In vitro inhibition of alpha amylase and glucosidase of digestive snail juice by crude extracts of cashew cakes

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ABSTRACT

The goal of this work was to study the effect of some solvents as extractants of total polyphenols from cashew cakes and test the ability of the extract with highest level of polyphenols to inhibit alpha amylase and alpha glucosidase from snail (*Achatina ventricosa*) digestif tract. For this purpose, water, water-methanol (50:50 v/v), water-ethanol (50:50 v/v) and water-acetone (55:45 v/v) were used as solvents. Extract with highest level of polyphenols was obtained using water-acetone (55:45 v/v). The average total phenols content varied respectively from 9179.89 ± 0.154 mgGAE / 100g for the water-acetone extract to 55439.02 ± 0.117 mgGAE/100g for the aqueous extract. The average flavonoid content ranged from 370.86 ± 0.015 to 200.88 ± 0.001 mg/100g and that of condensed tannins ranged from 1852.09 ± 0.023 to 857.45 ± 0.050 mg/100g. The in vitro inhibition of alpha-amylase and alpha-glucosidase enzymes of the snail digestive tract allowed to determine the concentration of the extract that inhibits 50% of the enzymes (IC_{50}). The IC_{50} of alpha-amylase was 0.24 mg / ml and that of alpha glucosidase was 1.44 mg / ml.
The results showed that cashew apple residue is a natural source that has potential application in the management of diabetes mellitus.

Keywords: Solvents, polyphenols, α-amylases, α-glucosidases, diabetes

1. Introduction

In 2000, the global population of diabetics was estimated at about 171 million people. This population is projected to increase to 366 million by 2030 [1]. The main cause of this disease is an elevation of blood glucose. In fact, the hydrolysis of starch by pancreatic amylases and the breakdown of glucose by intestinal glucosidases cause a sudden rise in blood glucose (hyperglycemia), which causes diabetes.

Thus, there are two types of diabetes, type 1 diabetes or insulin-dependent diabetes which is treated with insulin and type 2 diabetes. The latter is responsible for a public health problem because it affects nearly 90% of the diabetic population and the treatment of it is laborious, being non-insulin dependent [2].

To solve this public health problem, the inhibition of amylases and glucosidases is one of the effective strategies.

Alpha-amylases (EC 3.2.1.1) catalyze the hydrolysis of α-1,4-glucosidic bonds of

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starch, glycogen and various oligosaccharides. Inhibition of their digestive activity is considered effective in controlling obesity or diabetes by decreasing the absorption of glucose released from starch by these enzymes [3]. The use of synthetic hypoglycemic agents such as acarbose, miglitol and voglibose is possible [4]. However, in developing countries most people have limited resources and do not have access to modern treatment. In addition, these synthetic hypoglycaemic agents would often cause gastrointestinal side effects [5].

Faced with this situation, the use of tropical fruits, identified as health agents is possible. Among these fruits, one can quote the cashew apple (*Anacardium occidentale*). Indeed, the cashew apple is a fruit rich in bioactive compounds such as total polyphenols, flavonoids and tannins. These secondary metabolites are a large group widely distributed in fruits and vegetables and identified as antioxidants. In addition, polyphenols are known for their high affinity to peptides or proteins and as amylase inhibitors [6]. Thus, it is known that fruit extracts rich in polyphenols are effective against the main enzymes for the management of hyperglycemia promoting type 2 diabetes [7].

The purpose of this study is to evaluate the effect of solvents on the extraction of phenolic compounds from cashew cakes and to evaluate the effect of extracts on alpha amylase and alpha glucosidase from snail digestive tract.

2. Material and methods

2.1 Plant material

The plant material consists of cashew apple cakes. The apples are harvested from cashew plantations in central Ivory Coast, specifically in the region of Aries (Yamoussoukro). After juice production, the cashew cakes are collected, dried and crushed. The powder obtained is used as biological material.

2.2. Solvents used for extraction

Four solvents are used for the extraction of phenolic compounds from the powder of the cashew apple cake. These solvents are composed of aqueous solvent and hydro-organic solvents, namely water-methanol, water-ethanol and water-acetone solvents.

For the extraction with the aqueous solvent, 100 g of cashew seed cake powder is treated in 1.5 liters of boiling distilled water for 2 hours, cooled to room temperature and allowed to stand for 24 hours. The extracts are then cold filtered through Whatman filters. The filtrate is evaporated to dryness under reduced pressure at 40°C. using a rotary evaporator [8].

Extractions with water-acetone and water-methanol solvents are carried out by the method described by Andrade and al., [9] using respectively 55% acetone and 50% methanol as solvent. These solvents are added to the cashew cake powder. The mixture is shaken for 30 minutes in the first extraction solvent and centrifuged at 4000 rpm. The supernatant is collected, the precipitate resuspended in the second solvent, stirred for 30 minutes and centrifuged at 4000 rpm. The supernatants are combined and concentrated under reduced pressure at 40 °C.

As for the water-ethanol extract, it is produced by the modified protocol of Romani and al., [10]. A quantity of 20 to 30 g of cashew seed cake powder is macerated at room temperature for 2 hours in 100 ml of an ethanol / water mixture, in a proportion of 50/50 (v/v). The mixture is centrifuged for 20 min at 4000 rpm at room temperature, filtered through Whatman filter paper and stored at 4 °C.

2.3 Determination of total phenol content

The total phenol content is determined according to the method of Singleton and Rossi [11]; Wood et al. [12]. Appropriate dilution of the plant extracts is oxidized with 2.5 ml of Folin-Ciocalteau 10% (v / v) reagent and neutralized with 2 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45 °C and the absorbance measured at 765 nm in the UV-Visible spectrophotometer (model 6305,

Jenway, Barlo World Scientific, Dunmow, UK). Then, the total content of phenolic compounds is expressed in gallic acid equivalent (GAE).

2.4. Determination of flavonoid content

The total flavonoid assay is performed by the method of Marinova et al. [13]. To a quantity of 0.3 ml of 5% (w / v) $NaNO_2$ is added 0.3 ml of 10% (w / v) aluminum chloride ($AlCl_3$) and 1 ml of vegetable extract. After 5 minutes of reaction at ambient temperature (30 ± 2 ° C.), 2 ml of NaOH (1M) are added to the mixture. The volume of the mixture is finally adjusted to 10 ml with distilled water. After vigorous stirring of the mixture, the absorbance is measured spectrophotometrically at 510 nm.

2.5 Determination of condensed tannins content

Determination of condensed tannins content is performed using the spectrophotometric method [14]. The principle of the assay is based on the fact that tannic acid (more particularly flavan-3-ol) in the presence of the reagent consisting of vanillin (0.1 mg / ml) in an acid medium (70% sulfuric acid (v/v) gives a red color whose absorption maximum is at 500 nm.

2.6 Determination of enzymatic activity

2.6.1 Extraction of the digestive juice of snail

The digestive juice of the snail Archachatina ventricosa is extracted according to the method described by Colas [15]. Enzymatic digestion was carried out on batches of snails kept on an empty stomach for 3 days. The shell of the mollusk is carefully broken and the brown colored digestive tract is isolated with forceps. The raw digestive juice containing mucus is filtered on a sterile medical compress. The filtered digestive juice is then centrifuged at 10,000 rpm for 15 min using a refrigerated centrifuge (ALRESA) at 4°C to obtain the crude enzyme extract.

2.6.2 Determination of the specific activity of alpha amylase and alpha glucosidase

The protein assay is done according to the method of Lowry and al., [16] using the following reagents:
solution A: Folin-Ciocalteu reagent diluted by half in 0.1 N sodium hydroxide solution;
solution B: sodium carbonate (2%, w/v) prepared in 0.1 N sodium hydroxide;
solution C1: Copper sulphate (0.5%, w/v) prepared in distilled water;
solution C2: sodium and potassium double tartrate (1%, w/v) prepared in distilled water;
solution D: prepared extemporaneously from 100 µl of solution C1, 100 µl of solution C2 and 10 ml of solution B.

2.6.3 Dosage

Different dilutions (1/50, 1/100, 1/150, 1/200, 1/250) are made from the crude extract. One hundred (100) µl of each dilution is diluted in 2 ml of solution D. The mixture is stirred and incubated for 15 min in a 37 °C water bath. Then, a quantity of 200 µl of solution A is added. The reaction medium is stirred and allowed to stand for 30 min in the dark to allow the development of the coloring. One (1) ml of distilled water is added, and then the absorbance of the test is measured at 660 nm in the SPECTRONIC spectrophotometer against a control made under the same conditions, but not containing a protein extract. Absorbance is converted to protein levels using a calibration line obtained from a stock solution of bovine serum albumin (0.2 mg/ml).

2.6.4 Amylase activity

In a tube containing 100 µl of sodium acetate buffer (100 mM, pH 5.0) is added 50 µl of enzymatic extract diluted 1/200. The whole is pre-incubated for 10 min at 37°C and a quantity of 100 µl of starch paste is added. The reaction mixture thus obtained is incubated at 37 °C. In a water bath for 15 minutes. After 15 minutes, 300 µl of DNS is added to stop the reaction. The reducing sugars released are assayed according to the method of Bernfeld [17]. Control tubes containing no enzyme are made under the same conditions. Absorbance is measured at 540 nm using a UV-Force spectrophotometer (Cyberlab, Uv-100, USA).

2.6.5 Invertatic activity

In a tube containing 100 µl of sodium acetate buffer (100 mM, pH 5.0) is added 50 µl of enzymatic extract diluted 1/200. The whole is preincubated for 10 min at 37°C. and a quantity of 100 µl of sucrose is added. The reaction mixture thus obtained is incubated at 37°C. In a water bath for 15 minutes. After 15 minutes, 300 µl of DNS is added to stop the reaction. The reducing sugars released are assayed according to the method of Bernfeld [17]. Control tubes containing no enzyme are made under the same conditions. Absorbance is measured at 540 nm using a UV-Force spectrophotometer (Cyberlab, Uv-100, USA). The inhibition percentages of the amylase and invertase activity are calculated according to the following formula:

$$\%\text{Inhibition} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})}{\text{Abs}_{\text{Control}}}$$

2.7 Statistical analysis

The results obtained during this study were the subject of a one-way statistical analysis of variance (ANOVA) and the significance of the differences between the extraction techniques was determined at the risk of error of α = 0.05 using the STATISTICA 7.1 software. Multivariate exploratory techniques such as Principal Component Analysis (PCA) and Hierarchical Classification (CAH) are used to process the data generated by the different extractions. The objective of these multivariate analyzes is to classify individuals with similar behavior on a set of variables [18].

3. Results

Table 1 reports results of polyphenol contents of cashew seed cake extracts.

Table 1: Polyphenol content of the various extracts of cashew cakes

Extracts	Aqueous	Water-methanol	Water-ethanol	Water-acetone
Polyphenols (mg/100g)	5439.02±0.12^{a}	6815.47±0.07^{b}	**7**169.31±0.08^{c}	9179.89±0.15^{d}
flavonoids (mg/100g)	200.88±0.001^{a}	215.96±0.01^{a}	237.30±0.01^{ab}	370.86±0.02^{c}
Tannins (mg/100g)	857.41±0.05^{b}	1010.71±0.07^{a}	1068.59±0.09^{a}	1852.09±0.02^{c}

Numbers in the same line with different letters are statistically different at P <0.05.

3.1 Principal Component Analysis

Figure 1 shows the principal component analysis (PCA) of the different extractions and their phenolic compound contents.

Principal component analysis correlated all the studied traits with 3 factors. However, according to Kaiser's rule, only the first factor, having an eigenvalue greater than or equal to 1, is considered for the interpretation of PCA data. It totals 81.42% of the total variability. Nevertheless, the second eigenvalue factor 0.55 and total variability 18.55% was associated with the first factor for representation of the PCA.

The factor (F1) has an eigenvalue of 2.44 and is mainly formed by all the characteristics related to the content of total phenols, tannins and flavonoids. These phenolic compounds are superimposed on the factor F1 in its negative part.

The projection of characters and individuals from extraction solvents is made in the plane formed by the factors (1 and 2), which record 99.95% of the total variability. She divided the individuals into 3 groups.

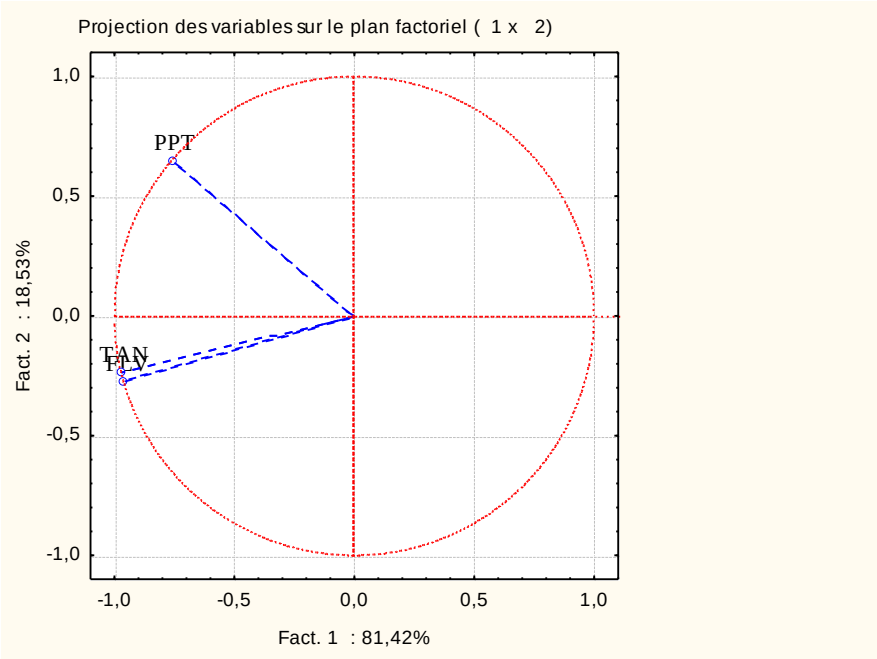
Group 1 consists essentially of the individual from the water-acetone extraction which is superimposed on characters negatively correlated to factor F1. Thus, it is characterized by high values in flavonoids, tannins and total phenols.

The second group contains individuals from water-based and water-methanol extraction that overlap with the F1-positively correlated traits. Thus these elements are characterized by lower levels of phenolic compounds.

The third class contains the sample of water-ethanol extraction. This individual is distinguishable from other samples by a higher phenol content than the phenolic content of individuals in the second group, but lower than that of the group 1 individual.

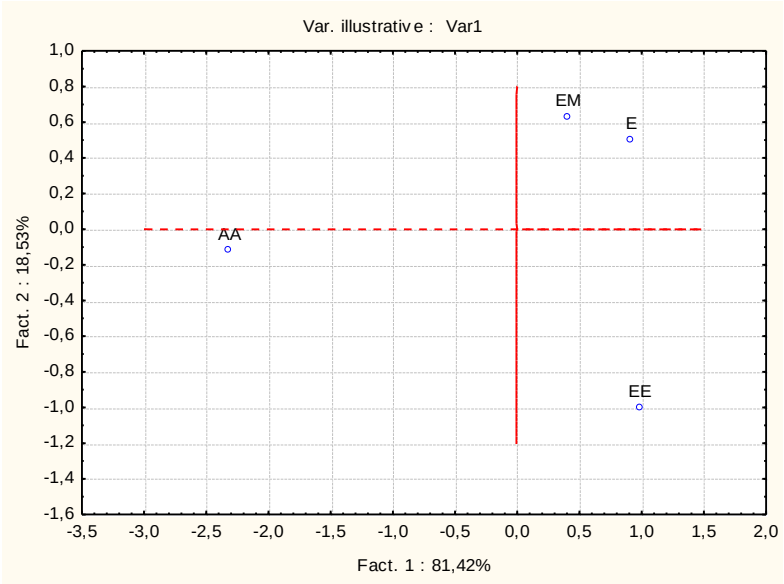
3.2 Hierarchical Ascending Classification

The hierarchical ascending classification (CAH) established by the Euclidean distance method confirms the variability observed at the ACP level. In fact, the truncation of the dendrogram at a Euclidean aggregation distance of 2000 reveals three classes observed during the extraction of the phenolic compounds studied (Figure 2). The first-class individual comes from water-acetone extraction. The sample of this class is distinguished by a high content of tannins, flavonoids and total phenols compared to the sample of other solvents. It is the good solvent for extracting phenolic compounds from the cashew cakes studied. The second group contains individuals from water-based extractions; water-methanol and the third group contains the individual from the water-ethanol extraction. These individuals are characterized by lower levels of phenolic compounds compared to the group 1 individual.



(a) Projection of the variables

TAN: Tannins; **PPT:** total phenols**; FLV:** Flavonoids



(b) Projection of individuals

E: Aqueous extract;**; EM :**water-methanol**; EE :** water-ethanol**; AA :** acetone water

Figure 1: Projection of Biochemical Characteristics (a) and Individuals (b)

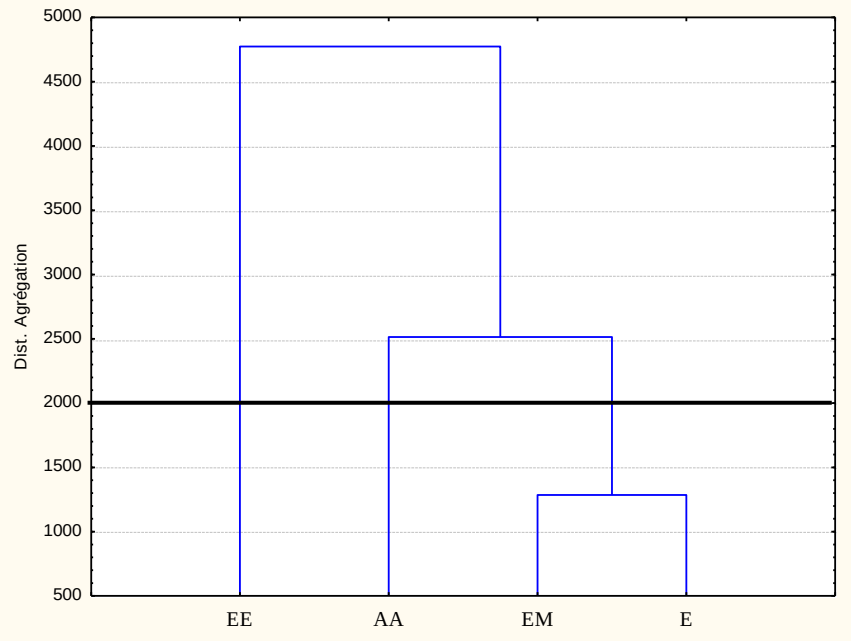


Figure 2: Hierarchical ascending classification (dendrogram) of phenolic compounds

3.3 Enzymatic inhibition

Several dilutions of the crude snail extract were made in order to obtain the best dilution. The 1/200 dilution with the highest specific activity (4.05 µmol/min/mg) was selected for enzymatic inhibition tests with different extracts of cashew cake.

Figures 3 and 4 show the results of the inhibition of α-amylase and α-glucosidase of the snail digestive juice of the water-acetone extract of the powder of the cashew cake. The results obtained show that the water-acetone extract inhibits 50% of α-amylases at a concentration (IC 50) of 0.24 mg / ml. The percentage inhibition of this extract with the concentration tested reached 97.98 ± 0.47% at a concentration of 10 mg / ml. As for the concentrations inhibiting 50% of invertases (IC_{50}), the water-acetone extract has an inhibitory concentration of 1.44 mg / ml and the percentage inhibition of the concentrations tested reaches 88.27%.

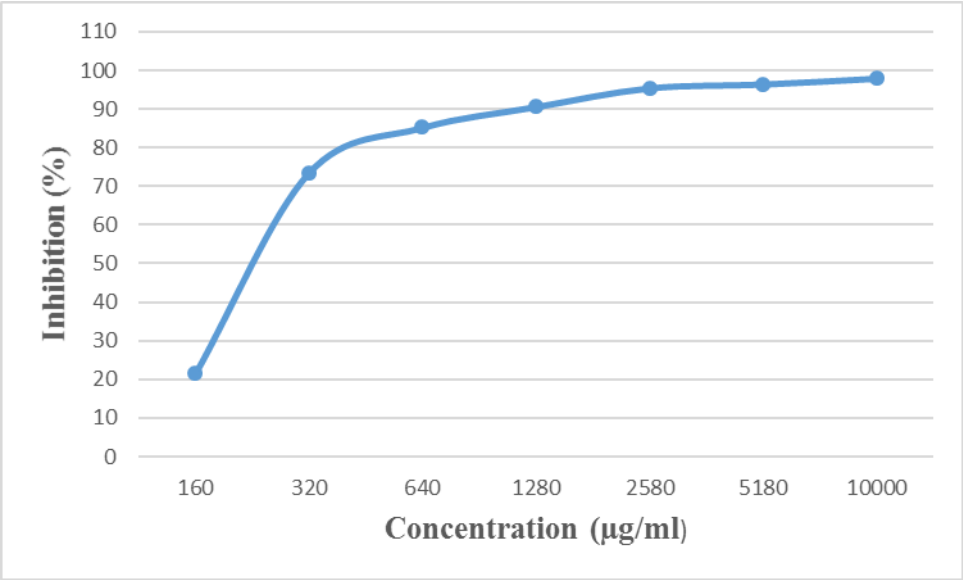


Figure 3: Inhibition of alpha-amylase digestive snail juice by water-acetone extract of cashew cake.

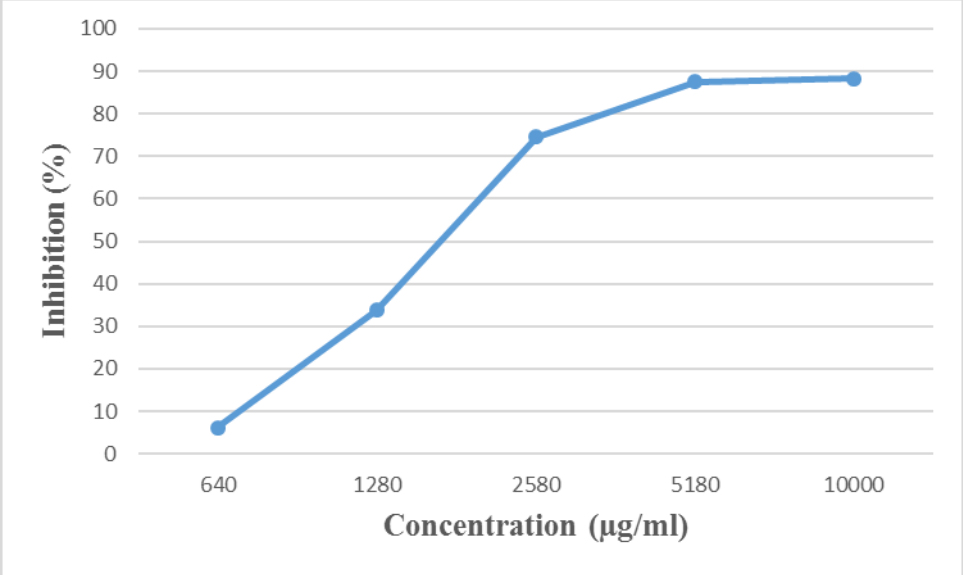


Figure 4: Invertase activity of snail digestive juice by water-acetone extract of cashew cake.

4. Discussion

This study investigated the use of flour extracts from cashew cakes for the enzyme inhibition test of *Achatina ventricosa* snail digestive juice. In this study, solvents including water, water-methanol, water-ethanol and water-acetone were used. The water-acetone solvent extracted the maximum of total phenols with a significantly high value

(9179.89 ± 0.15 mg / 100g) at p <0.05. The water-ethanol extract at a content of 7169.31 ± 0.08 mg / 100g; the water-methanol extract has a content of 6815.47 ± 0.069 mg / 100g whereas the aqueous extract has a content of 5439.02 ± 0.12 mg / 100g. These values are significantly higher than those obtained by Andrade and al., [9] when evaluating the polyphenol content of agro-industrial cashew apple residues. This extraction was performed with the use of 55% acetone. These results are superior to those of Ruffino and al. [19] who used the sequential extraction method (50% methanol followed by 70% acetone) and observed 830 mg / 100g.

The flavonoid content of the water-acetone extract is significantly higher (370.86 ± 0.02 mg/100g) at p <0.05 than that of the water-ethanol extract, which is 237.30. ± 0.02 mg / 100g. The water-methanol extract at a content of 215.96 ± 0.013 mg / 100g and the aqueous extract meanwhile, has a content of 200.88 ± 0.001 mg / 100g. There is no significant difference between the flavonoid content of the aqueous extract and the water-ethanol extract. The different flavonoid contents are lower than those obtained by Sulaiman et al., [20] whose values were 930mg / 100g and 2170 mg / 100g when it used 70% ethanol and 70% acetone as extraction solvent. The values obtained are higher than that of Andrade et al., [9] which was 109.03 using 80% methanol to extract flavonoids from agro-industrial cashew apple residues.

The tannin content of the water-acetone extract (1852.09 ± 0.023 mg / 100g) was significantly higher at p <0.05 compared to the tannin content of the 1068 ethanol extract. , 60 ± 0.091 mg / 100g. The tannin content of the water-methanol extract is 1010.72 ± 0.069 mg / 100g and 857.45 ± 0.05 mg / 100g for the aqueous extract.

The contents of phenolic compounds vary depending on the extraction solvent. These results are similar to those of Naczk and Shahidi [21]. According to these authors, the type and polarity of the solvent, the time and temperature of extraction, and the physical characteristics of the sample affect the extraction of polyphenols. For Zhao et al., [22], different polarities of solvents can influence the solubility of the chemical components in a sample. In addition, intrinsic and extrinsic factors, such as genetic variety, stage of maturation, type of cultivar, weather and crop conditions; harvesting and post-harvest conditions can contribute to the variability of the amounts of photochemical compounds extracted [23].

These bioactive photochemical compounds have recognized antioxidant activity on mechanisms such as the complexation of metal ions, the capture of free radicals, the decomposition of peroxides, the donation of electrons and hydrogen, the inactivation of the reactive species of the oxygen and UV absorption. Polyphenols are of paramount importance because there is a positive correlation between plant phenolic compounds and antidiabetic activities [24]. Thus the presence of phenolic compounds in cashew cakes could positively influence the hypoglycemic activity of these.

Indeed, many bioactive compounds of plants are known for their hypoglycaemic effect [25], [26]. These compounds include alkaloids [27], [28], flavonoids [29], [30], phenolic compounds [31] and triterpenoids [32].

With regard to the extraction solvents, several solvents are used for the extraction of the phenolic compounds. In fact, the phenolic compounds of plants are often associated with other biomolecules (proteins, polysaccharides, terpenes, chlorophyll, lipids and inorganic compounds). Thus it is necessary to find a suitable solvent to extract them.

The water-acetone extract, whose composition in phenolic compounds was significantly higher compared to the other extracts studied, was used for the inhibition test of the *Achatina ventricosa* digestive juice. This extract showed an inhibition of the digestive enzymes of the snail digestive juice. The IC_{50} (concentration that inhibits 50% of the studied enzymes) of the alpha-amylase of the digestive juice of this extract is 0.24 mg / ml and the IC_{50} of the alpha glucosidase of the digestive juice of snail of this same extract is 1.44 mg / ml. These IC_{50} values are higher than those obtained by [33] on the inhibition of alpha-amylase and alpha glucosidase in the ethanolic extract of cissus arnottina. The IC50 of the water-acetone extract of the cashew cake is higher than that obtained by [34] on the inhibition of extracts from 23 Ivorian plants using as an enzyme the raw extract of *Achatina ventricosa*.

Alpha-amylase catalyzes the hydrolysis of starch and alpha-glucosidase catalyzes the hydrolysis of the last stage of carbohydrate digestion which leads to postprandial

hyperglycemia. Thus, alpha-amylase and alpha-glucosidase inhibitors are useful in controlling hyperglycemia by delaying carbohydrate digestion and reducing the rate of glucose uptake. These inhibitors have been shown to be useful in controlling diabetes mellitus for many years [35], [36].

5. Conclusion

The results of this study show that cashew cakes are a source of natural bioactive compounds. Among the solvents studied, the water-acetone mixture would make it possible to extract the maximum of bioactive compounds studied (total polyphenols, flavonoids and tannins), since these bioactive compounds were more easily extracted in the water-acetone solvent. In addition, these cakes would also represent an antidiabetic potential in vitro. Indeed, the water-acetone extract was able to inhibit in vitro alpha amylase and alpha-glucosidase, key enzymes of carbohydrates metabolism. These results prompt us to identify the molecules responsible for these inhibitory effects that can be used in the management of diabetes. The use of these cakes would be a rational strategy that would result in economic gain and environmental benefit.

RERERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes Estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27: 1047–1053.
2. Apostolidis E, Lee CM. In vitro potential of *Ascophyllum nodosum* phenolic antioxidant-mediated α-glucosidase and α-amylase inhibition. J. Food. Sci. 2010; 75: 97-102.
3. Yukiliko H and Honda M. The inhibition of α-amylase by tea polyphenols. Agric. Biol. Chem. 1990; 54 (8): 1939-1945.
4. Bailey CJ. New approaches to the pharmacotherapy of diabetes. In: Pickup, J.C., William, G. (Eds.), Textbook of Diabetes, vol. 2, third ed. Blackwell Science Ltd., UK. 2003; 73.1–73.21.
5. Cheng AYY, Fantus IG. Oral antihyperglycemic therapy for type 2 diabetes mellitus. Canadian Medicinal Association Journal. 2005; 172(2): 213-226.
6. Ani V and Naidu K. Antihyperglycemic activity of polyphenolic components of black/bitter cumin Centratherum anthelminticum (L.) Kuntze seeds. European Food Research and Technology. 2007; 226 (4): 897-903.
7. Fujita A, Borges K, Correia R, Franco B, Genovese M. Impact of spouted bed drying on bioactive compounds, antimicrobial and antioxidant activities of commercial frozen pulp of camu-camu (Myrciaria dubia Mc. Vaugh). Food Res. Int. 2013; 54: 495 500.
8. Majhenic L, kerget MS, Knez Z. Antioxidant and antimicrobial activity of guarana seed extracts. Food Chemistry. 2007; 104: 1258–1268.
9. Andrade RAM, Maciel MIS, Santos A.M.P. Melo, E.A. Polyphenols extraction cashew residues. 2015; 35(2), http://dx.doi.org/10.1590/1678-457X.6585.
10. Romani AP, Pinelli C, Cantini A, Cimato D, Heimler. Characterization of Violetto di Toscana, a typical Italian variety of artichoke (Cynara scolymus L.). J. Food Chem. 2006; 95: 221-225.
11. Singleton VL and Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 1965; 16: 144-158.
12. Wood JE, Senthilmohan ST, Peskin AV. Antioxydant activity of prodyanidin containing plant extracts at different pHs. Food Chem. 2002; 77:155-161. DOI: http://dx.doi.org/10.1016/S0308-8146(01)00329-6.

13. Marinova D, Ribavora F, Antanassova M. Total phenolics in bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy. 2005; 40(3): 255-260.
14. Bainbridge Z, Tomlins K, Westby A. Methods for assessing quality characteristic of non – grains starch (Part 3. Laboratory methods). Natural Ressources Institute, Chathom, UK. 1996.
15. Colas B. Purification et caractérisation des propriétés cinétiques et moléculaire de β glycosidases du suc digestif de *Archachatina ventricosa*. Thèse de doctorat d'état ès Sciences, Université de Provence, France. 1977 ; 155 p.
16. Lowry OA, Rosenbrough, NJ, Farr AL, Randal RJ. Protein measurement with th Folin Phenol Reagent, J Biol Chem. 1951; 193: 265-275.
17. Bernfeld P. Alpha and beta-amylases. In, Methods in Enzymology, Colowick SP, Kaplan N., eds. Academic Press, New York. 1955; 1:149-158.
18. Chessel D, Thioulouse J, Dufour AB. Introduction à la classification hiérachique. 2004.
19. Rufino MSM, Alves RE, de Brito ES, Pérez-Jiménez J, Saura-Calixto F, Mancini, JF. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. Food Chemistry. 2010; 121(4): 996-1002.
20. Sulaiman SF, Sajak AAB, Ooi KL, Supriatno, Seow EM. Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. Journal of Food Composition and Analysis. 2011; 24(4-5) : 506-515.
21. Naczk, M., & Shahidi, F. Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. Journal of Pharmaceutical and Biomedical Analysis. 2006; 41(5):1523-1542.
22. Zhao H, Dong J, Lu J, Chen J, Li Y, Shan L et al. Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (Hordeum vulgare L.). Journal of Agricultural and Food Chemistry. 2006; 54(19), 7277-7286.
23. Capecka E, Mareczek A, Leja M. Antioxidant activity of fresh and dry herbs of some Laminaceae species. Food Chemistry. 2005; 93(2), 223-226.
24. Anam K, Widharna RM, Kusrini D. α-glucosidase inhibitor activity of Terminalia species. Int J Pharmacol. 2009; 5:277-280.
25. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol. 2002; 81: 81-100.
26. Oboh G, Ademiluyi AO, Faloye YM. Effect of combination on the antioxidant and inhibitory properties of tropical pepper varieties against α-amylase and α-glucosidase activities in vitro. J Med Food. 2011; 14:1152-1158.
27. Dineshkumar B, Mitra A, Mahadevappa M. Antidiabetic and hypolipidemic effects of mahanimbine (carbazole alkaloid) from Murraya koenigii (rutaceae) leaves. Int J Phytomed. 2010; 13: 22-30.
28. Sharma B, Salunke R, Balomajumder C, Daniel S, Roy P. Anti-diabetic potential of alkaloid rich fraction from Capparis decidua on diabetic mice. J Ethnopharmacol. 2010; 13: 457-462.
29. Panda S and Kar A. Apigenin (4′,5,7-trihydroxyflavone) regulates hyperglycaemia, thyroid dysfunction and lipid peroxidation in alloxan-induced diabetic mice. J Pharm Pharmacol. 2007; 13: 1543-1548.
30. Yoshikawa M, Wang T, Morikawa T, Xie H, Matsuda H. Bioactive Constituents from Chinese Natural Medicines. XXIV. Hypoglycemic Effects of Sinocrassula indica in Sugar-Loaded Rats and Genetically Diabetic KK-Ay Mice and Structures of New Acylated Flavonol Glycosides, Sinocrassosides A1, A2, B1, and B2. Chem Pharm Bull. 2007; 55: 1308-1315.
31. Manickam M, Ramanathan M, Jahromi MA, Chansouria JP, Ray AB. Antihyperglycemic activity of phenolics from Pterocarpus marsupium. J Nat Prod. 1997; 13: 609-610.

32. Tan MJ, Ye JM, Turner N, Hohnen-Behrens C, Ke CQ, Tang CP. Antidiabetic activities of triterpenoids isolated from bitter melon associated with activation of the AMPK pathway. Chem Biol. 2008; 13: 263-273.
33. Kavitha S, Kamaraj M, Rajeshwari S. In vitro alpha amylase and alpha glucosidase inhibition activity of crude ethanol extract of Cissus arnottiana. Asian Journal of Plant Science and Research. 2012; 2 (4):550-553.

34. Koffi E, Sea T, Dodehe Y, Soro S. Effect of solvent type on extraction of polyphenols from twenty three Ivorian plants. Journal of Animal & Plant Sciences, 2010; 5(3): 550-558.

35. Layer P, Rizza RA, Zinsmeister AR, Carlson GL, Di Magno EP. Effect of a purified amylase inhibitor on carbohydrate tolerance in normal subjects and patients with diabetes mellitus. Mayo Clin Proc. 1986; 61(6): 442-447.
36. Tundis R, Loizzo MR, Menichini F. Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycemic potential in the treatment of diabetes: an update. Mini Rev Med Chem. 2010; 10(4): 315-331.