Research Article ISSN 2641-4295

Food Science & Nutrition Research

In vitro Antioxydant Potential of Two Formulated Antidiabetic Powders by Using Nutraceutic Plants of Cameroon

Charles Falang Doumta[1]*, Bebbe Fadimatou[2*], Marlyne Josephine Mananga[2] and Eyenga Mougnol Jean Sylvain[2]

[1]*Department of Biomedical Sciences, Faculty of Health Sciences (FHS), University of Buea, Buea, Cameroon.*

[2]*Department of Biochemistry, University of Yaounde I, Yaounde, Cameroon.*

[*]Correspondence:
Doumta Charles Falang, Department of Biomedical Sciences, Faculty of Health Sciences (FHS), University of Buea, Buea, Cameroon, Tel: 00237 699 370 660.

Received: 09 Sep 2023; **Accepted:** 22 Oct 2023; **Published:** 29 Oct 2023

Citation: Doumta CF, Fadimatou B, Mananga JM, et al. *In vitro* Antioxydant Potential of Two Formulated Antidiabetic Powders by Using Nutraceutic Plants of Cameroon. Food Sci Nutr Res. 2023; 6(2): 1-6.

ABSTRACT

Many modes of action have been explored in the fight against type 2 diabetes, including the use of drugs. But these drugs, in addition to their relatively high cost, are not without side effects. As an alternative to these difficulties, traditional pharmacopoeia use several nutraceutic plants in the treatment of type 2 diabetes including: Vernonia amygdalina, Tetrapleura tetraptera, Leptadenia lancifolia decne and Gum Arabic (Acacia Senegal sap). The fact that these plants are used for the same treatment means that they contain bioactive contents, which can be different depending on the plant.

For this reason production of formulated powders (JE1 and JE2) of these plants with good anti-diabetic effects can help in the treatment of some chronic diseases. To assess the efficacy of the formulated powders a study of antioxidant activity of two formulated powders was evaluated.

Formulation of powders and comparative analyses of bioactive compounds of different formulated powders obtained was done by using classical methods.

The results revealed some difference in the phytochemical contents of the both formulated powders. JE1 is rich in mineral and anti-nutrition contents and JE2 is rich in vitamin C. JE1 and JE2 possess strong antiradical activities, but in general JE1 has the better free radical scavenging efficacy and antiradical activity compared to JE2.

Indeed the results reveal that JE1 and JE2 can have an impact on the control of oxidative stress on patients suffering from type 2 diabetes and this explains why Vernonia amygdalina, Tetrapleura tetraptera, Leptadenia lancifolia decne and Gum Arabic (Acacia Senegal sap) are used in the traditional pharmacopoeia for treatment of type 2 diabetes. For this reason consumption of these plants need to be encouraged.

Keywords

Nutraceutic plants, Formulated powders, Phytochemical analyses, Antioxidant activities, Treatment of type 2 diabetes.

Introduction

Type 2 diabetes is a chronic disease that occurs when a person's blood sugar level is high because their body cannot effectively use the insulin it produces. It usually affects people aged 20-79 years and accounts for about 90% of diabetes cases worldwide. Type 2 diabetes is the fifth leading cause of death in the world raising the alarm and classifying it as a public health problem [1]. This problem needs a specific management, which consists of lifestyle changes followed by pharmacological treatment including insulin if necessary [2]. Many modes of action have been explored to

fight against type 2 diabetes including blocking the potassium-dependent ATP pump in pancreatic β-cells (Sulfonylureas-Glipizide); stimulation of Peroxisome Proliferator-Activated Receptor-γ (Thiazolidinediones-Rosiglitazone); stimulation of adenosine mono-phosphate-activated protein kinase (Biguanides-Metformin) and modulation of Glucagon like Peptide-1 activity (Incretins-Exematide). These agents act either by stimulating insulin secretion by β-pancreatic cells (sulphonamides), or by decreasing hepatic glucose production (metformin) or the reduction of post prandial blood glucose by inhibiting the activity of intestinal enzymes (α-amylases and α-glucosidases) [2]. However, the drugs with its relatively high cost are not without side effects (fatal lactic acidosis, buformin and penformin, nausea, vomiting and diarrhoea (metformin), visual disturbances, upper respiratory infection, sinusitis and weight gain); as a result of this, many of them in the USA/Europe, have limited uses, are not marketed, have almost restricted prescribing and are sometimes even withdrawn from the market [3]. It has been reported that only 3 out of 20 patients are able to buy prescribed drugs in hospitals and only 1 out of every 1000 patients is able to consult a specialist [4]. As a result of this, there is a rich tradition in the use of herbal medicines for the treatment of several ailments and plans are on the way to integrate traditional medicine in the health care system even though the plans have not been put into action yet [5].

Cameroon however has a rich biodiversity, with ~8,620 plant species [6,7], some of which are commonly used in the treatment of several chronic diseases [8] and are ranged of neglected tropical diseases including malaria, trypanosomiasis, leishmaniasis, diabetes, tuberculosis, etc. [4]. As an alternative to these difficulties, Cameroonians are using nutraceutical foods. Which are ordinary foods that have components or ingredients incorporated in them to give a specific medicinal or physiological benefit other than a purely nutritional effect [9-11]. The economic production and availability of nutraceutical foods are highly desirable objective to improve the health of people in the country especially that of the poor [9]. Now, the nutraceuticals related research for improving its quality and quantity is an important area for ongoing biotechnological investigations [12]. Moreover, the Covid 19 pandemic has proven that in Africa and especially in Cameroon, due to the strong ethnobotanical potential, it is possible to overcome many diseases such as type 2 diabetes. In the traditional pharmacopoeia, several nutraceutic plants are used in the treatment of type 2 diabetes, including: *Vernonia amygdalina, Tetrapleura tetraptera, Leptadenia lancifolia* decne and Gum Arabic (*Acacia Senegal sap*) [13]. Since nutraceuticals or functional foods can be classified on the basis of their natural sources, pharmacological parameters or according to their chemical constitution, the combination of those nutraceutical plants would help to improve their efficacies in the treatment of type 2 diabetes. Therefore, the goal of this study targets comparative assessment of two combinations of nutraceutic plants on their antioxidant activities in view of the treatment of type 2 diabetes. To overcome this, two formulated powders are produced and are assessed on their phytochemical characterization and antioxidant activities.

Material and Methods

Collection and Processing of Plant Material

The leaves of *Vernonia amydalina* were collected from a field in the Nkolmesseng district of Yaounde V. The fruits of *Tetrapleura tetraptera* and Gum Arabic (*Acacia Senegal sap*) were purchased at the Mfoudi and Briqueterie markets (Yaoundé, Cameroon). *Leptadenia lancifolia* leaves and vines were collected in the Kaele area (Mayo-kani, Far-North Cameroon). The samples were then sent to the Laboratory of Food Science and Metabolism (LabSAM) where they were sorted, weighed, put under a stream of water, taken out and dried in a dehydrator at 45°C until a constant weight was obtained. The dried samples were then crushed and sieved through a 160 micron sieve and the resulting powder was packaged and labelled for analysis.

Formulation and Preparation of Powders

Formulation was done as shown in table 1. The objective in this constraint was to be able to reach as much as possible in the formulations of the recommended contents of some important molecules in the management of type 2 diabetes. Table 1 presents different formulations ingredients.

Table 1: Different formulations ingredients.

Formulation		Formulation 1 (JE1) (g/100g)	Formulation 2 (JE2) (g/100g)
Ingredients	*V.amygdalina*	0.41	0
	T.tetraptera	54.29	54
	G. arabic	39.38	39.38
	L. lancifolia	6.18	6.62
Composition	Carbohydrates (g)	52.89	49.90
	Fibres (g)	19.69	19.69
	Vit C (mg)	5.25	5.91
	Mg (mg)	254.66	253.35
	Ca (mg)	196.90	196.90
	Zn (mg)	5.25	5.91

Phytochemical Characterisation

The vitamin C content was evaluated by Harris et al. [14] method. The minerals Zn^{2+}, Ca^{2+} and Mg^{2+}, these contents were analysed according to the method described by Horwitz [15]. Extraction and determination of total phenolic compounds was carried out using the Folin - Ciocalteu reagent as described by Marigo [16]. Flavonoid content was done as described by de Vinson et al. [17]. Total tannins was assessed by Ndhlala et al. [18] method. Phytate content was done base on Olayeye et al. [19] method. Oxalate content was determined by the modified titration method of Aina et al. [20]. Saponin content was measured by Koziol [21] method.

Evaluation of *In Vitro* Antioxidant Potentials

Global antioxidant capacity: FRAP (Ferric Reducing Antioxidant Power) method is done as described by Benzi et al., [22]; Total antioxidant capacity was assessed according to the method of Prieto et al., [23]; DPPH radical scavenging test was done according to the method described by Lopes-Lutz et al., [24].

Statistical Analysis

Results were expressed as means ± standard deviation. The result obtained was the mean for three tests. All results were analyzed using a one-way analysis of variance. Duncan's Multiple Range Test was carried out to test for the means that are significantly different ($p < 0.05$) from each other, which are represented by letters in superscripts.

Results and Discussion

Mineral and the Theoretical Bioavailability of the Mineral Contents

Table 2 is the mineral and the theoretical bioavailability of the minerals (Anti-nutrient/mineral ratios).

Table 2: Mineral and the theoretical bioavailability of the mineral contents of JE1 and JE2 formulated powders.

Samples	JE1	JE2	Critical values
Vitamine C	44.51 ± 0.10^{a}	118.29 ± 0.79^{b}	/
Ca	404.15 ± 0.21^{b}	340.25 ± 0.3^{5}a	/
Zn	4.105 ± 0.13^{b}	3.27 ± 0.32^{a}	/
Mg	125.90 ± 2.6^{9}b	117.78 ± 0.6^{1}a	/
Oxalates/Ca	0.012	0.008	2.5
Oxalates/(Ca + Mg)	0.009	0.006	2.5
Phytates/Zn	1.912	1.538	10-15
Phytates/Ca	0.019	0.015	0.4

JE1: JE1 formulation; JE2: JE2 formulation. Values with different letters in the same row are significantly different ($p<0.05$).

Table 2 reveals that the highest vitamin C content is 118.29 ± 0.79 mg/100g DM (JE2) and the lowest is 44.51 ± 0.10 mg/100g DM (JE1), with a significant difference at the 5% level between these different values. This vitamin C helps to lower blood sugar levels in diabetic patients as high blood sugar levels lead to the production of free radicals, which in excess cannot be neutralised by the antioxidants present in the body. In addition, diabetics have a lower blood concentration of vitamin C than healthy people so it can be seen that the content of JE1 and JE2 formulations corresponds to the FAO-WHO [25] recommended dietary allowance of vitamin C for children and adolescents of 20-40 mg/day and for adults of 45-70 mg/day. These ideal levels are explained by the high vitamin C content of the ingredients.

Regarding calcium content, table 2 shows that JE1 has the highest calcium content (404.15 ± 0.21 mg/100g DM) and JE2 the lowest (340.25 ± 0.35 mg/100g DM) with a significant difference at the 5% level between these different values. The calcium content of JE1 is roughly equivalent to the recommended daily allowance of calcium, which is 400mg/day to 1200mg/day. Calcium helps to stimulate insulin secretion by the β-cells of the pancreatic islets, which helps to improve the management of type 2 diabetes [26]. The high calcium content of the formulation is explained by the high calcium contents of the ingredients and that of JE1 is even higher as it contains the ingredient *Vernonia amygdalina* something which JE2 does not have and which has the highest calcium content compared to the other ingredients. Table 2 reveals that the bioavailability of calcium is not hindered by phytate and oxalate contents.

Concerning zinc, it appears in table 2 that the highest zinc content is JE1 (4.10 ± 0.13 mg/100g DM) and the lowest is JE2 (3.27 ± 0.32 mg/100g DM), with a significant difference at the 5% threshold between these different values. The effect of zinc on LDL-cholesterol and HDL-cholesterol has also been reported in a meta-analysis, several trials showed that zinc supplementation in diabetic patients resulted in a slight reduction of glycated haemoglobin (HbA1C) [27]; in addition, zinc supplementation could prevent oxidative damage to the heart and prevent or delay diabetic complications [25]. The high zinc content of the formulation is explained by the high zinc content of the ingredients, Table 2 shows that the bioavailability of zinc is not hindered by the phytate and oxalate content.

Assessment of magnesium reveals (table 2) that the highest magnesium content is 125.90 ± 2.69 mg/100g DM (JE1) and the lowest is 117.78 ± 0.61 mg/100g DM (JE2). Statistical analysis shows a significant difference at the 5% level between these different values. Magnesium acts as a cofactor in many enzymatic reactions such as the antioxidant enzyme superoxide dismutase. It plays an important role in glucose and insulin homeostasis. The high magnesium levels in JE1 and JE2 formulations, which are within the recommended daily intake (80-420 mg/day) are explained by the high magnesium content of the ingredients. Table 2 shows that the bioavailability of magnesium is not impaired by the phytate and oxalate content.

Secondary Metabolites and Anti-nutrients

Table 3 shows the contents of secondary metabolites and anti-nutrients.

Table 3: Secondary metabolite and anti-nutrient contents of formulated powders.

Samples	JE1	JE2
Polyphenols totaux (mg eq AG/100g DM)	183.88 ± 0.33^{b}	146.06 ± 0.49^{a}
Flavonoids (mg eq Q/100g DM)	44.49 ± 0.41^{b}	37.62 ± 0.46^{a}
Saponin (mg/100g DM)	4.35 ± 0.22^{b}	3.24 ± 0.28^{a}
Tannin (mg eq leu/100g DM)	42.77 ± 0.69^{b}	10.90 ± 0.11^{a}
Oxalate (mg/100g DM)	4.92 ± 0.098^{b}	2.69 ± 0.13^{a}
Phytate (mg eq AP/100g DM)	7.85 ± 0.16^{b}	5.03 ± 0.28^{a}

GA: gallic acid; Q: quercetin; PA: phytic acid; leu: leucocyanidin; JE1: JE1 powder; JE2: JE2 powder. Values assigned to different letters on the same line are significantly different ($p<0.05$).

From results in table 3, it is observed that JE1 formulation showed a higher total polyphenol content (183.88 ± 0.33 mg eq GA/100g DM) than JE2 (146.06 ± 0.49 mg eq GA/100g DM). Statistical analysis shows a significant difference at the 5% level between these different values. Like vitamin C, total polyphenols help to lower blood sugar levels in diabetic patients as high blood sugar levels can lead to the production of free radicals which when in excess can no longer be neutralised by the body's antioxidants [28].

When regard flavonoids content, JE1 (44.49 ± 0.41 mg eq EQ/100g DM) presents the highest flavonoid content compared to JE2 (37.62 ± 0.46 mg eq EQ/100g DM). Between the two values a significant difference ($p< 5\%$) is noted. These levels are

lower than the recommended daily value of flavonoid, which is 897 mg/day. Just like vitamin C and total polyphenols, flavonoids can neutralise the free radicals produced by excess glucose found in type 2 diabetics [28]. For saponin content, the highest value is obtained with JE1 (4.35 ± 0.22 mg/100g DM) and the lowest with JE2 (3.24 ± 0.28 mg/100g DM). According to the statistical analysis, there is a significant difference (p< 5%) between both values. This difference could be due to the fact that JE2 doesn't contain *V. amydalina* thus the reduction of saponin compared to JE1 which has *V. amydalina* in its formulation. Saponins have positive health effects and are only toxic at a dose above 200 mg/Kg [29]. They are involved in digestion by increasing the permeability of cell membranes, lowering cholesterol levels by reacting with bile acids to form micelles leading to an acceleration of its metabolism in the liver [30]. Saponins act in type 2 diabetics by lowering blood glucose levels and reducing oxidative stress via several mechanisms namely activation of glycogen synthesis, regeneration of insulin action, suppression of glucogenogenesis, suppression of disaccharide activity and modulation of insulin signalling [31].

Investigation on tannin content reveals that JE1 has the highest tannin content (42.77 ± 0.69 mg leu eq/100g DM) as compared to JE2 (10.90= ± 0.11 mg leu eq/100g DM). Statistically, there is a significant difference (p< 5%) between both values. It is well known that tannins inhibit the activities of digestive enzymes and these nutritional effects are related to their interactions with proteins and minerals. However, the levels found in those formulations are largely lower than the dose of tannin considered toxic and which is 150-200 mg/100g DM [32].

The result of oxalate content of the formulations are 4.92 ± 0.09 mg/100g DM and 2.69 ± 0.13 mg/100g DM respectively for JE1 and JE2. Statistical analysis shows a significant difference at the 5% threshold between these different values. These values are much lower than the daily dose (200 to 500 mg) of oxalate considered as toxic [33].

The phytate content of the formulations are 7.85 ± 0.16 mg eq AP/100g DM (JE1) and 5.03 ± 0.28 mg eq AP/100g DM (JE2), with a significant difference (p< 5%) between them. High phytate levels are detrimental to health by the fact that they form complexes with minerals leading to a decrease in their solubility and reduce their accessibility in the gut [34]. However, the phytate contents of the various powders formulated are far below the safe dose, which is between 2000 and 500 mg/day [35]. Therefore, they are an antinutrient of interest for the prevention and management of type 2 diabetes insofar as the same phytates reduce the formation of advanced glycation products in type 2 diabetic patients [36]. In general, observation, absence of *V. amygdalina* leads to the decrease of bioactives compounds (polyphenol, tannin, flavonoid, phytates) and mineral contents (Ca, Zn, and Mg).

In vitro Antioxidant potentials of formulated powders

Type 2 diabetes is a disease characterised by a major metabolic disorder of glucose, and oxidative stress contributes even more to the evolution of this disease. This is why the powders formulated as JE1 and JE2 based on *Vernonia amygdalina, Tetrapleura tetraptera, Leptadenia lancifolia* and Gum Arabic have undergone *in vitro* antiradical tests. The difference of bioactives contents, described above can be explained by the variation of soils and therefore the terroir effect. Table 4 shows the different values of antioxidant activities of formulated powders.

Table 4 : *In vitro* antioxidants activities of formulated powders JE1 and JE2.

Samples	JE1	JE2	Gallic acid
FRAP (mg $FeSO_4$/100gDM)	164.86 ± 0.56^a	210.44 ± 0.56^b	
CI_{50} (ug/ml)	0.03 ± 0.00^a	0.04 ± 0.00^b	0.07 ± 0.00^c
Mo VI (mg eq AG/100g Ms)	202.74 ± 0.83^b	165.15 ± 0.26^a	

JE1: JE1 formulation; JE2: JE2 formulation. Values are expressed as mean ± standard deviation from the mean (percentage change from the mean); Values assigned different letters on the same line are significantly different (p<0.05).

Ferric reducing antioxidant power (FRAP) measures its ability to give up electrons and reduce the oxidised intermediates of the lipid peroxidation process. It also indicates that powders can act as a primary and secondary antioxidant [37]. Analysis of the data by the paired sample T Test reveals that the overall antioxidant activity of JE1 is lower than that of JE2 and correspond to 164.86 ± 0.56 (mg FeSO4/100g DM) and 210.44 ± 0.56 (mg FeSO4/100g DM) respectively. There is a difference at the 5% significance level (P<0.05). This can be explained by the fact that the absence of *V. amygdalina* in JE2 increases the antioxidant activities. This absence leads to the decrease of bioactives compounds (polyphenol, tannin, flavonoid, phytates) and mineral contents (Ca, Zn, and Mg). In the other hand increase in vitamin C is observed. Reversible and significant correlations were observed between bioactives compounds, mineral and FRAP which are ranged from -0.99 to -0.89. While positive and significant correlation (0.99) is noted between vitamin C and FRAP. Based on this observation it can be concluded that the FRAP of a formulated powder is linked to the activity of vitamin C. In addition, the FRAP test, which does not involve oxidants or oxidizable substrates, but the ability to reduce Fe^{3+} to Fe^{2+}, shows results that can be explained by the electron donating capacity of the flavonoids in JE1 and JE2. These results are not only due to the flavonoid content of the powders, but also to the configuration and glycosylation of the hydroxyl groups [38].

The free radical scavenging activity by the DPPH test performed is represented by the inhibitory concentration 50% (IC50). The IC50 value indicates the concentration of the antioxidant required to inhibit the DPPH radical by 50%. However, it should be noted that lower the IC50 is, higher the antioxidant capacity of a compound is. The ANOVA data show that (Table 3) the free radical scavenging efficacy of JE1 and JE2 powders using total DPPH shows a significant difference at the 5% level for each powder formulated. JE1 has the smallest IC50 (0.03 ± 0.00) and therefore has the best anti-free radical activity compared to JE2 (0.04 ± 0.00) and to the gallic acid used as a reference (IC50 (0.07 ± 0.00). In addition the activity of JE1 is better than that of Fofack's Beverage 851 (0.77),

[39]. This can be explained by the fact that the presence of *V. amygdalina* in JE1 increases the inhibition activities. This presence leads to the increase of bioactives compounds (polyphenol, tannin, flavonoid, phytates) and mineral contents (Ca, Zn, and Mg). In the other hand decrease in vitamin C is observed. Reversible and significant correlations were observed between bioactives compounds, mineral and inhibition, which are ranged from -1 to -0.93. While positive and significant correlation is noted between vitamin C and Inhibition. Based on this observation it can be concluded that the IC50 of a formulated powder is linked to the activity of bioactive compounds and mineral identified.

The total antioxidant capacity quantifies all substances in the formulated powders that have an ability to prevent oxidation. The data processed by paired sample T Test show total antioxidant capacities that are significantly different at the 5% level for each formulated powder. These contents are 202.74 ± 0.83 mg eq AG/100g DM and 165.15 ± 0.26mg eq AG/100g DM respectively for JE1 and JE2. JE1 has a higher total antioxidant activity than JE2. This can be explained by the fact that the presence of *V. amygdalina* in JE1 increases the total antioxidant activities. This presence leads to the increase of bioactives compounds (polyphenol, tannin, flavonoid, phytates) and mineral contents (Ca, Zn, and Mg). In the other hand increase in vitamin C is observed. Reversible and significant correlations (-0.99) were observed between bioactives compounds, mineral and inhibition. While negative and significant correlation (-0.99) is noted between vitamin C and total antioxidant activities. Based on this observation it can be concluded that total antioxidant activities of a formulated powder is linked to the activity of bioactive compounds and mineral identified. It must be said that the polyphenols contained in JE1 and JE2 have free hydroxyphenolic groups and conjugated double bonds in their structures capable of providing a hydrogen or an electron to a free radical or a metal which is also the case for vitamin C [40]. The total antioxidant capacity of a powder is strongly associated with its polyphenol content; this could explain why JE1 has the highest total antioxidant activity because its polyphenol content is the highest.

Conclusion

From these results, it can be observed that both formulated powders, JE1 and JE2 possess strong antiradical activities, but in general, JE1 has the better free radical scavenging efficacy and antiradical activity compared to JE2. all these are made possible thanks to their high contents on bioactive compounds and vitamin C; indeed the results reveal that JE1 and JE2 can have an impact on the control of oxidative stress in type 2 diabetes patient. The presence of those bioactive compounds in *Vernonia amygdalina, Tetrapleura tetraptera, Leptadenia lancifolia* decne and Gum Arabic (*Acacia Senegal sap*) can explain their use in the traditional pharmacopoeia for treatment of type 2 diabetes. For this reason, consumption of those plants needs to be encourage. More investigations is needed to assess antidiabetic and hypoglycaemia effects of formulation of nutraceutic drugs.

References

1. World Health Organization., International Program on Chemical Safety., International Labour Organisation., & United Nations Environment Programme. Pyrrolizidine alkaloids health and safety guide. 1989; 20.
2. Williams R. L'atlas Du Diabète De La Fid 9ème Édition 2019. In Atlas du diabète de la FID. 2019; 9.
3. ADA-EASD. Position des experts ADA-EASD sur la prise en charge de l'hyperglycémie chez les patients diabétiques de type 2 : une stratégie centrée sur le patient. 2012; 6.
4. Kuete V, Efferth T. Cameroonian medicinal plants: pharmacology and derived natural products. Front Pharmacol. 2010; 1: 123.
5. Nkongmeneck BA, Mapongmetsem PM, Pinta YV, et al. Etat des lieux des plantes médicinales importantes à conserver et des jardins des plantes médicinales à promouvoir. Geneva. 2007.
6. Mbatchou GPT. Plant diversity in Central African rain forest: Implications for biodiversity and conservation in Cameroon. Wageningen University, Department of Plant Sciences: PhD thesis. 2004.
7. Earth Trends: Evolution of Cameroon Protected Areas (1995 - 2008). Washington DC: World Resource Institute. 2003.
8. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Med. 2010; 76: 1479-1491.
9. Brower V. Nutraceuticals: poised for a healthy slice of the healthcare market? Nat Biotechnol. 1998; 16: 728-731.
10. Elizabeth AC. Over-the-counter products: nonprescription medications, nutraceuticals, and herbal agents. Clin Obstet Gynecol. 2002; 45: 89-98.
11. Whitman M. Understanding the perceived need for complementary and alternative nutraceuticals: lifestyle issues. Clin J Oncol Nurs. 2001; 5: 190-194.
12. Bickford PC, Tan J, Shytle RD, et al. Nutraceuticals synergistically promote proliferation of human stem cells. Stem Cells Dev. 2006; 15: 118-123.
13. Effoe S, Gbekley EH, Mélila M, et al. Étude ethnobotanique des plantes alimentaires utilisées en médecine traditionnelle dans la région Maritime du Togo. International Journal of Biological and Chemical Sciences. 2020; 14: 2837-2853.
14. Harris LJ, Ray SN. Determination of ascorbic acid in urine. Method using titration with 2, 6 dichlorophenol indophenol. Lancet. 1935; 1: 462.
15. Horwitz W. Official Method of Analysis of AOAC. International. 17th Edition. AOAC International, Maryland, USA. 2000.
16. Marigo G. Méthode de fractionnement de d'estimation des composes phénoliques chez les végétaux. Analusis. 1973; 106-110.
17. Vinson J, Yong Hao, Xeuhui Su, et al. Phenol antioxidant qauntity and quality in foods: vegetables. Journal of Agricultural and Food Chemestry. 1998; 46: 3630-3634.

18. Ndhlala, Kasiyamhuru, Mupure, et al. Phenolic composition of Flacourtia indica, Opuntia megacantha and sclerocarya birrea. Food Chemistry. 2007; 103: 82-87.
19. Olayeye, Owolabi, Adesina, et al. Chemical composition of red and white cocoyam (Colocasia esculenta) leaves. International journal. Science Research. 2013; 11: 121-125.
20. Aina V, Sambo B, Zakari A, et al. Détermination Of Nutrient And Antinutritional Content Of Vitisvinifera (Grapes Grown In Bomo (Areac)) Zaira. Nigeria. Food Technology. 2012; 4: 225-228.
21. Koziol M. Afrosimetric estimation of threshold saponin concentration for bitterness in quinoa (Chenopodium quinoa Willd). Journal of the Science of Food and Agriculture. 1990; 54: 211- 219.
22. Benzie IF, Strain JJ. The Ferric Ability of Plasma (FRAP) as a measure of antioxidant power: The FRAP Assay. Analytical Biochemistry. 1996; 239: 70-76.
23. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemestry. 1999; 269: 337-341.
24. Lopes-Lutz D, Alviano SD, Alviano C, et al. Screening Of Chemical Composition, Antimicrobial And Antioxidant Activity Of Artemisia Essential Oils. Phytochemestry. 2008; 69: 1732-1738.
25. FAO-OMS. Programme mixte FAO-OMS sur les normes alimentaires, Commission du Codex Alimentarius vingt quatrième session, Genève (Suisse) 2-7 juillet. 2001; 91.
26. Benmeziane F. Nutrition et pathologies de la nutrition. 2010; 1-13.
27. Liévin S. Syndrome métabolique et compléments alimentaires. Universite De Rouen. 2016; 62-71.
28. Grosso G, Stepaniak U, Topor-Madry R, et al. Estimated dietary intake and major food sources of polyphenols in the Polish arm of the HAPIEE study. Nutrition. 2014; 30: 1398-1403.
29. Diwan FH, Abdel-Hassan IA, Mohammed ST. Effect of saponin on mortality and histopathological changes in mice. In Eastern Mediterranean Health Journal. 2000; 6: 345-351.
30. Das TK, Banerjee D, Chakraborty D, et al. Saponin: Role in Animal system. Veterinary World. 2012; 5: 248-254.
31. Ragab R, Evans JG, Adriano Battilani, et al. Towards accurate estimation of crop water requirement without the crop coefficient Kc : New approach using modern technologies: Estimation Of Crop Water Requirement. Irrigation and Drainage. 2017; 66: 469-477.
32. Frédéric S. Determination D ' Une Dose Efficace Et D ' Une Dose Toxique De Tanins. 2012.
33. Ekop AS, Obot IB, Ikpatt EN. Anti-nutritional factors and potassium bromate content in bread and flour samples in Uyo Metropolis, Nigeria. E-Journal of Chemistry. 2008; 5: 736-741.
34. Priyodip P, Balaji S. An in vitro chicken gut model for the assessment of phytase producing bacteria, 3 biotech. 2019; 9: 294.
35. Danso J, Francis A, Reindorf B, et al. Effect of drying on the nutrient and anti-nutrient composition of bombax buonopozense sepals. African Journal Of Food Sciences. 2019; 13; 21-29.
36. Sanchis P, Rivera R, Berga F, et al. Phytate decreases formation of advanced glycation end-products in patients with type ii diabetes: randomized crossover trial. Sci Rep. 2018; 8: 9619.
37. Iris F.F. Benzie, Siu-Wai Choi. Chapter One - Antioxidants in Food: Content, Measurement, Significance, Action, Cautions, Caveats, and Research Needs. Advances in Food and Nutrition Research. 2014; 71: 1-53
38. Panche A, Diwan A, Chandra S. Flavonoids: An overview. Journal of Nutritional Science. 2016; 5: E47.
39. Focfack. Formulation à base des rhizomes de Zingiber officinale, feuilles de Moringa oleifera Lam, feuilles de Stevia rebaudiana Bertoni, fruits de Spydium guava et des fleurs d'hibiscus sabdariffa. Univ. 2021; 1-90.
40. Takuisssu NG, Ngondi JL, Oben Julius E. Antioxidant and Glucose Lowering Effects of Hydroethanolic Extract of Baillonella toxisperma Pulp. Journal of Food Research. 2020; 9: 1-12.