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ARTICLE

Phytochemical Study and Anti-nutritional Factors in Stems of *Dioscorea praehensilis* Benth (*Dioscoreaceae*)

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ABSTRACT

The aim of this research was to find and assay phytochemical compounds and various biological macromolecules of the tender stems of *Dioscorea praehensilis* benth and evaluate their antioxidant activity and to compare the content of oxalates and cyanogenetic glucosides between raw and cooked tender stems. The plant collection and identification, phytochemical evaluation: phytochemical screening, preliminary (qualitative) analyses and *in vitro* assays. Phytochemical screening was performed by qualitative methods. The estimation of the content of secondary metabolites was evaluated by spectrophotometry-UV. Antioxidant activity was evaluated using the ABTS and DPPH assays and preliminary composition by the gravimetric method. The results obtained show that the stems of *Dioscorea praehensilis* are devoid of certain important chemical groups, the flavonoids were not detected and they were rich in total polyphenols (17.22 ± 0.16), tannins (19.32 ± 0.52) and anthocyanins (25.22 ± 0.04). Our extracts showed a lower antioxidant activity than that of positive controls. The samples are rich in carbohydrates and fiber, with low levels of proteins, lipids and ash. *Dioscorea praehensilis* has a high toxicity in HCN, but after a good cooking of about 1 hour, 99.97% of the cyanide are eliminated and does not have many oxalates. The results obtained show that *Dioscorea praehensilis* has a high dietary value and can therefore be used as a nutritive food.

1. Introduction

Agricultural innovation in Sub-Saharan Africa strongly focused on food security [1]. According to the FAO [2], vegetable crops play an important role in improving food security in urban and peri-urban areas. Its identity today makes it an essential part of the city landscape and an economic and cultural heritage [3].

Dioscorea spp. is a major nutrient plant in West Africa where it actively contributes to food security and poverty reduction [4,5]. Among the food species, the most

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consumed are those of the *Dioscorea cayenensis* Lam-*D. rotundata* Poir complex, which represent more than 95% of the world production of this genus [6,7]. These species are the result of a long process of domestication of wild yams, especially *Dioscorea praehensilis* Benth. [8-12]. Face to rapid global population growth and accelerating climate change, this Wild Crop-Allied Species (WACS) constitutes a huge reservoir of genetic variability that can be used in plant breeding programs and is essential to improve food security, boost agricultural production and maintain productivity [13-15].

In recent time, considerable attention has been focused on the use of *Dioscorea praehensilis* benth (*Dioscoreaceae*) in human food and food components. However, the nourishment of monogastric animals with certain protein-rich plants is hampered by the existence of anti-nutritional factors. These factors, which are regularly present in phytonutrients, such as saponins, tannins and phytates, have been shown to reduce nutrient availability and cause growth inhibition. Among them, some contribute to the production of flatulence in consumers. Others like lectins and alkaloids can be toxic to the plants themselves and to consumers [16].

The present research aims to provide an overview and evaluation of the content of phytochemicals and their antioxidant activity, to identify and evaluate anti-nutritional factors (cyanogenetic glycosides, tannins and oxalate) in tender stems of *Dioscorea praehensilis* benth (*Dioscoreaceae*).

2. Material and Methods

2.1 Plant Material

The plant material consists of tender stems (aerial part of the growing plant) of *Dioscorea praehensilis* benth (*Dioscoreaceae*), harvested in October 2020 in Kingantoko, in the township of Mont-Ngafula, City Province of Kinshasa where the plant is called Kisadi and in Kikwit in the Province of Kwilu (the province of where we find a great number of consumers of this plant species), where it is called Bandindi in vernacular tongue (Figure 1).

The plant was identified and confirmed by the herbarium of the Institut National d'Etudes et de Recherches Agronomiques (INERA/UNIKIN) of the Faculty of Sciences at the University of Kinshasa.

The harvested tender stems were cut into pieces of between two and three cm and dried in the shade and in the ambient air [25-27 °C] until a constant weight was obtained. The dry samples were ground to powder using the MOULINEX mill and stored in a 500 mL glass bottle in a dark place.

(a)

(b)

Figure 1. (a) and (b) Stems of *Dioscorea praehensilis* benth (*Dioscoreaceae*).

2.2 Methods

2.2.1 Phytochemical Analysis

Phytochemical screening

We used standard qualitative methods as decreed by Bruneton [17], Harborne and Baxter [18] and Kokate *et al*. [19] in order to study the characteristic phytochemicals.

Total polyphenols content

The total phenol content of the tender stem extract was determined according to the Folin-Ciocalteu method [20]. Briefly, the reaction mixture consisted of 0.5 mL of methanolic extract of each of the plant extracts at 1 mg / mL in 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent. Then, 1 mL of a 20% saturated Na_2CO_3 solution is added three minutes later. Homogenize the mixture then incubate at laboratory temperature protected from light for one hour. The absorbances were taken at 725 nm. Each assay was performed in triplicate. For the seven different dilutions of gallic acid standard (5 to 150 µg / mL), the same procedure was followed to obtain a straight line standard

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curve. For the blank, the same procedure was also followed but the extract was replaced by 80% methanol. The results were expressed as mg GAE / g of dry plant material using the following equation y = 0.0098x - 0.0195 (R^2 = 0.967).

Flavonoids content

The flavonoid content of *D. praehensilis* benth extracts was determined by UV-Vis spectrophotometry [21]. The mixture contained 1 mL of methanolic extract from each yam extract at 1 mg / mL concentration and 1 mL of 2% $AlCl_3$ dissolved in 80% methanol. Then, the mixture is stirred well and incubated for one hour at room temperature and protected from light. The absorbances are taken at 425 nm. Quercetin solution (50-200 µg / mL) was used as a standard. 80% methanol was used as a control (blank). The results are expressed in mg QE/g of dry plant matter according to the following equation y = 0.0232x + 0.1535 (R^2 = 0.945).

Tannins dosage

The tannin content was carried out according to the modified method [22]. 2.5 g of powder were extracted from 50 mL of acetone / distilled water (35/15, v / v) at room temperature for three days. The solution was filtered and then evaporated at 40 ° C. in a water bath to remove the acetone, then the aqueous phase was rinsed with dichloromethane (15 mL) in order to remove the pigments and the lipids. Then it was extracted with ethyl acetate (2 x 15 ml). The organic phase is evaporated to dryness at 40 °C. and the extract is weighed and taken up in 3 ml of methanol.

Condensed tannins have been quantified by the vanillin method in acidic medium [23]. 50 L of the crude extract was added to 1500 L of 4% vanillin solution (methanol) and subsequently mixed. Then 750 L of concentrated hydrochloric acid was added. The mixture obtained is incubated at room temperature for 20 mins. Compared to a blank, the absorbance was measured at 550 nm using the UV-Vis spectrophotometer. The results are expressed in mg catechin equivalent per gram of dry plant material (mg EC / g) using the following equation y = 0.006x - 0.0032 (R^2 = 0.857).

Anthocyanes dosage

0.5 mL of the 1 mg/mL solution of extract prepared in MeOH/H_2O (80%) was mixed with 3 mL of methanolic vanillin solution (4%) and 1.5 mL of concentrated hydrochloric acid. The mixture was then incubated for one hour at room temperature and the absorbance is read at 540 nm [24].

The anthocyanin content of the extracts is expressed as mg of D-catechin equivalent (EC/g) of the corresponding dry matter using the equation from the calibration curve: y= 0.0728x + 0.0171 and R2 = 0.944 where x is the absorbance and y is the catechin equivalent (mg/g).

2.2.2 Radical Scavenging Activity

The extracts were dissolved in a DMSO-Water mixture (1: 1), then their effect was compared to a control test carried out with the mixture alone. The antioxidant activity was assayed by ABTS and DPPH spectrophotometry which were carried out according to the method described by [25].

2.2.3 Qualitative Composition

a) Bromatological analyses of Dioscorea praehensilis benth

Determination of moisture, total lipids, total ash, fibers, total proteins and total carbohydrates was performed according to the methods described by Mbemba and Remacle [26], Degroote [27], Vervack [28], AOAC [29], Sadasivam [30], Mbemba [31] and Makengo *et al*. [32]. Each experiment was carried out in triplicate.

b) Anti-nutritional factors

The following treatments were applied separately to the tender stems in triplicate: raw and cooked samples. Portions of the tender stems were cooked in a 1 liter conical flask equipped with a condenser. Tap water was added (stem / water ratio, 1: 3 w/v), and the tender stems were cooked on a heat cap until soft when squeezed with the stems. fingers. The cooking juices and tender stems were separated using a sieve; and the tender stems were air dried and then stored in an oven at 60 °C for complete drying. Cooked samples were stored in airtight bottles and stored at 4 °C for later analysis.

Determination of Total Cyanide contents

The cyanide contents were obtained according to the method (n ° 26.151) described by the AOAC [33]. The crude and dried samples were placed in two Kjeldhal glass vials (autolysis) with vapor distillate collected in NaOH solution and titrated against standard $AgNO_3$ solution in the presence of NH4OH and KI. The concentration of HCN was calculated from the amount of $AgNO_3$ used for the titration.

Oxalate determination

The oxalate assay was carried out using the method

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described by Oke [34]. 2 g of the raw and dried samples were dissolved in 10 ml of HCl (6 M) for one hour and completed at 250 ml in a volumetric flask. The pH of the filtrate was adjusted with a concentrated NH_4OH solution until the solution color changed from salmon pink to pale yellow. Then, the filtrate was treated with 10 ml of a 5% $CaCl_2$ solution to precipitate insoluble oxalate. The suspension was centrifuged at 2500 rpm. Then the supernatant was decanted and precipitated completely dissolved in 10 ml of H_2SO_4 at 20 % (v/v). The total filtrate resulting from dissolution in H_2SO_4 was increased to 300 mL. A 125 ml aliquot of the filtrate was heated to near the boiling point and then titrated against 0.05 M of standardized $KMnO_4$ solution to a pale pink color that persisted for about 30 s after which the burette reading was taken. The oxalate content was assessed from the value of the security. The overall oxidation reaction is:

$2MnO_4^- + 5C_2O_4^{2-} + 16H^+ \rightarrow 2Mn^{2+} + 8H_2O + 10CO_2$

2.2.4 Statistical Analysis

All the data were reported as mean ± standard deviation of three replicates. The IC_{50} values were calculated using Graph Pad Prism version 6.0 Software (Graph Pad Software, San Diego California, USA), The analysis of variance Diego California, USA). Statistical analysis was performed using Microsoft Excel.

3. Results and Discussion

3.1 Moisture Content

The measures of moisture are shown below (Figure 2). Our results show that *D. praehensilis* tender stems have a very high moisture content (86.7%).

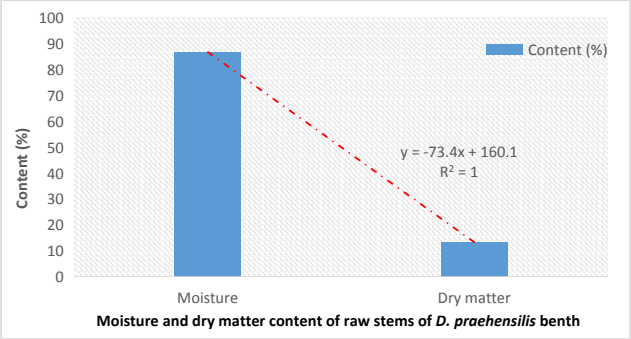

Figure 2. Moisture and dry matter content of raw stems of *D. praehensilis* benth

The variation of moisture between fresh and dried matters is very significant at P>5% ($R^2 = 1$).

We can notice that *D. praehensilis* tender stems have a very high moisture content compared to *D. praehensilis* yams and *D. alata* liana and D. dumetorum yams, whose moisture turn around 10% [35].

3.2 Phytochemical Screening

The results in Table 1 show that the tender stems of

Table 1. Phytochemical screening of *bark of Dioscorea praehensilis* benth

a. Results of chemical screening on the aqueous phase			**Results**
Chemical Groups Sought	***Reagents used***	***Observations***	**Bark**
1. Phenolic compounds			
Anthocyanins	HCl et NH_4OH	Red (Acid) and blue or greenish (Alca) colouring	+
Catechetical	Stiasny	Pink precipitate	+
Flavonoids	Cyanidine of Shinona	Colouring orange, red, violet	-
Gallic	Stiasny+$FeCl_3$ + CH_3COONa	Blue or black tint	+
Linked Quinones	Bornträger	Pink to purplish-red colouring	+++
Tannins	$FeCl_3$ 1%	Dark blue to black green colouring	-
Total polyphenols	Burton	Blue coloration with blue precipitate	+
2. ***Alkaloids***	Mayer	Precipitation	-
3. ***Saponines***	Foam test	Persistent foam of more than 1 cm after 15 min.	+++
4. Cardiotonic Heterosides	Killer - Killiani	Brownish-red ring at the interface of 2 solutions	+++
b. Results of chemical screening on the organic phase (Methanol)			
Chemical Groups Sought	***Reagents used***	***Observations***	**Bark**
1. Triterpenoids	Liebermann	Purple colouring	+
2. Steroids	Liebermann	Purple colouring	+
3. Free quinones	Bornträger and NaOH 10%	Blackish red coloration with precipitate	+
4. Anthocyanosids	HCl 20% + Diethyl ether + NaOH	No fluorescence under UV light	+

Legend: +++: Very abundant of the desired substance +: Presence of the desired substance; - : Absence of the desired substance

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Dioscorea praehensilis benth although having many phytoconstituents, are devoid of the main compounds as indicated in Table 1. The tender stems were found to be a rich source of polyphenols, anthocyanins and saponins. The same phytochemical compounds such as polyphenols [36] and tannins [37] have been reported in *Dioscorea* yams by Bukatuka *et al*. [35], Sherman [36] and Bray and Taylor [37]. The secondary metabolites generated in the plant systems act as major sources of dietary supplements and medicinal components for human body.

Phytochemical compounds are known to be biologically active constituents and are responsible for various activities such as antioxidants, antimicrobials, antifungi and anticancer [39-40]. Phenolic compounds have various activities including antioxidant, antidiabetic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities.

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The qualitative results of the content of secondary metabolites shown in Table 2 indicated that gallic tannins were present in the methanolic extract; their presence was confirmed by a positive reaction with a solution of ferric chloride and that flavonoids were not detected. These results confirm those of obtained by Bukatuka *et al*. [35]. In the table above, the samples were rich in total polyphenols (17.22 ± 0.16), tannins (19.32 ± 0.52) and anthocyanins (25.22 ± 0.04). Previous studies carried out on yams species of the same family have shown similar results [35,43,44]. The fact that this species has a high content of polyphenols could confer it considerable antioxidant properties.

Table 3 presents the antioxidant activity of *D. praehensilis*, as determined by ABTS and DPPH, expressed in IC_{50} values. Radical scavenging activity, as an indicator of antioxidant capacity, the antioxidant response of extracts does not appear to correlate with total phenolic content.

In addition, it should be noted that the IC_{50} values obtained by the ATBS test are lower than those of the DPPH test. This difference in activity could be attributed to their reaction mechanism. ABTS reacts simultaneously with both hydrophilic and lipophilic compounds while DPPH reacts only with hydrophilic compounds [45]. In an order of hundredths, our extracts showed a lower antioxidant activity than that of ascorbic acid (vitamin C), gallic acid and Quercetin used as positive controls. Nevertheless, this extract showed an interesting antioxidant activity compared to other plants [35,46-49].

3.3 Biochemical Analyses

3.3.1 Bromatological Analyses

The biochemical composition of ecotypes of *Dioscorea praehensilis* benth consumed in Kinshasa and Kwilu provinces (Democratic Republic of the Congo) is shown in the Figure 3.

As shown in this figure, the tender stems of *Dioscorea praehensilis* benth are rich in carbohydrates (67.16 g/100 g), with low levels of protein (8.4 g/100 g), fiber (14.25 g/100 g), lipid (2.88 g/100 g) and ash (7.31 g/100 g). These results corroborate with those obtained by several other studies [35,42-44]. As about the results obtained on dry matter, it can be stated that for all nutrients, the values we obtained are not in the range of the values given by Mbemba and Remacle [26] because they worked on fresh yam matter.

Table 2. Secondary metabolites content of methanolic extracts from *D. praehensilis*

Tender stems	Total polyphenols (mg GAE/g)	Flavonoids (mg QE/g)	Tannins (mg CE/g)	Anthocyanins (mg CE/g)
D. praehensilis	17.22 ± 0.16	nf	19.32 ± 0.52	25.22 ± 0.04

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

Table 3. IC_{50} (µg/mL) values of *D. praehensilis* extracts (Means ± SD, n=6)

Tender stems extract	IC_{50} (µg/mL)	
	ABTS	DPPH
D. praehensilis	97.26 ± 7.52	142.53 ± 11.61
Vitamin C	2.36 ± 0.60	3.24 ± 0.96
Acide gallique	0,71 ± 0,08	1,07 ± 0,10
Quercétine	1,42 ± 0,04	3,21 ± 0,99

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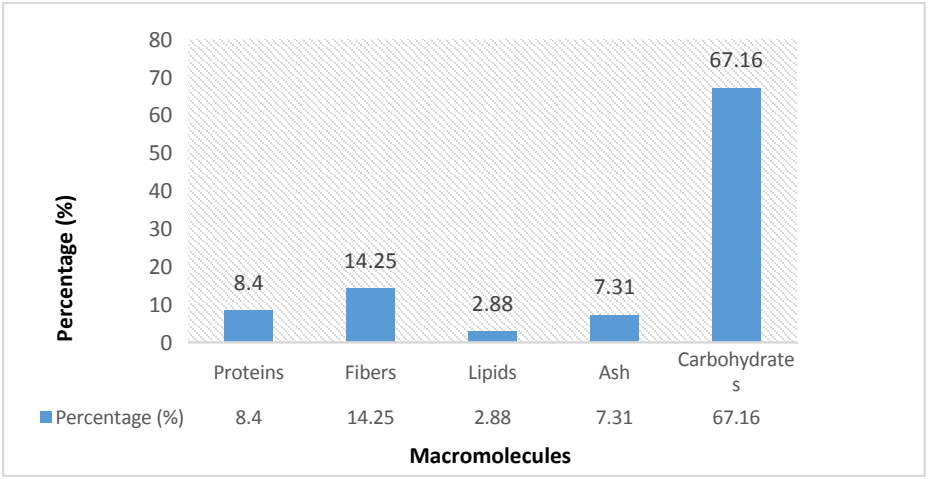

Figure 3. Macronutrient content in *Dioscorea praehensilis* benth stems

3.3.2 Anti-nutritional Factors

Cyanide content before and after cooking

The physico-chemical tests of our samples showed that *Dioscorea praehensilis* benth contains a significant amount of hydrocyanic acid in fresh tender stems. Hence 30 g of sample contains 30 mL of hydrocyanic acid. At this quantity, crude *Dioscorea praehensilis* benth used as food has a high toxicity in hydrocyanic acid. But after a good cooking, about 1 hour, the cyanide is eliminated in 99.97%. According to the procedure, after cooking we obtained 0.03 mL of hydrocyanic acid. Which gives us 0.011 mg/Kg. So after cooking, the Cyanide content is three times less than the no observed effect level (NOEL). NOEL in international way = 0.3 mg/Kg.

Oxalate content before and after firing

The tender stems of *Dioscorea praehensilis* benth do not contain a high quantity of oxalates, according to the result of our study. 10g of our sample gave a result of 0.025 mol/L in oxalate. So *Dioscorea praehensilis* benth as food has a low concentration of oxalic acid.

An excessive amount of soluble oxalate in the body prevents the absorption of soluble calcium ions, as oxalate binds to calcium ions to form an insoluble calcium oxalate complex. Therefore, people with a tendency to form kidney stones are advised to avoid foods rich in oxalate. On the other hand, people suffering from coronary heart disease are encouraged to consume rich foods that are moderately oxalic because this contributes to the reduction of blood cholesterol [50].

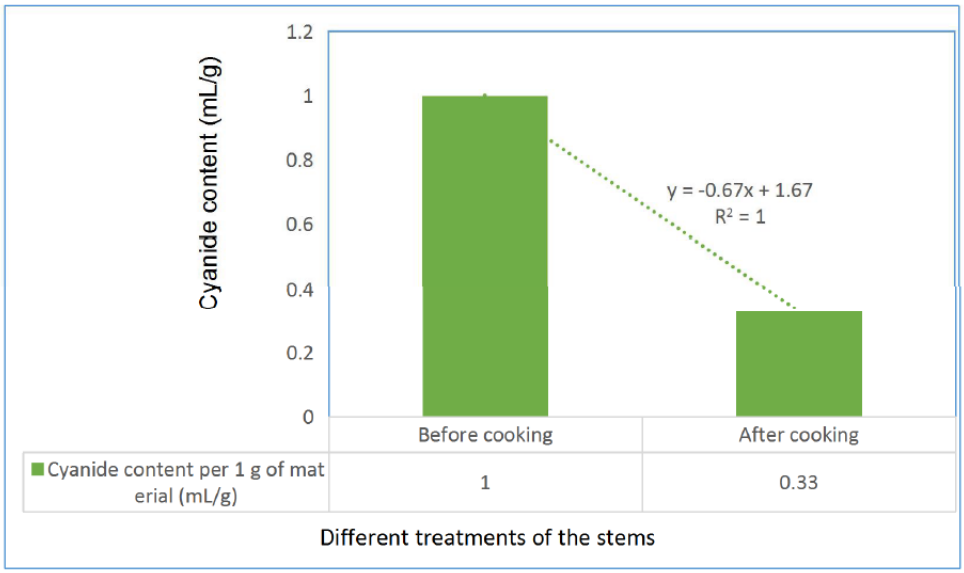

Figure 4. Cyanide content in the stems of *Dioscorea praehensilis* benth before and after cooking

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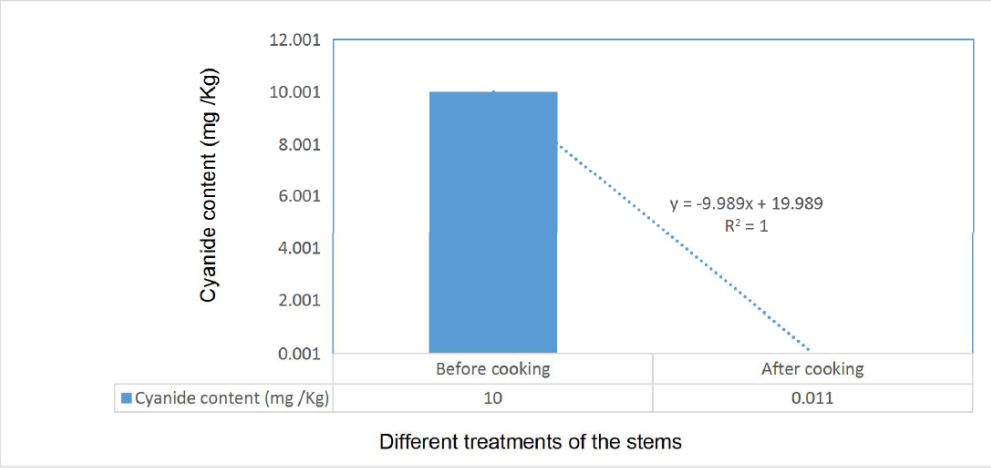

Figure 5. Cyanide content in the stems of *Dioscorea praehensilis* benth before and after cooking

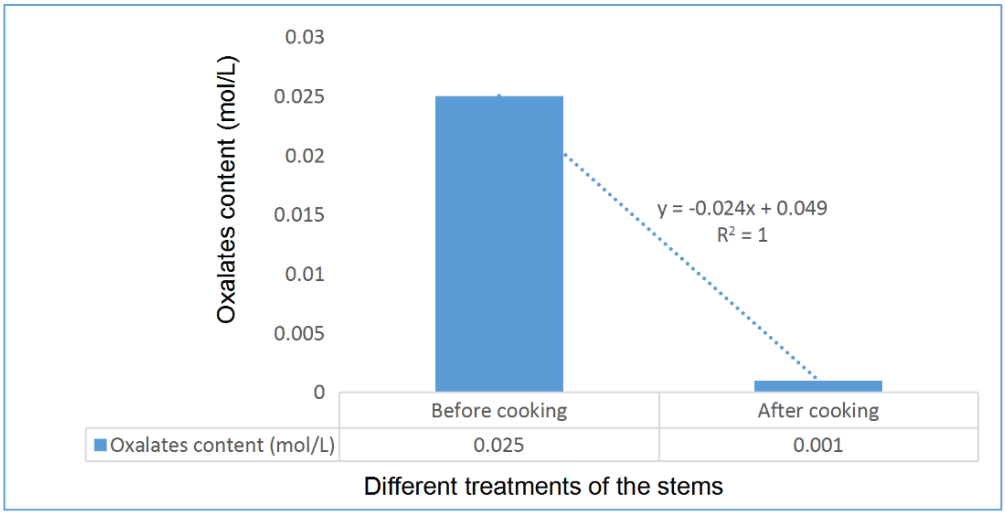

Figure 6. Oxalate content in the stems of *Dioscorea praehensilis* benth before and after firing

4. Conclusions

Nutrient rich plants are very important for a growing world population. Biodiversity is at the center of countless of these foods, but we only use a few of them. Among these plants, Dioscorea species play a vital role in supplementing food and medicine needs of rural populations. The results of the present study revealed various phytochemical compounds in this species and that the tender stems of *Dioscorea praehensilis* benth (Dioscoreaceae) were rich in carbohydrates, proteins and ash.

The results of the oxalates and cyanides measures in cooked food show an average value that does not present a significant risk to public health. Tubers and cereals contain a small amount of oxalate and can therefore be eaten moderately on a regular basis, while legumes are rich in oxalate and should be reduced in the diet to avoid the formation of kidney stones. The public should also be informed of the danger of excessive consumption of oxalate-rich foods.

The ethno-medical practices of traditional healers use *Dioscorea praehensilis* benth in the treatment of various diseases; in particular, the tuber is used against diarrhea, dysentery, stomachaches, dyspepsia, leukoderma, bronchitis and applied on ulcer; it is also used as a tonic, aphrodisiac and improves appetite.

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Availability of data and materials

All data and materials are presented in the manuscript.

Authors' contributions

Authors Patience M. Ngelinkoto, André-Marie K.

Lokassa, Bernadin Bulumuka, Johnny B. Mukoko, Myriam M. Ngondo and Florent B. Mukeba. Bernadin Bulumuka, Dorcas M. Kabasele, Ruth L. Mbuli and Florent B. Mukeba collected, dried and reduced in powder and perfomed the extractions. Patience M. Ngelinkoto, André-Marie K. Lokassa, Bernadin Bulumuka, Jeff K. Maliani, Johnny B. Mukoko and Florent B. Mukeba did the literature study, participated in experimental works. Johnny B. Mukoko, Patience M. Ngelinkoto, André-Marie K. Lokassa and Florent B. Mukeba interpreted the data, performed statistical analysis and prepared the manuscript. Patience M. Ngelinkoto, André-Marie K. Lokassa and Florent B. Mukeba designed the study. Patience M. Ngelinkoto, Myriam M. Ngondo and Florent B. Mukeba approved the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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