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

Gédéon Ngiala Bongo¹, Koto-te-Nyiwa Ngbolua¹,²,³, Colette Masengo Ashande², Kevin L. Karume¹, Janvier Mukiza³, Dorothée D. Tshilanda⁵, Damien S.T. Tshibangu⁵, Nadège K. Ngombe⁶, Théophile F. Mbemba¹ and Pius T. Mpiana⁵

¹Département de Biologie, Faculté des Sciences, Université de Kinshasa, B.P. 190 Kinshasa XI, République Démocratique du Congo
²Département des Sciences de l’Environnement, Faculté des Sciences, Université de Gbadolite, B.P. 111 Gbadolite, Province du Nord Ubangi, République Démocratique du Congo
³Institut Supérieur Pédagogique d’Abumombazi, Province du Nord Ubangi, République Démocratique du Congo
⁴Department of Biochemistry, Faculty of Medicine and Surgery, University of Gitwe, Nyanza, Republic of Rwanda
⁵Département de Chimie, Faculté des Sciences, Université de Kinshasa, B.P. 190 Kinshasa XI, République Démocratique du Congo
⁶Faculté des Sciences Pharmaceutiques, Université de Kinshasa, Kinshasa XI, République Démocratique du Congo

*Corresponding Author: jpngbolua@unikin.ac.cd [Accepted: 25 November 2017]

Abstract: In Democratic Republic of the Congo (DRC), it was reported a rare association in a patient, of two genetic diseases namely sickle cell anemia and diabetes which have a common denominator that is to make patients susceptible to infections. They constitute a serious public health problem in Africa. Given the difficult and limited management of these diseases, the use of Traditional Medicine and medicinal plants can be an effective alternative. The leaves of both Gymnanthemum coloratum and Terminalia ivorensis were collected in 2014 in Kinshasa city and Gbadolite city (Nord Ubangi province) respectively and were selected through chemotaxonomic approach. The bacterial strains used for assessing the antibacterial were Staphylococcus aureus and Escherichia coli and mice for the antidiabetic activity. The phytochemical screening showed the presence of total polyphenols, tannins, flavonoids, linked quinones, saponins, leucoanthocyanins, alkaloids and anthocyanins. The organic extracts of G. coloratum and T. ivorensis showed an antisickling activity, Only S. aureus was sensitive to the leaves of T. ivorensis (MIC < 62.5 µg.mL⁻¹) and G. coloratum (MIC ≤ 250 µg.mL⁻¹) while no effect was observed on E. coli. The mean values for glycemia in treated and untreated mice after 2 hours were 62±14.3 mg.dL⁻¹ (Glibenclamide 20 mg.Kg⁻¹) and 70.4±16.6 mg.dL⁻¹ (ethyl acetate extract of T. ivorensis 500 mg.Kg⁻¹). To our knowledge, it is for the first time that the antisickling activity of G. coloratum and T. ivorensis is reported thus validating the chemotaxonomic approach used as a criterion for selecting these two plants. It is also for the first time that anti-hyperglycaemic activity of T. ivorensis is reported.

Keywords: Sickle cell anemia - Diabetes - Chemo-taxonomic approach.

INTRODUCTION

In Democratic Republic of the Congo (DRC), it has been recently reported that a rare association in a patient,
of two genetic diseases, namely sickle cell disease and diabetes, diseases that constitute a serious public health concern worldwide (Kamba et al. 2014). Sickle cell anemia is characterized by the presence of hemoglobin S in the blood (Ngbolua 2012, Mpiana et al. 2013), and it is the first genetic disease in Africa by the number of patients and it is clinically manifested by a vaso-occlusion and hemolytic anemia, resulting from the polymerization of hemoglobin S molecules into tactoids (Ngbolua 2012).

Diabetes, on the other hand, is characterized by hyperglycaemia (sugar content > 1.2 g.dL\(^{-1}\) on an empty stomach), hyper-ketone (keto acid > 3 mmol.L\(^{-1}\)), an acid-base and hydro-electrolytic imbalance. At the muscular level, there is a real competition between free fatty acids and glucose to be oxidized: free fatty acids are oxidized in priority, leading to an increased production of acetyl CoA which in turn inhibits glycolysis enzymes (Declerck 2002). Thus, the storage and use of glucose are decreased at the muscular level while there is a stimulation of gluconeogenesis in the hepatic level and all of this contributes to increase the blood glucose (Declerck 2003, De boeck et al. 2012).

Given the difficult and limited management of sickle-cell anemia and diabetes in DRC, the use of traditional medicine and medicinal plants can be an effective alternative in the management of both diseases in emergency situations. According to the World Health Organization (WHO), more than 80% of the population living in poor areas in Africa uses medicinal plants to treat themselves (Kolling et al. 2010, Mangambu et al. 2012). Several scientific works carried out in DRC and elsewhere highlight the antisickling and antidiabetic properties of various plants (Katemo et al. 2012, Ngombe et al. 2013, Masunda et al. 2014, Ngbolua et al. 2014a, b, c, d, e, Kasali et al. 2016).

In the present study Gymnanthemum coloratum and Terminalia ivorensis were used which were selected using a chemo-taxonomic approach. In fact, some species of Vernonia and Terminalia genera are known for their antisickling and antidiabetic properties (Mboj 2003). Following this approach, it should therefore be expected that T. ivorensis shows antisickling and antidiabetic activities while G. coloratum (Syn Vernonia colorata) with antidiabetic properties is expected to show an antisickling activity. In addition, both hypotheses (antisickling and antidiabetic activities) are validated, given that diabetes and sickle-cell anemia have a common denominator which is to make patients susceptible to infections (Ngbolua 2012), henceforth both plants have to show an in vitro antibacterial activity. The main objective of this study was to provide the scientific rationale of what the ethnomedical use of these two plants would represent. And, the specific objectives were to perform a phytochemical screening of aqueous extracts of these two selected plants, to subject the leaf powders to fractional extraction using increasingly polar solvents (petroleum ether, ethyl acetate and methanol) and last to assess the antisickling, antibacterial and anti-hyperglycemic activities of organic extracts. The significance of this research is to value the use of medicinal plants in the treatment and management of sickle cell disease and diabetes.

MATERIALS AND METHODS

Plant materials

Plant materials used were leaves of Gymnanthemum coloratum and Terminalia ivorensis A. Chev. collected at Makala commune in Kinshasa and Gbadolite in Nord Ubangi province respectively. G. coloratum was identified in the Herbarium of Faculty of Sciences, University of Kinshasa while T. ivorensis was identified a botanist from “Centre de Surveillance de la Biodiversité”, University of Kisangani. Blood samples used for assessing the antisickling activity of plant extracts were taken from a sickle cell adolescent patient at 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 were provided by the National Institute of Biomedical Research (NIRB).

Methods adopted

A. Collection and conditioning of plant material

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

B. 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 apparatus at 37°C.

C. Phytochemical Screening

The phytochemical screening was carried out according to the standard protocol as described by Bruneton (1999), and it can be performed in aqueous or organic phases.

D. Biological experiments

a. Emmel test

The total SS blood previously diluted in saline solution (NaCl 0.9%) by reason of four to eight drops of the saline solution. On a slide was placed a drop of diluted blood brought into contact with a drop of the drug along with a drop of sodium meta-bisulfite (Na$_2$S$_2$O$_5$ 2%).

The mixture obtained constitutes the microscopic preparation, and it is then covered with a coverslip and is super-cooled by paraffin put on the edges of the slide. The solutions of our extracts (petroleum ether, ethyl acetate and methanol) are prepared by dissolving a few mg of these extracts in the saline solution. Different preparations obtained were observed with the OLYMPUS optical microscope at 10X and 40 X magnifications after 24 hours (Ngbolua et al. 2014a, b, c, d).

b. Antibacterial assay

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

i. Preparation of the stock solution

In a sterile test tube, place 0.020 g of each extract diluted in 250 μL of DMSO then stir for 10 minutes and add 5 mL of Mueller Hinton culture medium using a pipette and mix.

ii. Preparation of the bacterial suspension

Place 2 mL of the saline solution into two sterile test tubes. Using a sterile platinum loop, take two isolated colonies of two strains to be tested namely Escherichia coli ATCC 27195 and Staphylococcus aureus ATCC 33591 and place each colony in the saline solution in both tubes. This bacterial suspension is diluted in the appropriate culture medium at a rate of 9 mL.

iii. 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. Then each well was filled with 100 μL of culture medium as follows: $A_2$ to $A_8$, $B_2$ to $B_8$, $C_2$ to $C_8$, $D_2$ to $D_8$, $E_2$ to $E_8$ and $F_2$ to $F_8$ and then the 11th and 12th wells served as controls. Using a micropipette, place 200 μL of the stock solution of extract 1 in A1 and B1 wells, 200 μL of the stock solution of extract 2 in C1 and D1 wells while 200 μL of the stock solution of extract 3 in E1 and F1 (i.e. 2 wells were used for each extract). Meanwhile, 100 μL of each stock or control solution were taken and later the serial dilutions of 2 to 2 were performed.

Afterwards, 100 μL of $A_8B_1$, $C_8D_1$ and $E_8F_1$ wells were taken and transferred to $A_8B_4$, $C_8D_4$ and $E_8F_4$ wells, then from the previous wells 100 μL were taken and were transferred to $A_8B_7$, $C_8D_7$, $E_8F_7$ wells. These solutions were thoroughly mixed and the same procedure continued till we reached $A_8B_8$, $C_8D_8$ and $E_8F_8$ wells. The last 100 μL taken from these wells were removed and thrown away (Ngbolua et al. 2014e, f).

iv. Determination of the minimum inhibitory concentration

The growth observed in different wells containing extracts or controls was compared to that in the bacterial growth control well (well-having 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 μL of TCC 2% (2, 3, 5-triphenyltetrazoliumchloride) to the control wells then the minimum inhibitory concentration can be read. The principle of this method is based on the ability of living cells to reduce the tetrazolium salt to a red or formazan precipitate. The minimum inhibitory concentration was then read from the first wells showing no bacterial growth after 48 hours (Ngbolua et al. 2014e, f).

c. Oral glucose tolerance test

The study was carried out on an animal model consisting of 10 NMRI mice (male and female) subjected to temporary hyperglycemia by gavage of a glucose solution (200 mg.mL$^{-1}$). These 10 mice were divided into 3 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 (Gibencamid 10 mg.Kg$^{-1}$) and the third group of five mice to be tested with the ethyl acetate extract (500 mg.Kg$^{-1}$). Blood glucose testing was performed using a contour TS blood glucose meter from the tail (Williamson et al. 1996).
d. 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.
e. Antihyperglycaemic activity
In our study, we caused a temporary hyperglycemia in mice by oral administration of glucose (diluted to 10% in distilled water) at a dose of 4 g.Kg⁻¹ of body weight. 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 Glibenclamid at 10 mg.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.

RESULTS AND DISCUSSION
A. Phytochemical Screening
The result of the phytochemical screening is presented in the table below.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gynnanthemum coloratum</th>
<th>Terminalia ivorensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total polyphenols</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Leucoanthocyanins</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Bound Quinones</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

**Note**: -, Absence of the researched substance; +, Low concentration of the researched substance; ++, High concentration of the researched substance; ++++, The highest concentration of the researched substance.

From table 1 above, it is shown that *G. coloratum* leaves are rich in total polyphenols, tannins, flavonoids, linked quinones, saponins, leucoanthocyanins and anthocyanins while the total absence of alkaloids was noted. On the other hand, the leaves of *T. ivorensis* are rich in total polyphenol, tannins, flavonoids, linked quinones, saponins, leucoanthocyanins and alkaloids; however they are devoided of anthocyanins.

B. Biological activities
a. Antisickling activity
Figure 1 shows the phenotype of untreated (1a) and treated (1b) sickle cells respectively.

![Figure 1](https://www.tropicalplantresearch.com)

**Figure 1.** A, Optical micrographic of untreated SS blood; B, red blood cells treated with petroleum extract of *Terminalia ivorensis* (50 µg.mL⁻¹) [NaCl 0.9% ; Na₂S₂O₅ 2%, X500].
As it can be noticed in the above figures, the red blood cells are of sickle cell phenotype showing that, the blood used was taken from a sickle cell patient. However, in the presence of petroleum extracts (b), sickle-cells revert to the normal circular and biconcave form. These results are consistent with previous observations (Ngbolua 2012, Mpiana et al. 2010).

The rate of normalization (Emmel test) of sickled cells in the presence of various extracts under conditions of isotonic hypoxia (NaCl 0.9%, Na₂S₂O₃ 2%) is presented in table 2 below.

<table>
<thead>
<tr>
<th>Concentration (µg.mL⁻¹)</th>
<th>Gymnanthemum coloratum</th>
<th>Terminalia ivorensis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>4000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1000</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>500</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>250</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>125</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>62.5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MIC</td>
<td>&gt; 4000</td>
<td>&gt; 2000</td>
</tr>
</tbody>
</table>

Note: -, Inactive; +, 10 < RN < 50 % (low activity); ++, 50 < RN < 70 % (higher activity); ++++, RN > 70 % (the highest activity) [Source: Mpiana et al. 2010]

As described in table 2, the presence of organic extracts (petroleum ether, ethyl acetate and methanol) in G. coloratum and T. ivorensis demonstrate the normalization of the sickle-cells under conditions of hypoxia. This normalization of SS erythrocytes under conditions of hypoxia constitutes partially scientific evidence that justifies the integration of these two plants on the database of antischickling plants. The normalization of the SS blood erythrocytes treated with the extracts of these plants results in the reappearance of the circular form of sickled cells. These results are therefore similar with those of previous works (Mpiana et al. 2007) as shown in figures 1a and 1b. However, it has to be noted that the extract of the ethyl acetate of G. coloratum showed a high antischickling activity with a normalization rate of between 10 and 50%.

b. Antibacterial Activity

Table 3 and 4 present the results of the antibacterial test.

<table>
<thead>
<tr>
<th>Concentration (µg.mL⁻¹)</th>
<th>Escherichia coli ATCC 27195</th>
<th>Staphylococcus aureus ATCC 33591</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EEP</td>
<td>EAE</td>
</tr>
<tr>
<td>4000</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2000</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1000</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>500</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>250</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>125</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>62.5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MIC</td>
<td>&gt; 4000</td>
<td>&gt; 2000</td>
</tr>
</tbody>
</table>

Note: +, bacterial growth (appearance of red color, conversion of colorless TCC to red formatzan); -, 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.

This table shows that only S. aureus is susceptible to T. ivorensis leaf extracts (MIC <62.5 µg.mL⁻¹) while no effect was observed on E. coli.

<table>
<thead>
<tr>
<th>Concentration (µg.mL⁻¹)</th>
<th>Escherichia coli ATCC 27195</th>
<th>Staphylococcus aureus ATCC 33591</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EEP</td>
<td>EAE</td>
</tr>
<tr>
<td>4000</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2000</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>1000</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>500</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>250</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>125</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>62.5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MIC</td>
<td>&gt; 4000</td>
<td>1000</td>
</tr>
</tbody>
</table>

Note: +, bacterial growth (appearance of red color, conversion of colorless TCC to red); -, 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 this table, it is shown that only S. aureus is sensitive to G. coloratum extracts (MIC ≤ 250 μg.mL⁻¹). In fact, it is well known that extracts having a MIC less than 500 μg.mL⁻¹ are considered active and it is the case for these two plant extracts used in this study with respect to S. aureus (Molina-salinas et al. 2007). Contrary to E. coli, it should be noted that S. aureus is a positive gram bacterium, and its wall is thick (having several layers superimposed) while in E. coli the additional outer membrane would prevent the entrance of chemical compounds in the bacterial cell (Maligan & Martiniko 2007). The results obtained in this work indicated that the extracts of the two plants used in this study may constitute a source of new drugs against S. aureus, knowing that the sickle cell anemia patients are prone to repeated tissue infarction leading to a functional asplenia and are therefore exposed to frequent infections due to immune deficiency and phagocytic cell abnormality to cytokines (Bégué 2009). These two plants could be very useful in clinical trials.

C. Antidiabetic activity

The hyperglycemic test carried out with the ethyl acetate extract of T. ivorensis is presented in table 5 below.

<table>
<thead>
<tr>
<th>Drug/Extract</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>% RG</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl 0.9% (Control)</td>
<td>96±14.1</td>
<td>106.5±14.8</td>
<td>122.5±17.6</td>
<td>108±1.41</td>
<td>98.5±3.5</td>
<td>-</td>
</tr>
<tr>
<td>Glibencilamid (10 mg.kg⁻¹)</td>
<td>68±10.5</td>
<td>111±19.8</td>
<td>185±15.08</td>
<td>67±10.9</td>
<td>62±14.3</td>
<td>37.05</td>
</tr>
<tr>
<td>EAE (500 mg.kg⁻¹)</td>
<td>45.4±9.2</td>
<td>93.4±17.7</td>
<td>97.4±14.2</td>
<td>74.2±10.08</td>
<td>70.4±16.6</td>
<td>28.53</td>
</tr>
</tbody>
</table>

Following the above table, it is clearly displayed that the mean values of blood glucose after 120 minutes in untreated and treated mice were 98.5±3.5 mg.dL⁻¹ (NaCl 0.9%), 62±14.3 mg.dL⁻¹ (Glibencilamid 10 mg.Kg⁻¹) and 62±14.3 mg.dL⁻¹ (ethyl acetate extract of T. ivorensis 500 mg.Kg⁻¹) respectively. These results show that T. ivorensis has an antihyperglycaemic (hypoglycaemic) activity. In fact, the reduction rate of glycaemia is of 28.53% versus 37.05% for Glibencilamid. Up to date, numerous hypoglycemic plants have been identified by Fezan et al. (2008). The results on animal models showed that plant extracts could act through various mechanisms to lower blood glucose levels, thus reinforcing our results. To our knowledge, it is for the first time that the antiscickling activity of G. coloratum and T. ivorensis 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 T. ivorensis is reported. Henceforth, this validates the hypothesis that an antidiabetic plant would potentially possess an antiscickling activity.

CONCLUSION AND SUGGESTIONS

The aim of this study was to assess the antiscickling, antibacterial and anti-hyperglycemic potential of two taxonomic Congolese plants of which Gymnanthemum coloratum and Terminalia ivorensis. Both plants contain secondary metabolites capable of conferring the antiscickling, antibacterial and anti-hyperglycaemic activities. Only S. aureus was sensitive to T. ivorensis and G. coloratum extracts. The extract of ethyl acetate of T. ivorensis is endowed with anti-hyperglycaemic properties. Therefore, it would be necessary to carry out a toxicological study on both plants in order to include them in the traditional antiscickling pharmacopoeia and also to consider carrying out an advanced phytochemical study on ethyl acetate extract of T. ivorensis in order to isolate bioactive molecules.

ACKNOWLEDGEMENTS

The authors thank The World Academy of Sciences (TWAS) for Grant No. 15-156 RG/CHE/AF/AC_G-FR3240287018 and the Switzerland embassy at Kinshasa (DRC) for providing financial assistance to RESUD (Research for sustainable development/NGO).

REFERENCES


