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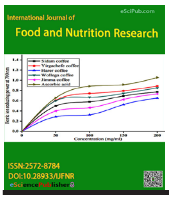

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Nutritional potential of anti-anemic drinks based on *Manihot esculenta* L. or *Graptophyllum pictum* L. leaf extracts consumed in Yaoundé Cameroon

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ABSTRACT

Anemia is a global public health problem. In Cameroon, the most vulnerable to anemia are children under 5 years of age (60%) and pregnant women (40%). To reduce prevalence of anemia, several approaches have been adopted, including promoting the production and consumption of iron-rich foods/products. The objective of this work was to study the nutritional potential of anti-anemic drinks based on extracts from the leaves of either *Manihot esculenta* or *Graptophyllum pictum* consumed by the populations of the city of Yaoundé in Cameroon. Macronutrient contents were determined using the standard A.O.A.C. methods, while mineral contents were analyzed using the atomic absorption spectrophotometry. Bioactive compounds such as total polyphenols, flavonoids, and saponins were analyzed. Vitamin C was determined by the 2,6 dichlorophenol indophenol spectrophotometric method (DCPIP) and the contents of anti-nutrients (tannins, phytates, oxalates, saponins, hydrocyanic acid) quantified using standard methods. The results of these analyses show that the mean protein contents in the studied anti-anemic drinks ranged from 0.64 ± 0.08 g/100 mL to 1.84 ± 0.02 g/100 mL (M. esculenta drink); and 0.25 ± 0.01 g/100 mL (*G. pictum* drink). Sugar contents ranged from 0.30 ± 0.02 g/100 mL to 0.45 ± 0.01 g/100 mL (M. esculenta drink), and 0.29 ± 0.01 g/100 mL (*G. pictum* drink). As concerns iron contents, and vitamin C contents were inversely proportional across M. esculenta drinks containing 25% milk (2.29 ± 0.15 mg/100 mL iron; 57.9 ± 0.2 mg/100mL vit C) through 50% milk (1.70 ± 0.03 mg/100 mL iron; 147.19 ± 16.05 mg/100 mL vit C) to 75% milk (1.01 ± 0.11 mg/100 mL iron; 221.1 ± 16.96 mg/100 mL vit C). Likewise, iron and vit C levels in the *G. pictum* drink were 0.18 ± 0.01 mg/100 mL and 999.1 ± 41.2 mg/100 mL respectively. In general, although some anti-nutrients were detected in the studied anti-anemic beverages, the levels were not significant to pose harm. The principal component analysis revealed that the M. esculenta drink containing 75% milk was relatively the most nutritious of all the studied drinks viewing its contents in Vitamin C, protein and soluble sugars, thus, facilitating intestinal absorption of non-heme iron. Similarly, the *G. pictum* drink may require optimization of preparation method. Finally, the need for iron-bioavailability studies based on these anti-anemic drinks cannot be over emphasized.

Keywords: Anemia; anti-anemic drinks; nutritional contents; *Manihot esculenta; Graptophyllum pictum*

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INTRODUCTION

Anemia affects 1.62 billion people worldwide. It is a global public health problem. The most affected regions of the world are South-East Asia and Africa (Apouey *et al.,* 2016). In Cameroon, it affects 60% of children under the age of five and 40% of pregnant women (INS international, 2012). There are two types of anemia: lack of red blood cell production (iron deficiency anemia, aplastic anemia) and abnormal destruction of red blood cells (hemolytic anemia, hemorrhages). Iron deficiency anemia is the most common type of anemia. In general, anemia is characterized by a deficiency of red blood cells or hemoglobin in the blood, which disrupts oxygen transport (Kushwaha et *al.,* 2017). The poor cognitive development of young children and their subsequent impaired social and economic integration are some of the consequences (Louison, 2013). In women, iron-deficiency anemia can either lead to fatigue or increase the risk of maternal and fetal mortality (INS international, 2012).

In order to contribute to the fight against anemia, the Cameroon government together with international organizations such as WHO, UNICEF and FAO have adopted several strategies. These include promoting the production and consumption of micronutrient-rich foods, promoting the fortification of foods with micronutrients and promoting micronutrient (iron and vitamin A) supplementation programs for at-risk groups (pregnant and breastfeeding women, adolescent children and other vulnerable groups) (Minsanté, 2011, WHO, 2017).

In Cameroon, the prevalence of anemia is constantly rising. However, several studies have shown that plants are potential sources of iron, such as *Solanum nigrum* (26.85 ± 2.01 mg/100 g DM), *Manihot esculenta (*35.10 ± 2.25 mg/100 g DM) and *H. sabdariffa (*167.42 ± 18.63 mg/100 g DM) (Tchiéngang 2004, Ejoh *et al.,* 2017). Empirically, people therefore resort to the use of natural plants to combat iron deficiency. Indeed, Kavira in 2011 showed that the leaves of *Manihot esculenta* have anti-anemic properties when obtained by manual pressing followed by the addition of unsweetened condensed milk. Decoctions and infusions from the petals of *Hibiscus sabdariffa Linn* (Djomou, 2014), and drinks made from the leaves of *G. pictum* (Nga *et al.,* 2016), are used in Cameroon for their anti-anemic properties. More recently, Djuikwo *et al.* (2020) identified several anti-anemic drinks prepared from the plants used in Cameroon and the percentage of satisfaction with the use of these drinks by respondents was also high for drinks made from *G. pictum* and *M. esculenta*. Following this survey, we evaluated the nutritional potential of some of the most popular anti-anemic drinks consumed in Yaoundé, Cameroon.

MATERIALS AND METHODS

Preparation of anti-anemic drinks

Following a survey (Djuikwo *et al.* 2020), the two plants most frequently used by the populations of the city of Yaoundé were selected for the preparation of anti-anemic drinks in the framework of this study. Indeed, in September 2018 in Yaoundé, *Manihot esculenta* leaves were collected from the Mendong district in the 6th sub-division of the city and those of *Graptophyllum pictum* from the Etoudi district in the 1st sub-division. The plants had been identified at the Cameroon National Herbarium in Yaoundé.

Based on the leaves of *Manihot esculenta,* three anti-anemic drinks in different concentrations were prepared. After harvesting, washing, trimming, crushing, extraction of the juice by simple manual pressing followed by sieving, different volumes were taken, and the addition of milk was carried out according to determined proportions:

- The first anti-anemia drink named BL25 (contains 25% milk and 75% leaf extract)
- The second anti-anemic drink called BL50 (contains 50% milk and 50% leaf extract).
- The third anti-anemic drink called BL75 (contains 75% milk and 25% leaf extract).

- The resulting beverages were packaged, labelled and stored at -18 °C.
- NB: for 1 kg of leaves we obtained 450 mL of extracts.

In addition, an anti-anemic drink was prepared from the leaves of *G. pictum.* Indeed, after harvesting, washing and trimming, the young leaves were weighed (332.5 g) and infused in 2L of water (BI) for 10 minutes. After cooling, the drink obtained was packaged, labelled and stored at -18°C. For further analysis, all four drinks were freeze-dried and stored.

Determination of the nutritional potential of drinks

The water contents were determined by the method described by A.O.A.C., (1980). Approximately 2 g of lyophilizate were weighed using "Sartorius". a precision balance They were dried in a "Memmert" oven at a temperature of 105 ℃ for 24 hours. Weighing was carried out regularly until a constant weight was obtained. The dry residue was cooled in the atmosphere of a desiccator containing P_2O_5 as a desiccant for 1 hour and weighed. The results were expressed in % (g/100 mL anti-anemic drink) as a mean ± standard deviation. Three tests were performed and the water content was the average of the determined contents.

For crude protein, total nitrogen (N) was determined after digestion of the samples according to the Kjeldahl method (Buondonno et *al.,* 1995), according to the colorimetric technique of Anderson and Ingram, (1993). Total nitrogen (N) was determined from a wet acid lyophilizate, and was detected by colorimetric analysis using a UVVIS spectrophotometer. The crude protein was then calculated using the conventional nitrogen to protein conversion factor 6.25 (A.O.A.C, 1975). Soluble sugars were extracted and determined by the DNS (3,5-dinitrosalycilic acid) described by Fischer and Stein (1961).

The ash contents were determined by simple incineration at 550 °C according to A.O.A.C. (1980). The mineral content (Ca, Mg, Fe, Zn Cu) was evaluated using the flame atomic absorption spectrophotometric method described by Benton and Vernon (1990). The apparatus gives the results in ppm (mg/L) and, in this study, the results were expressed in mg/10 ml for each drink.

The determination of vitamin C was carried out by the spectrophotometric method of 2,6-dichlorophenol indophenol (DCPIP) described by Harris and Ray (1935). The ODs were read at 515 nm against the white. The calibration line was used to determine the vitamin C concentration (mg/100 mL) of each sample by projecting absorbances onto the curve.

Determination of secondary metabolites and anti-nutritional factors in drinks

The analysis of total polyphenols, flavonoids, and tannins was performed on the extracts prepared as follows: approximately 0.5 g of lyophilizate was placed in a beaker to which 30 mL of 70% (v/v) ethanol was added. After 30 min of stirring, the whole was filtered on Whatman N°. 1 paper. Two extractions were repeated in the same way on the residue and the volume of the extract was made up to 100 mL with distilled water.

The determination of total polyphenol contents was made by Singleton and Rossi 1965. To 200 µL of extract, gallic acid solution (standard) or 70% ethanol (white) contained in a 10 mL tube, 2 mL of a 10% *Folin Ciocalteu* solution was added. After 5 minutes, 2 mL of a freshly prepared sodium carbonate solution is added to the mixture which is then stirred with a "PYROMULTI MAGNESTIR" brand blender before being left to stand for 30 minutes. The absorbance of the colored complex was determined at 765 nm against the white. Gallic acid solutions were made at concentrations ranging from 0.03125 to 0.25 mg/mL in a geometric sequence. The content of phenolic compounds is deduced from the calibration line and expressed in mg gallic acid equivalent/100 mL of beverage.

The experimental protocol followed for the determination of total flavonoid contents is that described by Zhishen *et al.* (1999). Catechin was used as the standard optical density was read at 510 nm against the blank. The flavonoid content was deduced from the calibration line and expressed in mg catechin equivalent/100 mL beverage. Total tannins were determined using the spectrophotometric method described by Bainbridge *et al.* (1996). The standard solution consisted of catechin (2 mg/mL) prepared in 70% (v/v) ethanol. After 20 min in the dark, the optical density was read at 500 nm against the blank. The tannin contents were deduced from the calibration line and expressed as mg catechin equivalent/100 mL beverage. Phytic acid in the beverage samples was determined by the method of Gao *et al.* (2007), while oxalate levels were determined by the method described by Aina *et al* (2012). 1 g of lyophilizate anti-anemic drinks was weighed and introduced into an Erlenmeyer flask; 75 mL of H_2SO_4 (3 mol/L) was added. The mixture was magnetically stirred for 1 h followed by filtration. 25 mL of the filtrate obtained was collected and heated to 80-90°C and kept above 70°C at all times; the hot sample was titrated continuously with 0.05mol /L $KMNO_4$ until a persistent pale pink color was obtained (15 seconds minimum). The oxalate content was then calculated by taking 1 mL of 0.05 mol/L of $KMNO_4$ as equivalent to 2.2 mg of oxalates. The results were expressed in mg/100 mL of beverage. The determination of the saponin content was carried out by the method described by Koziol, (1990). 0.5 g of anti-anemic drinks lyophilizate was introduced into a test tube, then 5 mL of distilled water was added. The tube was shaken vigorously for 30 seconds. Immediately afterwards (5 to 10 s), the height of the foam formed was measured with a ruler graduated to the nearest 0.1 cm. The determination of the hydrocyanic acid content was described by the A.O.A.C. (1995). Approximately, 10 g of sample was soaked in a mixture of 200 mL of water and 10 mL of orthophosphoric acid was added. The mixture was left for 12 h to release all the bound hydrocyanic acid (HCN) and then the mixture was distilled. Approximately, 150 mL of the distillate was collected. Next, 20 mL of the distillate was placed in a conical flask and diluted with 40 mL of water, 8 mL of NH_4OH (6 M) and 2 mL of potassium iodide solution or KI (8%) were added. The mixture was titrated with 0.02 M $AgNO_3$ using a micro burette until a permanent turbidity was obtained (1 mL $AgNO_3$ (0.02 M) = 1.08 mg HCN). The results were expressed in mg HCN/100 mL of drink.

Statistical analysis of the results

The statistical analyses were carried out using *Statistical Package for Social Science* (*SPSS)* version 20.0 for Windows. The graphs were plotted using Microsoft Excel 2016 for Windows. The results of the analyses were represented as mean ± standard deviation; the tests were performed in triplicate. The significance threshold was set at 5% based on an Analysis of Variance (*ANOVA*) coupled with a *Post Hoc* test (*Turkey*). The *XL STAT* trial software for Windows was used to carry out the principal component analysis which allowed the anti-anemic drink to be seen to contain the elements that promote intestinal iron absorption.

RESULTS AND DISCUSSION

Macronutrients

Table 1 shows the water, protein and soluble sugar contents of the anti-anemic drinks studied.

The results show that the water content of anti-anemic drinks made from *M. esculenta* leaf extracts varies from 82.44 ± 3.97% to 85.87 ± 0.05%. The ANOVA test revealed that there is no significant difference (p > 0.05) between these values. The water content of the anti-anemic drink made from *G. pictum* was much higher (99.06 ± 0.37%) than the previously reported levels. The slightly lower values observed in the leaves of *M. esculenta are* partly because the leaves contain milk in addition to leaves, so the dry matter is important.

Protein contents of anti-anemic drinks range from 0.64 ± 0.08 g/100 mL to 1.84 ± 0.02 g/100 mL for beverages prepared with *M. esculenta* extracts. The addition of milk increases the protein content of the drinks. The milk used in this study contains 8.7 g/100 mL of protein. The drink made from *G. pictum* leaves, on the other hand, had a relatively lower protein content of 0.25 ± 0.01 g/100 mL. Regardless of the origin of the extract, the significant amounts of protein in the drinks help in the absorption of iron from the duodenum (Minihane and Rimbach, 2002).

As with proteins, soluble sugar contents increase with the addition of milk. In fact, the milk used in this study contains 12.6 g/100 mL of soluble sugars. Values of 0.30 ± 0.02 g/100 mL, 0.38 ± 0.03 g/100 mL and 0.45 ± 0.01 g/100 mL were obtained for the 25, 50 and 75% milk drinks respectively. The drink made from the infusion of *G. pictum* leaves gave a value of 0.29 ± 0.01 g/100 ml. Doudi and Atia, in 2015 demonstrated that soluble sugars promote intestinal iron absorption.

Table 1: Macronutrient contents of anti-anemic drinks based on extract leaves of *M. esculenta or G. pictum*

	Sample code	Moisture content (%)	Protein (g/100 mL)	Soluble sugar (g/100 mL)
	BL75	82,44 ± 3,97[a]	1,84 ± 0,02[a]	0,45 ± 0,01[a]
***M. esculenta* drinks**	**BL50**	84,57 ± 2,54[a]	1,12 ± 0,05[b]	0,38 ± 0,03[b]
	BL25	85,87 ± 0,05[a]	0,64 ± 0,08[c]	0,30 ± 0,02[c]
***G. pictum* drink**	**BI**	99,06 ± 0,37	0,25 ± 0,01	0,29 ± 0,01

The values are expressed as mean ± standard deviation over the mean (percentage variation of the mean);

BL25: Drink with extracts of M. esculenta leaves with 25% milk;

BL50: Drink with extracts of M. esculenta leaves with 50% milk;

BL75: Drink with extracts from the leaves of M. esculenta with 75% milk;

BI: Drink with extracts of G. pictum leaves obtained by infusion;

The values assigned different letters on the same column are significantly different

Total ash content

Analysis of the total ash in the table above shows that beverages made with *M. esculenta* extracts contained 0.28 ± 0.00 g/100 mL (BL75); 0.37 ± 0.00 mg/100 mL (BL50) and 0.41 ± 0.01 g/100 mL (BL 25) ash. The *ANOVA* test shows that there is a significant difference at the 5% threshold between these ash contents. The total ash content of the *G. pictum* drink is significantly smaller than that of the cassava leaves. Indeed, these ash contents are 0.16 ± 0.01g/100 mL. This suggests that these drinks could be good sources of mineral. Minerals

Contents of minerals in the studied drinks are recorded in Table 2. The calcium contents in Table 2 show that there is a significant difference at the 5% threshold ($p < 0.05$) between these contents in drinks made from cassava leaf extracts. The calcium levels are 25.75 ± 0.25 mg/100 mL (BL75), 44.25 ± 0.5 mg/100 mL (BL50) and 63.90 ± 0.75 mg/100 mL (BL25). For the drink made from the infusion of *G. picum* leaves,) a calcium content of 6.10 ± 0.25 mg/100 ml was obtained. All these values are lower than those obtained by Imelda *et al* (2017) who worked on anti-anemic fortifications based on fruit juice. (77 mg/100 mL). This could be due to the differences in matrices. Drinks based on cassava leaf extracts have magnesium contents that are significantly different ($p < 0.05$) from each other. They are 3.17 ± 0.25 mg/100 mL (BL75), 8.00 ± 0.50 mg/100 mL (BL50) and 14.17 ± 0.75 mg/100 mL (BL25). The drink based on *G. pictum* extracts showed a magnesium content of 10.60 ± 0.10 mg/100 mL. The phosphorus results show that there is a significant difference at $p < 0.05$ between BL75, BL50 and BL25. These phosphorus levels are 20.45 ± 0.25 mg/100 mL (BL75), 37.25 ± 0.50

mg/100 mL (BL50) and 53.85 ± 0.75 mg/100 mL (BL25) for cassava leaf drinks. The phosphorus content of *G. pictum* was only 3.25 ± 0.5 mg/100 mL.

Like calcium, phosphorus is involved in skeletal development and various other physiological processes (Mezajoug, 2010). Calcium, magnesium and phosphorus are the most important minerals involved in the construction of the body's rigid structures (Aletor et *al.,* 2001). However, it is the divalent ions that complex the iron in the small intestine and thus prevent its absorption (Cousins and Liuzzi, 2018).

The *ANOVA* test in the statistical analysis of the samples showed a significant difference at $p < 0.05$ between the K contents of the different beverages. These levels are 26.5 ± 0.25 mg/100 mL (BL75), 64.5 ± 0.50 mg/100 mL (BL50) and 78.11 ± 0.75 mg/100mL (BL25). These results are of decreasing order because the milk used in this study contained small amounts of potassium and sodium. The potassium content of the *G. pictum* drink is 29.10 ± 0.5 mg/100mL.

The sodium contents are 2.14 ± 0.00 mg/100 mL (BL75), 4.31 ± 0.01 mg/100 mL (BL50), and 6.53 ± 0.01 mg/100 mL (BL25) for drinks based on *M. esculenta* extracts. The sodium content is much lower 1.93 ± 0.1 mg/100 mL for the *G. pictum* drink.

Na and K are necessary to maintain the osmotic balance of body fluids, the

body's pH, to control glucose absorption and to improve normal protein retention during growth. Their presence in the breakdown is therefore desirable.

The iron content of beverages made from cassava leaf extract is 1.01 ± 0.11 mg/100 mL (BL75),

1.70 ± 0.03 mg/100 mL (BL50) and 2.29 ± 0.15 mg/100 mL (BL25). These levels are significantly different at the 5% threshold. This indicates that the milk used would contain trace amounts of iron. On the other hand, for the *G. pictum* leaf drink, the iron content is 0.18 ± 0.01 mg/100 mL. Iron is important in the diet of pregnant and lactating women as well as children (Kadwe *et al.,* 1995; WHO, 2017). With these iron levels, many women would benefit from consuming these anti-anemic drinks during pregnancy and even after delivery, subject to good bioavailability studies. Hem iron is well absorbed by the body, while iron from plant sources (non-hem iron) requires vitamin C. In addition, several sources (Théau, 2017) state that hem iron in addition to non-hem iron (plant iron) is easily assimilated by the body.

Zinc analysis shows that drinks made from cassava leaf extracts contain low levels of zinc (Table 2). These levels are 0.005 ± 0.001 mg/100 mL (BL75); 0.008 ± 0.007 mg/100 mL (BL50) and 0.021 ± 0.003 mg/100 mL (BL25). Since zinc is present in the beverages in this study, regular consumption of these drinks would help prevent the adverse effects of zinc deficiency, including growth retardation. The zinc content of the drink based on *G. pictum* extracts is only 0.003 mg/100 mL. This would be interesting because in order to be absorbed in the small intestine, iron does not need to be with ions such as Zn^{2+} which is one of the mineral elements which prevent its absorption at this level.

In general, minerals are represented in the anti-anemic drinks analyzed. Iron is the mineral par excellence in this study is indeed present. However, its intestinal absorption, especially the nonheme iron in foods of plant origin, requires other nutrients here, namely proteins, mainly vitamin C. However, several other minerals make iron complex, especially divalent minerals such as calcium, zinc, copper and phosphorus. Studies by Cousins and Liuzzi, (2018) show that zinc and copper, when present in large quantities in extracts, complex non-heme iron and thus prevent its intestinal absorption because the two minerals compete for the same carriers, such as DMT1. Calcium and phosphorus would act with the iron to prevent its absorption by the formation of the chelate Fe: Ca: PO_4 in the small intestine. Alternatively, the inhibitory effect of calcium on iron absorption

Major micronutrient content in mg/100mL of anti-anemic drinks

Table 2: Major micronutrient contents of anti-anemic drinks based on extracts of *M. esculenta* or *G. pictum* leaves.

	Sample code	Ash (g/100 mL)	Ca	Mg	k	Na	P	Fe	Cu	Zn
	BL75	0,28± 0,00[c]	25,75 ± 0,25[c]	3,17 ± 0,25[c]	26,5 ± 0,25[c]	2,14 ± 0,00[c]	20,45± 0,25[c]	1,01 ± 0,11[c]	ND	0,005 ± 0,001[b]
***M. esculenta* drinks**	**BL50**	0,37± 0,00[b]	44,25± 0,5[b]	8,00 ± 0,50[b]	64,5± 0,50[b]	4,31± 0,01[b]	37,25± 0,50[b]	1,70± 0,03[b]	ND	0,008 ± 0,007[b]
	BL25	0,41 ± 0,01[a]	63,90±0,75[a]	14,17± 0,75[a]	78,11± 0,75[a]	6,53 ± 0,01[a]	53,85± 0,75[a]	2,29 ± 0,15[a]	ND	0,021 ± 0,003[a]
***G. pictum* drink**	**BI**	0,16± 0,01	6,10± 0,25	10,60 ± 0,10	29,10± 0,5	1,93 ± 0,1	1,32± 0,25	0,18± 0,01	ND	0,003 ± 0,000

The values are expressed as mean ± standard deviation over the mean (percentage variation of the mean);
BL75: Drink with extracts from the leaves of M. esculenta with 75% milk;
BL50: Drink with extracts of M. esculenta leaves with 50% milk;
BL25: Drink with extracts of M. esculenta leaves with 25% milk;
BI: Drink with extracts of G. pictum leaves obtained by infusion;
The values assigned different letters on the same column are significantly different.

Secondary metabolites and anti-nutritional factors

Table 3: Anti-nutrient contents of levels of secondary metabolites and antinutrients anti-anemic drinks based on extracts of *M. esculenta* or *G. pictum* leaves

	Sample code	Total polyphenols (mg eq AG/100 mL)	Flavonoids (mg eq cat. /100 mL)	Tannins (mg eq. Cat. /100 mL)	Phytates (mg eq AP/100 mL)	Oxalates (mg /100 mL)	Saponins (mg /100 mL)	HCN(mg/1 mL)
***M.esculenta* drinks**	**BL75**	1,37 ± 0,13[b]	3,76 ± 0,00[c]	44,01 ± 0,88[c]	66,56 ± 1,36[c]	55,21 ± 0,38[b]	1,05 ± 0,15[a]	6,02±0,11[c]
	BL50	1,47 ± 0,03[b]	8,02 ± 1,26[b]	74,54 ± 5,55[b]	92,27 ± 1,37[b]	46,82 ± 5,33[b]	1,17 ± 0,44[a]	16,21±0,94[b]
	BL25	4,97 ± 0,15[a]	14,79 ± 0,00[a]	162,08 ± 25,47[a]	153,39 ± 5,22[a]	77,45 ± 3,46[a]	1,65 ± 0,30[a]	20,62±1,10[a]
***G.pictum* drinks**	**BI**	0,39 ± 0,01	12,74 ± 0,81	20,78 ± 2,81	2,74 ± 0,22	5,03 ± 0,11	0,83 ± 0,06	1,11 ± 0,11

AG: gallic acid; cate: catechin; AP: phytic acid.
The values are expressed as mean ± standard deviation over the mean (percentage variation of the mean);
BL75: Drink with extracts from the leaves of M. esculenta with 75% milk;
BL50: Drink with extracts of M. esculenta leaves with 50% milk;
BL25: Drink with extracts of M. esculenta leaves with 25% milk;
BI: Drink with extracts of G. pictum leaves obtained by infusion;
The values assigned different letters in the same column are significantly different ($p < 0.05$).

may be in the intestinal cells at a stage in the transport of iron that is common for heme and non-heme iron. Reductions in iron absorption have been shown with milk intake (Hallberg *et al.,* 1997). Thus, those who are iron deficient and need to maximize iron absorption from a supplement should not take the iron supplement with a calcium source. In view of this, anti-anemic drinks with low levels of these minerals would be considered better because iron absorption would not be affected and problems of iron deficiency anemia would also be solved.

Vitamin C content

From Figure 1 below, it can be seen that vit C contents (57.9 ± 0.2 mg/100mL for BL25; 147.19 ± 16.05 mg/100 mL for BL50 and 221.1 ± 16.96 mg/100 mL for BL75) of the anti-anemic drinks based on *M. esculenta* leaves were significant different at the 5% threshold (p < 0.05). This is thought to be due to the unequal proportion of ingredients used in the preparation of the beverages. In fact, it can be seen that the content of vit C increases with the addition of milk because the milk used was obviously fortified in vit C. As far as the drink made from the *G. pictum* infusion is concerned, the vit C content is 999.1 ± 41.2 mg/100 mL. The vitamin C contents of these drinks are much higher than those obtained (30.88 mg/100 mL) by Leahu et *al (*2013) for orange juice and those obtained (57.52 mg/100 mL) by Djomou in 2014 for the drink from the chalices of *Hibiscus sabdariffa Linn*. It should be noted that vitamin C plays an important role in the absorption of non-heme iron and for the immune system (Longo-Silva et *al.,* 2015).

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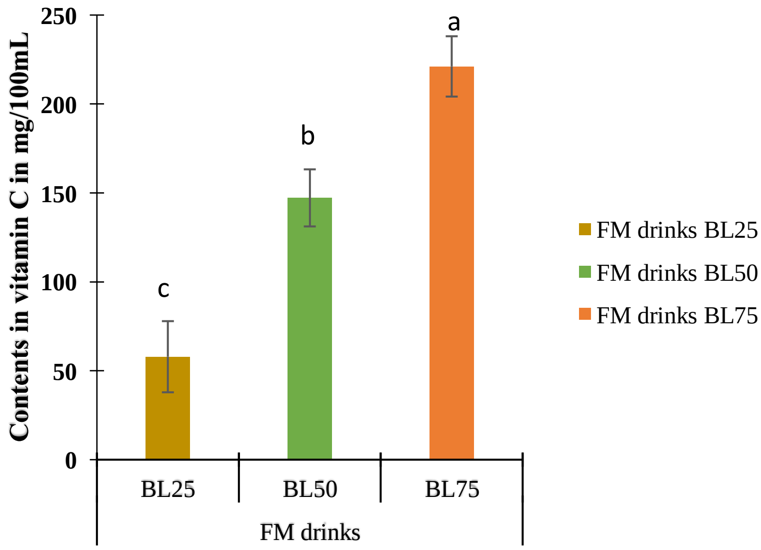

Figure 1: Vitamin C content of anti-anemic drinks based on extracts of *Manihot esculenta*

Secondary metabolites and anti-nutritional factors

Total polyphenols

The polyphenol contents of beverages based on extracts from the leaves of *M. esculenta* are

1.37 ± 0.13 mg eq AG/100 mL for BL75; 1.47 ± 0.03 mg eq AG/100 mL for BL50 and 4.97 ± 0.15 mg eq AG/100 mL for BL25 (Table 3). At 5% threshold (p < 0.05) there was no significant difference between BL75 and BL50, whereas there were significant statistical differences when comparing BL25 with either BL75 or BL50. The drink based on *G. pictum*, had a low polyphenol content (0.39 ± 0.01 mg Fatty Acid Eq/100 mL). Phenolic compounds in plants are known to be powerful antioxidants (Baptista et *al.,* 2015). Therefore, the presence of phenolic compounds in our extracts that decrease after the addition of milk could affirm that our extracts have antioxidant power. Studies by Zhang *et al (*2015) show that phenolic compounds in plants can act as radical chelators. So, the fewer radicals in the anti-anemic drink analyzed in this study, the more pronounced their antioxidant activities would be.

Flavonoids

The flavonoid contents of beverages based on cassava leaf extracts are 3.76 ± 0.00 mg/100 mL (BL75), 8.02 ± 1.26 mg eq/100 mL (BL50) and 14.79 ± 0.00 mg eq/100 mL (BL25). The reductions observed after the addition of milk could be explained by the presence of flavonoids, more specifically the catechin contained in these plant extracts. The drink resulting from the infusion of *G. pictum* gave a content of 12.74 ± 0.81mg eq cate/100 mL. Flavonoids have the ability to react with ferric iron by reducing it to ferrous iron (Baptista et *al.,* 2015).

Tannins

Tannin analysis (Table 3) shows that beverages obtained by infusion have a low tannin content of 20.78 ± 2.81 mg eq/100 mL. Those made from cassava leaves range from 44 to 162 mg eq/100 ml. The nutritional effects of tannins are related to their interaction with proteins and minerals (Ejoh *et al.,* 2017). However, tannins are considered to be responsible for the decrease in growth rate, feed efficiency, net metabolizable energy and protein digestibility in humans. Therefore, tannin-rich foods are considered to have low nutritional value (Chung *et al.,* 2018).

Phytates

The phytate content of the infusion drink is very low (2.74 ± 0.22 mg AP eq/100mL). For drinks made from cassava leaves, the phytate levels are 66.56 ± 1.36 mg AP eq/100mL for BL75, 92.27 ± 1.37 mg AP eq/100mL for BL50 and 153.39 ± 5.22 mg AP/100mL for BL25. Phytates chelate metal ions, thereby compromising their bioavailability. Their reduction would improve the bioavailability of proteins and minerals such as iron, calcium and zinc (Shashi and Salil, 1999). In addition, the phytate levels in the drinks in this study are below the safe dose range of 2000-2600 mg/day (Danso *et al.,* 2019).

Oxalates

The oxalate content of the drink infused with *G. pictum* is very low (5.03±0.11) (Table 3). For drinks based on *M. esculenta* leaves extracts, the oxalate contents are 55.21 ± 0.38 mg/100 mL (BL75); 46.82 ± 5.33 mg/100 mL (BL50) and 77.45 ± 3.46 mg/100 mL (BL25). Phytates and oxalates use oxygen to bind with many minerals, including not only iron but also zinc, copper, and calcium. Mineral phytate and oxalate complexes are insoluble and poorly absorbed (Frontela et *al.,* 2008; Mitchikpe *et al.,* 2008).

Saponines

As for saponin contents, statistical analysis shows that there is no significant difference at $p < 0.05$ between drinks based on cassava leaf extracts (Table 3). They are 1.05 ± 0.15 mg/100 mL (BL75); 1.17 ± 0.44 mg/100 mL (BL50) and 1.65 ± 0.30 mg/100 mL (BL25). Once the milk is added, we can see that there is a leaching of the foams there by reducing the saponin content of our extracts. The saponin content of the drink infused with *G. pictum* is only 0.83 ± 0.06 mg/100 mL. The contact between the leaves and hot water favors the rupture of the cell wall and thus the saponins would be leached and drained in the bleaching water (Shashi and Salil 1999).

Hydrocyanic acid (HCN)

The addition of milk has significantly lowered the level of hydrocyanic acid in drinks made from cassava leaf extracts. These values are 6.02 ± 0.11 mg/100 mL (BL75); 16.21 ± 0.94 mg/100 mL (BL50) and 20.62 ± 1.10 mg/100 mL (BL25). The drink made from *G. pictum* infusion has a very low hydrocyanic acid content (1.11 ± 0.11 mg/100 mL). All these values are far lower than 10 g/100 g DM which is the critical threshold for HCN in blood according to Regnier, (2011).

In general, the dosages of antinutrients in anti-anemic drinks based on *Manihot esculenta* leaf extracts show that the more milk is added, the more these antinutrients decrease significantly. The drink made from the infusion of *G. pictum* has very low levels of antinutrients. In all cases, these drinks are below the safety limits, except for BL25 which exceeded the dose in tannins and phytates (table 4). However, it is important to study the bioavailability of iron in these beverages analyzed in this study to better address the problem of iron deficiency anemia.

Table 4: Safe Dose (SD) of anti-anemic drinks according to antinutritional factors (FANs**)**

FANs / DS	Tannins (mg) 150 - 200 mg (Gafar *et al.*, 2012)	Phytates (mg) 2000 - 2600 mg (Danso *et al.*, 2019)	Oxalates (mg) 200 - 500 mg (Gafar *et al.*, 2012)	HCN (mg) 1000 mg anti-anemic drink (Regnier, 2011)
BL75	176	1845	226	241
BL50	147	2045	325	304
BL25	216	2640	436	394
BI	115	548	106	222

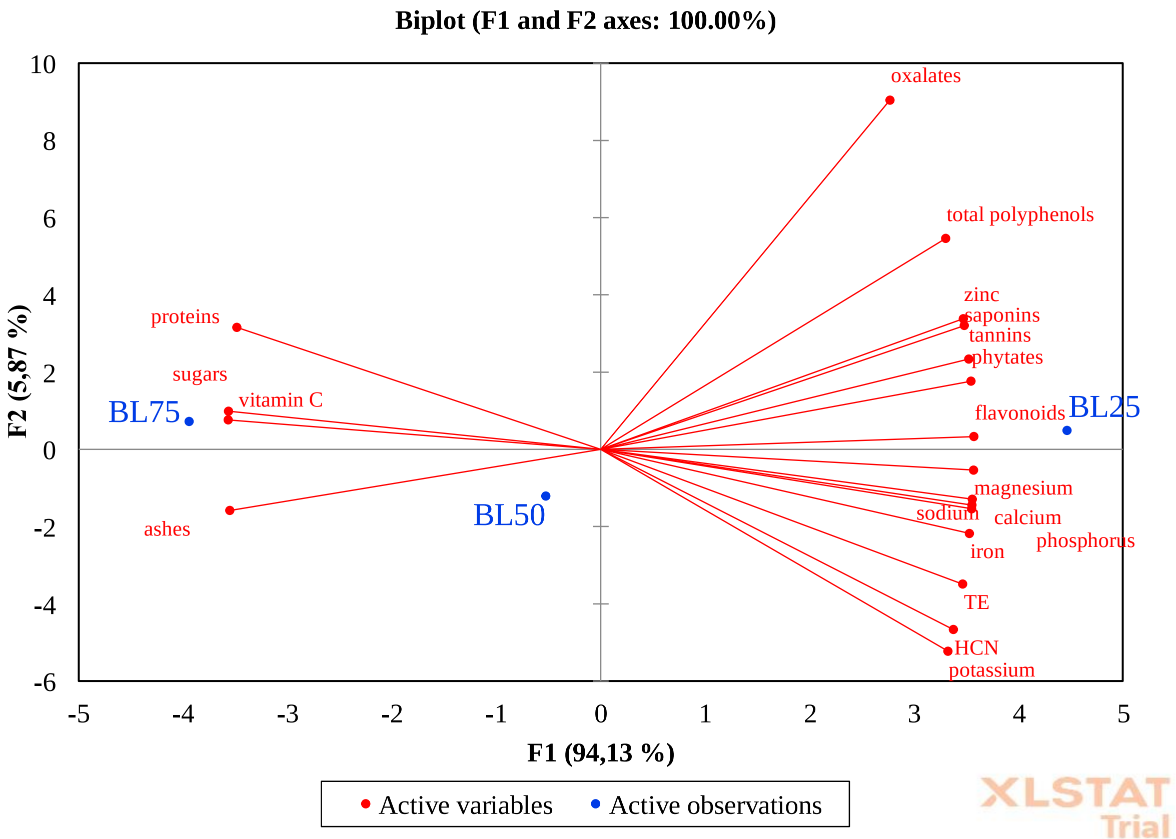

Figure 2: Principal Component Analysis of *M. esculenta* extract-anti-anemic drink.

Principal Component Analysis (PCA):

Principal Component Analysis (PCA) aims to help understand the major characteristics of a sample and to see the correlations (strong/weak). For example, PCA in drinks prepared from the *M. esculenta* leaf extracts (Figure 2) with 75% milk (BL75) is highly correlated with protein, sugars and vitamin C. On the other hand, the drink with 25% milk (BL25) is highly correlated with the minerals, antinutrients (phytates, oxalates, tannins, cyanides, saponins) and secondary metabolites (polyphenols, flavonoids) analyzed in this study.

It can be said that the added milk made it possible to reduce undesirable compounds to a maximum, this is the case of phytates, and oxalates which are metal chelators (iron, copper, zinc). Considering these correlations, the drink with 75% milk (BL75) would be the one that facilitates the intestinal absorption of non-heme iron because to be absorbed, it needs to be linked to vitamin C, proteins and soluble sugars. In addition, a study by Théau in 2017 proves that hem iron combined with non-hem iron is easily absorbed.

CONCLUSION

In view of the data obtained, the populations are encouraged to consume these anti-anemic drinks, in particular the drink prepared with 75% milk for *Manihot esculenta* and the one infused for *Graptophyllum pictum* while waiting to study the bioavailability of iron. Also, it would be wise to vary the concentration and time of the infusion in order to optimize the preparation of this drink which seems to have potential to be explored.

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