



Bioactivity and Nutritional Values of Some *Dioscorea* Species Traditionally Used as Medicinal Foods in Bandundu, DR Congo

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FCB, KPM, MDM and UM designed the study, performed the statistical analysis and wrote the protocol. Authors KNN, KTNN and FTM wrote the first draft of the manuscript. Authors BMM, KGM, LAP, NGB and MPM managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To valorize traditional foods of the Democratic Republic of Congo in general and the province of Bandundu in particular by evaluating the antioxidant activity of five species of *Dioscorea*, the determination of their chemical composition as well the determination of their glycemic index.

Study Design: Survey; plant collection and identification, phytochemical and biological evaluation:

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phytochemical screening, proximate analyses, in vitro and in vivo assays.

Place and Duration of Study: "Université de Kinshasa" (DR Congo), from February 2013 to December 2015.

Methodology: The anti-diabetic activity was carried out in vivo using an animal model (NMRI mice), by administration of a solution of glucose (200 mg/mL). Antioxidant activity was evaluated using the ABTS and DPPH assays. The micrographic analysis of the flours of the studied species showed that their starch grains are characteristic of each species by their size, their form and the position of the hila and the presence of the scratches.

Results: All extracts of *Dioscorea* displayed highest ABTS and DPPH radical-scavenging activities ($IC_{50} < 1$ mg/mL) related to their appreciable amount of total phenolic contents. Total phenolic contents expressed as mg of gallic acid equivalent per gram of dried extract are ranged from 9.85 ± 0.098 to 51.12 ± 0.500 . Only *Dioscorea bulbifera* contains flavonoids expressed as 3.33 ± 0.23 mg of quercetin equivalent. These yams showed a good antihyperglycemic activity, *Dioscorea praehensilis* and *Dioscorea bulbifera* showed a glucose reduction rate of 54.0% and 51.3% after 30 min respectively.

Conclusion: The results obtained show that *Dioscorea* tubers species studied have a high food value and thus can be developed as functional food.

Keywords: *Diabetes mellitus; free radicals; traditional foods; Dioscorea genus; Democratic Republic of the Congo.*

1. INTRODUCTION

Human traditions in ages have developed empirical knowledge concerning the use of plants in order to relieve the pain and thus get better the human health. Nowadays due to the ineffectiveness of modern drugs, two-third of the pharmacopeia resorts to plant curative properties. The use of medicinal plants is turning to a high scale in Africa (where more or less 80% of population resort to traditional medicine) than in Europe [1,2]. Thanks to their known biopharmaceutical interest, currently, medicinal plants are searched for their secondary metabolites. Among these secondary metabolites, polyphenols are subjected to a lot of research owing to their capacity to halt the radical reactions in neutralizing free radicals [3].

Several studies have shown that diabetes mellitus induces glucose self-oxidation leading to free radicals formation responsible of oxidative stress that disrupt insulin-secretion balance in favoring insulin-resistance and cardiovascular complications related to that [4]. Therefore, polyphenols have shown interesting results at once as hypoglycemic and antioxidant [5]. Lately, it has been shown that a therapeutic complement constituted of plant extracts is necessary for diabetes mellitus treatment optimization [6].

Regarding to that, edible medicinal plants show a particular interest for they can be given to sicklers, not only for the fact that they do not

require toxicological trials prior to their utilization. In fact, the best approach for chronic diseases would be not to go straight to molecules and synthesize many other but trying to integrate the antisickling drug in his daily diet. Hence forth, the relevance of dietary supplement (or nutraceuticals) as well as the nutritherapy for chronic pathologies that remains with the SCD people all their lifetime [7].

Traditional foods and foodstuffs could be a significant trail in the search for solution in the fight against chronic diseases such as diabetes mellitus. The scientific validation of these nutraceuticals pharmacological properties could set a solid statement out in order to promote traditional food and foodstuffs for the medicine based on scientific evidences is a priority in Africa.

The aim of the present study is the promotion of five different species of yams consumed in the province of Bandundu (*Dioscorea alata*, *Dioscorea bulbifera*, *Dioscorea dumetorum*, *Dioscorea burkilliana* and *Dioscorea praehensilis*) and known to be relevant in traditional medicine for diabetes mellitus treatment [8,9]. Thus, a micrographic and phytochemical study of the different yam powder has been undertaken in order to determine the different yam powder phytochemical structure, followed by a phytochemical screening of powders, of polyphenols dosage contained in the powders and at last the determination of

antioxidant and anti-hyperglycemic of different powders.

warmed up to boiling and then observed to an optical microscope [11].

2. MATERIALS AND METHODS

2.1 Study Area

Plants have been harvested in Kenge (S04° S1' E16° S8'), an area in Pelende-Nord sector, Kwango district, Bandundu Province in Democratic Republic of the Congo. According to Koppen classification, its climate is of AW4 type. The annual average pluvometry is around 1500 mm. The monthly average temperatures vary between 22 and 24°C; during the rainy season the average maxima rise around 28°C and in the dry season around 31°C whereas the average minima get down between 17 and 13°C. Geologically, Kenge-Kwango formations belong to two systems, which are: Kharroo and Kalahari. This latter covers another one, and the Kalahari system is made of layer superposing of Bateke series and of soft polymorphics and stones upon hard rock's (silicious sand stones). The Kharroo is mainly made by Kusango series (upper cretaceous). These are soft clay sandstones, red brick with argillite (clay stone) and conglomerate. The soft sandstones are made of average dimension quartz grains, well rolled, spread in a mass of slender grains [10].

2.2 Biological Materials

Harvested in October 2014, tubers of *D. alata*, *D. bulbifera*, *D. burkilliana*, *D. praeheasilis* and *D. dumetorum* (Fig. 1) constituted the biological material. For identification, their corresponding herbaria have been sent into the herbarium of University of Kinshasa where they are kept under the following voucher numbers: IUKO 9291, IUKO 9292, IUKO 9293, IUKO 9294, and IUKO 9295.

2.3 Yam Treatment and Powder Micrography

Before being ground, the uprooted yam tubers were washed, peeled and cut into thin slices then dried at the oven (HERAEUS T5050 EK brand) at 60°C for 48 hours. The received flours were sieved and then kept in plastic flasks far from the light and moisture for further analyses. For the micrography, two drops of Steimetz reagent dropped on the slide were mixed with a small quantity of powder and then covered with a cover glass. The obtained microscopic preparation was



Fig. 1. Young leaves and tubers of *Dioscorea alata* (A and B), *Dioscorea bulbifera* (C and D), *Dioscorea burkilliana* (E) and *Dioscorea praeheasilis* (F)

2.4 Phytochemical Analysis

2.4.1 Phytochemical screening

The phytochemical screening was performed following the standard techniques [12,13].

The TLC analysis of 10 µl of solution for 10 mg/ml of aqueous extracts were performed in normal phase using Silicagel 60F₂₅₄ plates (Merck) with Ethyl Acetate/Formic acid/Acetic acid/water (100: 11:11:27 v/v) as mobile phase. For organic extracts, butanone-2/toluene (4; 6; v/v) was used as mobile phase. Before and after adding specific reagents, the TLC plate observation was carried out using the visible light and underneath UV light (254 and 366 nm). Flavonoids and phenolic acids were revealed due to the presence of Neu reagent, quinones owing to Borntrager reagent (NaOH 10% or NH₄OH 10%), alkaloids due to Dragendorff reagent; terpenoids and steroids were revealed by using sulphuric anisaldehyde and antimony 20%.

2.4.2 Total polyphenols content

Total phenols content of yam tuber extracts was determined by spectrophotometry UV-Vis using Follin-Ciocalteu's method [14]. Briefly, the reaction mixture was made of 0.5 mL of methanol extract of each of the yams prepared at 1 mg/mL, 5 mL of distilled water and 0.5 mL of Folin Ciocalteu's reagent. Then 1 mL of a saturated solution of Na₂CO₃ 20% was added three minutes later. The mixture was shaken and then incubated at the ambient temperature far from the light for an hour. The absorbencies were read thru the spectrophotometer GENESYS 10S UV-Vis at 725 nm. Each dosage was performed as a triplicate. For the eight different dilutions of gallic acid standard solution (5 to 150 µg/mL), the same procedure was followed in order to the calibration straight line. For the control (blank), we followed the same procedure as well but the extract was replaced by methanol 80%. Results are expressed into mg equivalent of gallic acid per g (mg GAE/g) of dry vegetal material using the following equation $y = 0.0098x - 0.0195$ ($R^2 = 0.967$).

2.4.3 Flavonoids content

The content of flavonoids of yam extracts was determined by spectrophotometry UV-Vis [15]. The reaction mixture contained 1 mL of methanolic solution of each yam extract at 1 mg/mL of concentration and 1 mL of AlCl₃ 2% dissolved in methanol 80%. Then, the mixture is well homogenized and incubated for an hour at the ambient temperature and in the dark. Absorbencies reading are carried out by using the spectrophotometer GENESYS 10S UV-Vis at 425 nm. Quercetin solution (50-200 µg/mL) was used as a standard. For the control (blank), the extract was replaced by methanol 80%. Results are expressed in mg equivalent of quercetin per g (mg QE/g) of dry vegetal material using the following equation $y = 0.0232x + 0.1535$ ($R^2 = 0.945$).

2.4.4 Tannins dosage

The extraction of tannins was carried out according the adapted method used by [16]. Then 2.5 g of powder was extracted from 50 mL of acetone/distilled water mixture (35/15, v/v) during three days at the ambient temperature. The solution is filtrated then evaporated at 40°C in the water bath in order to discard acetone, then the aqueous phase is washed using dichloromethane (15 mL) in order to eliminate

pigments and lipids. Afterwards, the aqueous phase was extracted with ethyl acetate (2x15 mL). The organic phase is evaporated to dry at 40°C. Thus the extract is weighed and taken back in 3 mL of methanol.

The condensed tannins were quantified by the method of vanillin in acid medium [17]. Then, 50 µL of the raw extract was added to 1500 µL of vanillin solution 4% (methanol) and mixed later on. Afterwards, 750 µL of concentrated hydrochloric acid were added. The mixture obtained was incubated at the ambient temperature for 20 minutes. Versus a blank, the absorbance was measured at 550 nm using the spectrophotometer GENESYS 10S UV-Vis. The results were expressed in mg equivalent catechine per gram of the dry vegetal material (mg EC/g) using the following equation $y = 0.006x - 0.0032$ ($R^2 = 0.857$).

2.5 Proximate Analysis of *Dioscorea* spp.

Determination of moisture, total lipids, total ash, fibers, total proteins, total carbohydrates as well the energy value was performed according to [18-20].

2.6 Radical Scavenging Activity

2.6.1 DPPH radical scavenging capacity

DPPH assay was performed according to the method described by Floegel et al. [21] slightly modified. A solution of 0.004% of DPPH in 80% (v/v) methanol was prepared 1 hour before use and its absorbance adjusted to 0.70±0.03 at 517 nm using 80% (v/v) methanol. Samples (20 µL for each concentration) were analysed after mixing with DPPH[•] solution (1980 µL) for 30 min in the dark. The decrease of absorbance at 517 nm was compared to the control and standard with a Spectrophotometer GENESYS 10S UV-Vis. Antiradical capacity analysis was performed on dry extracts. Ascorbic acid was used as a positive control. For ABTS and DPPH assays, extracts were diluted in methanol. Each sample was measured in triplicate.

2.6.2 ABTS radical scavenging capacity

ABTS assay was based on the method of Re et al. [22] and performed as reported by Franck et al. [23]. The extract (5 mg/mL) was diluted with methanol for the obtention of different trial solutions of respective concentrations at 0.5, 1, 2, 3, 4 and 5 mg/mL. Afterwards, 20 µL of extract

are mixed with 1980 μL of ABTS radical in microtubes then incubated into darkness for 30 minutes. The decrease of absorbance at 734 nm was compared to the control and standard with a Spectrophotometer GENESYS 10S UV-Vis. Each sample was measured in triplicate.

ABTS^{•+} and DPPH[•] scavenging activity of extracts were expressed in IC₅₀ values. Different values of IC₅₀ for samples are determined using Graph Pad Prism version 6.0 Software.

2.7 Hypoglycemic Activity

For the purpose of this study, the animal model was used made of 20 rats of NMRI strains of 3 months old of which the weigh varies between 18 and 24, were subjected to a temporary hyperglycemia by forced feeding (gavage) a glucose solution of 200 mg/mL. These mice were divided into six groups as follows: the first batch of five mice considered as negative control (saline solution), the second batch of five mice as positive control (Glibenclamide 10 mg/kg) and the two remaining batches of each five mice were for testing 1 g of flours of *D. bulbifera* and *D. praeheasilis* dissolved in 5 mL of water. The glycemia dosage was performed with an empty stomach then each half of a hour for three hours just after feeding, using a glycemia reader Contour TS without code on total blood taken from the tail [3,5].

2.8 Data Analysis

Data were analyzed with Graph Pad Prism version 6.0 Software (Graph Pad Software, San Diego California, USA). The analysis of variance having a criterion of classification (One Way ANOVA) allowed us in the comparison of means. The significance level is $p \leq 0.05$.

3. RESULTS AND DISCUSSION

The result of micrography of different *Dioscorea* powders selected in order to determine the morphological characteristics of starch grains were given in the following pictures. It can be noticed from the Fig. 2 that *D. alata* starch is formed of average grains almost circular, the hila is located in the grain central part. *D. bulbifera* is formed of average grains almost triangular, having the hila located in the enlarge part of the

grain; whereas *D. dumetorum* is presented like the small round grains of the same size; then *D. burkilliana* is appear like big polyhedral and rounded grains with a central hila. The grains have striations. *D. praeheasilis* is made of average oval grains having neat striation.

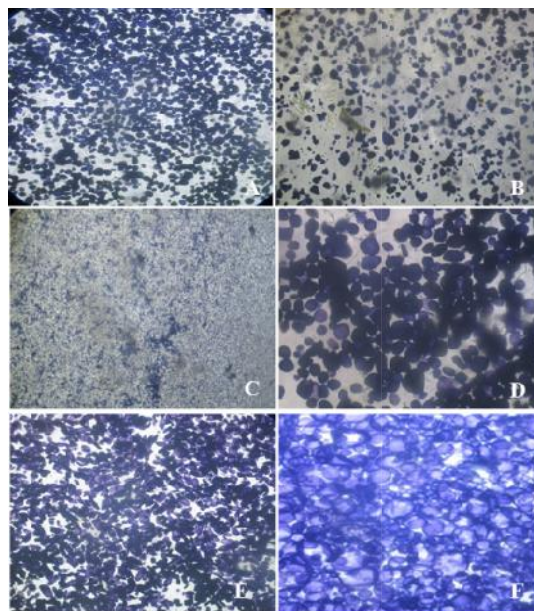


Fig. 2. Microscopic characteristic of starch grains of *Dioscorea alata* (A), *Dioscorea bulbifera* (B), *Dioscorea burkilliana* (C), *Dioscorea praeheasilis* (D) and *Dioscorea dumetorum* (E) compared to potato starch (F)

According to the results obtained starch is characteristic of each type of *Dioscorea* regarding the form, the height, the hila position and the presence of striations. These characteristics are sufficient for detecting any possible adulteration of flours from these species because they are known to be alternative flour that can be substituted to certain hyperglycemic flours, which are also deprived of certain mineral, like cassava flour [20].

Phytochemical screening results of different selected yams are given in Table 1. The table shows that only *D. dumetorum* contains alkaloids whereas *D. bulbifera* contains tannins while *D. praeheasilis* contains quinones. Yet, flavonoids, phenol acids, terpenoids and steroids as well free amines are present in each of the studied species.

Table 1. Phytochemical screening results thru colored and precipitation reactions

Compoundds	Reagents	Flours				
		<i>D. alata</i>	<i>D. bulbifera</i>	<i>D. burkilliana</i>	<i>D. dumetorum</i>	<i>D. praehensilis</i>
Alkaloids	<i>Drangerdoff</i>	-	-	-	+	-
Flavonoids	<i>Neu</i>	-	+	-	-	-
Quinones	<i>Borntrager</i>	-	-	-	-	+
Tannins	<i>Ferric chloride</i>	-	+	-	-	-
Terpenoids & steroids	<i>Sulfuric Anisaldehyde</i>	+	+	+	+	+
Cardiotonic heterosides	<i>Badjet</i>	+	+	+	+	+
	<i>Kedde</i>	-	-	-	-	-
Free amines	<i>Ninhydrine</i>	+	+	+	+	+

+: present; - : absent. Studied *Dioscorea* spp are without cardiotonic heterosides

TLC analysis showed the presence of flavonoids like quercetin and kaempferol derivatives in *D. bulbifera* whereas the phenolic acids were found in other species of studied *Dioscorea* genus. Sougata et al. [24] reported also the probable dominance of flavonoids in ethyl acetate fractions of *D. bulbifera* from India. TLC analysis showed also the presence of terpenoids in the extracts of different *Dioscorea* by the presence of blackish blue color spot with sulphuric anisaldehyde reagent.

Results of estimation of content of secondary metabolites given in Table 2 indicated that gallic tannins were present in the methanolic extract of *D. bulbifera*. Their presence was confirmed through a positive reaction with ferric chloride solution. Alkaloids were detected in *D. dumetorum* while quinones were present in *D. praehensilis* and flavonoids were detected only in *D. bulbifera*, this result confirms these of TLC analysis.

From the above table, it is noticed that *D. Bulbifera* and *D. dumetorum* were rich in total polyphenols and tannins. Previous studies have shown that *D. bulbifera* was rich in total polyphenols [25]. The fact that these two species have a high content of polyphenols could confer to them considerable antioxidant properties.

The qualitative evaluation of the radical scavenging ability on TLC plate revealed by DPPH 0.2% solution showed the wealth content

of different extracts in reducing compounds (reduction of DPPH radical purple into yellow). *D. bulbifera* extract is the richest species in radical scavenging compounds.

The antioxidant activity of tested *Dioscorea*, determined by ABTS and DPPH assay is presented in Table 3 and is expressed as IC₅₀ values. IC₅₀ is the amount of antioxidant necessary to decrease the initial concentration of radical by 50%. Lower IC₅₀ value indicates a higher antioxidant activity. Radical scavenging activity, as an indicator of antioxidant capacity, the antioxidant response of extracts appeared to be not correlated with the total phenolic content. *D. dumetorum* showed a less antioxidant activity than *D. praehensilis* which contains less total phenolic content.

For the ABTS radical assay, the IC₅₀ of different tested samples indicate *D. bulbifera* showed the greatest antioxidant activity then followed by *D. praehensilis*, *D. burkilliana*, *D. alata* and *D. dumetorum* respectively. On the contrary, DPPH radical test showed that *D. bulbifera* is the most active then followed by *D. praehensilis*, *D. burkilliana*, *D. dumetorum* and *D. alata* respectively. This difference of activity might be due to the qualitative and quantitative composition of the extracts. Also, it has to be noted that the IC₅₀ values obtained from the ATBS test are lower than the ones of DPPH assay. This difference of activity could be

Table 2. Secondary metabolites content of methanolic extracts from *Dioscorea spp*

Plants	Total polyphenols (mg GAE/g)	Flavonoids (mg QE/g)	Tannins (mg CE/g)
<i>D. alata</i>	9,85±0,098	nf	8,94±0,03
<i>D. bulbifera</i>	51,12±0,5	3,33±0,23	680,05±0,43
<i>D. dumetorum</i>	30,07±0,3	nf	88,11±0,07
<i>D. burkilliana</i>	16,58±0,164	nf	2,56±0,02
<i>D. praehensilis</i>	13,35±0,13	nf	12,28±0,03

(nf: not found), GAE: gallic acid equivalent, QE: quercetine equivalent, CE: catechine equivalent

Table 3. IC₅₀ (µg/mL) values of *Dioscorea* extracts and vitamin C used as positive control (Means ± SD, n=6)

Yam extracts	IC ₅₀ (µg/mL)	
	ABTS	DPPH
<i>D. alata</i>	181,55±30.77	1150,8±68.20
<i>D. bulbifera</i>	93,11±11.21	109,65±8,81
<i>D. dumetorum</i>	1164,13±28.32	916,22±65.74
<i>D. burkilliana</i>	97,5±9.4	510,51±45,97
<i>D. praehensilis</i>	83.34±8.74	115,88±10,93
Vitamin C	2.36±0,60	3.24±0,96

ascribed to their reaction mechanism. As a matter of fact, ABTS reacts at the same time with hydrophilic and lipophilic compounds while DPPH reacts only with hydrophilic compounds [21]. In an order of hundredths, our extracts have shown a lower activity compared to ascorbic acid (Vitamin C) activity used as a positive control. Nevertheless, these extracts showed an interesting antioxidant activity compared to other plants [26-29].

The biochemical composition of five species of *Dioscorea* traditionally consumed in Bandundu province (Democratic Republic of the Congo) is shown in the (Table 4).

Comparing the results of our five samples, we can notice that *D. alata* has a high content in water followed by *D. bulbifera*, *D. burkilliana* and of *D. dumetorum*. Regarding the ash content, *D. dumetorum* ash content is higher than the one of others. Our results show that the proteins content of *D. bulbifera* is slightly higher to the one of *D. dumetorum* and *D. praehensilis* and widely higher than the one of *D. alata* and *D. burkilliana*. Meanwhile, we note that *D. praehensilis* contains 12.57% of total lipids then followed by *D. dumetorum*, *D. alata*, *D. bulbifera* and *D. burkilliana*. The fibers content of *D. alata* was higher than others species. *D. burkilliana* contains 75.18% of total glucids against 70.57% for *D. bulbifera*, 63.39% for *D. alata*, 68.83% for *D. praehensilis* and 68.30% for *D. dumetorum*. With regard to the results obtained on the dry

matter, it can be stated that for all the nutrients the values that we got are not in the range of values as given by Mbemba and Remacle [20] for they worked on the fresh matter. Thus, the quantitative composition yam carbohydrates would vary according to the moisture content.

Results of Hypoglycemic activity test (Table 5) showed that the glycemia in the fasting stage (on empty stomach) is complying with the standard (value < 115 mg/dL) [30] and after 2 hours (value < 140 mg/dL).

30 minutes later after ingesting of the extracts, we got a glycemia of 126.4 mg/dL for *D. praehensilis* and of 133.9 mg/dL for *D. bulbifera*. This is related to a reduction rate of 54.0% and of 51.3% respectively, as noticed in Fig. 3. It can be noted from the above figure that *Dioscorea* extracts reduce the blood glucose level in mice.

These results showed that *D. praehensilis* and *D. bulbifera* possess a potent antihyperglycemic effect. This activity could be ascribed to polyphenols present in the tested samples [30]. Previous studies reported antihyperglycemic activity of *D. alata* and *D. bulbifera*. However, to the best of our knowledge, we report here for the first time the antihyperglycemic activity of *D. praehensilis* which is similar to that of *D. bulbifera*. The glycemic index (GI) of our samples (*D. bulbifera*: 69.8 and *D. praehensilis*: 64.9) is meeting the requirements of the standard for the range is between 50 and 70 [31].

Table 4. Biochemical composition and energy value of different species of *Dioscorea* expressed in mg per 100 g of dry matter

	<i>D. alata</i> (%)	<i>D. bulbifera</i> (%)	<i>D. dumetorum</i> (%)	<i>D. burkilliana</i> (%)	<i>D. praehensilis</i> (%)
Moisture	10.95	10.34	9.68	10.25	10.25
Ash	2.40	2.90	3.40	1.90	2.54
Proteins	0.53	2.12	1.91	0.32	1.72
Lipids	9.34	9.02	11.40	7.06	12.57
Fibers	7.39	5.05	5.31	5.29	4.09
Carbohydrates	69.39	70.57	68.30	75.18	68.83

Table 5. Glycemia mean values (mg/dL) in treated and untreated mice

Samples	Blood glucose level (mg/dL)						
	On empty stomach	30 min	60 min	90 min	120 min	150 min	180 min
T ₁	99,5±17,8	133,9±15,4	110,3±21,2	91,5±17,6	84,5±16,5	71,4±13,8	64,1±11,3
T ₂	98,8±10,2	126,4±24,8	104,9±19,8	83,0±15,5	70,0±12,7	65,8±9,6	63,5±12,0
T ₀	99,6±19,6	274,7±32,7	163,8±30,8	120,7±20,1	98,1±17,9	77,7±14,8	64,1±12,1

T₁: *D. bulbifera*, T₂: *D. praehensilis*, T₀: Glucose

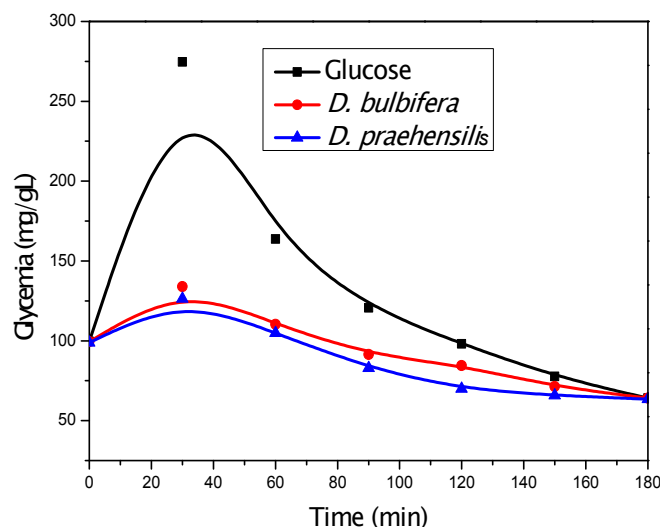


Fig. 3. Postprandial glycemia trend with time

4. CONCLUSION

The current study was initiated in order to promote traditional foods of Democratic Republic of the Congo and precisely of Bandundu province in view of the evaluation of the antioxidant activity of five species of *Dioscorea*, the determination of their chemical composition as well of the glycemic index of two species. The micrography of different studied species flours showed that their starch grains are characteristics to each species according to their height, form and position of hila as well as the presence of striation. The phytochemical screening revealed the presence of polyphenols, alkaloids and terpenoids. These yams have shown a good antioxidant and anti-hyperglycemic activities. The results also showed that the studied *Dioscorea* are tubers with a high nutritive value thus could be promoted as functional foods. However, in vitro findings are of uncertain relevance to the in vivo situation in healthy humans; further studies are needed to evaluate the cellular, in vivo antioxidant activity and to elucidate the mechanisms underlying antihyperglycemic activity of studied *Dioscorea*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that Principles of laboratory animal care" (NIH publication No. 85-

23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Department of Biology ethics committee, University of Kinshasa/democratic Republic of the Congo.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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