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 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-l-picrylhydrazyl (DPPH). **Results**: Plants whose leaves are higher in total phenols are *Mangifera indica, Parkia biglobosa and Bambusa* vulgaris while *Annona muricata* and *Parkia biglobosa* are the richest plants in flavonoids. The hydroethanolic extract of leaves of *Bambusa vulgaris* (IC_{50}=0.28mg/mL), *Parkia biglobosa* (IC_{50}=0.3mg/mL) and *Mangifera indica* (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 *Mangifera indica, Parkia biglobosa and Bambusa 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.

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, 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 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, Parkia biglobosa, Mangifera indica, Saccharum officinarum et Annona muricata,* 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. 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

III RESULTS AND DISCUSSION

III.1 Result

III.1-1 Secondary metabolites identified

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

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*, *Parkia biglobosa*, *Mangifera indica*, *Saccharum officinarum* and *Annona muricata* are 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

Figure 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 is 16.892 µgQE/gEx while that of *Parkia biglobosa* is 6.437µgQE/gEx. The flavonoid contents of *Mangifera indica*, *Saccharum officinarum* 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 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_{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, Parkia biglobosa* and Mangifera indica are 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 (IC50=3.20 mg/mL) which is a synthetic compound. The concentration of the hydroethanolic extract of *Annona muricata* is 11.00 mg/mL and 39.00 mg/mL.

III.2 DISCUSSION

The leaves of *Bambusa vulgaris*, *Parkia biglobosa, Mangifera indica*, *Saccharum officinarum* and *Annona muricata* 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 leuco anthocyanins which are absent in our sample.[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 *Parkia biglobosa* harvested in Nigeria, the absence of the sterols and terpenes are noted whereas that are 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 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 saponins possess antidiabetic activities.[25-26]

Our results showed that the leaves of *Mangifera indica*, *Parkia Biglobosa and Bambusa vulgaris* are the richest in total phenols while *Annona muricata* and *Saccharum officinarum* 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, Parkia biglobosa, Mangifera indica, Saccharum officinarum* and *Annona muricata*), the hydroethanolic extract of its leaves showed interesting activity. It should be noted that the hydroethanolic leaf extract of *Bambusa vulgaris, Parkia biglobosa and Mangifera indica* 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 *Annona muricata* 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, 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 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 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* 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*

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_{50} of the hydroethanolic plant extract.

Hydroethanolic extract	**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

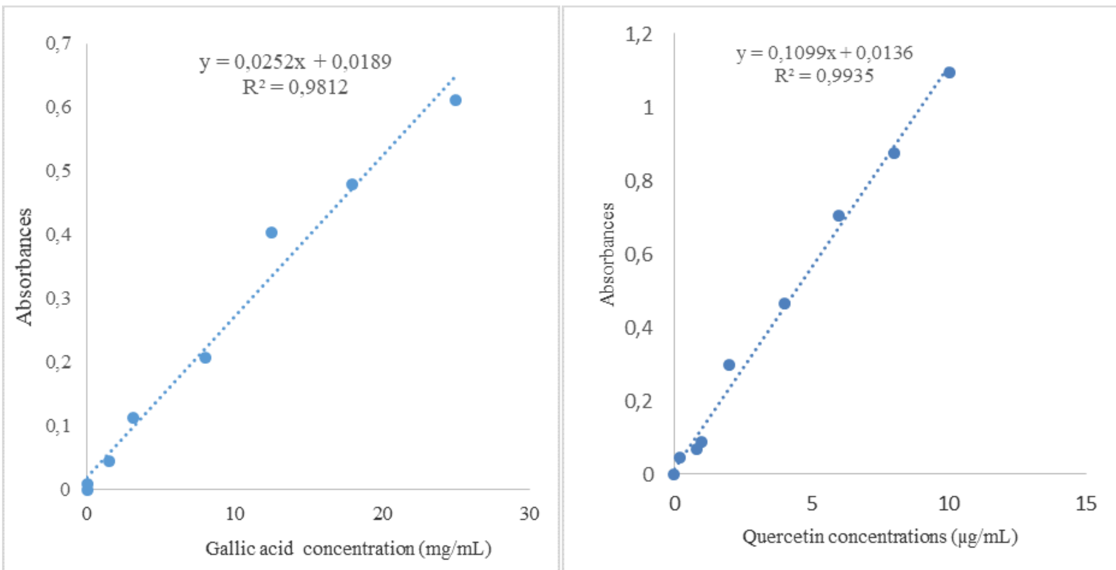


Figure 1: Calibration curves for the evaluation of the levels of phenolic compounds

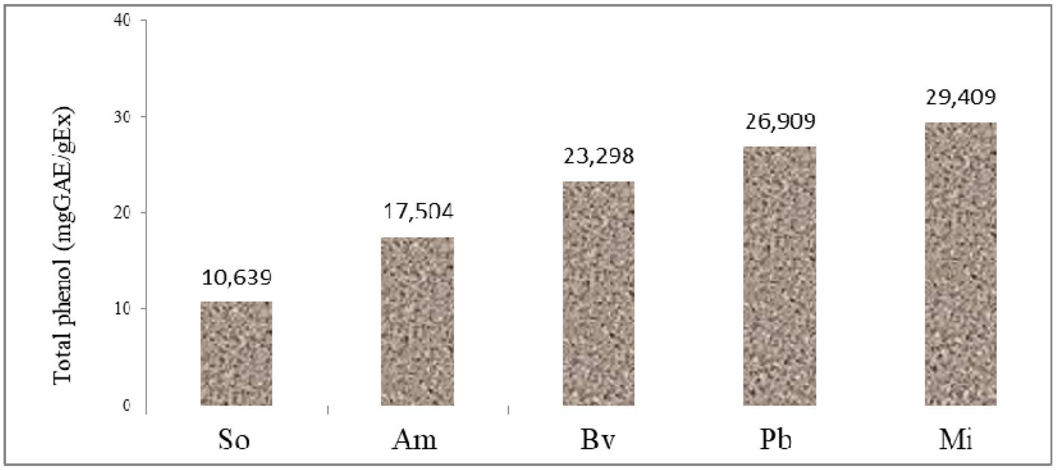


Figure 2: Total phenol content of hydroethanolic plant extract

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

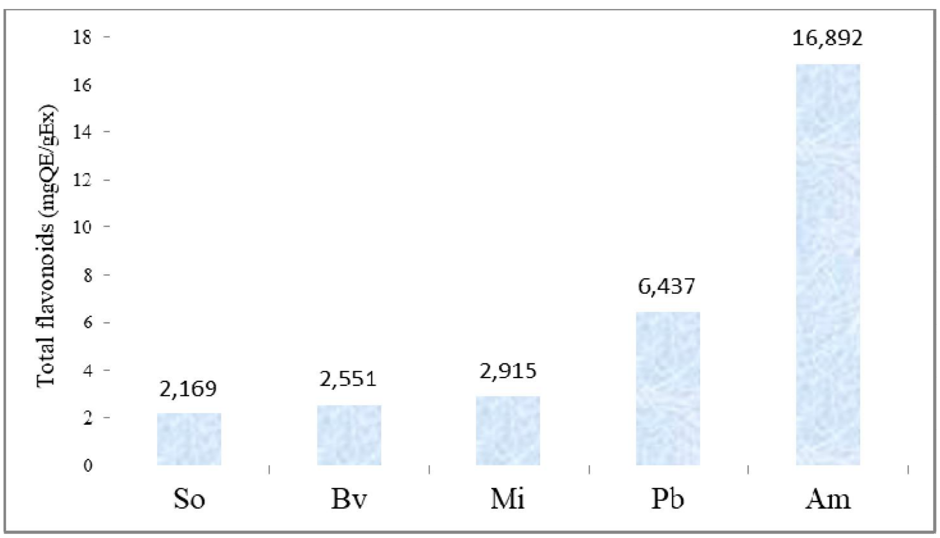
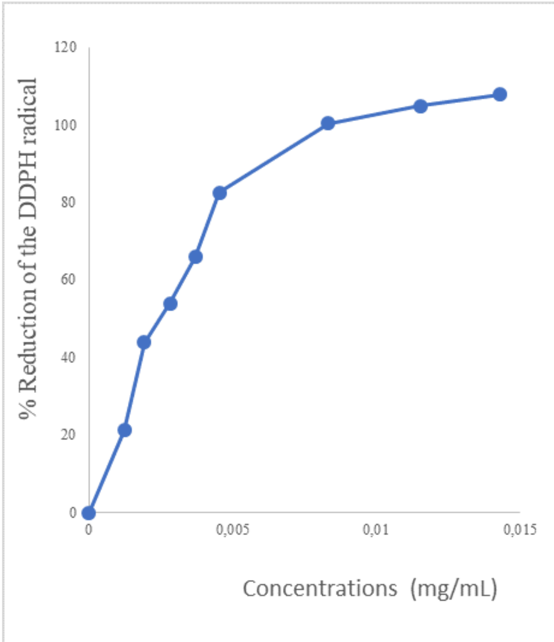
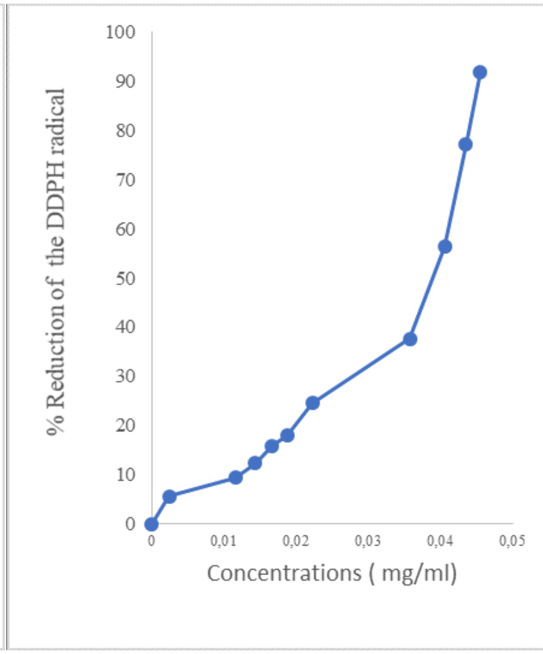


Figure 3: Total flavonoids content of hydroethanolic plant extract

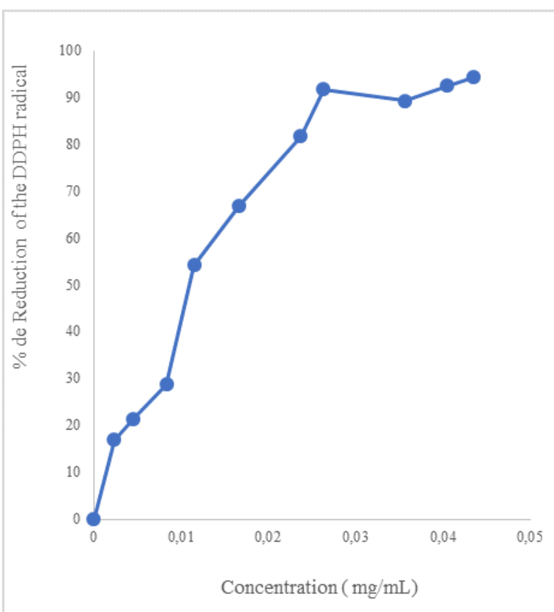
Legends: *Bv: Bambusa vulgaris ; Pb: Parkia biglobosa ; Mi: Mangifera indica; So :Saccharum officinarum; Am: Annona muricata*



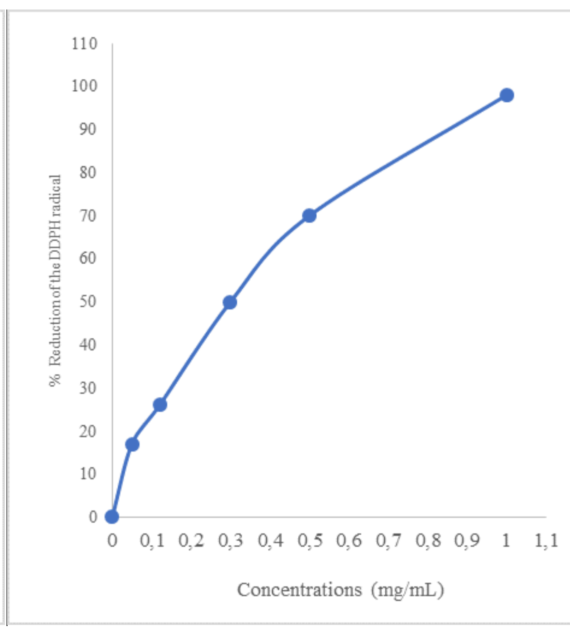
Mangifera indica



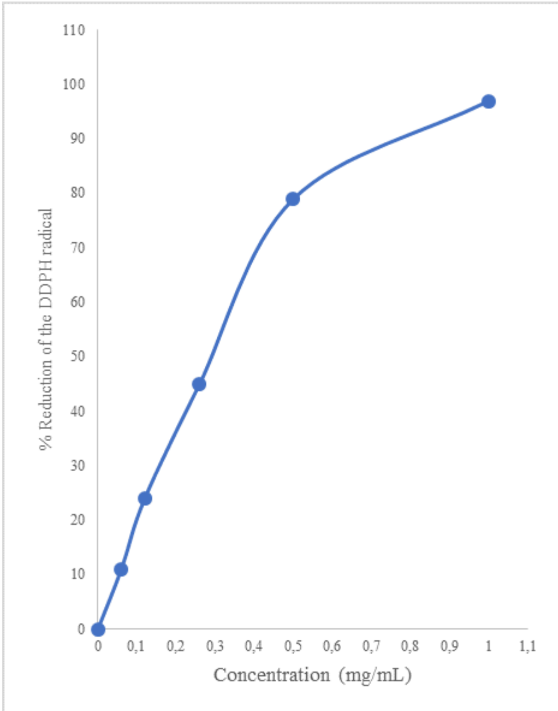
Saccharum officinarum



Annona muricata



Parkia viglobosa



Bambusa vulgaris

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

REFERENCES

1. Fédération Internationale du Diabète (FID/IDF). ATLAS du diabète 8e édition 2017 www.idf.org/sites/default /files/EN_6E_Atlas_Full_0. pdf.pdf
2. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4*4 million participants. Lancet 2016; published online April 7. http://dx.doi.org/10.1016/S0140-6736(16)00618-8.
3. Organisation Mondiale de la Santé (OMS). Rapport mondial sur le diabète. 2016, 88p ;
4. Houngla MFN. Pratiques alimentaires et gestion du diabète chez les diabétiques suivis au Centre National Hospitalier et Universitaire et à la Banque d'insuline de Cotonou au Bénin. Mémoire présenté à la faculté de médecine en vue de l'obtention du grade de maîtrise en nutrition. Université de Montréal. Département de nutrition Faculté de médecine, (2020).

5. Ministère de la santé du Bénin. Programme National de Lutte contre les Maladies Non Transmissibles (PNLMNT), Rapport final de l'enquête pour la surveillance des facteurs de risque des maladies non transmissibles par l'approche « STEPSwise» de l'OMS, 2015.
6. Bonnefont-Rousselot DJL, Beaudeux P, Thérond J, Peynet A, Le grand J, Delattre. Diabète sucré, stress oxydant et produits de glycation avancée. Annales Pharmaceutiques Françaises, 2004, 62(3), pp : 147-157
7. Berger MM, Manipulations nutritionnelles du stress oxydant : état des connaissances. Nutrition Clinique et Métabolisme, 2006, 20(1), 48-53.
8. Dagnoko S. Étude de la qualité des feuilles de Sclerocarya birrea (A. Rich) Hoscht. Utilisées dans le traitement du diabète. Thèse de Médecine, de Pharmacie et d'Odonto-Stomatologie. Université de Bamako, 2009.
9. Jayakumar G, Ajithabai MD, Sreedevi S, Viswanathan PK, Remeshkumar. Ethnobotanical survey of the plangts used in the treatment of diabetes. Indian Journal of Traditional Knowledge, 2010, 9(1);100-104;
10. Fah L, Klotoé JR, Dougnon V, Koudokpon, Fanou VB, Dandjesso C. Étude ethnobotanique des plantes utilisées dans le traitement du diabète chez les femmes enceintes à Cotonou et Abomey-Calavi (Bénin). Journal of Animal & Plant Sciences. 2013,18(1), pp: 2647-2658;
11. Seton S, Koukoui O, Ewedje E-EBK, Agbo KM, Betira. M, Amagbegnon JB. Ethnopharmacological study of phytomedicines used by traditional healers for the treatment of diabetes and hypertension in some municipalities of Benin, J. Rech. Sci. Univ. Lomé (Togo), 2021, 23(4): 273-284.
12. Houghton P.J., Raman A. Laboratory Handbook for the Fractionation of Natural Extracts. Chapman and Hall, New York, 1998, 5: 103-108.
13. Dohou R, Yamni K, Tahrouch S, Hassani L I, Badoc A, Gmira N. Screening phytochimique d'une endémique iberomarocaine, Thymelaea lythroides. Bulletin-Société de Pharmacie de Bordeaux, 2003, 142(1/4): 61-78.
14. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999; 299: 152-178.
15. Koudoro YA, Daye ER, Dassou HG, Atindehou M, Agbangnan DCP, Alitonou GA. Phytochemical screening, antioxidant capacity, antibacterial and anti-inflammatory

activities of ethanolic extract of Cordia senegalensis leaves, a plant used in Benin to treat skin diseases. Chemistry Research Journal. 2021 ; 6(2): 137-146 ;

16. Djeridane A, Yous M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem. 2006; 97: 654-660.
17. Brand-Willams W., Cuvelier M.E., Beret C. ‘’Use of a free radical method to evaluate antioxidant activity, Lebensm.Wiss.U. Technol, 1995, 28, pp. 25-30;
18. Koudoro YA, Mahudro Y, Yehouenou B, Agbangnan DCP, Tchobo FP, Alitonou GA, et al. Chemical study, antiradical and antibacterial potential of the extracts of Ximenia americana and Cussonia arborea of Benin, World Journal of Pharmaceutical Sciences 2014, ISSN (Print): 2321-3310; ISSN (Online): 2321-3086.
19. Hessavi BFM., Arlette A, Aimé A, Micheline A, Djikpo T. Investigation ethnobotanique, profil phytochimique et cytotoxicité de Bambusa vulgaris Schrad. Ex J.C. Wendl. (Poacee), une espèce à usages multiples et sous-utilisée au Bénin Journal of Animal & Plant Sciences, 2019, 39(2): 6435-6453;
20. Appy SA, Kouao AA, Kacou MJ D and Kesse PN. Phytochemical and acute toxicity study of aqueous extract of Bambusa vulgaris leaves on Wistar rats. African Journal of Biological Sciences, 2020, Page 107 of 114.
21. Osuala FN, Kehinde SF and Philippe PEM. Pharmacognostic and antidysentery screening of mixed ethanol leaf extract of Parkia biglobosa and Acanthus montanus (50:50). Magna Scientia Advanced Research and Reviews, 2021, 03(02), p: 017–039;
22. Yang C, Gundala SR, Mukkavilli R, Vangala S, Reid MD, Aneja R. Synergistic interactions among flavonoids and acetogenins in graviola (Annona muricata) leaves confer protection against prostate cancer. Carcinogenesis, 2015. 36(6): 656-665;
23. Jhaumeer SL, Bhowon MG, Soyfoo S, and Chua LS. Nutritional and Biological Evaluation of Leaves of Mangifera indica from Mauritius, Journal of Chemistry Volume 2018, Article ID 6869294, 9 pages https://doi.org/10.1155/2018/6869294.
24. Daddona PE, wright JL, Hutchinson CR. Alkaloid catabolism and mobilization in Catharanthusroseus. Phytochem, 1976, 15 : 941-945 ;
25. Manolaraki F. Propriétés anthelmintiques du sainfoin (Onobrychisviciifoliae) : Analyse des facteurs de variations et du rôle des composés phénoliques impliqués. Thèse de Doctorat de l’université de Toulouse III, 2011.

26. Owolabi OJ, Ayinde BA, Iwogu ZA, Ogbonna OO. Properties evaluation of the ethanol extract of Musanga cecropioides. Methods and findings in experimental and clinical pharmacology, 2010, 32 (6): 407-411 ;
27. Mangano S, Williamson G. Polyphenols and phenolic acid from decrease glucose uptake and transport by human intestinal caco-2 cells. Molecular nutrition and food research, 2010, 54 (12): 1773-1780.
28. Cano-Marquina A, Tarim JJ, Cano A. The impact of coffee on health. Pub Med, 2013 75 (1): 7-21.
29. Aouissa Itiann., Wen Rehaba. Etude des activités biologiques et de la Toxicité aiguë de l’extrait aqueux des Feuilles de mangifera indica. (anacardiaceae). Universite de Bamako, Faculté de Médecine de Pharmacie et d’Odonto-Stomatologie, 2002.
30. Nawwar M, Ayoub N, Hussein S, Hashim A, El-Sharawy R, Wende K, Harms M, Lindequist U. Flavonol triglycoside and investigation of the antioxidant and cell stimulating activities of Annona muricata linn. Arch Pharm Res. 2012; 35:761-767.
31. Millogo-Kone H, Guissou IP, Nacoulma O, Traore AS. Comparative study of leaf and stem bark extracts of Parkia biglobosa against enterobacteria, African Journal of Traditional, Complementary Alternative Medicines, 2008, 5, 238-243.
32. Kaou SF. Etude de la phytochimie et des activités biologiques de Musa Acuminata L., de Mangifera Indica L., de Boerhavia Erecta L. et de Eclipta Prostrata L. rapport de mémoire de thèse, 2012.
33. Akibou OML, Pascal ADC, Annick BH, Paul Y, Félicien A, And. Dominique KCS. Activités antiradicalaires et étude des composés volatils de trois plantes de la médecine traditionnelle du Bénin : Anchomanes difformis, Parkia biglobosa et Polyalthia longifolia, Journal of Innovation and Applied Studies, 2014, 9(4), pp. 1609-1619.
34. Betira Mansouratou, Koukoui Omédine*, Houngbeme Alban, Seton Santorin, Amagbegnon Jean-Baptiste, Sonounameto Fidèle and Sezan Alphonse. Combination of the Leaves of Annona muricata, Launaea taraxacifolia and Tridax procumbens: An Herbal Medicine for the Prevention and Treatment of Hypertension, Atherosclerosis and Cardiovascular Diseases. Acta Scientific Pharmaceutical Sciences, 2021, 5(10): 2-8
35. Francesca Santilli., et al. Oxidative stress in chronic vascular disease: From prediction to prevention. Vascular Pharmacology, 2015 (74): 23-37.

36. Khurana S., et al. Oxidative stress and cardiovascular health: therapeutic potential of polyphenols". Canadian Journal of Physiology and Pharmacology, 2013 (91): 198-212.

37. Haber, C.A., Lam, T.K., Yu, Z., et al. N-acetylcysteine and Taurine Prevent Hyperglycemia-Induced Insulin Resistance in Vivo: Possible Role of Oxidative Stress. The American Journal of Physiology Endocrinology and Metabolism, 2003, (285): 744-753.

38. Scott, J.A. and King, G.L. Oxidative Stress and Antioxidant Treatment in Diabetes. Annals of the New York Academy of Sciences, (2004), (1031): 204-213