the financial difficulties of populations in paying for treatment linked to this chronic disease. Production of very little or no insulin and resistance to insulin action are the cause of this pathological condition. The resulting permanent hyperglycemia will affect the body in different ways. There are significant disorders in the metabolism of carbohydrates but also lipids and proteins with hyperglycemia and dyslipidemia as the main manifestations.[2]

Circulating and intracellular proteins undergo a glycation reaction by glucose, which will modify their structure and function. These glycated proteins do not properly exert their different functions, forming cross-linking aggregates, and accumulating in tissues.[3] Their degradation products called advanced glycation products AGEs, are linked to deleterious effects of diabetes such as retinopathy, nephropathy, neuropathy and vascular damage.[4,5] Moreover, hyperglycaemia causes the production of reactive oxygen species (ROS) which in a reaction cascade will result in a negative effect on insulin regulation, further aggravating hyperglycaemia.[6]

The therapy of diabetes is multifaceted, reflecting the complexity of the disease. Measures such as sport and adapted diets are combined with therapeutic molecules, which intervene at different levels with varied mechanisms of action. Apart from the administration of insulin which remains one of the major therapeutic interventions, the inhibition of the glycation of biological macromolecules, the delay of the digestion of polysaccharides, the inhibition of protein tyrosine phosphatase constitute approaches to counteract the deleterious effects of hyperglycemia due to diabetes. Alpha-amylase catalyses the hydrolysis of dietary starch to maltose which is then digested by α-glucosidase to release glucose molecules. Therefore, inhibiting digestive enzymes like α-amylase is one of the most effective ways to alleviate

hyperglycaemic condition. Culinary herbs and spices have been used for seasoning but also for their therapeutic benefit against various pathologies. Synthetic molecules, natural compounds isolated from medicinal plants and extracts from these plants are tested by researchers around the world to expand the range of therapeutic solutions. Numerous studies have demonstrated the biological activities of natural compounds found in spices and aromatic herbs, including their antidiabetic effects.[7] This work was initiated in the aim to investigate the antidiabetic effects through the inhibition of glycation and alpha-amylase activity by 14 spices and aromatic herbs purchased in a market in southern Benin.

MATERIAL AND METHODS

Chemical and reagents

Porcine alpha-amylase was procured from FischerSci (Illkirch-Graffenstaden, France). The reagent for determination of alpha-amylase activity was the kit from ELITechGroup (Puteaux, France) using chloronitro-3-phenol as substrate. All other reagents were obtained from MolyCHEM (Badiapur, India).

Vegetal and biological materials

The plant material consisted of fourteen spices and aromatic herbs widely used in south Benin for seasoning. 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 various herbs and spices thyme (*Thymus vulgaris L*.), parsley (*Petroselinum crispum Mill*.), clove (*Syzygium aromaticum L*.), ginger (*Zingiber officinale*), black pepper (*Piper nigrum L*), anise (*Pimpinella anisum L*), coriander (*Coriandrum sativum*), cumin (*Cuminum cyminum*), turmeric (*Curcuma longa*), celery (*Apium graveolens*), laurel (*Laurus nobilis*), basil (*Ocimum basilicum*), mint (*Mentha spicata*) and garlic (*Allium sativum*) were purchased in February 2021 at a market in Abomey-Calavi, southern Benin. The herbs and spices were identified by a botanist from the University of Abomey-Calavi.

The study required human serums from anticoagulant-free tubes. Venous blood samples were collected from normoglycemic subjects after overnight fast. Glycaemia and determination of serum proteins concentration were carried out using respectively GOD/POD method and biuret. A pool of the serums was realised to perform the serum proteins glycation reactions.

After the proteins concentration was adjusted to 50 g/l, the average glucose value of the serum pool was 0.86 g/l.

Preparation of plants extracts

The plant organs were carefully cleaned and dried at a temperature of 25°C for two weeks. The plants parts used were leaves of thyme, parsley, celery, laurel, basil and mint, the seeds of clove, black pepper, anise, coriander and cumin, rhizomes of ginger and turmeric and bulbs of garlic. They were then grounded and served for the preparation of the aqueous extracts. A decoction of the plant parts was performed by adding 1L of distilled water to 50 g of the powdered spice or aromatic herbs. The mixture was boiled and after cooling at room temperature, it was filtered using Whatman® paper. The filtrates were dried in an oven at 45°C until the water evaporates completely. They are then stored in airtight tubes and will be used to carry out biological tests.

In vitro glycation of serum proteins

The glycation of total serum proteins was carried out with glucose according to a modification of the method described by Singh.[8] The reaction medium contains 1 ml of 2%, 5%, 10%, 20% or 30% glucose solution. 1 ml of human serum (50g/l), 100 µl of 0.1 M phosphate buffer, pH 7.4 and 50 µl of a sodium azide solution as antimicrobial agent (20 mg/L) are added. All solutions were prepared in phosphate buffer. The tubes containing the reaction media were incubated at 37°C in the dark for 7 days. Then the fructosamines formed by the glycation of serum proteins were determined by the thiobarbituric acid method. The reactions were performed in triplicate.

Effect of aromatic herbs and spices on serum proteins glycation

The concentration of 15% glucose was chosen for the evaluation of the glycation inhibition by the aqueous extracts. For the glycation inhibition tests, 1 ml of the extract of aromatic herbs or spices at a concentration of 150 mg/ml was added to the glycation reaction medium described above. After the preliminary tests, the duration of the incubation of the reaction media for the determination of the level of glycated serum proteins is set at 6 days. A reaction medium without plant extract which glycation rate was set at 100% served as a negative control. The experiments were repeated three times and their averages were reported.

Determination of the level of proteins glycation

Total serum glycated proteins or fructosamines were determined by the thiobarbituric acid (TBA) colorimetric method.[9,10] To 1 ml of the serum sample subjected to glycation, 1 ml of trichloracetic acid (TCA) was added. The solution was centrifuged for 10 minutes at 3000 RPM. The supernatant was removed and the operation was repeated twice. Then, 1 ml of phosphate buffer and 0,5 ml of 0,3 N oxalic acid was added to the pellet. The mixture was incubated for 3 minutes in boiling water bath, then left to stand until cooled. After adding 0,5 ml of 40% TCA to each sample, the mixture was centrifuged for 10 minutes at 3000 RPM. The supernatant is separated and 1 ml is taken to which 0,5 ml of 5% thiobarbituric acid (TBA) was added. The solution was incubated in water bath at 40°C for 30 minutes. The absorbance of the samples was determined using a Genesys spectrophotometer (Illkirch Cedex, France) at 440 nm.

The inhibition percentage was calculated as follow:

$$\text{Percentage of inhibition} = \frac{\text{OD of sample without extract} - \text{OD of the sample}}{\text{OD of sample without extract}} \times 100$$

Alpha-amylase inhibition assay

The inhibition of porcine pancreas alpha-amylase by aqueous extracts of aromatic herbs and spices was evaluated. The principle of the reaction is based on the degradation of the substrate chloronitro-3-phenol under the catalytic action of alpha-amylase with the release of 2-chloro-4-nitrophenol whose absorbance is determined at 405 nm. The reaction medium contains 25 UI/L of alpha-amylase, 1 ml of the chloronitro-3-phenol substrate and 200 µL of aromatic herb or spice extracts at a concentration of 50 mg/mL prepared in 0.02 M phosphate buffer. (pH = 6.6). A reaction medium produced without plant extract (negative control) constitutes the basic value for alpha-amylase activity. The reaction media were incubated for 20 min at room temperature and then the absorbance was determined. The percentage inhibition of enzymatic activity was calculated according to the formula below.

$$\text{Percentage of inhibition} = \frac{\text{OD of sample without extract} - \text{OD of the sample}}{\text{OD of sample without extract}} \times 100$$

The samples were determined in triplicate.

Statistical Analysis

Variance was calculated using ANOVA software to compare glycation inhibition and alpha-amylase 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 Kruskal-Wallis test. The significance level was set at 5%. Graphs were produced using Excel and Sigmaplot V14.5.

RESULTS AND DISCUSSION

Level of protein glycation depending on the glucose concentration

Glycation reaction is mostly carried out in vitro with BSA at concentration of 10 mg/ml to 50 mg/ml. Other proteins such as haemoglobin, lens protein, HSA have also been used. In this study we chose to determine the glycation of total serum proteins or fructosamine since all proteins are affected by glycation with glucose, the main circulating monosaccharide in human body. Concerning the determination of fructosamines which are the early products of the reaction between sugar and protein, the literature suggests a duration of 3 to 7 days of incubation of the glycation reaction medium. In this study, an incubation period of the reaction medium of 6 days was used. Different concentrations of glucose were used in the aim to determine a single concentration that will serve for the further glycation and inhibition tests. The results showed that the amount of glycated proteins (fructosamines) was proportional to the concentration of glucose detected by the thiobarbituric acid method. The glycated proteins are formed in a dose dependant manner of glucose concentration as depicted in figure 1. At the end of this experiment the glucose concentration of 15% was retained for the further glycation tests.

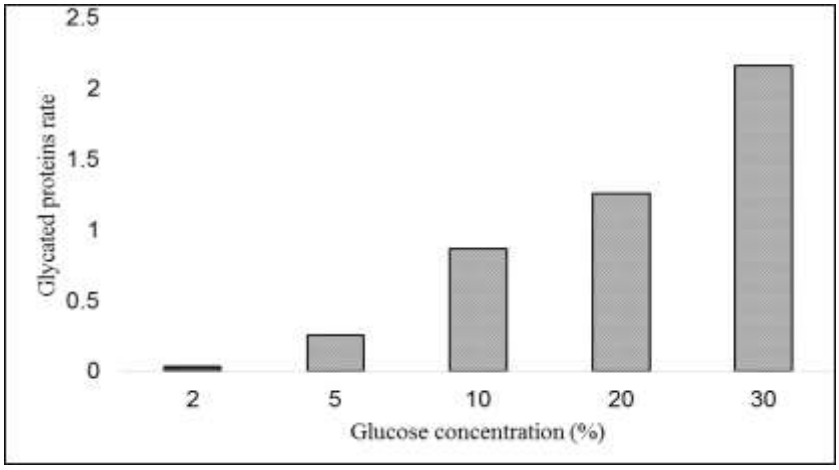

Fig 1: Glycated proteins rate depending on concentration of glucose.

Inhibition of proteins glycation by the aqueous extracts of herbs and spices

After 6 days of incubation with serum proteins and the 15% glucose solution, all the aqueous extracts of the herbs and spices tested inhibited the glycation of serum proteins with glucose as indicated by the graph of figure 2. The inhibition rates were all over 50% and ranged from

55% for thyme to 79% for laurel in the order thyme < celery < anise < mint < cumin < clove < parsley < black pepper < garlic < basil < turmeric < coriander < ginger < laurel. The reference molecule aminoguanidine with an inhibition percentage of 69% was between garlic and basil.

The work of Starowicz[11] on the glycation of bovine serum albumin with glucose showed that among the spices and herbs tested, clove and anise exerted the strongest inhibitions of 88 and 81% respectively. In the present study, clove and anise showed inhibition rates of respectively 64% and 59% which are lower than those of Starowicz. According to a study conducted by Ramkissoon, garlic (*Allium sativum*), ginger (*Zingiber officinale*), thyme (*Thymus vulgaris*), parsley (*Petroselinum crispum*), mint (*Mentha spicata*), turmeric (*Curcuma longa*), curry leaves (*Murraya koenigii*), onion (*Allium cepa*), spring onion (*Allium fistulosum*) and coriander (*Coriandrum sativum*) all showed inhibition percentages of glycation varying from 23.8% to 67.8%.[12] The inhibition rates of the present study varying between 55 and 79% were generally higher than Ramkissoon's results. It should be noted that this author used herbst and spices that were not included in our study. Yoshika's work on inhibition of glycation showed similar results regarding the antiglycative properties of thyme, parsley, ginger, celery, laurel, basil, mint and garlic, 08 herbs and spices included in the present study[13] by using the model Human Serum Albumin (HSA)-glucose.

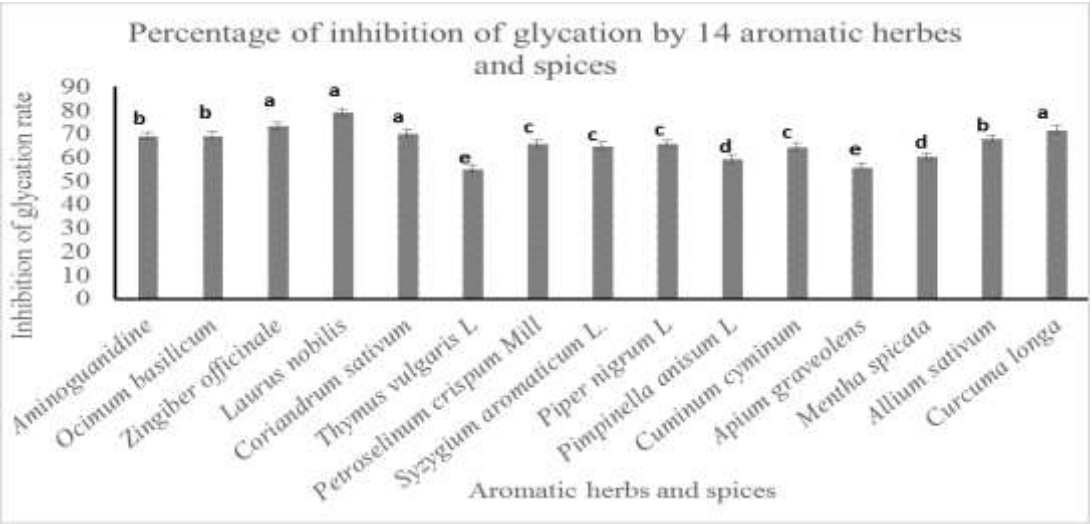

Fig 2: Percentage of inhibition of proteins glycation by aqueous extracts of spices and herbs

The values are expressed as percentages and the histogram bars not bearing the same letters are significantly different AG).

Our results suggest that aqueous extracts of ginger, turmeric, laurel, basil and coriander have the highest inhibition rate of proteins glycation. A study carried out in 2014, focusing on the antidiabetic effect of spices and aromatic herbs showed similar findings with ginger, coriander, laurel and turmeric having the strongest inhibitory activity on protein glycation.[14] Thus, the spices tested in our study also showed antiglycative properties in work carried out elsewhere.

Protein glycation is one of the consequences of permanent hyperglycemia due to diabetes. Glycation inhibition is a therapeutic approach in the fight against the deleterious effects of diabetes. Indeed, it is admitted that the accumulation of glycation products in different tissues and organs is the basis of most of the complications of diabetes. Authors have shown the antiglycative properties of plant species used in the treatment of diabetes and bioactive molecules involved in the inhibition of different stages of the Maillard reaction have been identified. Among the phytochemical components found in herbs and spices flavonoids and polyphenols are known to be responsible for their antiglycative properties. Natural compounds eugenol, quercetin, kaempferol, gingerols, shogaols, resveratrol, curcumin, catechin, gallic acid are linked to the antiglycative properties of herbs and spices.[7,15]

Effect of the aqueous extract of the herbs and spices on the alpha-amylase

Alpha-amylase is an enzyme found in the salivary, intestinal mucosal and pancreatic secretions, functioning in the breakdown of the α-1-4-glycosidic bonds in starch. Thus, this enzyme increases the bioavailability of glucose in the blood. All the aqueous extracts of the herbs and spices have shown inhibitory activity on alpha-amylase with percentages ranging from 61.21% to 93.18% as showed in figure 3. Out of the 14 herbs and spices included in this study, 07 extracts namely garlic, parsley, cumin, anise, mint, laurel and ginger revealed inhibition rates higher than that of the reference molecule aminoguanidine. Hemlata have evaluated the alpha-amylase inhibitory effects of six dietary spices including clove and cumin. At the concentration of 2.5 mg/ml the aqueous extract of cumin showed no inhibition whereas clove exhibited an inhibition of 21.83%.[16] It should be noted that the herbs and spices concentration used by this author are lower than the 50 mg/ml of our study.

A study on antidiabetic plants revealed 40 to 60% alpha-amylase inhibition but no activity of garlic.[17] It has been demonstrated that the phytochemical composition and biological properties of different garlic species varied and some species have no effect of alpha amylase activity.[18] This could explain the differences observed between the studies. Similar to our

findings, alpha amylase inhibitory activities were described for celery, coriander and parsley.[19] Another author in India reported alpha-glucosidase and alpha-amylase inhibition by cumin, black pepper and laurel.[20] The extracts tested in the present study inhibited alpha-amylase, indicating that they may produce a postprandial antihyperglycemic effect by slowing down carbohydrate catabolism and the resulting release of glucose. Inhibition of alpha-amylase and alpha-glucosidase enzymes to delaying the digestion of carbohydrates, leads to a reduction in the rate of glucose absorption and therefore suppress postprandial hyperglycaemia, which plays a central role in the development and the progression of diabetic complications.[21,22] Oyedemi et al reported in a study carried out in Nigeria, the inhibitory properties of alpha -amylase by 08 medicinal plant extracts with antidiabetic properties.[23] Similarly, another author has shown a reduction in alpha-amylase activity of more than 70% due to the action of medicinal plant extracts.[24]

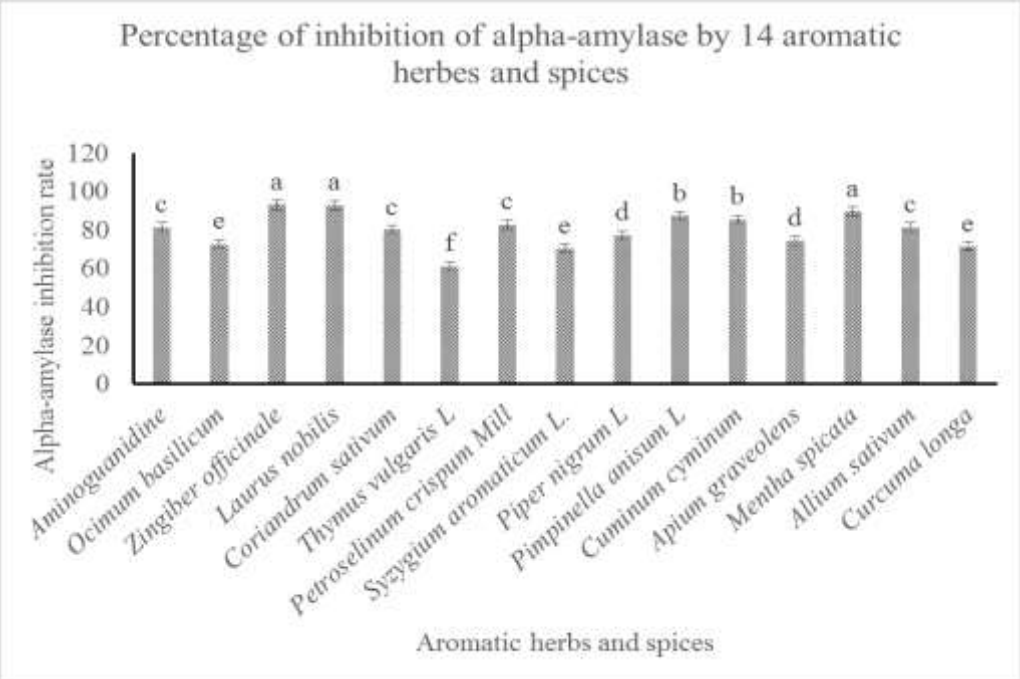

Fig 3. Percentage of inhibition of alpha amylase by aqueous extracts of spices and aromatic herbs.

The values are expressed as percentages and the histogram bars not bearing the same letters are significantly different.

For a substance to be antidiabetic, it should be able to reduce the amount of glucose in the blood or increase the efficacy of insulin. Among the few drugs are voglibose, miglitol, and acarbose. These drugs are used by most people around the world to prevent the breaking of

macromolecules into simple sugar units. Acarbose is one of the major drugs used to inhibit both α-amylase and α-glucosidase, while as voglibose and miglitol, are only effective against α-glucosidase. However, all these drugs have some serious gastrointestinal side effects.[25,26] The drugs are very expensive, while most people in the world live below the poverty line.[27] Phytotherapy remains an important approach in the therapy of many chronic diseases, including diabetes and cardiovascular diseases due the fewer undesirable side effects of medicinal plants and their easy accessibility.[28] Postprandial hyperglycaemia is an important initial feature of diabetes mellitus. Therefore, inhibiting digestive enzymes like α-amylase is one of the most effective ways to alleviate hyperglycemic conditions.[29]

Combination of antidiabetic effects of plant species

Diabetes being a complex disease process which involves several metabolic pathways, it is necessary to approach its therapy through the use of various compounds which will act on their specific targets. Antidiabetic medicinal plants, herbs and spices meet this requirement perfectly due to the presence of several bioactive compounds. Sharma and Khanal showed the inhibitory effect of ginger extracts on alpha-amylase and alpha-glucosidase. According to them, this action is due mainly to the presence of the phenolic compounds gingerols and shogaols.[30] Bioactive compounds responsible to alpha amylase and alpha glucosidase inhibition have been identified in herbs and spices. They include piperine for black pepper, dehydrodieugenol, oleanolic acid and maslinic acid for clove, cuminaldehyde for cumin, diosmin, [6]-gingerol, carvacrol and thymol for ginger and tumerin for turmeric.[31] Inhibition of alpha-amylase in vitro by the extracts may be correlated with enzyme inhibition in vivo, which is capable of decreasing glucose release from starch.[16] The main anti-diabetic effects observed in the literature were a reduction in hyperglycemia, reduction in hyperlipidemia, regulation of insulin secretion through its release, increase of the insulin sensitivity or the insulin-like activity of the plant extract.[31,32]

In this study, the herb and spice extracts tested have revealed inhibitory properties of alpha-amylase and protein glycation which constitute two aspects of diabetes treatment. Other works by researchers shown the combination of several antidiabetic properties of spices and herbs. Thus, Cazzola reported in 2011 antioxidant and antiglycative properties as well as inhibitory activity against alpha-amylase and alpha-glucosidase by 07 herbs and spices include basil, garlic and parsley which were also tested in our work.[33] Similar findings have been proved through a study that highlighted alpha-glucosidase, alpha-amylase, aldose

reductase and advanced glycation end-products formation inhibition of *Peltophorum pterocarpum*, a medicinal plant with antidiabetic properties.[34]

It has been proved in trials that ginger enhanced insulin sensitivity, lowered C-reactive protein (CRP), improved lipid profile, as well as total antioxidant capacity in patients with type 2 diabetes mellitus. The antidiabetic effects of turmeric are linked to its capacity to lower glycaemia, to reverse insulin resistance in fat cell cultures, to increased glucose uptake into skeletal muscle and to trigger pancreatic beta-cell function.[7] Cumin has been shown to possess an inhibitory effect against alpha-glucosidase and aldose reductase, in addition to its ability to reduce calcium dependent glucose transport.[35]

Chronic hyperglycemia promotes protein glycation and contributes to the formation of AGEs. It plays an important role in the development of long-term diabetic complications such as diabetic retinopathy, nephropathy, cataract and atherosclerosis.[15] There is indeed a growing interest in the development of medicinal plant extracts as an alternative and complementary natural therapy for the medical care of diabetes. Thus, natural inhibitors of α-amylase and protein glycation become promising therapeutic options to complement or even replace existing synthetic drugs.[36]

CONCLUSION

The present study was carried out to evaluate the potential of spices and culinary herbs used in southern Benin to inhibit proteins glycation and alpha-amylase. Herbs and spices have gained particular interest because of their historical widespread use in the human diet. Their safety is therefore taken for granted compared to antidiabetic medicinal plants, some of which could prove toxic, especially in long-term use. In this study, 14 spices and aromatic herbs, namely thyme (*Thymus vulgaris L*.), parsley (*Petroselinum crispum Mill*.), clove (*Syzygium aromaticum L*.), ginger (*Zingiber officinale*), black pepper (*Piper nigrum L*), anise (*Pimpinella anisum L*), coriander (*Coriandrum sativum*), cumin (*Cuminum cyminum*), turmeric (*Curcuma longa*), celery (*Apium graveolens*), leaves of bay leaf (*Laurus nobilis*), basil (*Ocimum basilicum*), mint (*Mentha*) and garlic (*Allium sativum*) were tested. All of them displayed interesting antiglycative properties and inhibit alpha-amylase. These results may lead to the consideration of gallenic preparations with a view to their therapeutic use.

REFERENCES

1. Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2019 (GBD 2019) Disease and Injury Burden 1990-2019 [Internet]. 2020 [cité 2024 mars 21]; Available from: http: //ghdx.healthdata.org/record/ihme-data/gbd-2019-disease-and-injury-burden-1990-2019.
2. Chen X, Yang W. Branched-chain amino acids and the association with type 2 diabetes. J Diabetes Investig, 2015; 6(4): 369‑70.
3. Ashraf JM, Ahmad S, Choi I, Ahmad N, Farhan M, Tatyana G, et al. Recent advances in detection of AGEs: Immunochemical, bioanalytical and biochemical approaches. IUBMB Life, 2015; 67(12): 897‑913.
4. Singh VP, Bali A, Singh N, Jaggi AS. Advanced Glycation End Products and Diabetic Complications. Korean J Physiol Pharmacol Off J Korean Physiol Soc Korean Soc Pharmacol, 2014; 18(1): 1‑14.
5. Vishva N, Vishnupriya M, Vasanth A, Rajapandi R, Gopikrishnan S. A pilot study: Drug utilization pattern and prevalence of micro and macrovascular complication in type 2 diabetes patient. World J Adv Res Rev, 2023; 20: 384‑92.
6. Moussa S.A. Oxidative stress in diabetes mellitus. Romanian J Biophys, 2008; 18(3): 225‑36.
7. Jiang TA. Health Benefits of Culinary Herbs and Spices. J AOAC Int, 2019; 102(2): 395‑411.
8. ingh P, Jayaramaiah RH, Agawane SB, Vannuruswamy G, Korwar AM, Anand A, et al. Potential Dual Role of Eugenol in Inhibiting Advanced Glycation End Products in Diabetes: Proteomic and Mechanistic Insights. Sci Rep, 2016; 6(1): 18798.
9. Naderi GH, Dinani NJ, Asgary S, Taher M, Nikkhoo N, Boshtam M. Effect of Some High Consumption Spices on Hemoglobin Glycation. Indian J Pharm Sci, 2014; 76(6): 553‑7.
10. Seri A, Khorsand M, Rezaei Z, Hamedi A, Takhshid MA. Inhibitory Effect of Bunium Persicum Hydroalcoholic Extract on Glucose-Induced Albumin Glycation, Oxidation, and Aggregation In Vitro. Iran J Med Sci, 2017; 42(4): 369‑76.
11. Starowicz M, Zieliński H. Inhibition of Advanced Glycation End-Product Formation by High Antioxidant-Leveled Spices Commonly Used in European Cuisine. Antioxidants, 2019; 8(4): 100.

12. Ramkissoon JS, Mahomoodally MF, Subratty AH, Ahmed N. Inhibition of glucose- and fructose-mediated protein glycation by infusions and ethanolic extracts of ten culinary herbs and spices. Asian Pac J Trop Biomed, 2016; 6(6): 492‑500.
13. Yoshika Ishioka, Masayuki Yagi, Mari Ogura, Yoshikazu Yonei. Antiglycation effect of various vegetables: Inhibition of advanced glycation end product formation in glucose and human serum albumin reaction system. Glycative Stress Res, 2015; 2(1): 022‑34.
14. Cazzola R, Cestaro B. Chapter 9 - Antioxidant Spices and Herbs Used in Diabetes [Internet]. In: Preedy VR, éditeur. Diabetes: Oxidative Stress and Dietary Antioxidants. San Diego: Academic Press; 2014 [cité 2024 mai 2]. page 89‑97. Available from: https: //www.sciencedirect.com/science/article/pii/B9780124058859000097
15. Song Q, Liu J, Dong L, Wang X, Zhang X. Novel advances in inhibiting advanced glycation end product formation using natural compounds. Biomed Pharmacother, 2021; 140: 111750.
16. Hemlata B, Pornima G, Tukaram K, Pankaj B. In vitro anti-amylase activity of some Indian dietary spices. J Appl Biol Biotechnol, 2019; 7(4): 70‑4.
17. Firdhouse M J, Lalitha, P. Assessment of Î ±- amylase inhibitory action of some edible plant sources. Innovare J Sci, 2016; 1‑7.
18. Wu H, Xu B. Inhibitory Effects of Onion Against α-Glucosidase Activity and its Correlation with Phenolic Antioxidants. Int J Food Prop, 2014; 17(3): 599‑609.
19. Maser WH, Maiyah N, Nagarajan M, Kingwascharapong P, Senphan T, Moula Ali AM, et al. Effect of different extraction solvents on the yield and enzyme inhibition (a-amylase, a-glucosidase, and lipase) activity of some vegetables. Biodiversitas J Biol Divers [Internet] 2023 [cité 2024 mai 2]; 24(6). Available from: https: //smujo.id/biodiv/article/view/14763
20. Mehrotra N, Jadhav K, Rawalgaonkar S, Khan S, Parekh B. In vitro evaluation of selected Indian spices for α-amylase and α-glucosidase inhibitory activities and their spice-drug interactions. Ann Phytomedicine Int J., 2019; 8.
21. Ashwini S, Anitha R. Antihyperglycemic activity of *Caralluma fimbriata*: An In vitro Approach. Pharmacogn Mag, 2017; 13(Suppl 3): S499‑504.
22. Kaur N, Kumar V, Nayak SK, Wadhwa P, Kaur P, Sahu SK. Alpha-amylase as molecular target for treatment of diabetes mellitus: A comprehensive review. Chem Biol Drug Des., 2021; 98(4): 539‑60.

23. Oyedemi SO, Oyedemi BO, Ijeh II, Ohanyerem PE, Coopoosamy RM, Aiyegoro OA. Alpha-Amylase inhibition and antioxidative capacity of some antidiabetic plants used by the traditional healers in Southeastern Nigeria. Sci World J., 2017; 2017: 1‑11.
24. Odhav B, K T, asamy, Khumalo N, Baijnath H. Screening of african traditional vegetables for their alpha-amylase inhibitory effect. J Med Plants Res, 2013; 4(14): 1502‑7.
25. Etxeberria U, De La Garza AL, Campión J, Martínez JA, Milagro FI. Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase. Expert Opin Ther Targets, 2012; 16(3): 269‑97.
26. Laar F. Alpha-glucosidase inhibitors in the early treatment of type 2 diabetes. Vasc Health Risk Manag, 2008; 4: 1189‑95.
27. Silva JAD, Souza ECFD, Echazú Böschemeier AG, Costa CCMD, Bezerra HS, Feitosa EELC. Diagnosis of diabetes mellitus and living with a chronic condition: participatory study. BMC Public Health, 2018; 18(1): 699.
28. Governa P, Baini G, Borgonetti V, Cettolin G, Giachetti D, Magnano A, et al. Phytotherapy in the Management of Diabetes: A Review. Molecules, 2018; 23(1): 105.
29. El-Abhar HS, Schaalan MF. Phytotherapy in diabetes: Review on potential mechanistic perspectives. World J Diabetes, 2014; 5(2): 176‑97.
30. Sharma KR, Khanal N. Comparative investigation on antihyperglycemic and antioxidant activity of Zingiber officinale growing in different regions of Nepal. Sci World, 2022; 15(15): 33‑43.
31. Pereira ASP, Banegas-Luna AJ, Peña-García J, Pérez-Sánchez H, Apostolides Z. Evaluation of the anti-diabetic activity of some common herbs and spices: Providing new insights with inverse virtual screening. molecules, 2019; 24(22): 4030.
32. Mamun-or-Rashid ANM, Hossain MS, Hassan N, Dash BK, Sapon MA, Sen MK. A review on medicinal plants with antidiabetic activity. J Pharmacogn Phytochem, 2014; 3(4): 149‑59.
33. Cazzola R, Camerotto C, Cestaro B. Anti-oxidant, anti-glycant, and inhibitory activity against α-amylase and α-glucosidase of selected spices and culinary herbs. Int J Food Sci Nutr, 2011; 62(2): 175‑84.

34. Manaharan T, Teng LL, Appleton D, Ming CH, Masilamani T, Palanisamy UD. Antioxidant and antiglycemic potential of Peltophorum pterocarpum plant parts. Food Chem, 2011; 129(4): 1355‑61.
35. Ani V, Varadaraj MC, Naidu KA. Antioxidant and antibacterial activities of polyphenolic compounds from bitter cumin (*Cuminum nigrum L.*). Eur Food Res Technol, 2006; 224(1): 109‑15.
36. Magaji UF, Sacan O, Yanardag R. Alpha amylase, alpha glucosidase and glycation inhibitory activity of *Moringa oleifera* extracts. South Afr J Bot, 2020; 128: 225‑30.