



## **Comparative Phytochemical Analysis and Antiradical Activity of Five Plants Used for the Treatment of Type 2 Diabetes in Benin**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors OK, SS, YK and CPA designed the study. Authors SS and YK wrote the protocol, and wrote the first draft of the manuscript. Authors OK, SS, YK and CPA managed the analyses of the study. Authors SS, JBA, MB and FS managed the literature search. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Background:** Diabetes remains a real public health problem in the world today. Although considered a disease of rich countries, today diabetes is increasingly a major concern in developing countries and particularly in sub-Saharan Africa. In Benin, its prevalence in 2015 was 12.4%.

**Objective:** This work aimed to compare the secondary metabolites, the content of phenolic compounds (total phenol, flavonoid) and the antiradical power of five plants (*Bambusa vulgaris* Schrad. Ex Wendel; *Parkia biglobosa* (Jacq.) R. Br. Ex G. Don; *Mangifera indica* L.; *Saccharum officinarum* L.; and *Annona muricata* L.) used in Benin by traditional healers to treat type 2 diabetes.

**Materials and Methods:** Secondary metabolites were identified by coloration and precipitation reactions specific to each family of metabolites. Total phenols were determined by Folin Ciocalteu method. The aluminum trichloride method has been used to quantify total flavonoids. The antiradical capacity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH).

**Results:** Plants which leaves were higher in total phenols were *Mangifera indica*, *Parkia biglobosa*

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and *Bambusa vulgaris* while *Annona muricata* and *Parkia biglobosa* were the richest plants in flavonoids. The hydroethanolic extract of leaves of *Bambusa vulgaris* ( $IC_{50}=0.28\text{mg/mL}$ ), *Parkia biglobosa* ( $IC_{50}=0.3\text{mg/mL}$ ) and *Mangifera indica* ( $IC_{50}=2.5\text{mg/mL}$ ) showed more pronounced antiradical activity than vitamin C ( $IC_{50}=3.2\text{ mg/mL}$ ) which were synthetic antioxidant.

**Conclusion:** Our results showed that among the five plants studied *Mangifera indica*, *Parkia biglobosa* and *Bambusa vulgaris* were the richest in total phenols and also those whose leaf extracts had the highest antiradical activities. These three plants could therefore be considered as potential remedies for type 2 diabetes and its complications.

**Keywords:** Antiradical activity; diabetes; medicinal plants; phenolic compounds.

## 1. INTRODUCTION

Diabetes is a global public health problem [1]. Over 422 million adults were living with diabetes in 2014 [2]. In 2012, 1.5 million deaths worldwide were directly attributable to diabetes, the eighth leading cause of death in humans [3]. More than 80% of diabetes deaths occur in low-income countries.[1] The number of diabetics in sub-Saharan Africa were estimated at 19.8 million in 2013 [4]. Diabetes is on the rise in Benin and its prevalence increased from 2.9% in 2008 to 12.4% in 2015 and generated a mortality rate of 2% [5]. Type 2 diabetes and its associated complications are accompanied by oxidative stress, production of free radicals and reactive oxygen species. The chronicity of hyperglycaemia characteristic of type 2 diabetes generates oxidative stress responsible for increased glycolysis which, by increasing mitochondrial membrane potential, increases the production of radicals and inhibits glyceraldehyde-3-phosphate dehydrogenase [6-7]. Oral antidiabetics lead to the normalization of blood sugar only in less than 50% of cases. They have no regressive effect on established lesions and they are contraindicated in renal and hepatocellular insufficiencies [8]. In addition, there are the problems of intolerance, side effects, hypersensitivity and resistance related to antidiabetic drugs [9]. Moreover, in developing African countries and particularly in Benin, the medical care of diabetes is limited by the inaccessibility of certain populations to health centers and the high cost of conventional medicine drugs. In these conditions, populations often resort to medicinal plants for treatment. The Beninese flora is rich and diversified in plants used to treat type 2 diabetes [10]. *Bambusa vulgaris*, *Parkia biglobosa*, *Mangifera indica*, *Saccharum officinarum* and *Annona muricata* are some of the plants that are used to treat type 2 diabetes in Benin. It therefore seems essential to direct scientific studies on these plants, which are in high demand in traditional medicine to

treat diabetes. It was in this sense that this work compared the quantity of phenolic compounds and the antiradical activity of these five plants.

## 2. MATERIALS AND METHODS

### 2.1 Material

The plant material consisted of the leaves of *Bambusa vulgaris* Schrad. Ex Wendel POACEAE; *Parkia biglobosa* (Jacq.) R. Br. Ex G. Don. FABACEAE ; *Mangifera indica* L. ANACARDIACEAE; *Saccharum officinarum* L. POACEAE; And *Annona muricata* L. ANNONACEAE, plants selected on the basis of selection criteria resulting from an ethnopharmacological survey carried out in three departments (Oueme, Atlantique, Collines) of Benin among traditional healers [11].

### 2.2 Methods

#### 2.2.1 Pretreatment of plants

After harvesting, five batches of the leaves of the five plants were dried at laboratory temperature until their plant mass stabilized and then reduced to powder.

#### 2.2.2 Plant extraction

The extraction was made with hydroethanolic. 5g of powdered biomass were mixed with 100 mL solvent for 72 hours. Further, all the extracts were filtered through Whatman No.1 filter paper and concentrated. The residues were dried to constant weight and stored in the darkness at 4°C to avoid the degradations until use.

#### 2.2.3 Preliminary phytochemical screening

Secondary metabolites were carried out by coloration and precipitation reactions specific to each family (Table1: Methods for the identification of secondary metabolites of plants) [12-13].

## 2.2.4 Determination of phenolic compounds

**Total phenol content:** Total phenolic content was determined using the Folin-Ciocalteu colorimetric method. This method consisted of using a mixture of phosphotungstic and phosphomolybdic acids, which were reduced during the oxidation of phenols into a mixture of tungsten blue oxide and molybdenum. Finally, the absorbance was measured at 765 nm using a spectrophotometer and the total phenol content are expressed in micrograms of gallic acid equivalence per milligram of dry matter (mgGAE/gEx) [14-15].

**Total flavonoids content:** The method of aluminum trichloride (AlCl<sub>3</sub>) was used to quantify the total flavonoids. This technique was based on the formation of the aluminum complex flavonoids. The absorbance was read at 415 nm using a spectrophotometer and the Total flavonoid content are expressed in micrograms quercetin equivalence per milligram of dry matter (µgQE/gEx) [16].

## 2.2.5 Evaluation of antiradical activity

The antiradical activity was evaluated by the DPPH method. The principle of this method was based on measuring the trapping free radicals in a solution of DPPH. This trapping was indicated by the disappearance of the purple color of DPPH. The mixture of DPPH solution and the sample was left in the darkness for an hour and the absorbance measured at 517 nm. [17-18]. The trapping percentage was determined by the formula:  $P = ((Ab W - Ab S) / Ab) \times 100$ .

P: percentage of trapping; Ab W: absorbance of the white; Ab S: Absorbance of the sample

## 3. RESULTS AND DISCUSSION

### 3.1 Results

#### 3.1.1 Secondary metabolites identified

Table 2 showed the secondary metabolites of five plants (*Bambusa vulgaris*, *Parkia biglobosa*, *Mangifera indica*, *Saccharum officinarum* and *Annona muricata*). The five plants contained tannins, alkaloids, sterols and terpenes. Among the five plants studied, only *Annona muricata* does not contain saponosides and anthocyanins, while mucilages were absent only in *Parkia biglobosa*. On the other hand, coumarins and reducing compounds were identified only in *Annona muricata*. Flavonoids were present in the leaves of *Bambusa vulgaris*, *Parkia biglobosa*

and *Saccharum officinarum* while leucoanthocyanins had been reported only in the leaves of *Parkia biglobosa*, *Mangifera indica* and *Annona muricata*.

#### 3.1.2 Phenolic compound content

The calibration curves for determining the contents of total phenols and total flavonoids were shown in Figure 1. The calibration curves for measuring the total phenols content was obtained with the equation  $y = 0.0252x + 0.0189$  with the coefficient of determination of  $R^2 = 0.98$ . The calibration curves for determining the content of total flavonoids was  $y = 0.1099x + 0.0136$  with the coefficient of determination  $R^2 = 0.99$ .

**Total phenols:** The total phenol contents of leaves of *Bambusa vulgaris*, *Parkia biglobosa*, *Mangifera indica*, *Saccharum officinarum* and *Annona muricata* were shown in figure 2. The highest content is obtained at the level of *Mangifera indica* (29.409 mgGAE/gEx) accompanied by that of *Parkia biglobosa* (26.909mgGAE/gEx) of the *Bambusa vulgaris* content (23.298 mgGAE/gEx). The lowest total phenol content was noted in the hydroethanolic extract of *Saccharum officinarum* (10.639mgGAE/gEx) and *Annona muricata* (17.504mgGAE/gEx).

**Total flavonoids:** Fig. 3 indicates the flavonoid contents of the hydroethanolic extract of leaves of *Bambusa vulgaris*, *Parkia biglobosa*, *Mangifera indica*, *Saccharum officinarum* and *Annona muricata*. The total flavonoid content of the hydroethanolic extract of *Annona muricata* leaves was 16.892 µgQE/gEx while that of *Parkia biglobosa* was 6.437µgQE/gEx. The flavonoid contents of *Mangifera indica*, *Saccharum officinarum* and *Bambusa vulgaris* were respectively 2.915 µgQE/gEx, 2.169µgQE/gEx and 2.551 µgQE/gEx.

#### 3.1.3 Anti-radical activity of plant extracts

The curves in Figure 4 showed the change in trapping percentage as a function of the concentrations of the hydroethanolic plant extract. At the level of the five curves, there was a gradual increase in trapping percentage with the increase in the concentration of the hydroethanolic extract of leaves of *Bambusa vulgaris*, *Parkia biglobosa*, *Mangifera indica*, *Saccharum officinarum* and *Annona muricata*. The concentrations (IC<sub>50</sub>) of the hydroethanolic extract trapping 50 of the DPPH radical were determined. The IC<sub>50</sub> were listed in Table 3.

From the analysis of this table, it appeared that the IC<sub>50</sub> of *Bambusa vulgaris*, *Parkia biglobosa* and *Mangifera indica* were respectively 0.28 mg/mL, 0.3 mg/mL and 2.50 mg/mL. The hydroethanolic extract of *Bambusa vulgaris*, *Parkia biglobosa* and *Mangifera indica* showed a more interesting antiradical activity than vitamin C (IC<sub>50</sub>=3.20 mg/mL) which was a synthetic compound. The IC<sub>50</sub> of the hydroethanolic extract of *Annona muricata* and *Saccharum officinarum* were 11.00 mg/mL and 39.00 mg/mL.

### 3.2 Discussion

The leaves of *Bambusa vulgaris*, *Parkia biglobosa*, *Mangifera indica*, *Saccharum officinarum* and *Annona muricata* were rich and diversified in secondary metabolites. However, it should be noted that it had been identified in the leaves of *Bambusa vulgaris* collected in Benin, coumarins and leuco anthocyanins which were absent in our sample [19]. On the other hand, our results were in agreement with those who studied the sample from Ivory Coast [20]. Concerning the leaves of *Parkia biglobosa* harvested in Nigeria, the absence of the sterols and terpenes were noted whereas that were present in the sample from Benin [21]. The leaves of *Annona muricata*, contain flavonoids in accordance with other results [22]. Regarding the leaves of *Mangifera indica* harvested in Mauritius, the presence of flavonoids and coumarins were noted which were absent in that of Benin [23]. The variation in secondary metabolites observed in our samples compared to previous work could be related to the harvest period, the nature of the soil or climatic factors [24]. The diversity in secondary metabolites of these plants could explain their uses in traditional medicine. Some results had shown that tannins,

flavonoids, alkaloids, and saponins have antidiabetic activities [25-26].

Our results showed that the leaves of *Mangifera indica*, *Parkia Biglobosa* and *Bambusa vulgaris* were the richest in total phenols while *Annona muricata* and *Saccharum officinarum* were the least rich. It was reported that phenolic compounds were useful in the prevention of type 2 diabetes [27-32].

The hydroethanolic extract of the leaves of the five plants (*Bambusa vulgaris*, *Parkia biglobosa*, *Mangifera indica*, *Saccharum officinarum* and *Annona muricata*) showed interesting antiradical activities. It should be noted that the hydroethanolic leaf extract of *Bambusa vulgaris*, *Parkia biglobosa* and *Mangifera indica* showed more pronounced antiradical activity than vitamin C, which is a synthetic antioxidant. Our results were consistent with previous work on the antiradical activity of these plants [29-33]. The plants which had the weakest IC<sub>50</sub> and therefore had the highest antioxidant activities are those which contained the significant quantities of total phenols. With the exception of *Annona muricata* which contains few total phenols but was rich in vitamin C a good antioxidant [34]. The role of polyphenols as an antioxidant was no longer to be demonstrated, [35,36;37,38] so it was consistent that these plants have high antioxidant activities. Oxidative stress had also been shown to induce insulin resistance which leads to type 2 diabetes [39-40]. Hence *Bambusa vulgaris*, *Parkia biglobosa*, *Mangifera indica* and *Annona muricata* leaves with their high antioxidant activities could prevent insulin resistance and therefore prevent type 2 diabetes and its complications which were also linked to oxidative stress.

**Table 1. Methods for the identification of secondary metabolites of plants**

Secondary metabolites	Chemical test
Alkaloids	Mayer's test and Drangendorffs test
Anthocyanins	test with hydrochloric acid and ammonia
Anthraquinones	Borntranger's test
Coumarins	365 nm fluorescence test
Flavonoids	Shibita's reaction test
Tannins	stiasny test, ferric chloride and sodium acetate test
Saponins	Frothing test
Leuco anthocyanins	Bate-Smith and metcalf
Mucilage	flaky test
Cyanogenic derivatives	picric acid test
Reducing compound	Fehling's test
Sterols and terpenes	Liebermann-Burchard's test)

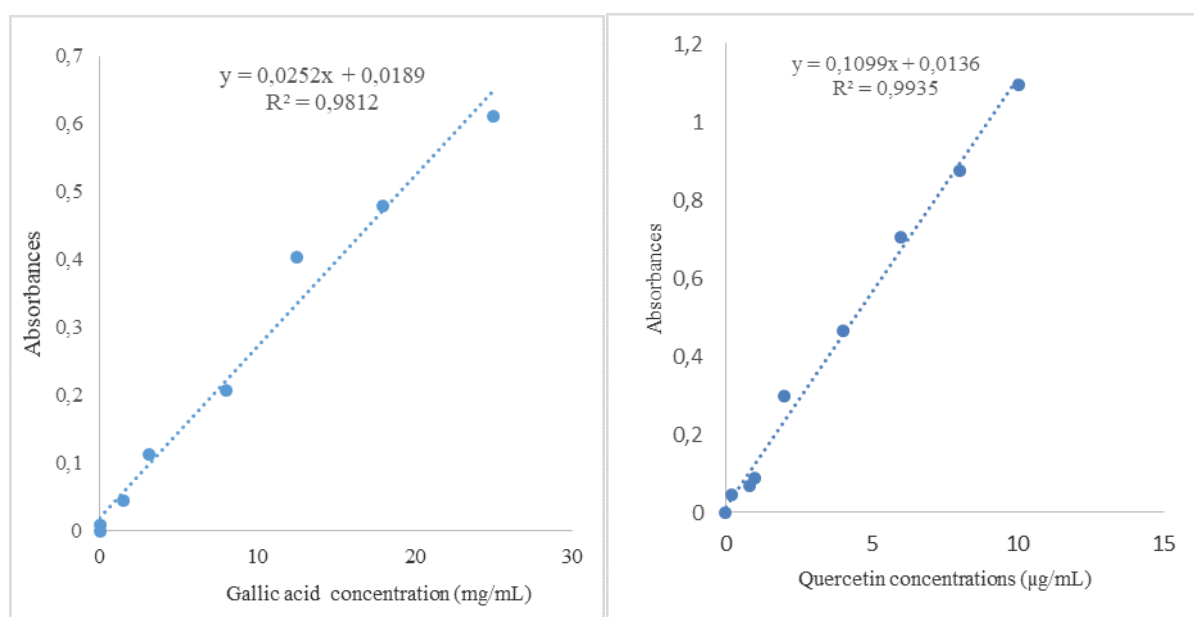
**Table 2. Secondary metabolites of plants**

Secondary metabolites	Bv	Pb	Mi	So	Am
Tanins	+	+	+	+	+
Flavonoids	+	+	-	+	-
Anthocyanins	+	+	+	+	-
Leuco anthocyanins	-	+	+	-	+
Alkaloids	+	+	+	+	+
Reducing compound	-	-	-	-	+
Mucilages	+	-	+	+	+
Saponins	+	+	+	+	-
Cyanogenic derivatives	-	-	-	-	-
Sterols and terpenes	+	+	+	+	+
Coumarins	-	-	-	-	+
Quinone derivatives	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Cardiotonic derivatives	-	-	-	-	-

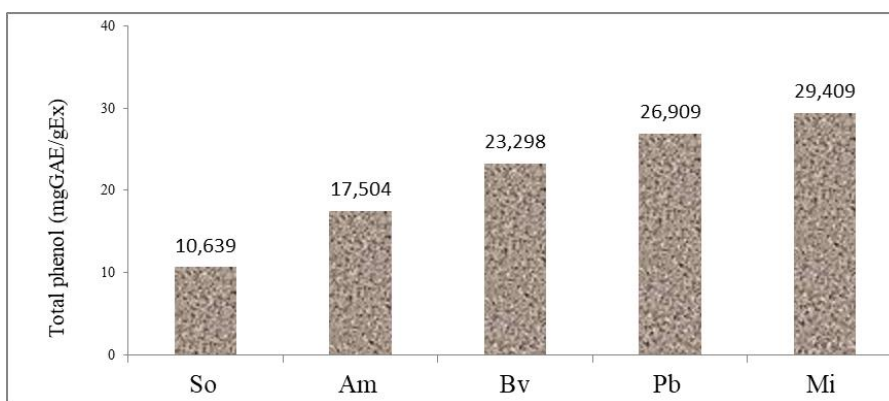
Legends: + : Presence; - : Absence ; Bv : *Bambusa vulgaris* ; Pb : *Parkia biglobosa* ; Mi : *Mangifera indica* ; So : *Saccharum officinarum* ; Am: *Annona muricata*

**Table 3. IC<sub>50</sub> of the hydroethanolic plant extract**

Hydroethanolic extract	IC50 (mg/mL)
<i>Mangifera indica</i>	2.50
<i>Parkia biglobosa</i>	0.30
<i>Bambusa vulgaris</i>	0.28
<i>Annona muricata</i>	11.00
<i>Saccharum officinarum</i>	39.00
Vitamine C	3.20

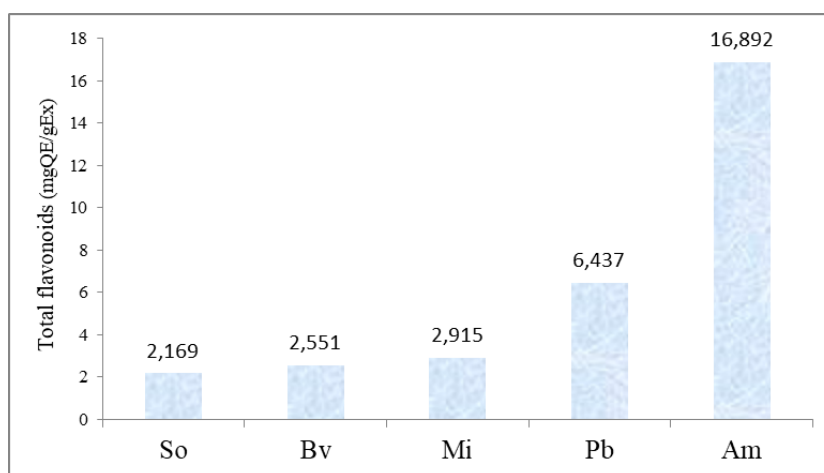


**Fig. 1. Calibration curves for the evaluation of the levels of phenolic compounds**



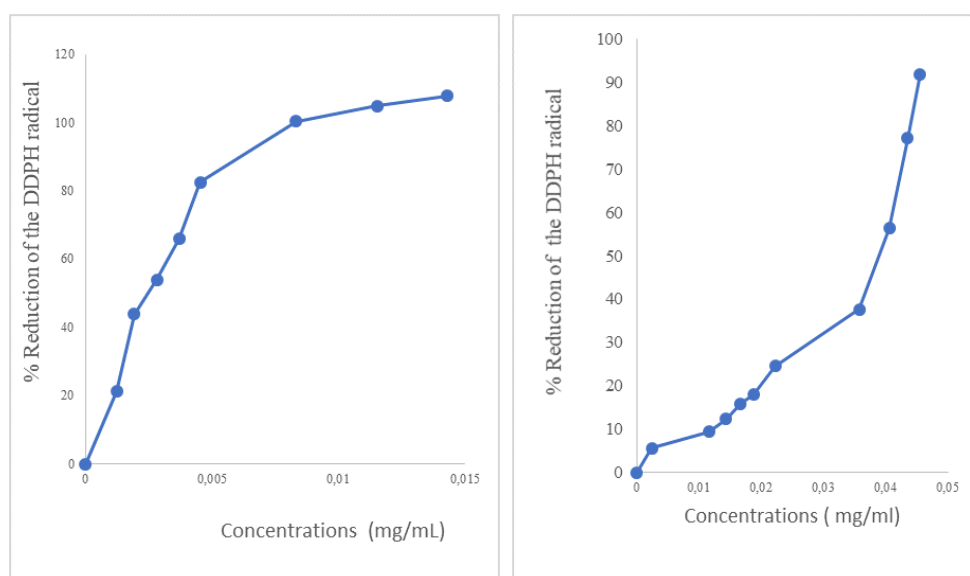
**Fig. 2. Total phenol content of hydroethanolic plant extract**

**Legends:** Bv : *Bambusa vulgaris* ; Pb : *Parkia biglobosa* ; Mi : *Mangifera indica* ; So : *Saccharum officinarum* ; Am: *Annona muricata*



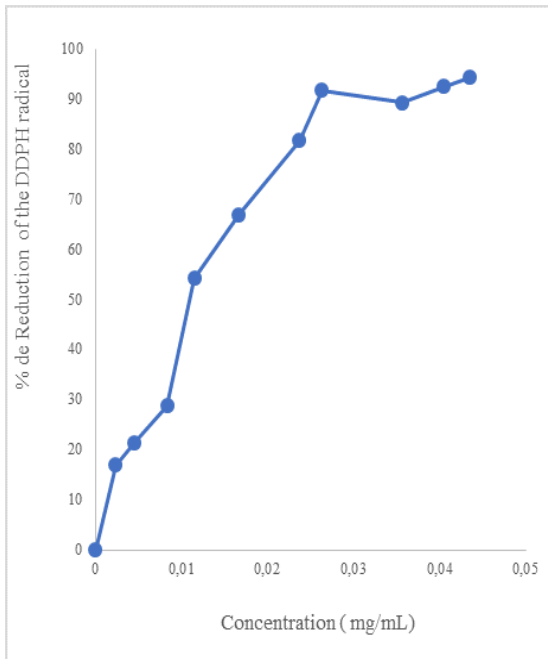
**Fig. 3. Total flavonoids content of hydroethanolic plant extract**

**Legends:** Bv : *Bambusa vulgaris* ; Pb : *Parkia biglobosa* ; Mi : *Mangifera indica* ; So : *Saccharum officinarum* ; Am: *Annona muricata*

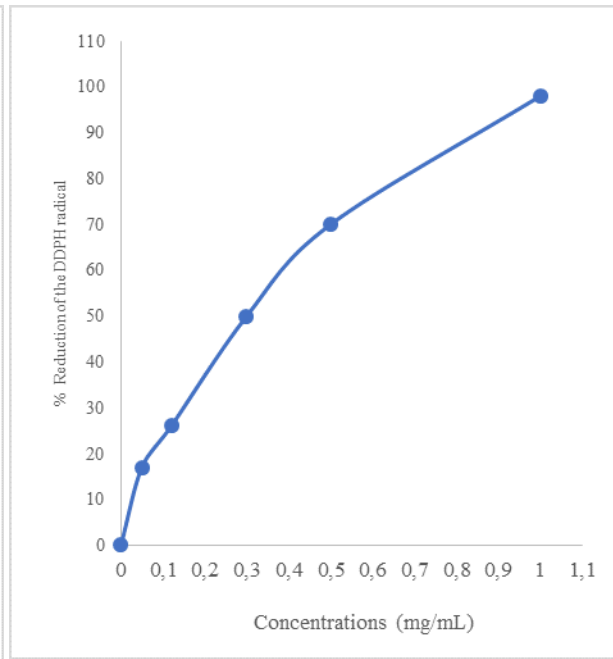


***Mangifera indica***

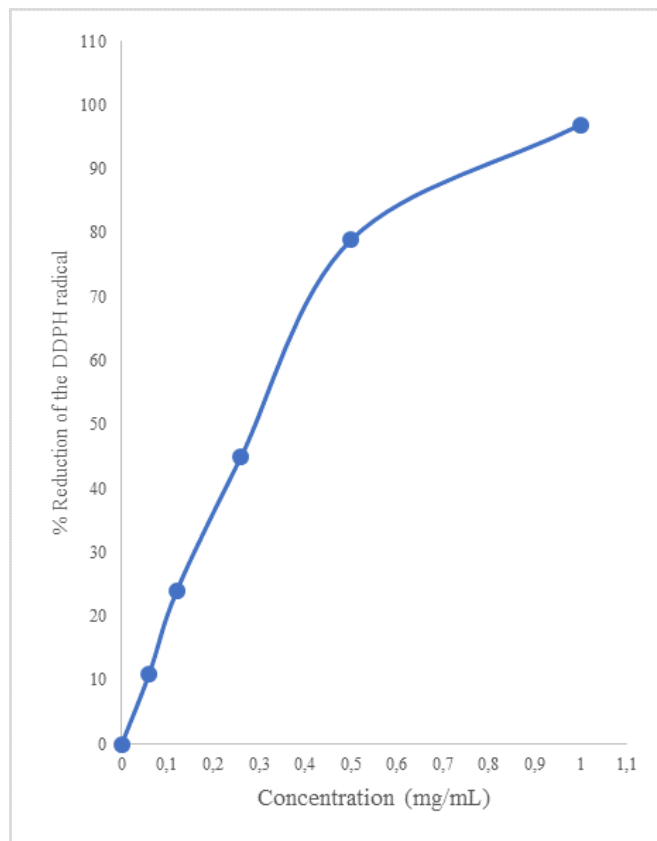
***Saccharum officinarum***



***Annona muricata***



***Parkia viglobosa***



***Bambusa vulgaris***

**Fig. 4. DPPH radical scavenging rate as a function of the concentration of the hydroethanolic extract of plants**

#### 4. CONCLUSION

Diabetes is emerging acutely as the silent pandemic of sub-Saharan Africa. As part of the development of medicinal plants used for the treatment of Diabetes, we have chosen 5 plants from our ethno pharmacological survey including *Bambusa vulgaris*, *Parkia biglobosa*, *Mangifera indica*, *Saccharum officinarum* and *Annona muricata*. Phytochemical screening revealed the presence of tannins, alkaloids, anthocyanins, flavonoids, mucilages, sterols and terpenes in the plant leaves. Quantitative analysis and antiradical study revealed that among the five plants studied *Mangifera indica*, *Parkia biglobosa* and *Bambusa vulgaris* were the richest in total phenols and also those whose leaf extracts had the highest antiradical activities. These three plants could therefore be considered as potential remedies for type 2 diabetes and its complications.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### ACKNOWLEDGEMENT

All participants in this work are authors.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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