Original Research Article

Comparative phytochemical analysis and antiradical activity of five plants used for the treatment of type 2 diabetes in Benin

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 aims to compare the secondary metabolites, the content of phenolic compounds (total phenol, flavonoid) and the antiradical power of five plants (*Bambusa vulgarisSchrad. Ex Wendel;Parkiabiglobosa (Jacq.) R. Br. Ex G. Don;Mangiferaindica L.;Saccharumofficinarum L.; And Annonamuricata 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 FolinCiocalteu method. The aluminumtrichloride method has been used to quantify total flavonoids. The antiradical capacity was evaluated by 2,2-diphenyl-l-picrylhydrazyl (DPPH). **Results**: Plants whose leaves are higher in total phenols are*Mangiferaindica, Parkiabiglobosa and Bambusa*vulgaris while*Annonamuricata* and *Parkiabiglobosa*are the richest plants in flavonoids. The hydroethanolic extract of leaves of *Bambusa vulgaris* (IC_{50}=0.28mg/mL), *Parkiabiglobosa* (IC_{50}=0.3mg/mL) and *Mangiferaindica* (IC_{50}=2.5mg/mL) showed more pronounced antiradical activity than vitamin C (IC_{50}=3.2 mg/mL) which is a synthetic antioxidant. **Conclusion**: Our results showed that among the five plants studied *Mangiferaindica, Parkiabiglobosa andBambusa vulgaris*arethe richest in total phenols and also those whose leaf extracts have the highest antiradical activities. These three plants could therefore be considered as potential remedies for type 2 diabetes and its complications.

Keywords: Diabetes, Medicinal plants, Phenolic compounds,Antiradical activity

I 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 is 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, Parkiabiglobosa, Mangiferaindica, Saccharumofficinarum and Annonamuricata*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 is in this sense that this work compares the quantity of phenolic compounds and the antiradical activity of these five plants.

II MATERIALS AND METHODS

II.1 Material

The plant material consists of the leaves of *Bambusa vulgaris, Parkiabiglobosa, Mangiferaindica, Saccharumofficinarum et Annonamuricata,* 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]

II.2 Methods

II.2-1 Pretreatment of plants

After harvesting, the samples were dried at laboratory temperature until their plant mass stabilized and then reduced to powder.

II. 2-2 Plant extraction

The extraction was made with hydroethanolic. About 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℃ to avoid the degradations until use.

II.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]

II.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 ($AlCl_3$) 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]

II.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) X100

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

Statistical Analysis

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III RESULTS AND DISCUSSION

III.1 Result

III.1-1 Secondary metabolites identified

Table 2 shows the secondary metabolites of five plants (*Bambusa vulgaris, Parkiabiglobosa, Mangiferaindica, Saccharumofficinarum and Annonamuricata*). The five plants contain tannins, alkaloids, sterols and terpenes. Among the five plants studied, only *Annonamuricata*does not contain saponosides and anthocyanins, while mucilagesare absent only in *Parkiabiglobosa*. On the other hand, coumarins and reducing compounds were identified only in *Annonamuricata*. Flavonoids are present in the leaves of *Bambusa vulgaris*, *Parkiabiglobosa* and *Saccharumofficinarum* while leuco-anthocyaninshave been reported only in the leaves of *Parkiabiglobosa*, *Mangiferaindica* and *Annonamuricata*.

III.1-2 Phenolic compound content

The calibration curves for determining the contents of total phenols and total flavonoids are shown in Figure 1. The calibration curves for measuring the total phenols content is 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 is 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*, *Parkiabiglobosa*, *Mangiferaindica*, *Saccharumofficinarum* and *Annonamuricata* are shown in figure 2. The highest content is obtained at the level of *Mangiferaindica* (29.409 mgGAE/gEx) accompanied by that of *Parkiabiglobosa*(26.909mgGAE/gEx) of the *Bambusa vulgaris* content (23.298 mgGAE/gEx). The lowest total phenol content was noted in the hydroethanolic extract of *Saccharumofficinarum* (10.639mgGAE/gEx) and *Annonamuricata* (17.504mgGAE/gEx).

Total flavonoids

Figure 3 indicates the flavonoid contents of the hydroethanolic extract of leaves of *Bambusa vulgaris*, *Parkiabiglobosa*, *Mangiferaindica*, *Saccharumofficinarum* and *Annonamuricata*. The total flavonoid content of the hydroethanolic extract of *Annonamuricata* leaves is 16.892 µgQE/gEx while that of *Parkiabiglobosa* is 6.437µgQE/gEx. The flavonoid contents of

Mangiferaindica, *Saccharumofficinarum* and *Bambusa vulgaris*are respectively 2.915 µgQE/gEx, 2.169µgQE/gEx and 2.551 µgQE/gEx.

III.1-3 Anti-radical activity of plant extracts

The curves in Figure 4 show the change in trapping percentage as a function of the concentrations of the hydroethanolic plant extract. At the level of the five curves, there is-what do you mean??a gradual increase in trapping percentage with the increase in the concentration of the hydroethanolic extract of leaves of *Bambusa vulgaris*, *Parkiabiglobosa*, *Mangiferaindica*, *Saccharumofficinarum* and *Annonamuricata*. The concentrations (IC_{50}) of the hydroethanolic extract trapping 50 of the DPPH radical were determined. The IC_{50} are listed in Table 3.

From the analysis of this table, ~~it appears that~~ the IC_{50} of *Bambusa vulgaris*, *Parkiabiglobosa* and Mangiferaindicaare respectively 0.28 mg/mL, 0.3 mg/mL and 2.50 mg/mL. The hydroethanolic extract of *Bambusa vulgaris*, *Parkiabiglobosa* and *Mangiferaindica* showed a more interesting antiradical activity than vitamin C (IC50=3.20 mg/mL) which is a synthetic compound. The concentration of the hydroethanolic extract of *Annonamuricata*is 11.00 mg/mL and 39.00 mg/mL, respectively.

III.2 DISCUSSION

The leaves of *Bambusa vulgaris*, *Parkiabiglobosa*, *Mangiferaindica*, *Saccharumofficinarum* and *Annonamuricata* are rich and diversified in secondary metabolites. However, it should be noted that it has been identified in the leaves of *Bambusa vulgaris* collected in Benin,coumarins and leucoanthocyanins which are absent in our sample-is this statement from the previous study??.[19] On the other hand, our results are in agreement with those who studied the sample from Côte d'Ivoire.[20]Concerning the leaves of *Parkiabiglobosa* harvested in Nigeria, the absence of the sterols and terpenesare noted whereas that are present in the sample from Benin.[21] The leaves of *Annonamuricata*,contain flavonoids in accordance with other ~~results~~studies.[22] Regarding the leaves of *Mangiferaindica* harvested in Mauritius, the presence of flavonoids and coumarins[23]are noted which are 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 have shown that tannins, flavonoids, alkaloids, and saponinspossess antidiabetic activities.[25-26]

Our results showed that the leaves of *Mangiferaindica, ParkiaBiglobosaand Bambusa vulgaris* are the richest in total phenols while *Annonamuricata* and *Saccharumofficinarum*are the least rich. It was reported that phenolic compounds are useful in the prevention of type 2 diabetes.[27-22]

Regarding the antiradical activity of the five plants (*Bambusa vulgaris, Parkiabiglobosa, Mangiferaindica, Saccharumofficinarum*and *Annonamuricata*), the hydroethanolic extract of its leaves showed interesting activity. It should be noted that thehydroethanolic leaf extract of *Bambusa vulgaris, Parkiabiglobosa and Mangiferaindica* showed more pronounced activity than vitamin C, which is a synthetic antioxidant. Our results are consistent with previous work on the antiradical activity of these plants.[29-33]The plants which have the weakest IC_{50} and therefore capable of having the highest antioxidant activities are those which contain the significant quantities of total phenols. With the exception of *Annonamuricata* which contains few total phenols but which is rich in vitamin C a good antioxidant.[34] The role of polyphenols as an antioxidant is no longer to be demonstrated,[35-36] so it is consistent that these plants have high antioxidant activities. Oxidative stress has also been shown to induce insulin resistance which leads to type 2 diabetes.[37-38] Hence *Bambusa vulgaris, Parkiabiglobosa,Mangiferaindica*and *Annonamuricata*leaves with their high antioxidant activities could prevent insulin resistance and therefore prevent type 2 diabetes and its complications which are also linked to oxidative stress.

VI 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 five~~5~~ plants from our ethno pharmacological-what is this?survey including *Bambusa* vulgaris, *Parkiabiglobosa*, *Mangiferaindica*, *Saccharumofficinarum* and *Annonamuricata*. 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 *Mangiferaindica, Parkiabiglobosa andBambusa vulgaris*are the richest in total phenols and also those whose leaf extracts have the highest antiradical activities. These three plants could therefore be considered as potential remedies for type 2 diabetes and its complications.

TABLES AND GRAPHS

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

Secondarymetabolites	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'sreaction test
Tannins	stiasny test, ferric chloride and sodium acetate test
Saponins	Frothing test
Leuco anthocyanins	Bate-Smith and metcalf
Mucilage	flaky test
Cyanogenicderivatives	picricacid test
Reducing compound	Fehling's test
Sterols and terpenes	Liebermann-Burchard's test)

Table 2: Secondary metabolites of plants

Secondarymetabolites	***Bv***	***Pb***	***Mi***	***So***	***Am***
Tanins	+	+	+	+	+
Flavonoids	+	+	-	+	-
Anthocyanins	+	+	+	+	-
Leuco anthocyanins	-	+	+	-	+
Alkaloids	+	+	+	+	+
Reducing compound	-	-	-	-	+
Mucilages	+	-	+	+	+
Saponins	+	+	+	+	-
Cyanogenicderivatives	-	-	-	-	-
Sterols and terpenes	+	+	+	+	+
Coumarins	-	-	-	-	+
Quinone derivatives	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Cardiotonicderivatives	-	-	-	-	-

Legends: + : Presence; - : Absence ; *Bv : Bambusa vulgaris ; Pb : Parkiabiglobosa ; Mi : Mangiferaindica ; So :Saccharumofficinarum ;Am: Annonamuricata*

Table 3: IC_{50} of the hydroethanolic plant extract.

Hydroethanolicextract	**IC50 (mg/mL)**
Mangifera indica	2.50
Parkia biglobosa	0.30
Bambusa vulgaris	0.28
Annona muricata	11.00
Saccharum officinarum	39.00
Vitamine C	3.20

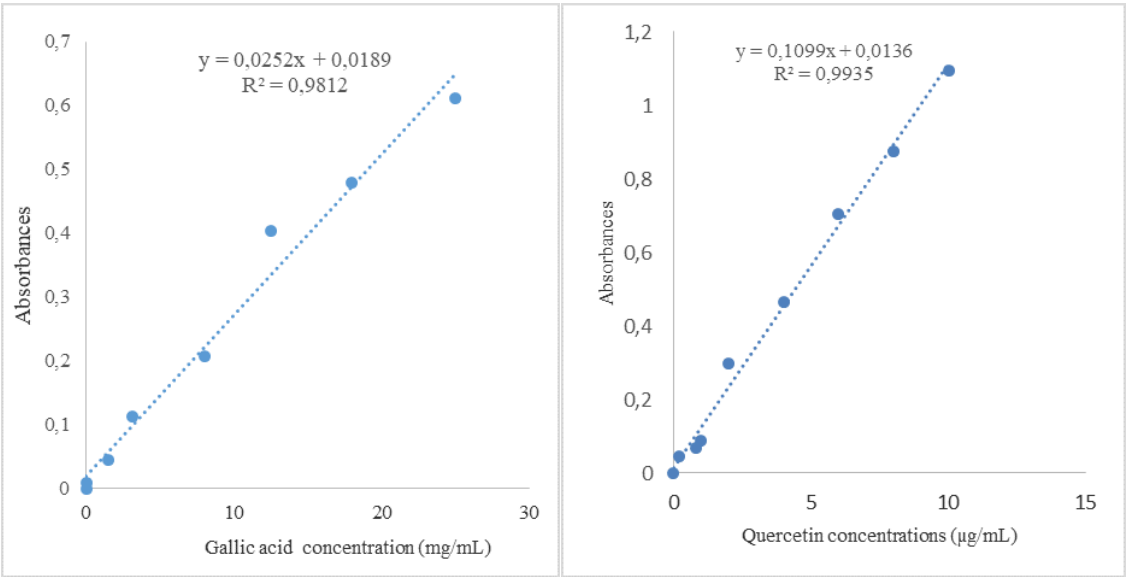


Figure 1: Calibration curves for the evaluation of the levels of phenolic compounds

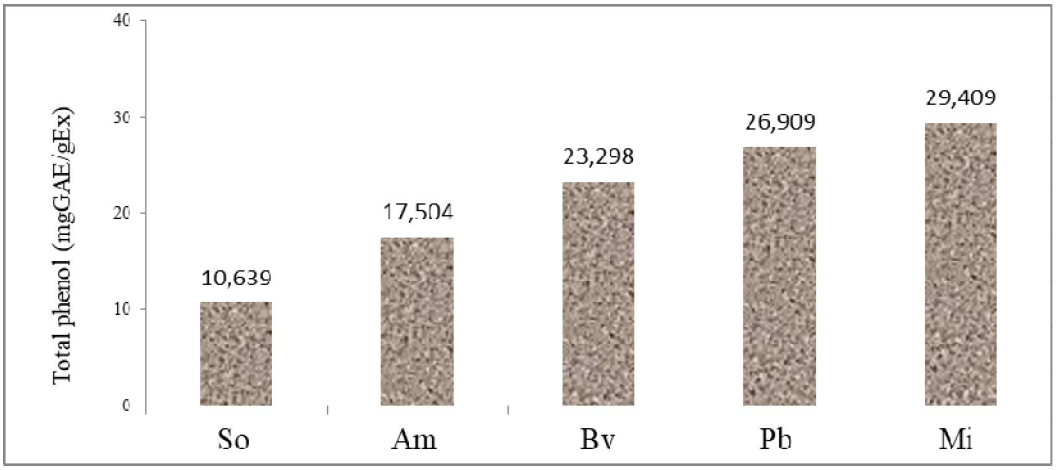


Figure 2: Total phenol content of hydroethanolic plant extract

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

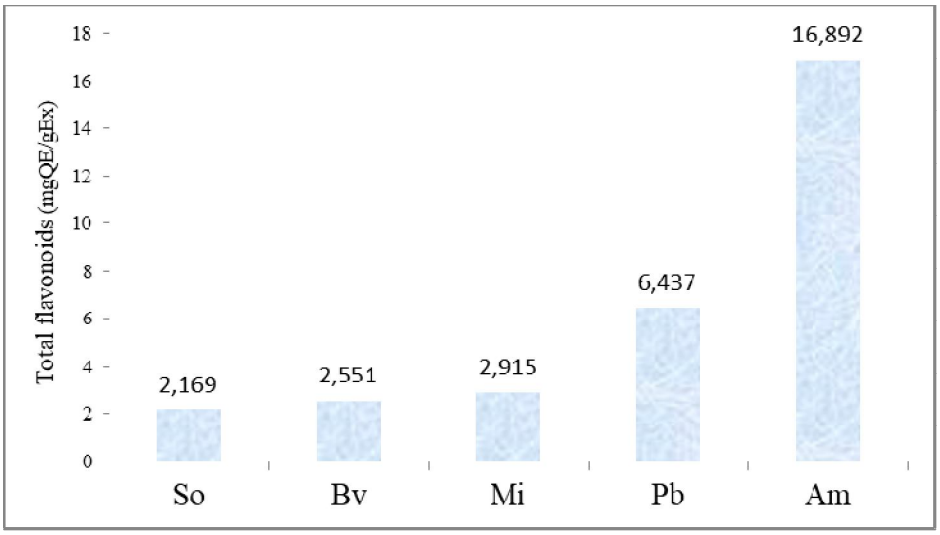
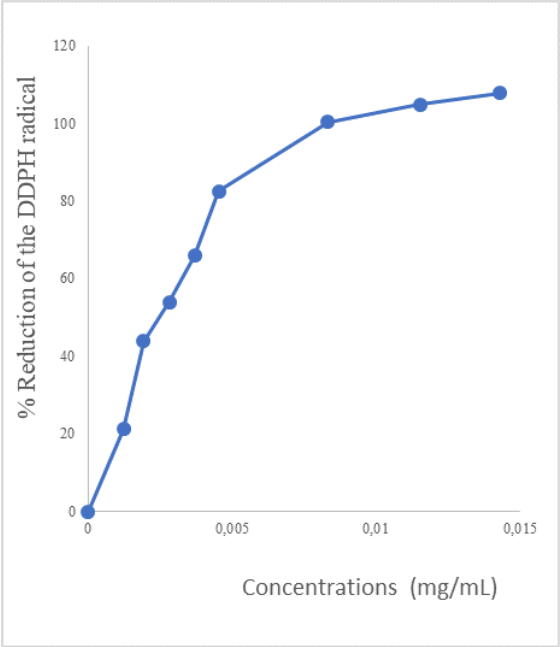
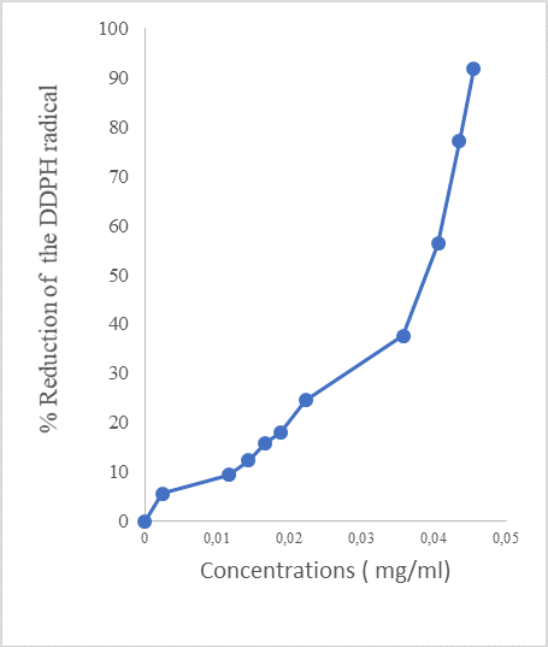


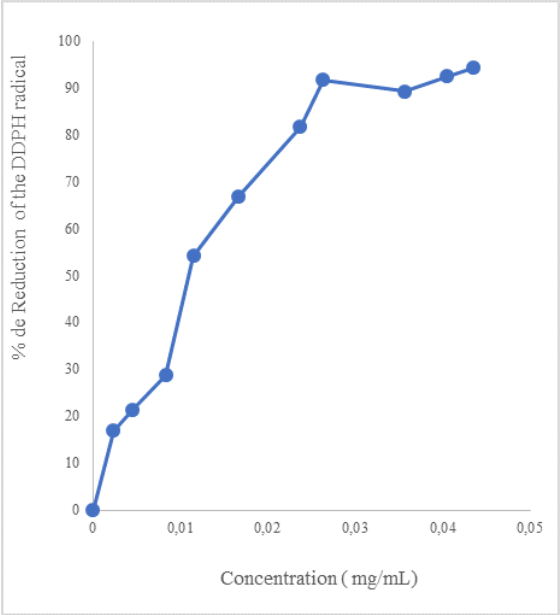
Figure3: Total flavonoids content of hydroethanolic plant extract

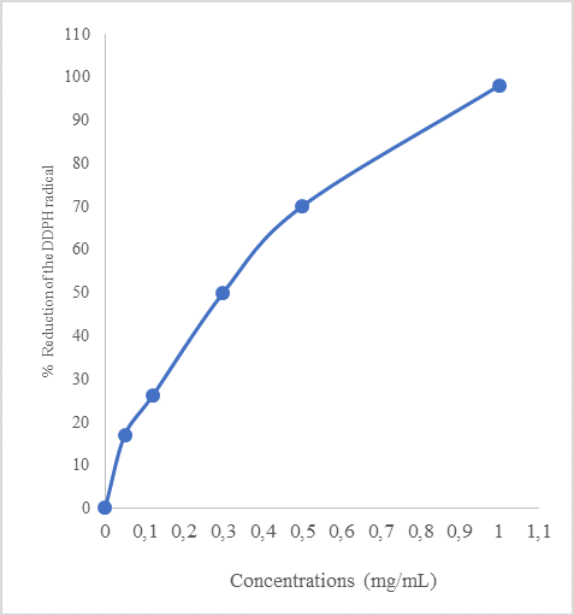
Legends: *Bv: Bambusa vulgaris ; Pb: Parkia biglobosa ; Mi: Mangifera indica; So :Saccharum officinarum; Am: Annona muricata*



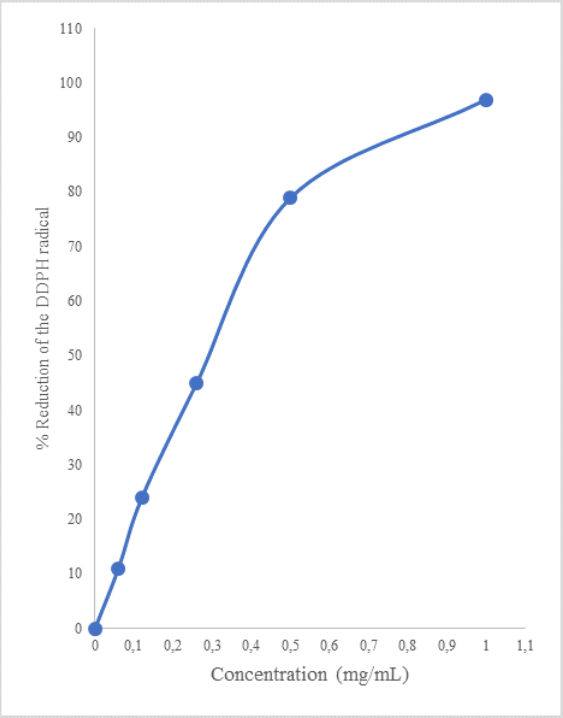


MangiferaindicaSaccharumofficinarum





AnnonamuricataParkiaviglobosa



Bambusa vulgaris-italic

Figure 4: DPPH radical scavenging rate as a function of the concentration of the hydroethanolic extract of plants

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