



Antidiabetic, Antisickling and Antibacterial Activities of *Anacardium occidentale* L. (Anacardiaceae) and *Zanthoxylum rubescens* Planch. Ex Hook (Rutaceae) from DRC

Gédéon Bongo¹, Koto-te-Nyiwa Ngbolua^{1,2,3,*}, Colette Ashande², Benjamin Gbolo¹, Claudine Tshiama⁵, Dorothée Tshilanda⁴, Damien Tshibangu⁴, Nadège Ngombe⁶, Théophile Mbemba¹, Pius Mpiana⁴

¹Department of Biology, University de Kinshasa, Kinshasa, Democratic Republic of the Congo

²Department of Environmental Sciences, University of Gbadolite, Nord Ubangi, Democratic Republic of the Congo

³Higher Pedagogical Institute of Abumombazi, Nord Ubangi, Democratic Republic of the Congo

⁴Department of Chemistry, University of Kinshasa, Kinshasa, Democratic Republic of the Congo

⁵Department of Teaching and Administration in Nursing Care, Higher Institute of Medical Techniques, Kinshasa, Democratic Republic of the Congo

⁶Faculty of Pharmaceutical Sciences, University of Kinshasa, Kinshasa, Democratic Republic of the Congo

Email address:

jpngbolua@unikin.ac.cd (Koto-te-Nyiwa N.)

*Corresponding author

To cite this article:

Gédéon Bongo, Koto-te-Nyiwa Ngbolua, Colette Ashande, Benjamin Gbolo, Claudine Tshiama, Dorothée Tshilanda, Damien Tshibangu, Nadège Ngombe, Théophile Mbemba, Pius Mpiana. Antidiabetic, Antisickling and Antibacterial Activities of *Anacardium occidentale* L. (Anacardiaceae) and *Zanthoxylum rubescens* Planch. Ex Hook (Rutaceae) from DRC. *International Journal of Diabetes and Endocrinology*. Vol. 3, No. 1, 2018, pp. 7-14. doi: 10.11648/j.ijde.20180301.12

Received: February 22, 2018; Accepted: March 27, 2018; Published: May 4, 2018

Abstract: It was recently reported a rare association of two genetic diseases notably sickle cell anemia and diabetes in one patient in the Democratic Republic of the Congo. Both diseases constitute a serious public health problem and have a common denominator that is to make patients susceptible to bacterial infections. Given the difficult and limited management of these diseases, the use of medicinal plants is considered as an effective alternative. Leaves of *Anacardium occidentale* and *Zanthoxylum rubescens* collected in the surroundings of University of Kinshasa and Gbadolite in Kinshasa and Nord Ubangi provinces respectively and these plants were selected through a chemo-taxonomic approach while a phytochemical screening was performed using a qualitative approach. Bacterial strains used to assess the antibacterial activity were *Staphylococcus aureus* and *Escherichia coli* and mice were used for the antidiabetic activity. The phytochemical screening revealed the presence of tannins, anthocyanins, leucoanthocyanins, flavonoids, bound quinones as well saponins and alkaloids. The aqueous extracts of *A. occidentale* and *Z. rubescens* showed an antisickling activity. Only *S. aureus* was sensitive to *A. occidentale* where petroleum ether extract (MIC = 125 µg.mL⁻¹) showed a good activity than other extracts and no activity was observed on *E. coli*. Meanwhile, *Z. rubescens* showed no antibacterial activity on both strains (MIC = > 500 µg.mL⁻¹). The mean values of blood glucose after 120 minutes in untreated and treated mice were 99.5 ± 7.77mg.dL⁻¹ (0.9% NaCl), 41.6 ± 10.07mg.dL⁻¹ (Glibenclamide 10mg.Kg⁻¹) and 64 ± 13.98mg.dL⁻¹ (methanolic extract of *A. occidentale* 500mg.Kg⁻¹). These findings show that *A. occidentale* plant possess an antihyperglycemic activity. To our knowledge, it is for the first time that the antisickling activity of *A. occidentale* and *Z. rubescens* is reported thus validating the chemotaxonomic approach used as a criterion of selection of these two plants. It is also for the first time that antidiabetic activity of *A. occidentale* is reported.

Keywords: Co-Morbidity, Sickle Cell Anemia, Diabetes, Chemo-Taxonomic Approach

1. Introduction

It was recently reported a rare association of two genetic diseases notably sickle cell anemia and diabetes in one patient in the Democratic Republic of the Congo (DRC). Both diseases constitute a serious public health problem worldwide as well in DRC [1]. Sickle cell anemia is characterized by the presence of hemoglobin S in the blood and its clinical manifestations are vaso-occlusion and hemolytic anemia [2]. On the other hand, Diabetes is characterized by a hyperglycemia (sugar level $> 1.2 \text{ g.dL}^{-1}$ of fasting), a hyperketonemia (keto acid level $> 3 \text{ mmol.L}^{-1}$), acid-base and hydro-electrolytic imbalance [3]. Considering the difficult and limited management of sickle cell disease and diabetes in the DRC, the use of traditional medicine and medicinal plants can be as an effective alternative in the management of these two diseases in emergency situations.

The World Health Organization (WHO) reported that more than 80% of African populations in poor regions use medicinal plants for their primary care [4, 5]. Several scientific studies carried out both in DRC and elsewhere highlight the antisickling and antidiabetic properties of medicinal plants as well as their antimicrobial activity [6-9]. It has to be noted that *Anacardium occidentale* used in the current study is well known to traditional healers for its antidiabetic potential. [10], while some species of the genus *Zanthoxylum* are cited in the literature as having an antisickling potential but both plants possess as well the antibacterial properties [11-13].

The main goal of the current research was to verify whether *A. occidentale* and *Z. rubescens* possess antisickling, antibacterial and anti-hyperglycemic activities. To this end, it should be expected that a plant having an antisickling property has also an antidiabetic property i.e. biochemically these two diseases lead to a disruption of glucose metabolism. Moreover, it is well established that the sickling of disease red blood cells is an inhibitory factor of glycolysis (inhibition of key enzymes by tactoids). The non-sequestered hemoglobin (Hb) fraction can then undergo non-enzymatic glycation to form glycated Hb generating oxygen free radicals [14]. Glycated Hb is a biomarker of diabetes while the formation of glycated Hb depends upon ambient glucose concentrations in which erythrocytes circulate as well the duration of exposure. A whole blood sample for glycated Hb is sufficient regardless of prandial state and clinical setting [3]. However, both diseases share the common denominator which is to make patients susceptible to infections [2, 15]. In addition, it should be expected that *Z. rubescens* possess an antisickling activity using a chemo-taxonomic approach. The staple objective of this research was to provide a scientific evidence that the ethnomedical use of these plants (*A. occidentale* and *Z. rubescens*) is based thru phytochemical screening and the assessment of their antisickling, antibacterial and antidiabetic activities of these plant extracts.

The significance of the current research is to promote the use of medicinal plants in the treatment of sickle cell disease

and diabetes in countries with limited health care. To our knowledge, it is for the first time that the antisickling activity of *A. occidentale* and *Z. rubescens* as well the validation of the hypothesis according to which an antidiabetic plant could potentially be antisickling is reported.

2. Materials and Methods

2.1. Materials

Plant materials used in the current study were leaves of *A. occidentale* L. (Anacardiaceae) and *Z. rubescens* PLANCH (Rutaceae) collected in the surroundings of University of Kinshasa in Kinshasa and Gbadolite in Nord Ubangi provinces respectively. *A. occidentale* was identified at the Herbarium of Faculty of Sciences, University of Kinshasa while *Z. rubescens* was identified at "Centre de Surveillance de la Biodiversité", University of Kisangani in Kisangani city. Blood samples used to assess the antisickling activity of plant extracts were collected from a sickle cell adolescent patient from the "Centre de Médecine Mixte et d'Anémie SS" of Kinshasa. Bacterial strains used were provided by the Laboratory of Microbiology, Faculty of Pharmaceutical Sciences (University of Kinshasa) and mice used for the assessment of the antidiabetic activity were provided by the Institut National des Recherches Biomédicales (INRB), Kinshasa, DRC.

2.2. Methods

2.2.1. Collection and Conditioning of Plant Material

Plant samples were collected in 2014 in Kinshasa and Gbadolite respectively. After collection, leaves were washed with tap water and dried under shade for one month, and then crushed separately in a grinder (Moulinex brand). The powders obtained were sieved in order to obtain fine powders for further analyses.

2.2.2. Extraction with Solvent of Increasing Polarity

Fifty grams of powder of each species were macerated in petroleum ether, ethyl acetate, and methanol (1:10, w/v) respectively for 48 hours. After filtration, various extracts were evaporated to dryness using a rotary evaporator device at 37°C.

2.2.3. Phytochemical Screening

The phytochemical screening is a chemical screening that includes a number of qualitative analysis that allows the identification of secondary metabolites present in a certain sample. The detection of these chemical groups is performed through color and precipitation reactions occurring with the addition of specific reagents [15-18]. The phytochemical screening was carried out according to the standard protocol as described by Bongo *et al.* [18] and it can be performed in aqueous or organic phases [19].

2.2.4. Biological Experiments

(i) Emmel Test

The hemoglobin S becomes insoluble and crystallizes

when the oxygen content decreases (hypoxia); it follows that, in case of sickle cell disease, the erythrocytes or red corpuscles containing hemoglobin S lose their normal circular shape, to assume a characteristic sickle shape. The evaluation of antisickling activity consists in seeing the effect of the drug on the sickling of red blood cells.

Four to eight drops of saline solution were used for the dilution of total SS blood (NaCl 0.9%) and on a slide was placed a drop of diluted blood, a drop of the drug/extract along with a drop of Na₂S₂O₅ 2%. The mixture obtained constitutes the microscopic preparation covered with a coverslip which is super-cooled with paraffin on the edges of the slide. The solutions of our extracts (petroleum ether, ethyl acetate and methanol) were prepared by dissolving a few mg of these extracts into the saline solution. Different preparations obtained were observed using an optical microscope (brand Olympus) at 10X and 40X magnifications after 24 hours [20-23].

(ii) Antibacterial Assay

The antibacterial activity was evaluated using the microdilution method in a liquid medium.

(iii) Preparation of the Stock Solution and Bacterial Suspension

In a sterile test tube, place 0.020 g of each extract diluted in 250 μ L of DMSO then stir for 10 minutes and add five mL of Mueller Hinton culture medium using a pipette and mix.

Afterwards, place two mL of the saline solution into two sterile test tubes. Using a sterile platinum loop, two isolated colonies of the two strains to be tested namely *Escherichia coli* ATCC 27195 and *Staphylococcus aureus* ATCC 33591 were collected and a colony of each strain was placed in the saline solution into both tubes. This bacterial suspension was diluted in the appropriate culture medium at a rate of 9 mL.

(iv) Dilution of Extracts and Inoculation of the Microplate

A 96-well sterile polystyrene microplate (8 rows A-H. x 12 columns) with a round bottom was used, whereby each well was filled with 100 μ L of culture medium as follows: from A₂ to A₈, B₂ to B₈, C₂ to C₈, D₂ to D₈, E₂ to E₈ and F₂ to F₈ while the 11th and 12th wells served as controls. Using a micropipette, 200 μ L of the stock solution of extract 1 was inoculated in A₁ and B₁ wells, 200 μ L of the stock solution of extract 2 in C₁ and D₁ wells while 200 μ L of the stock solution of extract 3 in E₁ and F₁ (*i.e.* 2 wells were used for each extract) respectively. Meanwhile, 100 μ L of each stock or control solution were collected and serial dilutions of 2 by 2 were performed afterwards. Moreover, 100 μ L of A₁B₁, C₁D₁ and E₁F₁ wells were collected and transferred to A₃B₃, C₃D₃ and E₃F₃ wells, then from the previous wells 100 μ L were collected and transferred to A₄B₄, C₄D₄, E₄F₄ wells. These solutions were thoroughly mixed and the same procedure continued till we reached A₈B₈, C₈D₈ and E₈F₈ wells. The last 100 μ L taken from these wells were removed and thrown away [24-25].

Aseptically, 15 μ L of the inoculum was collected and transferred to all wells of the microplate except for the wells of the 11th column (control of sterility of culture medium). Wells

of the 12th column served as a positive control (inoculum and culture medium). The microplate was incubated in an oven at 37°C for 24 hours. Later, five μ L of TCC 2% was added to each well and the observation occurred 15 min later.

(v) Determination of the Minimum Inhibitory Concentration

The principle of this method is based on the ability of living cells to reduce the tetrazolium salt to a red precipitate or formazan. The growth observed in different wells containing extracts or controls were compared to that in the bacterial growth control well (well-containing inoculum without extracts or antibiotics). For a test to be considered valid, an acceptable growth has to be observed in the control wells. If the growth is insufficient in these wells, there is a need to re-inoculate the microplate and after the addition of 5 μ L of TCC 2% (2, 3, 5-triphenyltetrazoliumchloride) to the control wells then the minimum inhibitory concentration can be read. The minimum inhibitory concentration was then read from the first wells showing no bacterial growth after 48 hours [24-25].

(vi) Oral Glucose Tolerance Test

The assessment of the antidiabetic activity was carried out using an experimental design on an animal model consisting of 10 NMRI mice (male and female) subjected to temporary hyperglycemia by gavage of a glucose solution (200mg.mL⁻¹) as per the protocol described by Williamson *et al.* [26]. These 10 mice were divided into three groups as follows: a first group of two mice as a negative control (saline solution), a second group of three mice as a positive control (Glibenclamide 10mg.Kg⁻¹) and the third group of five mice to be tested with the ethyl acetate extract (500mg.Kg⁻¹). Blood glucose testing was performed using a contour TS blood glucose meter from the tail.

The animal was immobilized by keeping its head raised and its mouth opened where a syringe loaded with the product along with the gastro-esophageal probe was introduced to the stomach, then by pushing the plunger of the syringe, the drug was administered to the animal as shown in figure 1 below. Before the administration of the drug, the extract was resolubilized in an appropriated solvent. For lipophilic extracts, the detergent such as DMSO was used.



Figure 1. Oral administration of extracts to mice.

(vii) Testing of Normal Blood Sugar

Mice were given a 24-hour pre-feed and then administered the extracts. Blood glucose was measured at T₀, T₁, T₂, T₃, and T₄.

(viii) Anti-Hyperglycemic Activity

In this research, a temporary hyperglycemia was caused in mice by oral administration of glucose (diluted to 10% in distilled water) at a dose of 4 g.Kg⁻¹ of body weight and the basic blood glucose of mice was first detected. The basal glucose level of mice was determined after 24 hours of fasting (Baseline) and then glucose was administered to the mice. After 30 minutes of glucose overload, the blood glucose was determined in order to note the temporary hyperglycemia (transient hyperglycemia should reach the maximal value 30 min after administration of glucose). And then three batches of mice according to sex, weight and especially temporary hyperglycemia were formed. Different batches of mice were treated as follows: A control batch treated with a saline solution at a dose of 0.9%. A reference batch treated with Glibenclamide at 10mg.Kg⁻¹ of body weight and a test batch treated with ethyl acetate extract at a dose of 500 mg.Kg⁻¹ of body weight.

3. Results

3.1. Phytochemical Screening

The phytochemical screening of the aqueous phase of *A. occidentale* and of *Z. rubescens* leaves is presented in table 1.

Table 1. Phytochemical screening of selected plants.

Phytochemical groups	Plant extracts	
	<i>A. occidentale</i>	<i>Z. rubescens</i>
Total polyphenols	+	+
Anthocyanins	+	+
Leucoanthocyanins	+	+
Bound quinones	+	+
Tannins	+	+
Flavonoids	+	+
Alkaloids	+	+
Saponins	+	+

Legend:- absence of the researched compound, +: presence of the researched compound.

As shown in the above table, the leaves of *A. occidentale* and *Z. rubescens* are enriched in polyphenols namely tannins, anthocyanins (phytochemical markers of the antisickling activity), leucoanthocyanins, flavonoids, bound quinones as well saponins and alkaloids. The presence of these secondary metabolites could confer on both plants the antisickling, antibacterial and antidiabetic properties.

3.2. Bioactivity of Our Extracts

3.2.1. Antisickling Activity

The phenotype of untreated (a) and treated (b) sickle cells respectively is presented in Figure 2 below.

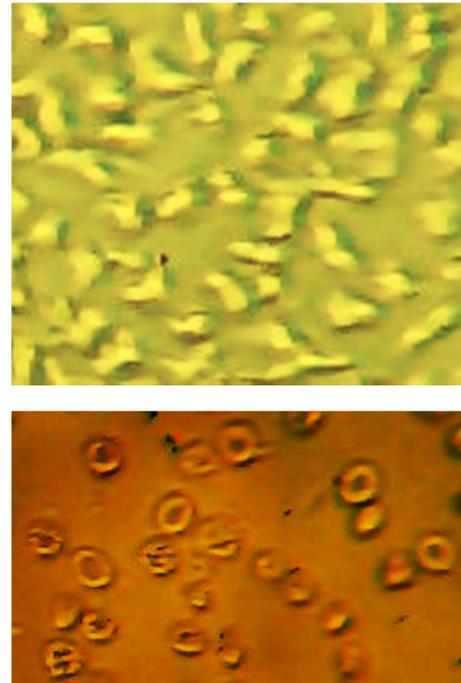


Figure 2. A, Optical micrographic of untreated SS blood; B, red blood cells treated with petroleum extract of *A. occidentale* (50 µg.mL⁻¹) [NaCl 0.9%; Na₂S₂O₅ 2%, X500].

The rate of normalization of red blood cells of our patient in presence of both plant extracts under hypoxic conditions (NaCl 0.9%; Na₂S₂O₅ 2%) is presented in the table below.

Table 2. Rate of normalization of sickled cells using Emmel test in presence of extracts.

Drugs/Extracts	Normalization rate	
	<i>A. occidentale</i>	<i>Z. rubescens</i>
Petroleum ether	+	++
Ethyl acetate	+++	+++
Methanol	+++	+++

Legend: -, Inactive; +, 10 < RN < 50% (low activity); ++, 50 < RN < 70% (higher activity); +++, RN > 70% (the highest activity)

Table 2 shows that in the presence of organic extracts (petroleum ether, ethyl acetate and methanol) of *A. occidentale* and *Z. rubescens*, the sickle cells are normalized under conditions of hypoxia. This normalization of SS erythrocytes in hypoxia conditions is a partial scientific evidence which can justify the integration of these two plants on the list of antisickling plants. The normalization of SS blood erythrocytes treated with extracts from these plants results in the reappearance of the circular form of sickle cells. These results are therefore in perfect agreement with those of previous studies [27]. However, it should be noted that the extract with petroleum ether of *A. occidentale* has shown a weak antisickling activity with a normalization rate between 10 and 50%.

3.2.2. Antibacterial Activity

The antibacterial activity of our plant extracts is presented in tables 3 and 4 below.

Table 3. Effects of *A. occidentale* on the bacterial growth in vitro using microdilution method with TCC dye 2%.

Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)	<i>E. coli</i> ATCC 27195			<i>S. aureus</i> ATCC 33591		
	EEP	EAE	MeOH	EPP	EAE	MeOH
4000	-	-	-	-	-	-
2000	-	-	-	-	-	-
1000	+	-	+	-	-	-
500	+	-	+	-	-	-
250	+	+	+	-	+	-
125	+	+	+	-	+	+
62.5	+	+	+	+	+	+
MIC	>1000	500	>1000	125	500	250

Note: +, bacterial growth (appearance of red color, conversion of colorless TCC to red formazan); -, Absence of visible growth (the staining of the well is that of the extract); MIC, Minimum inhibitory concentration; ATCC, American Type Culture Collection; EEP, Petroleum ether extract; EAE, Ethyl acetate extract; MeOH, Methanolic extract.

As shown in the above table, *A. occidentale* extracts displayed no activity against *E. coli* ATCC 27195 but active against *S. aureus*. The best activity is obtained with the petroleum ether extract (EEP, MIC = 125 $\mu\text{g}\cdot\text{mL}^{-1}$) followed by methanol (MeOH, MIC = 250 $\mu\text{g}\cdot\text{mL}^{-1}$) and ethyl acetate (EAE, MIC = 500 $\mu\text{g}\cdot\text{mL}^{-1}$).

Table 5. Antihyperglycemic test carried out with the methanolic extract of *A. occidentale*.

Drug (%)	Time (min)					% GR
	0	30	60	90	120	
NaCl 0, 9% (Control)	108.5±19.09	138±19.59	105.5±6.36	102.5±2.12	99.5±7.77	
Glibenclamide (10mg.kg ⁻¹)	85±13.07	109.6±13.4	94±7.54	48.6±11.07	41.6±10.07	58
MeOH _{A0} (500mg.Kg-1)	51±6.78	110±11.63	109.8±19.44	82±14	64±13.98	36

Note: MeOH: Methanolic extract of *A. occidentale*, GR: Glucose reduction.

From the above table, it is displayed that the mean values of blood glucose after 120 minutes in untreated and treated mice were 99.5 ± 7.77mg.dL⁻¹ (0.9% NaCl), 41.6 ± 10.07mg.dL⁻¹ (Glibenclamide 10mg.Kg-1) and 64 ± 13.98mg.dL⁻¹ (methanolic extract of *A. occidentale* 500mg.Kg-1). These results show that *A. occidentale* plant possess an antihyperglycemic (hypoglycemic) activity. In fact, the rate of reduction of glycemia is 36% for the extract versus 58% for Glibenclamide respectively.

4. Discussion

From the results obtained, we can observe that organic extracts (EEP, EAE, MeOH) of *A. occidentale* and *Z. rubescens* leaves normalize the sickled cells under hypoxia conditions created by the addition of Na₂S₂O₅ 2%. Given that all extractions were performed on increasing polarity, the active principles of these plants are probably polar compounds. The antisickling activity of polar compounds such as phenylalanine and anthocyanins was reported in the literature by several authors [27-34]. The phytochemical screening carried out on the leaves of *A. occidentale* and *Z. rubescens* revealed the presence of polyphenols notably anthocyanins. The presence of anthocyanins in these two plants may partially justify the observed antisickling activity.

Table 4. Effects of *Z. rubescens* extracts on in vitro bacterial growth (Microdilution method, dye: 2% TCC).

Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)	<i>E. coli</i> ATCC 27195			<i>S. aureus</i> ATCC 33591		
	EEP	EAE	MeOH	EPP	EAE	MeOH
4000	-	-	-	-	-	-
2000	+	-	+	-	-	-
1000	+	-	+	+	+	+
500	+	+	+	+	+	+
250	+	+	+	+	+	-
125	+	+	+	+	+	+
62.5	+	+	+	+	+	+
MIC	>2000	1000	>2000	>1000	>1000	>1000

Note: +, bacterial growth (appearance of red color, conversion of colorless TCC to red formazan); -, Absence of visible growth (the staining of the well is that of the extract); MIC, Minimum inhibitory concentration; ATCC, American Type Culture Collection; EEP, Petroleum ether extract; EAE, Ethyl acetate extract; MeOH, Methanolic extract.

From the above table, it is clearly shown that the extracts of *Z. rubescens* did not display any activity against the two bacterial strains tested because their MIC > 500 $\mu\text{g}\cdot\text{mL}^{-1}$.

3.2.3. Antidiabetic Activity

The hyperglycemic test carried out with methanolic extract of *A. occidentale* is presented in the table below.

Moreover, Mpiana and his collaborators from University of Kinshasa in DRC have recently demonstrated that the antisickling activity of the majority of Congolese medicinal plants was attributed to this chemical group [27-33].

However, other authors reported that phenylalanine, p-hydroxybenzoic acid and its derivatives as well as maslinic, oleanolic and betulonic acids are responsible of the antisickling activity of plant extracts [34-35]. It is not excluded that these compounds are present in *A. occidentale* and *Z. rubescens*. To our knowledge, it is for the first time that antisickling activity of *A. occidentale* and *Z. rubescens* has been reported. The present study also revealed that only the extract with petroleum ether has a MIC < 500 $\mu\text{g}\cdot\text{mL}^{-1}$ and therefore active against *S. aureus* as indicated by Rios and Recio [36], the plant extract can be considered active when its MIC is less than or equal to 500 $\mu\text{g}\cdot\text{mL}^{-1}$. This anti-infective activity (*S. aureus*) can be attributed to the alkaloids and polyphenols that are present in both plants [37]. The difference in activity observed is due to the presence in *E. coli*, a gram-negative bacterium, of an additional membrane layer impervious to the secondary metabolites contained in the various extracts. The results of this study are therefore similar to previous work. However, Ngbolua *et al.* [21-23] reported *E. coli* is less sensitive to plant extracts than *S. aureus*.

The anti-hyperglycemic activity observed in mice treated with methanolic extract of *A. occidentale* can be attributed to polyphenols of which antidiabetic properties are well established scientifically [38]. These results confirm those obtained in previous studies indicating that the values of the glycemic index and load of the whole fruit of *R. gentiliana* particularly low, justifying the use of this fruit as hypoglycemic by the Congolese population [2]. Regarding the mechanisms of action of polyphenols in general and tannins in particular on glucose, *in vitro* studies carried out on the membrane vesicles of the brush border of the intestines of small rabbits showed that Catechin green tea extract had hypoglycemic activity by a mechanism of prevention of glucose uptake. The results on animal models showed that plant extracts could act through various mechanisms to lower blood glucose levels, thus reinforcing our findings. To our knowledge, it is for the first time that the antisickling activity of *A. occidentale* and *Z. rubescens* is reported, thus validating the chemo-taxonomic approach used as a criterion for selecting these two plants while it is also for the first time that the anti-hyperglycaemic activity of *A. occidentale* is reported. Henceforth, this validates the hypothesis that an antidiabetic plant would potentially possess an antisickling activity.

5. Conclusion

The main aim of the current research was to assess the antisickling, antibacterial and antihyperglycemic properties of two Congolese medicinal plants namely *A. occidentale* and *Z. rubescens*. The findings of the current study showed that both plants possess secondary metabolites that may confer to them the antisickling, antibacterial, and anti-hyperglycemic activities. These secondary metabolites are polyphenols, anthocyanins, tannins, alkaloids, flavonoids and saponins. Indeed, both plants displayed a strong *in vitro* antisickling activity as well a good antibacterial activity on *S. aureus* which was sensitive to petroleum ether extract of *A. occidentale* and the methanolic extract of *A. occidentale* is endowed with anti-hyperglycemic properties. It would therefore be desirable for a toxicological study to be carried out on both plants in order to include them in the Congolese antisickling traditional pharmacopoeia control. A thorough phytochemical study on the methanolic extract of *A. occidentale* has to be conducted in order to isolate the bioactive molecule responsible of this anti-hyperglycemic activity.

References

- [1] Kamba A. S., Maznah I., Tengku A., Tengku A., Zuki I., Zakaria A.. Toxicity relationship of sorafenib in Japanese patients with Renal cell carcinoma and Hepatocellular Carcinoma *Journal of Clinical. Pharmacokinetics*, 2014, 53 (2): 185–196.
- [2] Mpiana P. T., Masunda A., Longoma F., Tshibangu D. S. T and Ngbolua K. N. Anti-hyperglycemic activity of *Raphia gentiliana* De Wild. *European Journal of Medicinal Plants*, 2013, 3 (2): 233-240.
- [3] Iftikhar A. A. S. Glycated haemoglobin; past, present, and future: are we ready for the change. *Journal of Pakistan Medical Association*, 2011, 61 (4):383-388.
- [4] Mangambu M., Van Diggelen R., Mwangi Mwangi J-C., Ntahobavuka H., Malaisse F. and Robbrecht E. Etude ethnobotanique, évaluation des risques d’extinction et stratégies de conservation aux alentours du Parc National de Kahuzi Biega en R. D. Congo. *Geo-Eco Trop*, 2013, 36 (2): 137-15.
- [5] Kolling M., Winkley K. and Von Deden M. “For someone who’s rich, it’s not a problem.” Insights from Tanzania on diabetes health-seeking and medical pluralism among Dar-es-Salam’s urban poor. *Globalization and Health*, 2010, 6: 8.
- [6] Katemo M., Mpiana P. T., Mbala B. M., Mihigo S. O., Ngbolua K. N., Tshibangu D. S. T., Koyange P. R.. Ethnopharmacological survey of plants used against diabetes in Kisangani city (D. R. Congo). *Journal of Ethnopharmacology*, 2012, 144: 39-43. DOI: 10.1016/j.jep.2012.08.022. PubMed ID: 22947387.
- [7] Ngombe K. N., Mbombo P. M., Maloueki U., Musuyu D. M., Mutwale K. P., Tujibikila A. M., Ngbolua K. N. and Kalenda D. T. Preliminary evaluation of antioxidant and antihyperglycemic activities of *Isolona hexaloba* (Pierre) Engl. & Diels (Annonaceae) leaves. *International Journal of Medicinal Plants*, Photon, 2013, 105: 242–249.
- [8] Masunda T. A., Mbala M. B., Kayembe S. J., Longoma B. F., Ngbolua K. N., Tshibangu D. S. T. and Mpiana P. T. Activité anti-hyperglycémique et antiradicalaire des extraits des fruits de *Raphia gentiliana* De Wild. (Arecaceae). *International Journal of Biological and Chemical Sciences*, 2014, 8 (6):2441-2451.
- [9] Mpiana P. T., Misakabu F. M., Tshibangu D. S. T., Ngbolua K. N. and Mwanangombo D. T. Antisickling activity and membrane stabilization effect of anthocyanins extracts from *Adansonia digitata* L. bark on sickle blood cells. *International Blood Research and Reviews*, 2014, 2 (5): 198-212.
- [10] Neuwinger H. D. African traditional medicine: a dictionary of plant uses and applications. Medpharm Scientific, Stuttgart, Germany, 2000, 945 pp.
- [11] Fukudo M., Ito T., Mizuno T., Shinsako K., Hatano E., Uemoto S., Kamba T., Yamasaki T., Ogawa O., Seno H., Chiba T. and Matsubara K. Exposure toxicity relationship of sorafenib in Japanese patients with renal cell carcinoma and hepatocellular carcinoma. *Clinical Pharmacokinetics*, 2014, 53 (2):185-96.
- [12] Bisnu J., Megh R. B., Dinata S, Janina J., Rajani M. and Lakshmaiah S. Lignocellulosic ethanol production: current practices and recent developments. *Biotechnology and Molecular Biology Review*, 2013, 68: 172–182.
- [13] Adenine, S. K. The Nigerian *Zanthoxylum*: chemical and biological values. *African Journal of Traditional, Complementary and Alternative Medicine*, 2005, 2 (3): 282– 401.
- [14] Ngbolua K. N. Evaluation de l’activité anti-drépanocytaire et antipaludique de quelques taxons végétaux de la République Démocratique du Congo et de Madagascar, Thèse de Doctorat. Université de Kinshasa, République Démocratique du Congo, 2012.

- [15] Bongo G. N., Ngbolua K. N., Ashande C. M., Karume K. L., Mukiza J., Tshilanda D. D., Tshibangu D. S. T., Ngombe N. K., Mbemba T. F. & Mpiana P. T. Pharmacological screening of *Gymnanthemum coloratum* (Willd.) H. Rob. & B. Kahn (Compositae) and *Terminalia ivorensis* A. Chev. (Combretaceae) from DR Congo: Spotlight on the antisickling, antibacterial and anti-diabetic activities. *Tropical Plant Research*, 2017, 4 (3): 441–448.
- [16] Gbolo B. Z., Asambo L. S., Bongo G. N., Tshibangu D. S. T., Kasali F. M., Memvanga P. B., Ngbolua K. N. and Mpiana P. T. Bioactivity and Chemical Analysis of *Drepanoalpa*: An Anti-Sickle Cell Anemia Poly-Herbal Formula from Congo-Kinshasa. *American Journal of Phytomedicine and Clinical Therapeutics*, 2017, 5 (1):1-7.
- [17] Mbadiko M. C., Ngbolua K. N., Mpiana P. T., Mutambel' H., Kikakedimau N. R., Makengo K. G., Pambu L. A., Kemfine L. L., Bongo G. N., Mbemba T. F. Phytochemical screening and assessment of anti-sickling activity of total methanolic extracts of different organs of *Curcuma longa* L. (Zingiberaceae). *The Pharmaceutical and Chemical Journal*, 2017, 4 (1):32-40.
- [18] Bongo G., Tuntufye H., Ngbolua K. N., Malakalinga J., Tshiana C., Pambu A., Mwanza F., Mbadiko C., Makengo G., Tshilanda D., Mpiana P., Mbemba T. and Kazwala R. Comparative Anti-mycobacterial Activity on Lowenstein-Jensen Slants of Selected Medicinal Plants Used in the Congolese Pharmacopeia. *Journal of Diseases and Medicinal Plants*. 2017 3 (5):88-96. doi: 10.11648/j.jdmp.20170305.12.
- [19] Bukatuka F. C., Ngombe K. N., Mutwale K. P., Moni B. M., Makengo K. G., Pambu L. A., Bongo N. G., Mbombo M. P., Musuyu M. D., Maloueki U., Ngbolua K. N. and Mbemba F. T. Bioactivity and Nutritional Values of Some Dioscorea Species Traditionally Used as Medicinal Foods in Bandundu, DR Congo. *European Journal of Medicinal Plants*, 2016, 14 (1):1-11.
- [20] Ngbolua K. N., Fatiany P. R., Robijaona B., Randrianirina A. Y. O., Rajaonarivelo P. J., Rasondratovo B., Raharisololalao A., Moulis C., Mudogo V. and Mpiana P. T. Ethno-botanical survey, Chemical composition and in vitro Antimicrobial activity of essential oils from the root bark of *Hazomalania voyroni* (Jum.) Capuron (Hernandiaceae). *Journal of Advancement in Medical and Life Sciences*, 2014a, 1 (1): 1–6.
- [21] Ngbolua K. N., Mubindukila R. N., Mpiana P. T., Tshibangu D. S. T., Ashande M. C., Nzongola W. X. K., Baholy R. and Fatiany P. R. Phytochemical screening, Antibacterial and Antioxidant activities of *Anthocleista liebrechtsiana* Wild & T. Durand (Gentianaceae) originated from Democratic Republic of the Congo. *Journal of Advancement in Medical and Life Sciences*, 2014b, 1 (3): 1–6.
- [22] Ngbolua K. N., Bishola T. T., Mpiana P. T., Mudogo V., Tshibangu D. S. T., Ngombe K. N., Tshilanda D. D. and Baholy R. *In vitro* antisickling and free radical scavenging activities of *Pentaclethra macrophylla* Benth. (Fabaceae). *Journal of Advancement in Medical and Life Sciences*, 2014c, 1 (2): 1–6.
- [23] Ngbolua K. N., Bishola T. T., Mpiana P. T., Mudogo V., Tshibangu D. S. T., Ngombe K. N., Ekutsu E. G., Gbolo Z. B. and Kabena O. N. Ethno-pharmacological survey, *in vitro* antisickling and free radical scavenging activities of *Carapa procera* DC. stem bark (Meliaceae). *Nova Journal of Medical and Biological Sciences*, 2014d, 2 (2): 1–14.
- [24] Ngbolua K. N., Bishola T. T., Mpiana P. T., Mudogo V., Tshibangu D. S. T., Ngombe K. N., Ekutsu E. G., Tshilanda D. D., Gbolo Z. B., Mwanangombo D. T., Fatiany P. R. and Baholy B. Ethno-botanical survey *in vitro* antisickling and free radical scavenging activities of *Garcinia punctata* Oliv. (Clusiaceae). *Journal of Advanced Botany and Zoology*, 2014e, 1 (2): 1–7.
- [25] Ngbolua K. N., Kabena O. N., Lukoki F. L., Mpiana P. T., Ngombe K. N., Fatiany R. P. and Baholy R. Phytochemical Screening of some medicinal plants traditionally used by African Women in Kinshasa city (DR Congo) for their intimate hygiene and Evaluation of the pH of derived recipes. *Journal of Modern Drug Discovery and Drug Delivery Research*, 2014f, 1 (3): 1–7.
- [26] Williamson E. M., Okpoko D. T. and Evans F. J. Pharmacological methods in phytotherapy research. John Wiley and sons, Inc. Third Avenue, New York, USA. 1996, pp. 155–167.
- [27] Mpiana P. T., Tshibangu D. S. T., Shetonde O. M., Ngbolua K. N. *In vitro* antidrepanocytary activity (anti-sickle cell anaemia) of some Congolese plants. *Phytomedicine*, 2007a, 14: 192-195.
- [28] Mpiana P. T., Mudogo V., Tshibangu D. S. T., Ngbolua K. N., Shetonde O. M., Mangwala K. P. and Mavakala B. K. *In vitro* Antisickling Activity of Anthocyanins Extracts of a Congolese Plant: *Alchornea cordifolia* M. Arg. *Journal of Medical Sciences*, 7 (7): 1182-1186.
- [29] Mpiana P. T., Mudogo V., Ngbolua K. N., Tshibangu D. S. T., Shetonde O. M. and Mbala M. B. *In vitro* Antisickling Activity of Anthocyanins from *Ocimum basilicum* L. (Lamiaceae). *International Journal of Pharmacology*, 2007c, 3 (4): 371-374.
- [30] Mpiana P. T., Mudogo V., Tshibangu D. S. T., Kitwa E. K., Kanangila A. B., Lumbu J. B. S., Ngbolua K. N., Atibu E. K., Kakule M. K. Antisickling Activity of Anthocyanins from *Bombax pentadrum*, *Ficus capensis*, *Ziziphus mucronata*: Photodegradation effect. *Journal of Ethnopharmacology*, 2008, 120: 413-418.
- [31] Mpiana P. T., Mudogo V., Ngbolua K. N., Tshibangu D. S. T., Atibu E. K., Kitwa E. K. and Kanangila A. B. *In vitro* antisickling activity of anthocyanins extracts of *Vigna unguiculata* (L.) walp., In *Recent Progress in Medicinal Plants: Chemistry and Medicinal Value*, Govil JN, Singh VK (eds). Daya Publishing House: New Delhi; 2009a, 91-98.
- [32] Mpiana P. T., Balangayi E. K., Kanangila A. B., Kalonda E. M., Ngbolua K. N., Tshibangu D. S. T., Atibu E. K. and Lumbu J. B. S. Activité antidrepanocytaire et thermodégradation des anthocyanes extraits de *Sterculia quinqueloba* et *Ficus capensis*. *International Journal of Biological and Chemical Sciences*, 2009b, 3 (3): 551-560.
- [33] Mpiana P. T., Mudogo V., Ngbolua K. N., Tshibangu D. S. T. and Atibu E. K. *In vitro* Antisickling Activity of Anthocyanins Extracts from *Morinda lucida* Benth (Rubiaceae), In: Gupta VK & Singh GD (eds) *Medicinal Plants: Phytochemistry, Pharmacology and Therapeutics*. Daya publishing house, Delhi, 2010, pp. 330–337.
- [34] Ekeke and Shoda, (1990). Phenylalanine is the predominant antisickling agent in *Cajanus cajan* seed extract. *Planta Medica*, 1990, 56, 41–43.

- [35] Tshibangu S. D. T, Shode F., Koorbanally N., Mudogo V., Mpiana P. T. and Ngbolua K. N. Antisickling triterpenoids from *Callistemon viminalis*, *Meulaleuca bracteata* var. Revolution Gold *Syzygium guineense* and *Syzygium cordatum*. *International Centre for Insect Physiology and Ecology* (ICIPE): Kasarani, Nairobi, Kenya. 2011; 296-300.
- [36] Rios J. L. and Récio M. C. Medicinal plants and antimicrobial activity. *Journal of ethnopharmacology*. 2005, pp 80– 84.
- [37] Fatemeh N. B. Antibacterial activities and antioxidant capacity of *Aloe vera*. *Organic and Medicinal Chemistry Letters*, 2013. DOI 10.1186/2191-2858-3-5.
- [38] Shimizu M., Kobayashi Y., Suzuki M., Satsu H. and Miyamoto Y. Regulation of intestinal glucose transport by tea catechins. *Biofactors*, 2000, 13: 61-65.