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Potential exploitation of Shea press cakes in glycaemia regulation: Inhibition of α-amylase and α-glucosidase by protein and methanolic extracts

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Abstract

Shea (*Vitellaria paradoxa)* tree is integrally used in traditional medicine for the treatment of several health disturbances. Its kernels fat is widely exploited for food, medicinal and cosmetic purposes. Nevertheless germinated kernels are considered as waste, whereas shea germinative power would be very high. Their anti-diabetic ability was evaluated *in vitro*, in ordre to highlight their pharmacological benefits. Therefore, both proteins (crude, digested and dialysed ones) and hydroalcoholic extracts, were prepared from germinated and ungerminated shea seed press cakes. The anti-diabetic essay was carried out by evaluating extracts inhibiting power on both α-amylase and α-glucosidase activities. Proteins were quantified by spectrophotometry (214 nm). Results revealed that the protein content of the extracts from germinated seed cakes was 450 mg/100 g and that of the extracts from ungerminated shea seed cakes was 410 mg/100 g. The percentage of inhibition of α-amylase by the dialysed extracts of germinated shea seeds, in this case the external dialysate of germinated seed, presented the best rate of inhibition with 30.21 %. Contrary to the percentage of inhibition of α-amylase, the highest rates of inhibition of α-glucosidase were recorded with the crude protein extracts of sprouted seeds (82.02 %) and unsprouted seeds (62.32 %). For methanolic extracts, the highest inhibition of α-amylase and α-glucosidase was recorded by the ungerminated seeds, with 42.61% for α-amylase and 97.47% for α-glucosidase. These results show that protein extracts of shea seed cakes may play a role in blood glucose regulation.

Keywords: Shea press cakes; Protein and methanolic extracts; Glycaemia; α-amylase; α-glucosidase

1. Introduction

Diabetes is a disease consisting in a disordered in carbohydrate metabolism; it would be caused by total or relative insufficiency of insulin [1], responsible for the spread of this disease, the main causes are urbanization, lifestyle, obesity and lack of physical activity [2]. Mowdays, diabetes is experiencing a very signifiant expansion. Indeed, according to WHO and IDF (2019), 463 million people worldwide would be diabetic ; this figure could reach 578 million by 2030. Despite the use of hypoglycaemic drugs as anti-diabetic drugs, diabetes and its complications constitute a major problem in the therapeutic management of diabetics. Moreover, regular administration of these curative/preventive drugs would generate undesirable effects [3] [4]. In such context, the emphasis is increasingly on finding new sources of antidiabetic drugs, including medicinal plants [5]. Such medicinal plants are excellent source of active molecules such as peptides, which would be used for the treatment of diabetes. In West Africa, more than 80 % of the population uses medicinal plants recognized for their lot of properties. Most of time, these medicinal plants (or plant parts), among which shea (*Vitellaria paradoxa*) tree, are used as infusats, macerats or digestats. shea tree, this oleaginous tree is fully exploited because of the wide therapeutic virtues of the whole tree and its fat called shea butter [6], [7]. Shea butter is also full of therapeutic virtues, but its production as well as its encreasing demand generate a lot of producing waste.

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In addition, the quality requirements of shea butter have led to the rejection of germinated seeds because they would lead to poor quality butter. This causes a real environmental problem related to germinated seeds management as reject. It is important to precise that if shea germinated seeds are considered as rejects, this is not the case for germinated seeds of soybean and sesame (for example); these latest would be widely exploited in the food sectors [8]. These oleagineous seeds would also be exploited for dietetical propose, when they are germinated. For instance, germinated soybean would be used dietetically for the treatment of diabetes [9]. Taking into account the potentials of these oleaginous seeds, shea seeds could also constitute an exploitable ressource with similar virtues. However, it would be rather avoiding any competitive interest for shea butter. Hence study was carried out on shea press cake (or delipidated seeds), which costitute production rejects. The aim of this study was therefore, to underline the pharmacological potential of shea press cakes by demonstrating their anti-diabetic ability. Therefore, the inhibiting effects of proteins and methanolic extracts of shea press cakes were tested on α-amylase and α-glucosidase activities.

2. Material and methods

2.1. Material

Germinated and ungerminated shea nuts and serval enzymes constituted the biological material of the present study. Shea nuts were collected under shea trees, in july 2020 during harvrest period. Ungerminated nuts served as control. Enzyms wich consisted in α-amylase and α-glucosidase, pepsin and pancreatin, were krindly offered by the laboratory of the UPR Biotechnology of University Felix Houphouët Boigny. Materiel also consisted in *arcabose* (drug for glycemia modulation) and pure distilled water; both were puchased in a pharmacy officine. If arcabose was used for positive control, pure distilled water and a semi-permeable hydrophilic membrane with a cut-off diameter of 10-12 KDa (SIGMA-ALDRICH, USA and Canada), as for them, served for dialysis process.

2.2. Methods

2.2.1. Shea seeds proteins extraction and quantification

Shea seeds were first, crushed and delipidated with aceton solution; a fatless powder was obtained after rotavaporation. Protein extraction from the previous power, consisted in a solubilization at pH 8 and isoelectric precipitation at pH 4.3, successively [10]. Hence, 2.5 g of the delipidated shea seeds powder were suspended in distilled water at a ratio of 1:10 (W/V, flour/water). The pH of the suspensions was adjusted to 8 with a 0.1 M NaOH solution. The resulting solution was centrifuged at 5000 rpm for 15 min at 4°C. The supernatant was recovered and its pH was adjusted to 4.3 with a 2 M HCl solution. The final mixture was centrifuged at 10000 rpm for 20 min at 4°C and the pellet was recovered and resuspended in distilled water (5%). Protein content was measured by spectrophotometry UV-visible at 214 nm. This method is based on the ability of proteins to absorb light in the ultraviolet range of light [11]. Bovine serum albumin (BSA) solution served for a standard line.

2.2.2. Preparation of shea seeds methanolic extract

1 g of shea seed fatless powder was dissolved in 10 mL of 70% (v/v) methanol. The mixture obtained after homogenization was centrifuged at 1000 rpm for 10 min. The pellet was recovered in 10 mL of 70% (v/v) methanol and centrifuged again. This operation will be repeated a third time. All three supernatants were recovered and stored for analysis [12].

2.2.3. In vitro digestion of shea seed protéins

Gastric and intestinal digestions of shea seeds proteins were simulated *in vitro* by using pepsin and pancreatin, successively. Hence, 50 µL of a 160 mg/ml pepsin solution (EC 3.4.23.1) prepared in 0.1 M HCl were mixted with 1200 µL of shea seeds proteins (5 % W/V) The mixture was homogenised and incubated at 37°C for 1 h, and adjusted to 7.5 adding with a 1 M $NaHCO_3$ solution. Pancreatin solution (4 mg/mL) was added at 4% W/V E/S ratio, the medium was homogenized and incubated at 37 °C for 2 h. enzymatic reaction was stopped by heating in a boiling bath at 100°C for 10 min. The mixture was kept for looking at room temperature and then centrifuged at 16000 rpm for 10 min. At this step, the resulting Supernatant of both germinated and ungerminated seeds proteins were recovered and stored at 4°C for further analysis [13].

2.2.4. Fractionnement of shea seeds protein extracts

Shea seeds proteins were fractioned by dialysis through a cellophane membrane (10 KD) Hence, 3 mL of protein extract introduced into a dialysis bag. This latest was firmly tied and plunged into a glass bottle containing pure distiller water, for hours. Both inside and outside contents of dialyse bag were concentrated and stored for future assay.

2.2.5. Essays for α-amylase and α-glucosidase inactivation by shea seeds proteins and methanolic extracts

α-amylase and α-glucosidase inhibiting potential of shea seeds extracts were evaluated and compaired to *arcabose* (as positive control) inhibiting power. Essays were performed on proteins (digested and undigested, and both dialysed fractions) and on methanolic extract of shea seeds.

α-amylase inhibition essay was performed following the method described by Hashim *et al*. [14]. Hence, a 90 µL mixture made of phosphate buffer (10 µL), shea seeds extract (30 µL; 5% W/V) or *arcabose* (5% W/V), amylase (50 µL ; 1.5 IU/mL) in a 0.02 M sodium phosphate buffer pH 6.9, was set up. The mixture was pre-incubated at 37 °C in a water bath for 5 min. The enzymatic reaction was started by adding 110 µL of gelatinized corn starch (1%, W/V in 0.02 M phosphate buffer pH 6.9) to the pre-incubated mixture. The enzymatic reaction went on at 37 °C in a water bath for 10 min, and then stopped by adding 200 µL of DNS (Dinitro salicylic acid).The whole set was placed in a boiling bath for 5 min and a 3.6 mL of distilled water was added for absorbance measurement at 540 nm (spectrophotometer *Pioway, China V5000*) against a blank (phosphate buffer) [15]. The inhibition rate was calculated according to the following formula:

$$\text{Inhibition (\%)} = \frac{(A_{EN} - A_{EEXt})}{(A_{EN})} X100$$

A_{EN} : Absorbance of normal test
A_{EExt}: Absorbance test with protein or methanolic extracts

3. Results and discussion

3.1. Resultat

3.1.1. Inhibiting power of shea press cake crude proteins extracts on α-amylase activity

Results illustrated on figure 1 revealed an α-amylase inhibiting effect of crude proteins extracted from both ungerminated and germinated shea press cakes. Moreover, both crude proteins demonstrated significantly the same inhibition level (21.83 and 18.01 %, respectively). However, these values were slighter than arcabose inhibiting power on the same enzyme. It is worth noting here, that the same inhibition test carried out with digested proteins (successively by pepsin and pancreatin) extracted from shea press cakes of both ungerminated and germinated seeds, did not show any inhibiting effect (0%) on α-amylase.

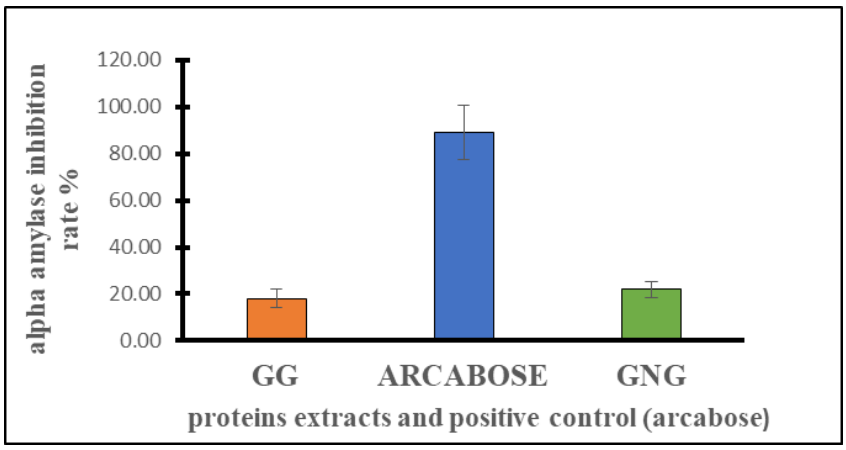

Figure 1 Inhibiting power of crude proteins extracted from ungerminated and germinated shea seeds and of arcabose, on α-amylase activity

3.1.2. Inhibiting power of dialyzed fractions of proteins extracted from shea press cakes, on α-amylase activity

Both protein fractions inside (≥ 10 KD) and outside (≤ 10 KD) the dialysis bag showed inhibition activity on α-amylase (figure 2). The highest (30.21 %) inhibition power were recorded with the outside (dialyzed) proteins fraction of germinated seeds press cake; the other values were significantly similar. However, the inhibiting percentage of the whole shea press cakes proteins fractions (22.48 - 30.21 %) was lower than arcabose one.

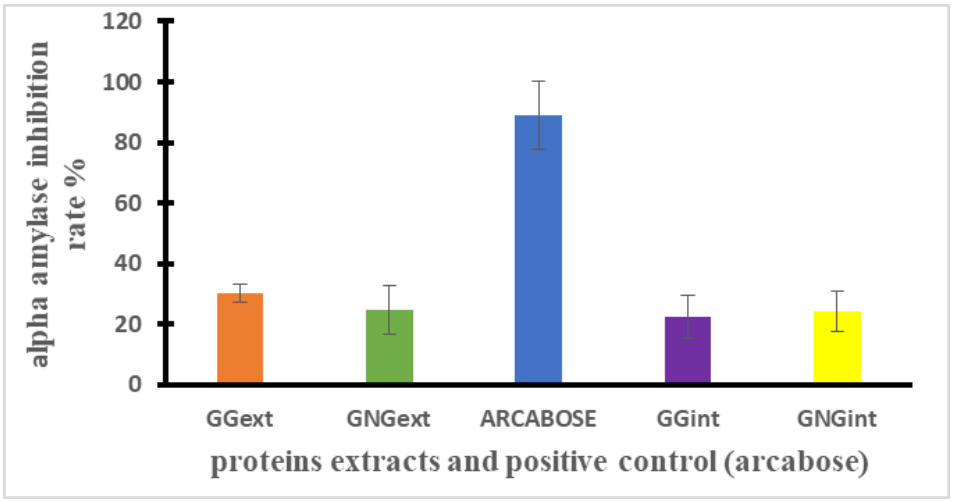

Figure 2 Inhibiting power of dialyses fractions of proteins extracted from ungerminated and germinated shea seeds and of arcabose, on α-amylase activity

3.1.3. Inhibiting power of shea press cakes methanolic extracts on α-amylase activity

Figure 3 revealed that methanolic extracts of shea press cakes also presented an inhibition effect on α-amylase activity. If both methanolic extracts of germinated and ungerminated shea press cakes exhibited lower inhibiting power than arcabose (33.24 and 42.61 % versus 89.65 %) did, their inhibiting effects on α-amylase remained nevertheless, effective and stronger than protein extracts ones.

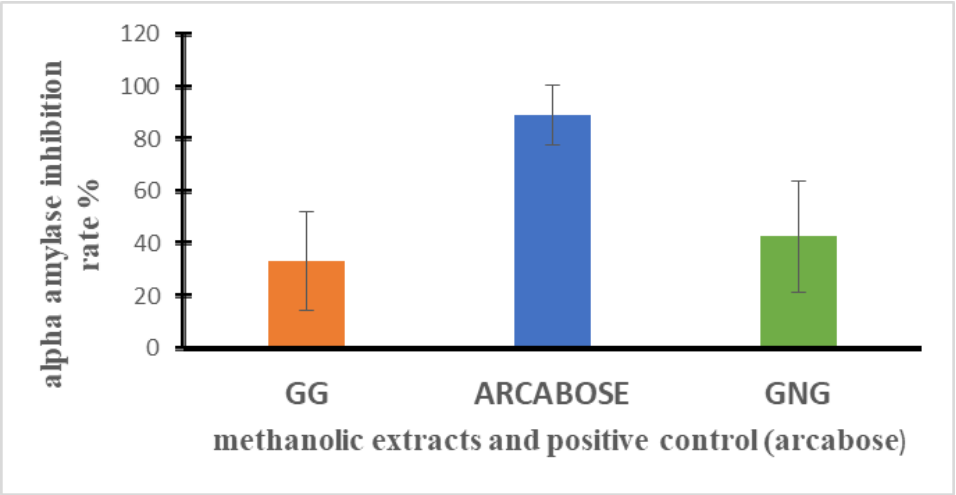

Figure 3 Inhibiting power of methanolic extracts from ungerminated and germinated shea seed press cakes and of arcabose, on α-amylase activity

3.1.4. Inhibiting power of shea press cakes crude proteins on α-glucosidase activity

The test concerning α-glucosidase inhibition by crude proteins extracted from shea press cakes showed an inhibition rate of 82.02 % for ungerminated seed press cakes and 62.32 % for germinated seed one. It is very interesting to underline that the inhibition power performed on α-glucosidase by arcabose (76 %) was lower than that of ungerminated seed press cake.

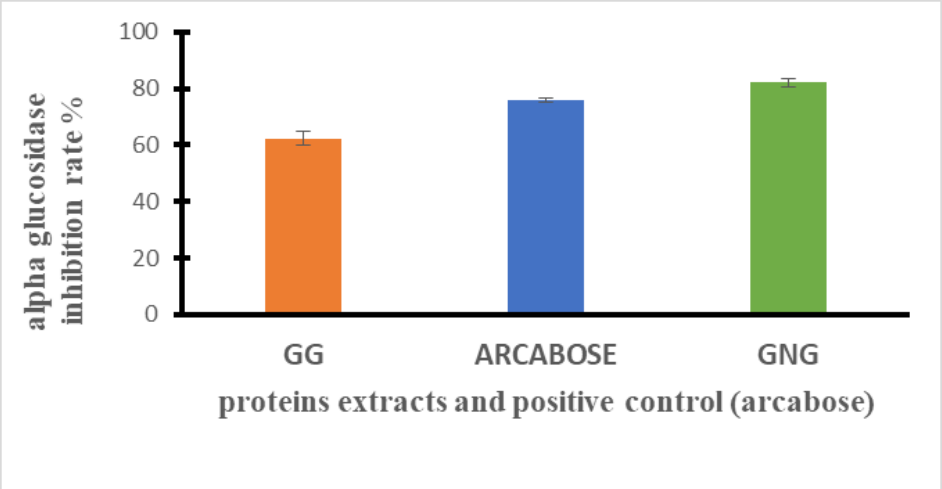

Figure 4 Inhibiting power of crude proteins extracted from shea press cake on α-glucosidase activity

3.1.5. Inhibiting power of digested proteins of shea press cakes on α-glucosidase activity

Results reported on figure 5 showed that the digested protein of both press cakes from germinated and ungerminated seeds, inhibited significantly α-glucosidase activity, like arcabose did. However, if the digested protein from germinated seeds press cake exhibited a higher inhibition power than the ungerminated one, arcabose as for it induced the strongest inhibition effect (76 % versus 60 % and 31 %,) on α-glucosidase.

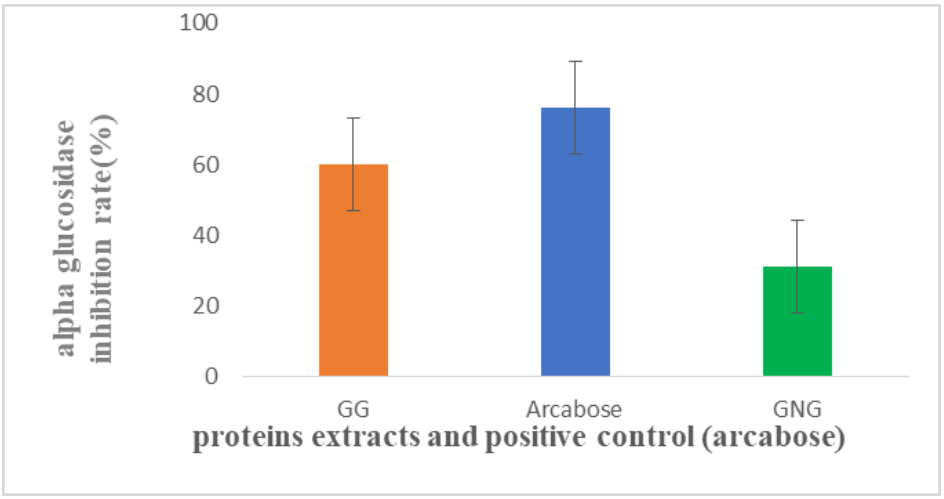

Figure 5 Inhibiting power of digested proteins of shea press cakes on α-glucosidase activity

3.1.6. Inhibiting power of dialysed protein fraction of shea press cakes on α- glucosidase activity

After dialyses of shea press cakes proteins and inhibition tests performing, outside (≤ 10 KD) protein fractions of both ungerminated and germinated shea press cakes recorded higher inhibition power on α- glucosidase activity than the inside (≥ 10 KD) proteins fractions did (45.54 and 42.31 % versus 33 and 27 %). At the whole, protein fractions of shea germinated seeds press cakes showed more important inhibition power on α-glucosidase.

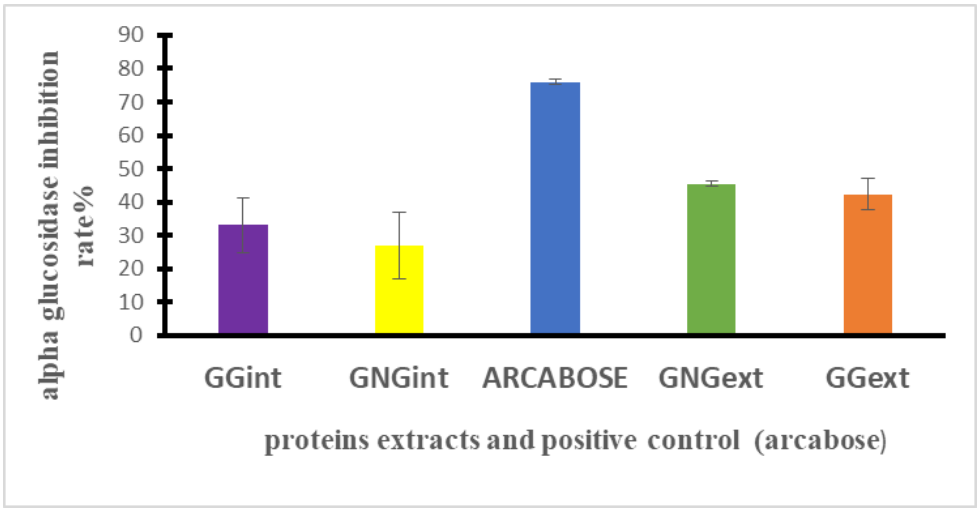

Figure 6 Inhibiting power of dialyses fractions of shea press cakes proteins on α-glucosidase activity

3.1.7. Inhibiting power of methanolic extracts of shea press cakes, on α-glucosidase activity

Inhibition test performed with methanolic extracts of shea press cakes, demonstrated an inhibition activity on α-glucosidase, like its proteins did, above. Moreover, this inhibiting activity was, at the whole stronger than arcabose. Indeed, if methanolic extract from germinated seeds press cake inhibited α-glucosidase activity at a level of 66.05 % (not so far from 76 %, for arcabose), the extract from ungerminated seeds press cake, as for it topped an inhibiting level of 97.47 % (almost 100 %).

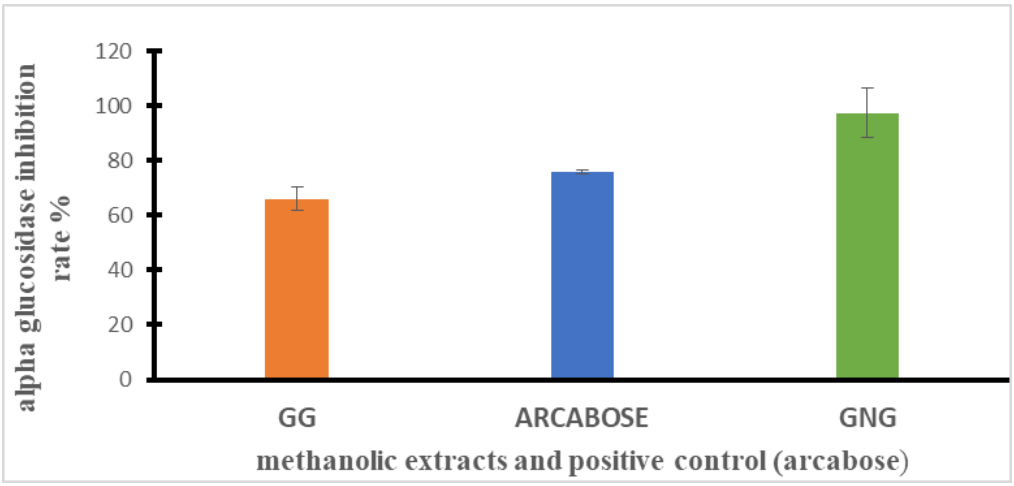

Figure 7 Inhibiting power of methanolic extracts of shea press cakes on α-glucosidase activity

3.2. Discussion

α -amylase and α-glucosidase are two key enzymes responsible for postprandial hyperglycemia. Indeed, during digestion α-amylase initiates the degradation of starch (large molecule) into molecules of intermediate sizes such as α-dextrins, malto-dextrin etc. Marze [16], which serve as a substrate for intestinal α-glucosidase, and will then be degraded into monosaccharides mainly consisting in glucose [16]. This massively released glucose will reach the bloodstream, and induce blood glucose to rise (hyperglycemia). According to the OMS, the search for peptides and other bioactive molecules able to inhibite the action of these enzymes could constitute an alternative pathway in the management of diabete type 2 [17].

The presence of peptids and the demonstration of the inhibiting effect of both proteinic and methanolic extracts of shea press cakes, on α -amylase and α-glucosidase activities, of the present study would constitute a very considerable advantage, and would then suggest their use in pharmacology for glycemia regulation (modulation). Futhermore, these extracts could also valuabily be exploited in prevention of many other diseases such as hypertension, obesity, diabetes,

etc. it is worth noting here that sheal seed are widely consumed in shae producing areas, as supplementary food. Some authors also reported that shea seeds would provide strenght and vogor during field-works

Above all, the main interest of the present study remain in shea press cake valorization in pharmacology where it could constitute an interesting organic and non-competitive (its is a subproduct, a reject of production) matrix for pharmacology purposes. Indeed, considering shea press cake proteins, their efficiency in the inhibition of both α -amylase and α-glucosidase activities, would suggest shea press cake benefic as edible matrix. Moreover, postprandial digestion similation through crude proteins digestion by pepsin and pancreatin (successively), and the relative important inhibition effect of the digestats, would suppose the garanty of their efficacity in glycemia regulation. About dialysis performed on crude proteins, it suggest that both fractions inside (more than 10 KD) and outside (less than 10 KD) the dialyse bag would be responsible for starch hydrolysis inhibition. However, another study might be performed for more precision.

The benefice of shea press cake in glycemia regulation also remain in its methanolic extract which also show its efficiency with a relatively important level. It is worth recalling here, that methanolic extracts would be mainly constituted by phenolic compounds. Hence, its ability as antioxydant might also be taken into account.

According to Auvray *et al*.[18], the hypoglycemic effect of biopeptides used in the treatment of diabetes are small peptides. In addition, the crude protein extracts from the germinated seeds showed the highest rate of inhibition (82.02%) of α-glucosidase unlike other protein extracts including arcabose (76 %). Germination would be the cause of these results, because shea seeds protein (reserves) would have been hydrolyzed, and thus releasing biopeptides able to inhibiting the activity of enzymes (α-glucosidase and α-glucosidase). As for methanolic extracts, the α-amylase inhibition test gave relatively low levels for both germinated and ungerminated seeds (33.24 % and 42.61 %) unlike arcabose (89, 65 %). Regarding the α-glucosidase inhibition test, very high percentage of inhibition were observed with 97.47 % for ungerminated seeds and 66.05 % inhibition for germinated seeds. These different results could be explained by the presence of the phenolic compound contained in the methanolic extract. Indeed, various *in vitro* and *in vivo* studies show that polyphenols could modulate carbohydrate metabolism and exhibit antidiabetic activities [19], [20], [21], Researchers have suggested that polyphenols could be involved in carbohydrate metabolism from the stages of digestion and intestinal absorption of sugars. And certain polyphenols such as anthocyanins and ellagitannins would be able to inhibit in vitro the enzymatic activity of α-glucosidase and α-amylase [22], [23], [24].

4. Conclusion

The aim of this study was, to enhance the pharmacological potential of shea press cake by demonstrating their aanti-diabetic ability potential. The study demonstrated that crude proteins extracted from the press cakes of shea germinated and ungerminated seeds can inhibite α-amylase and α-glucosidase activities, like *arcabose* which is a glycemia modulation drug. Moreover, despite of the postprandial digestion *in vitro* of these crude proteins, they still inhibited α--glucosidase activies. This would suggest to consider and to integrate shea press cake as an edible drug-food for glycaemia modulation. The methanolic extracts of the same press cake, also proved glycaemia modulation. Hence, in the present world context of metabolic diseases prevalence, press cakes of germinated and ungerminated shea seeds, could also be valorized and integrated in people feeding habit. Both proteins and methanolic extracts could also constitute bioactive principles for diabetic (type 2) drugs preparation. These extracts have to undergo purification of the active molecules. Hence, further research is needed to identify, isolate and purify the active constituents.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors have seen and approved the manuscript as submitted. The different mentioned authors participated in the work and are prepared to take public responsibility for the work. All authors of the manuscript have no conflict of interests to declare. The manuscript submitted to the journal is not copied or plagiarized version of some other published work. Mr Kouakou and me, Mr Djoman are PhD students working on the subject. All the data taken from other sources is written in authors own language and properly cited.

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