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Research Article

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Dietetic drink based on the use of local foodstuffs in the management of type 2 diabetes.

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Abstract

Oral antidiabetic drugs are widely used in the management of type 2 diabetes. Unfortunately, they do not allow for an efficient management of patients because of the high costs, and numerous side effects. Hence, this work aimed at formulating a low-cost diet drink with no side effects using several local plants (*Stevia rebaudiana* Bertoni, *Moringa oleifera* Lam, *Zingiber officinale*, *Spydium guava*, and *Hibiscus Sabdariffa* Lin) and evaluating the content of bioactive compounds, the nutritional, antioxidant and hypoglycemia potential of the drink Three drink formulations (512, 434 and 851) were made followed by sensory analysis, the nutritional and the bioactive compounds content were determined. The antioxidant potential was evaluated through 2 methods (FRAP test and DPPH° test). The *in vitro* anti-diabetic potential was done through the inhibition of -amylase (using Acarbose as a reference drug); the autophagic capacity and the stimulation of insulin sensitivity via glucose uptake by yeast cells (*Saccharomyces cerevisiae). In vivo*, acute toxicity of the selected beverage was performed. The different beverages showed a good sensory profile especially 851(score of 6.9 ± 0.95) and interesting nutritional potential (total carbohydrates =5.04±0.04, fibers =0.41 ± 0.28, zinc=3.40 ± 0.01, magnesium=13.15 ± 0.01). They also showed in vitro interesting antioxidant activities by DPPH radical scavenging with IC_{50}(0.7713 ± 0.31 µg/mL), and Fe^{3+} to Fe^{2+} reduction(1045.74 ± 3.00mg FeSO4/100ml) for 851. All beverages inhibited -amylase with the best IC_{50} of 0.098 mg/mL, higher than Acarbose (IC_{50} = 2.74 mg/mL). Subsequently, the glycosylation assay revealed that all beverages adsorbed glucose, reducing its availability with 851 having the best adsorption capacity (up to 43.65%).

In addition, all beverages stimulated glucose uptake by yeast cells, with a higher binding capacity observed with beverage 851 (32.91%, $p < 0.05$). In vivo, the evaluation of the acute toxicity was performed through protocol 423 of OCDE (2001) with the beverage with the best antidiabetic activity (851) using male rats.The results of the acute toxicity test revealed that the LD_{50} of the selected beverage (851) was greater than 2000 mg/kg. Therefore, beverage 851reduces blood glucose levels through combined actions on enzyme inhibition, glucose adsorption, and enhancement of insulin action via modes of action similar to oral antidiabetics.

Keywords: Type 2 diabetes, *Stevia rebaudiana Bertoni*, *Moringa oleifera* Lam, *Zingiber officinale*, *Spydium guava*, *Hibiscus Sabdariffa* Lin.

Introduction

Diabetes is a chronic disease that develops when blood glucose levels rise because the body fails to produce enough insulin and/or use it effectively (ADA, 2017). Several types of diabetes are identified; the most well-known being type 1 diabetes (T1D), type 2 diabetes (T2D) and gestational diabetes (WHO, 2016). According to the IDF, (2021), nearly 537 million of the adult population (20-79 years) have diabetes. In Africa, nearly 24 million adults suffer from it (IDF, 2021). In Cameroon, 615,000 diabetics aged between 20-79 years have been diagnosed (WHO, 2019). T2D alone accounts for 90% of the 537 million affected individuals worldwide (IDF, 2021). To reduce the associated morbidities and mortality, the main therapeutic target is the reduction of hyperglycemia. This can be achieved through non-pharmacological management on the one hand, relying on the consumption of high-fiber and low-fat diets, smoking cessation, reduction of alcohol consumption, and regular exercise (ADA, 2017). Therefore, the management of pathologies through diet is of public health importance (ADA, 2017). Exercise, on the other hand, leads to improved insulin action in the muscles via increased glucose transport, cellular uptake, and increased consumption (Pospiech *et al.,* 2017). When lifestyle changes are not sufficient to control and maintain blood glucose levels within acceptable thresholds, it is then necessary to turn to pharmacological treatments. This is usually done with a monotherapy, followed by a combination of several molecules, or even insulin if necessary. Many modes of action have been explored in the fight against type 2 diabetes and These agents act either by stimulating insulin secretion by -pancreatic cells (sulfonamides) or by decreasing hepatic glucose production (metformin) or either by the reduction of post prandial glycemia, by inhibiting the activity of intestinal enzymes (-amylases and -glucosidases) (Barau *et al.,* 2016). However, the drugs in addition to their relatively high cost, are not without side effects (fatal lactic acidosis (buformin, penformin), nausea, vomiting and diarrhea, visual disturbances, upper respiratory infection, sinusitis and weight gain) (ADA, 2017).Hence the use of herbal medicine (Amelie, 2013). Thus, several plants of the Cameroonian pharmacopoeia are used by the population for the management of type 2 diabetes like *Invirgiagabonensis*, *Portulacaoleracea, Garcinia Kola, Moringa oleifera Lam, Zingiber officinale, Stevia rebaudiana Bertoni, Hibiscus sabdariffa Linn* and many others. Some of them, due to their availability, accessibility and specific mechanisms of action, stand out. These as well as the aqueous extracts of *Moringa oleifera Lam* leaves have shown anti-diabetic, and antioxidant properties (Edoga *et al.,* 2013). The aqueous extract of *Zingiber officinale* rhizomes has shown its anti-diabetic effects by helping the liver and pancreas to decongest, function well (Semwal *et al.,* 2015), stabilize blood sugar levels by protecting pancreatic -cells, increasing insulin synthesis and sensitivity (Srinivasan, 2017). The aqueous extract of *Stevia rebaudiana Bertoni* leaves exerts these anti-diabetic effects by increasing hepatic glucokinase activity, which increases glucose utilization to promote energy storage in the form of glycogen, and promoting hepatic glucose production (Khaing *et al.,* 2019).These plants can be consumed in different forms such as teas, dietetic drinks, nectar etc. It has been shown that the demand for dietetic drinks has increased significantly in recent years

and has resulted in a multi-billion dollar business. Apart from the visual appearance, it is easy to consume, hence facilitating the intake of antidiabetic agents from the selected plants (Banga *et al.,* 2019).

Given their availability and their independent hypoglycemic, antioxidant, antidiabetic, blood sugar regulating and insulin synthesis improving effects, the combined use of these plants via the formulation of diet drinks would be a great asset for a low cost and undesirable effect free management of type 2 diabetes.

Materials and Methods

Plant material

The material for the preparation of the beverages consisted on the one hand of rhizomes of *Zingiber officinale, Guava (Spydium guajava L.)*, flower of *Hibiscus Sabdariffa* all bought at the market of Mfoundi and on the other hand of leaves of *Stevia rebaudiana Bertoni* collected in a field in the district of Manassa in the department of Mefou-Afamba district of Mfou and leaves of *Moringa oleifera Lam* collected in the district Ngoa-Ekellein Yaoundé. These plants were authentified at the National Herbarium of Yaounde. The identification was done through comparism with the botanic collection Leeuwenberg A.J.M.9497 for *Moringa oleifera* Lam recorded at the National Herbarium N°.49178SRFCam, Westphal 10107 for *Zingiber officinale* recorded at the National Herbarium N° .43146HNC and Westphal 9841 for *Hibiscus Sabdariffa Lin* recorded at the National HerbariumN°.42815 HNC.

Experimental animals

The experimental animals (rats of Wistar) were provided by the animal breeding unit of the Faculty of Science, Department of Animal Biology and Physiology of the University of Yaoundé .

Reagents

The kit for the determination of glucose (GOD-POD) came from CHEMELEX, S.A. (Pol.Ind.Castells.C / Industria 113, Nave J, Barcelona). The other chemicals were from Sigma-Aldrich Co. (St. Louis, MO, USA).

Preparation of beverages: The preparation of beverages was carried out according to the protocol below.

In brief, the leaves of *Stevia rebaudiana bertoni* and *Moringa oleifera Lam* were sorted, weighed, washed, and dried in an oven at 45°C until a constant weight was obtained and then powdered. The rhizomes of *Zingiber officinale* were washed, sorted, weighed, cleaned of pulp, and then ground. The fruits of guava (*Spydium guajava L*) were washed, weighed, de-pulped, and ground. *Hibiscus Sabdariffa Lin* flowers were sorted, weighed, and infused in distilled water for 30 minutes and filtered to obtain a liquid. Thanks to constraints previously defined on the basis of the recommended nutritional intake, these different products were then mixed via precise proportions obtained using Microsoft Excel 2016 software via its solver function.

Formulation constraints

As constraints, we set ourselves to formulate a diet drink per 100 g of formulated powder having a content of carbohydrate (g) 50, fiber (g) 20, vitamin C (mg) 90, magnesium (mg) 350 and zinc (mg) 2.4.

Different mixtures made: The different mixtures made for the formulation of the beverages are listed in Table 1 below.

Table 1: Different blends made for beverage formulation

Ingredients	Ginger grind (g)	Moringa powder (g)	Stevia powder (g)	Guava juice (g)	Hibiscus Calyx Infusion (g/L)	Water (litre)
Mixture 1	90,98	72,45	7,93	27,48	40	1
Mixture 2	65,85	69,68	13	52,98	40	1
Mixture 3	47,9	69,08	23,5	27,64	40	1

Moringa powder, Stevia powder, guava juice, ginger grind were mixed in a jar then homogenized for 10 minutes with deionized water and filtered through a 0.5 micron sieve to obtain a liquid. Then the infusion of hibiscus calyxes (20g in 500mL of distilled water for 30 minutes at 30°C) was carried out then the juice obtained was filtered, cooled and added to the liquid previously obtained. The whole constituted the different drinks formulated.

The beverages were filed into glass bottles, pasteurized at 65 °C for 30 minutes, and cooled to room temperature. Following the sensory analysis, the nutritional potential was evaluated. Then we evaluated in-vitro the antioxidant potential of the 3 beverages as well as the anti-diabetic potential and the acute toxicity of the selected beverage.

Sensory analysis: The organoleptic properties of the 3 prepared beverages were determined by a tasting panel of 70 evaluators (aged 20-40; 40 men and 30 women). The panel members were asked to evaluate the taste, color, smell, consistency, and general acceptability of each characteristic ranging from 9 (extremely pleasant) to 1 (extremely unpleasant).

***Proximal analysis*:** The **water content** was determined by oven drying according to **A.O.A.C (1980)**. A Mo mass of the fresh sample was dried at 105 °C to constant weight in an oven for subtracting the dry matter content for 100. For **crude protein**, the total nitrogen is determined after the mineralization of the samples according to the Kjeldahl method **(AFNOR, 1984)**, and the assay is performed according to the colorimetric technique of **Devani*et al*, (1989).** After the mineralization of beverage lyophilisate, the samples were assayed and the result was calculated using 6.25 as the nitrogen of the protein conversion coefficient. The **total lipids** were extracted by soxhlet according to the method of **Bourely (1982).** The test sample was dried in an oven at 105 °C, crushed, and placed in dried and tared filter paper bags. The oil was extracted with hexane in a soxhlet for 12 hours. The oil content was calculated at 0% moisture by the difference in the weight of the bag before and after lipids have been completely extracted. The **ash content** was determined by simple incineration according to **A.O.A.C (1980)**. This consist of completely incinerating the organic dried matter contained in the sample until white ash was obtained in a muffle furnace set at 550°C.

Crude fibre was determined by the method described by **A.O.A.C (1980).** This method is based on a sequence of digestion of a powder obtained from the feed with strong acid and strong bases. **Soluble sugar** were extracted and determined by **Fischer and stein (1961)** and **total carbohydrate** content was determined by the difference method described by **A.O.A.C (1984).** The **energetic value** was determined by the difference method described by **Livesey, (1995)**.

Mineral analysis: The content of Ca, Mg, K, Na, Fe, Cu, Mn and Zn were determined by flame atomic absorption spectrophotometry according to **Berton and Vernon (1990)**.

Analysis of bioactive compounds: The vitamin C content was determined by the method described by **Idah et al (2010)**. 2, 6 dichloro-phenol-indophenol (DCPIP) is used to directly determine the vitamin C present in the solution by redox titration. The end of the essay or equivalence is highlighted by an excess of DCPIP (pale pink hue).

Total phenolic compounds and total flavonoids were determined by the method of **Dhar et al (2012).** Extraction was done using 20mL water-methanol (v/v) solvent for 1 g of lyophylisate. After 30 min of stirring, the whole was filtered with Whatman N° 1 paper. From the extract obtained the determination of phenolic compounds was done using gallic acid as standard and the O.D. was read at 765 nm. For flavonoids the standard was quercetin and the O.D. was read at 430nm.

The tannin contents were determined according to **Ndhlala et al (2007).** In a 50 mL Erlenmeyer flask, 0.125 g of sample and 2.5 mL of 96% ethanol were added. The mixture was centrifuged at 3000 tr/min during 10 minutes and the collected supernatant is used for the tannin determination. The absorbance was read at 550nm. **Phytates** were assayed according to **Olayeye et al. (2013)**. 1g of beverage lyophylisate was introduced into a 150 mL flask to which 100 mL (2% HCl) was added. The resulting mixture was filtered. Titration was done with iron chloride solution (standard) until a persistent brownish yellow colour was observed for 5 min. **Saponin** where determined according to the method of **Koziol (1990)**. 0.5 g lyophylisate of the beverage was introduced into a test tube, 5mL of distilled water was added. The tube was shaken vigorously for 30 seconds. After 5-10 s, the height of the foam formed was measured with a ruler graduated to the nearest 0.1 cm.

Antioxidant Potential

The in vitro antioxidant potential of the beverages was evaluated through 2 mechanisms: radical scavenging and reducing properties.

Ferric reducing- antioxidant power: It was determined by the method described by **Benzie and Strain (1996).** To 0.1 mL of beverage, or FeSO4 solution (standard), were added 300 µL of freshly prepared FRAP reagent. After 5 min of incubation, the absorbance of the reaction medium was detected at 593 nm.

Capacity scavenged the 2, 2-phenyl-picryhydrazyl radical (DPPH°): The antioxidant activity of the beverages (1000; 500; 250; 125 and 62, 5 µg / mL) prepared in methanolic solution was measured in terms of radical scavenging ability, according to the DPPH method (**Hzounda*et al.,* 2014**). A volume of 75 µl of extract was introduced into 25 µl of a methanolic solution of DPPH (0, 01%) and kept in the dark for 30 min. Control experiments without the beverage but with equivalent amounts of methanol were conducted in a similar manner. The absorbance was then spectrophotometrically read at 517 nm. Gallic acid was used as reference.

In vitro antidiabetic potential of formulated beverages

The in vitro antidiabetic potential was evaluated through 3 mechanisms: glucophagic property through the glucose adsorption assay and insulino-sensitivity stimulating effects through glucose uptake by yeast cell assays.

Glucose Adsorption Capacity: Glucose adsorption capacity of the beverages was determined according to the method of **Ou, Kwok, Li & Fu (2001).** Briefly, 1 ml of beverage at different concentrations (5; 10; 15 and 20 mg/ml) was added to 1 mL of glucose solution concentration (25 mM), the mixture was well stirred, incubated in a shaking water bath at 37 °C for 1 hr, centrifuged at 4,000 g for 20 min and the glucose content in the supernatant was determined according to the method of Trinder (1959). Bound glucose was calculated using the following formula.

$$\text{Percentage bound glucose (\%)} = [(\text{Initial glucose} - \text{Final glucose})/\text{Initial glucose}] \times 100$$

Glucose Uptake by Yeast Cells: Yeast cells were prepared according to the method of **Cirillo (1962)**. Commercial baker's yeast was washed by repeated centrifugation (4,000 g for 5 minutes) in distilled water until the supernatant was clear and a 10% (v/v) suspension prepared in distilled water. Various concentrations of beverages (2.5; 5; 7.5 and 10 mg/ml) were added to 1 mL of glucose solution (25 mmol/L) and incubated for 10 minutes at 37°C. The reaction was started by adding 100 µL of yeast suspension, vortexed, and further incubated at 37°C for 60 minutes. After 60 minutes, the tubes were centrifuged (3,000 g for 5 minutes) and glucose in the supernatant was determined according to the method of **Trinder (1969)**. The reaction without the extract was used as control. The percentage increase in glucose uptake by yeast cells was calculated using the following formula.

Percentage increase in glucose uptake (%) = [(Abs control - Abs sample)/ Abs control] × 100

Inhibition of -amylase activity: The evaluation of -amylase activity (EC 3.2.1.1) was done using Chronolab kit by the method of **Ying and Renze (1998)**. The -amylase (0.31 mg/mL) was obtained as follows: 5 mL of the phosphate buffer was introduced into exactly 2 g of sprouted sorghum seed meal, then the whole was shaken (2h) and left from time to time to facilitate the extraction. The supernatant constituting the enzymatic extract (-Amylase) was taken and kept cool at 4°C (**Mamoudou H, 2006**). Subsequently a volume of 75µl of reagent R was diluted in 6ml of distilled water, then 550 µl of the diluted reagent was mixed with 100µl of drink at increasing concentrations (1.25 2.5, 5, 7.5 and 10 mg/mL). The control was performed by replacing the beverage with water. The whole was incubated in a water bath for 3 minutes at 37°c. Then 20µl of alpha amylase 0.31 mg/mL was introduced into the reaction medium. The mixture was incubated for 10 minutes at 37°C and then heated in a boiling water bath at 100°C for 5 minutes to stop the reaction. The absorbance of the solutions in each tube was read at 505nm against the blank.

After these different analyses, a beverage was selected for *in - vivo* evaluation of acute oral toxicity.

Acute oral toxicity: The acute toxicity of the selected beverage was evaluated according to the OECD (2001)method on two batches of four rats each (four females and four males). The drink was administered by gavage as a single dose of 2000 mg/kg body weight. The animals were observed for 14 days after treatment during which time they had free access to food and water.

Estimation of human dose and drinking volume: The human drinking amount was calculated according to the method described by (CDER, 2005). Indeed,the animal dose was obtained using the LD_{50} after acute toxicity study using the following formula:

Human equivalent dose (mg/kg) = [animal dose (mg/kg) * (animal safety factor/human safety factor)]. The administration volume was determined by a simple rule of 3 knowing the final volume of the formulated beverage and the mass of the lyophilisate of the beverage.

Statistical analysis: For statistical analysis of the data, IBM/SPSS 20.0 for Windows using ANOVA followed by a post-hoc test (LSD) was used to compare the means of the different groups. The results were presented as mean ± standard deviation with a significance level of 5%. Microsoft Office Excel 2016 software was used for graphical representations. Graph pad prism version 8.0 software was used for calculation of inhibitory concentrations 50 (IC_{50}).

Results and Discussion

Table 2: Results of sensory analysis of formulated diet drinks.

Drinks / Features	512	434	851
Color	6.21 ± 0.14 [a]	6.6 ± 0.17 [ab]	7.21 ± 0.26 [b]
Smell	5.63 ± 0.85 [a]	6.07 ± 0.83 [a]	6.16 ± 0,82 [a]
Texture	6,03 ± 0,79 [a]	5,79 ± 0,71 [a]	6,07 ± 0,79 [a]
Taste	5.61 ± 0.89 [a]	6.01 ± 0.74 [a]	6.29 ± 0.95 [a]
General Acceptability	6.21 ± 0.80 [a]	6.5 ± 0.63 [a]	6.9 ± 0.95 [a]

Of the sensory parameters studied (Table 2), only the colour showed a significant difference between the different drinks. This could be explained by the proportion of other ingredients in the formulations that influence the final colour of the beverages, as all received the same amount of burgundy hibiscus infusion at the same concentration used here as colouring agent. Drink 851 was the one that presented the best general acceptability.

Table 3: Proximal composition of formulated beverages

Parameter per 100 mL of beverage	512	434	851
Water content (%)	90.87 ± 0.77 [b]	89.41 ± 0.01 [a]	88.38 ± 0.03 [a]
Protein (g)	2.03±0.17 [a]	2.35± 0.00 [b]	2.52±0.06 [b]
Fat (g)	1.66 ± 0.14 [a]	2.03 ± 0.00 [b]	2.41 ± 0.01 [c]
Total carbohydrates (g)	4.13 ± 0.35 [b]	4.96 ±0.00 [b]	5.04±0.04 [b]
Soluble sugars (g)	2.14±0.02 [a]	2.83±0.01 [b]	3.93± 0.01 [c]
Energy (Kcal)	42. 64 ± 0.44 [a]	47.56 ± 0.05 [b]	51.93 ± 0.44 [c]
Ash (g)	0.96 ± 0.79 [a]	1.00 ± 0.00 [a]	1.20 ± 0.16 [a]
Fiber (g)	0.34 ±0.41 [b]	0.24 ± 0.00 [a]	0.41 ± 0.28 [b]

Means having different letters attached to them are significantly different ((P<0. 05). Where 512: Drink 512; 434: Drink 434 and 851: Drink 851.

The results in Table 3 show that: The water contents of the beverages are 90.87 ± 0.77% (512), 89.41 ± 0.01% (434) and 88.38 ± 0.03% (851) respectively. These values are higher than 81.43% obtained by Ariviani *et al.* (2018)with pigeon pea (*Cajanus Cajan*) drink. This suggests that these drinks are good sources of water for the body. The total carbohydrate contents were 4.13 ± 0.35 g/100 mL (512), 4.96 ±0.00 g/100 mL (434) and 5.04±0.04 g/100 mL (851), respectively, and were all lower than 5.9g/100 mL obtained by Sohrab *et al.* (2016).The fiber content of the beverages were all lower than 22.25 g/100 g DM obtained with pigeon pea (*Cajanus Cajan*) beverage by Ariviani *et al.* (2018). The energy values of the beverages were 42, 64 ± 0.44; 47.56 ± 0.05 and 51, 93 ± 0.44 / 100 mL for beverages 512, 434 and 851 respectively. These values are within the range of diet drinks containing fruit (0-50 Kcal/ 100 mL) (Banga *et al.,* 2019).

Table 4: Major microelements content of formulated beverages

	Mineral content in mg/100 mL of beverage			
	Calcium (Ca)	**Magnesium (Mg)**	**Potassium (k)**	**Sodium(Na)**
512	16.48 ± 0.57 [c]	13.15 ± 0.00 [a]	177.34 ± 1.73 [a]	2.41± 0.00 [b]
434	15.15 ± 0.00 [b]	13.16 ± 0.01 [a]	191.94 ± 2.85 [b]	1.86 ± 0.10 [a]
851	14.14 ± 0.01 [a]	13.15 ± 0.01 [a]	188.00 ± 2.31 [b]	2.86 ± 0.17 [c]

Means having different letters attached to them are significantly different ((P<0. 05). Where 512: Drink 512; 434: Drink 434 and 851: Drink 851.

Table 5: Minor microelements content of formulated drinks

	Mineral content in mg/100 mL of beverage			
	Zinc (Zn)	**Iron (Fe)**	**Copper (Cu)**	**Manganese (Mn)**
512	3.40 ± 0.01 [a]	16.48 ± 0.05 [c]	1.06 ± 0.00 [a]	2.71 ± 0.9 [c]
434	3.39 ± 0.01 [a]	15.16 ± 0.01 [b]	1.18 ± 0.00 [c]	2.26 ± 0.00 [a]
851	3.40 ± 0.01 [a]	14.17 ± 0.02 [a]	1.16 ± 0.11 [b]	2.56 ± 0.1 [b]

Means having different letters attached to them are significantly different ((P<0. 05). Where 512: Drink 512; 434: Drink 434 and 851: Drink 851.

Mineral analysis: Minerals are present in varying amounts in the formulated drinks (Table 4 and 5). The contents of magnesium and zinc are similar in the 3 formulated beverages. Magnesium is important for type 2 diabetics as hypomagnesemia is a common attribute of type 2 diabetes and occurs at an incidence of 13.5% to 47.7% in patients with T2DDubey *et al.*(2020).Zinc on the other hand plays a regulatory role at the level of gene expression and intracellular signaling and is an effective antioxidant (EFSA, 2014).

Table 6: Composition of bioactive compounds in formulated beverages

Parameter per 100 mL of beverage	512	434	851
Vitamin C (mg)	60.80 ± 0.28 [b]	59.76 ± 0.05 [a]	62.50 ± 0.10 [c]
Total polyphenols (mg GAE)	594.65 ± 1.67 [b]	486.07±1.92 [a]	598.65 ± 2.77 [b]
Flavonoids (mg EQ)	218.53 ± 2.84 [a]	257.36 ± 1.37 [c]	246.74 ± 1.75 [a]
Proanthocyanins (mg EAG)	138.72 ± 0.91 [a]	157.21 ± 0.78 [c]	154.64 ± 1.10 [b]
Tannins (mg ACE)	119.14 ± 0.00 [a]	120.27 ± 0.05 [a]	148.97 ± 2.20 [b]
Phytates (mg PAE)	4.36 ± 0.43 [a]	5.28 ± 0.07 [b]	5.81 ± 0.10 [b]
Saponins (mg)	4.43 ± 0.45 [a]	4.43 ± 0.45 [a]	4.74 ± 0.21 [a]

GAE: gallic acid equivalent; QE: quercetin equivalent; PAE: phytic acid equivalent; CCE: catechin equivalent. Means having different letters attached to them are significantly different ((P<0. 05). Where 512: Drink 512; 434: Drink 434 and 851: Drink 851.

Analysis of bioactive compound: Table 6 shows the bioactive compounds contained in the different formulated beverages. The contents of vitamin C, total polyphenols, tannins, phythates and saponins are higher in beverage 851 while beverage 434 has the highest content of flavonoids and proanthocyanins. The vitamin C content is very high compared to mandarin (32.06 mg/100mL) and Soursop (20.50 mg/100mL) nectar (Nwozole *et al.,* 2017). The total polyphenol content of all these beverages are higher than that of pomegranate juice (2125mg EAG/L) obtained by Sohrab *et al.* (2016)and pomegranate juice (1946 mg EAG/L) obtained by Sohrab *et al.* (2015). Flavonoid contents were higher than pomegranate juice (385 µg/ml ECA/L) obtained by Sohrab *et al.* (2016)and lower than pomegranate juice (345.87 mg ECA/mL) obtained by Sohrab *et al.* (2015). The proanthocyanin contents are all lower than that of grape skin-based juice i.e. 38.9 ± 8.3 mg cyanidin-3-glucoside equivalent/L (38.9 ± 8.3 mg ECG/L) obtained by Vicens. (2007).the phytate contents of the different beverages are all below the safe dose which is between 2000 and 2600 mg/day (Danso *et al.,* 2019).Therefore they are very important for the prevention and management of type 2 diabetes by their abilities to reduce the formation of advanced glycation products in type 2 diabetic patients (Sanchis *et al.,* 2018).The saponin contents of the different beverages formulated are all below the recommended safe dose of 12g/100g DM (ESFA, 2009). Hence their importance in that they intervene in digestion by increasing the permeability of cell membranes, lowering cholesterol and blood sugar levels (Das *et al.,* 2012). They also help lower cholesterol levels in the body by reacting with bile acids to form micelles leading to an acceleration of its metabolism in the liver (Ragab *et al.,* 2017).

Antioxidant potential of formulated beverages: Table 7 below shows the DPPH° inhibitory concentrations of the formulated beverages and gallic acid. Beverage 851 has the best efficacy as it has the lowest IC_{50} (0.7713 ± 0.31 µg/mL), followed by beverage 434 (0.8135 ± 0.22 µg/mL) and finally beverage 512 (0.8883 ± 0.01 µg/mL). However, the beverages are less effective than gallic acid which has a much lower IC_{50} (0.6229 ± 0.01µg/mL). This activity could nevertheless be explained by the presence in our drinks of molecules such as phenolic compounds. Indeed, these phenolic compounds due to their ideal structural chemistry seem to be efficient hydrogen donors to the DPPH radical, (Turkmen et al. 2007).These results can be also explained by the content of polyphenols and in particular of flavonoids in the beverage which, due to the redox potential of their OH groups, would be capable of yielding a proton and/or an electron thus trapping the DPPH- radicals to give the stable compound DPPH-H. Indeed, the low redox potential of flavonoids (FLOH) makes them thermodynamically capable of reducing free radicals (R-) by the transfer of a hydrogen atom or of electrons from hydroxyl groups (Procházková, Bousová & Wilhelmová, 2011).

Table 7: IC_{50}values for formulated beverages

	512	434	851	Gallicacid
IC_{50} (µg/ml)	0.8883 ± 0.01 ^{d}	0.8135 ± 0.22 ^{c}	0.7713 ± 0.31 ^{b}	0.6229 ± 0.01 ^{a}

Values assigned different letters in the same line are significantly different (P 0.05).Where 512: Drink 512; 434: Drink 434 and 851: Drink 851.

Table 8: Overall antioxidant capacity of the different beverages formulated

Different drinks	512	434	851
$FeSO_4$ content (mg $FeSO_4$/100ml drink)	947.03±2.61 [a]	941.29 ± 0.94 [a]	1045.74 ± 3.00 [b]

Values assigned different letters on the same line are significantly different (P 0.05).Where 512: Drink 512; 434: Drink 434 and 851: Drink 851.

From the above table 8it isevident that the overall antioxidant capacities of the different drinks are: 947.03±2.61, 941.29 ± 0.94 and 1045.74 ± 3.00 mg $FeSO_4$/100ml for 512, 434 and 851 drinks respectively. While between 512 and 434 there is no significant difference at the 5% threshold (P 0.05), statistical analysis shows that there is a significant difference between 851 and these at the 5% threshold (P 0.05). The differences in the values observed between the drinks could be due to the electron donating capacity that the flavonoids contained in each of our drinks possess. This property of flavonoids is due to the number, configuration and glycosylation of hydroxyl groups (Nabila *et al.,* 2009). More specifically, the results of the assay revealed the presence of flavonoids and proanthocyanins which could be compounds involved in the free radical scavenging and reducing activities of our beverages either by proton release or electron release. These results can still be linked to the flavonoid content of the beverages as confirmed by their ability to yield electrons. Khan, Khan, Sahreen& Ahmed (2012)had already demonstrated the ability of flavonoids to reduce phosphomolybdenum by electron transfer.

In vitro antidiabetic potential of formulated beverages

Glucose Binding Capacity

The glucose binding capacity of the beverages was evaluated through the glucose adsorption capacity and is shown in figure 1. The beverages could bind glucose effectively, and the glucose binding capacity was directly proportional to the beverage and glucose concentrations. The beverages was effective in adsorbing glucose at both lower and higher concentrations. The percentages of glucose binding rise to 25.70, 30.88, 36.15 and 36.57 % with 512; 39.95, 35.74, 37.05 and 43.48% with 434; 32.68, 37.55, 40.56 and 50.63 % with 851 respectively at beverage concentrations of 5, 10, 15 and 20 mg/ml.

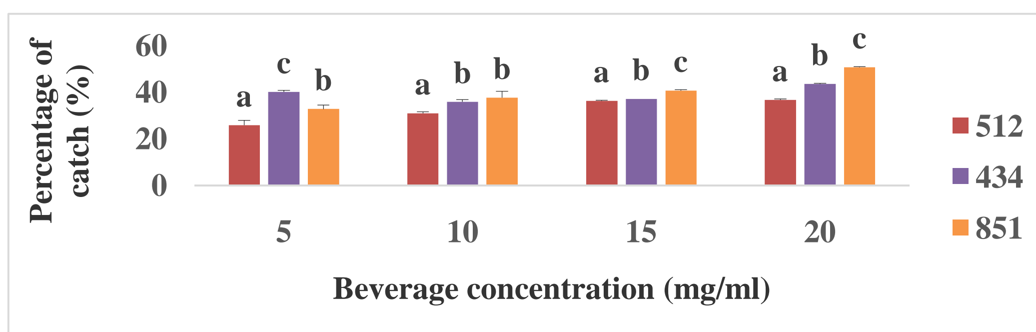


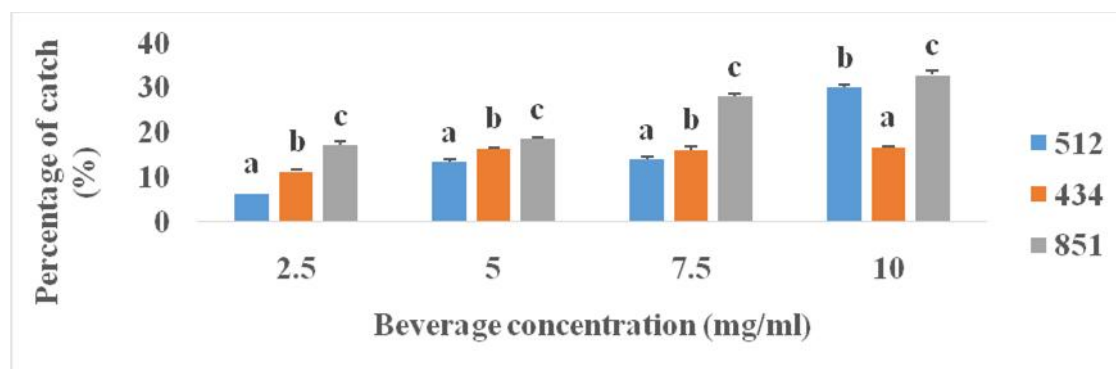
Figure 1: Percentage of glucose fixation by formulated beverages

The beverages demonstrated the ability to engulf glucose (Figure 1). This activity is attributed to the high flavonoid content. Flavonoids through condensation reactions complex with glucose molecules through hydroxyl groups forming glycosyl-flavonoids containing osidic bonds (Li *et al.,* 2014). This activity could also be attributed to the fiber content (0.34 ±0, 41 with 512; 0.24 ± 0.00 with 434 and 0.41 ± 0.28 g/100Ml with 851) of beverages. In effect, insoluble fibers are renowned for their ability to form complexes with glucose, thus rendering them unavailable, which contributes to the reduction in intestinal glucose absorption **(McRonie & McKeown, 2017; Somnath *et al.,* 2017).**

Insulino-sensitivity Stimulating Properties

The insulino-sensitivity stimulating properties of the extract was evaluated through glucose uptake by yeast cells as shown in figure 2.

The beverages promoted glucose uptake by yeast cells in a manner proportional to their concentrations. The increase of glucose uptake varied from 25.70 to 36.57 % with 512; 39.95 to 43.48% with 434 and 32.68 to 50.63 with 851.



512: drink 512; 434: drink 434; 851: drink 851. Values with different letters are significantly different ($p < 0.05$) when comparing the three drinks at each dose

Figure 2: Percentage of glucose uptake by yeast cells as a function of the concentration of the different beverages.

The extract stimulated the transport of glucose across the yeast cell membrane. These biological actions could be due to the presence in the extracts of compounds such as quercetins, -sitosterols and allyl. Indeed, quercetins and -sitosterol cause glucose uptake by yeast cells by stimulation of membrane transporters (Hexose Transporters (HXT1 to HXT17) and Snf3 and Rgt2) which act through a facilitated diffusion phenomenon **(Damsud *et al.,* 2017)**. Binding of glucose to these Snf3/Rgt2 sensors located in the plasma membrane of yeast cells allows phosphorylation of the two co-receptors (Mth1 and Std1) of Rgt1 (a transcriptional repressor that negatively regulates HXT gene activity). This binding thus exposes Rgt1 to phosphorylation by PKA (Protein Kinase A) allowing an interaction between the central region of Rgt1 and its Zinc finger **(Stefano et al., 2010)**. This inhibits the binding of Rgt1 to DNA which is forced to leave the HXT promoter and consequently the synthesis of HTX proteins will be responsible for the passage of glucose into the yeast cell membrane **(Dietvorst et al., 2010).**

Inhibition of alpha amylase

The effect of different beverage concentrations on -amylase activity is shown in **Figure 3**. Values are expressed as mean ± standard deviation on the mean (percentage change in the mean); Values assigned different letters on different histogram series has the same concentration are significantly different (P 0.05).

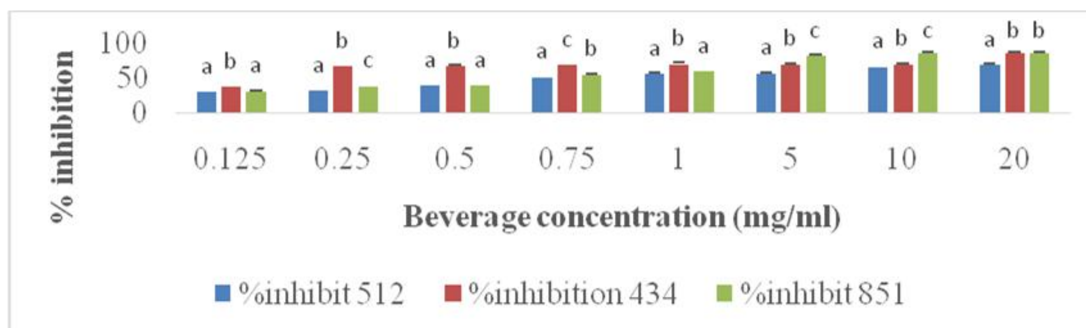


Figure 3: Effect of different beverage concentrations on -amylase activity.

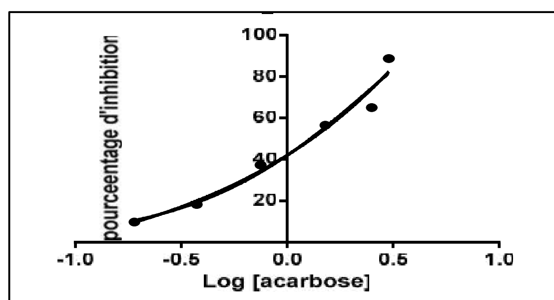


Figure 4: Effect of Acarbose on inhibition of -amylase activity.

Table 2: Inhibitory concentration 50 of beverages and Acarbose on -amylase activity.

Drinks	512	434	851	Acarbose
IC_{50} (mg/mL)	1.475 ± 1.02	0.6202 ± 0.65	0.098 ± 0.92	2.74 ± 0.80

Values are expressed as mean ± standard deviation. 512: beverages 512; 434: beverage 434; 851 beverage 851; IC_{50}: Inhibitory Concentration 50.

It can be seen from **Figure 3** that all beverages inhibited -amylase at the different concentrations chosen (0.125; 0, 25; 0.5; 0.75; 1; 5; 10 and 20 mg/mL). The inhibition was concentration dependent. The percentages of inhibition ranged from 28.82% to 68.27% with 512; 30.19% to 84.91% with 434; 36.60% to 85, 39% with 851. However, 851 showed the best inhibitory activity with an IC $_{50}$of 0.098 ± 0.92 mg/mL compared to 0.6202 ± 0.65 mg/mL for 434; 1.475 ± 1.02 mg/mL for 512. Moreover, the inhibitory activity achieved by these drinks was higher than that of the reference drug (Acarbose) which presented an IC $_{50}$of 2.74 mg/mL. In fact, the inhibition of -amylase by Acarbose was dose dependent on the different concentrations chosen (0.19; 0.375; 0.75; 1.25 and 2.5 mg/mL) with inhibition percentages ranging from 8.72% to 88.67% (**Figure 4**).

This inhibitory power can be explained by the fact that the different beverage formulations have compounds bearing functional groups close to those of the substrate which is starch, which occupies the active site of the enzyme. Indeed, studies on the reaction mechanisms of -amylase enzyme inhibition have shown that flavonoids and tannins can create conformational changes on the active site of the enzyme and form a complex between the latter and the enzyme which inhibits their biological activities. The inhibition potential for flavonoids and tannins is correlated with the number of hydroxyl groups in their B rings.

These compounds inhibit -amylase through the formation of hydrogen bonds between these hydroxyl groups and the active site residues of this enzyme (Sales *et al.,* 2012). Mojica *et al.* (2015)also showed that flavonols (myricetin, quercetin and kaempferol) or isoflavones (daidzein and genistein) are inhibitors of -amylase and -glucosidase.

Taking into account the results obtained so far, one beverage formulation was selected for the continuation of our work. This selection was made on the basis of sensory analysis with the 851 beverage being the most appreciated with a score of 6.9 on a scale of 9 higher than 6.5 and 6.21 obtained respectively with 434 and 512. The nutritional potential also showed that the nutrients of interest that we set in our nutritional constraints were higher in the 851 formulation beverage and therefore came closest to these constraints as did the contents of total carbohydrates (5.04; 4.96 and 4.13 mg/100mL for 851, 434 and 512 beverages respectively), Vitamin C (62.50 (851) ; 59.76 (434) and 60.80 (512) mg/100mL respectively), Magnesium (13.15; 13.16 and 13.15 mg/100mL for beverages 851, 434 and 512 respectively), Zinc (3.40 (851); 3.39 (434) and 3.40 (512) mg/100mL respectively) and Crude Fiber (0.41; 0.24 and 0.34 mg/100mL for beverages 851, 434 and 512 respectively). The antioxidant and anti-diabetic potential *in-vitro were* also found to be more promising with the 851 formulation compared to the other formulations. It is true that this drink had the highest carbohydrate content of 5.04 mg/100mL and the highest energy value of 51.93 Kcal/100mL. However, they are lower than the daily threshold values ordered in diabetic diet drinks, i.e. 89 Kcal/100mL for the energy value and 39 mg/100mL for total carbohydrates.

Acute oral toxicity: No specific signs of acute toxicity (change in coat, motility, tremors, mass, grooming, respiration, sensitivity to noise after metal shock, appearance of feces, mobility, and death) were revealed in rats for 14 days after dosing. The LD_{50} was greater than 2000 mg/kg. According to the Hodge and Sterner scale, its toxicity index is 4, thus low toxicity (Hodge and Sterner, 1980). This presages a safety of use for the said drink.

Estimating the dose and volume of the drink to be consumed

Estimation of the human drinking dose

Table 1: Humanequivalent dose

LD50 (mg/kg)	2000
Km Animal	6
Km adult male (60kg)	37
DEH (mg/kg)	800mg/kg
DEH for an adult 60Kg (mg)	48000

Estimated volume to be consumed

The 851 formulation selected had a volume of 1500 mL and the mass of lyophilisate obtained was 96.267g. The DEH for a 60 kg adult is 48,000 mg or 48g. By the rule of three, we obtain a volume of 747.92 mL, i.e. approximately 750 mL that can be administered in one day.

Mode of consumption

A patient could therefore take a 250 mL glass in the morning, 250 mL at noon and 250 mL at night. The intake could be before the meal (due to the drink's ability to inhibit carbohydrate digestion enzymes) and after the meal (due to its ability to reduce post-prandial hyperglycemia via its glucophagic, insulin-sensitizing and hypoglycemic effect).

Conclusion

Of the three beverages formulated, the 851 formulation presented the best general acceptability with a score of 6.09 on a scale of 0 to 9. *In vitro*, the three beverages showed an interesting nutritional and antioxidant potential and contents of bioactive compounds, but more significant in the 851 formulation. The 3 formulations showed an inhibition of enzymes involved in carbohydrate metabolism such as -amylase, a slowing down of glucose absorption (high Glucophagy) as well as an improvement of insulin sensitivity. *In-vivo* the formulation 851 drink presages a safety of use for the said drink with LD_{50}>2000 mg/kg.

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