**In vivo** antiplasmodial and toxicological studies of *Dialium angolense* Welw. Ex Oliv. (Fabaceae) leaves extracts, a medicinal plant from Eastern Congo

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**Abstract**

*Dialium angolense* is used in traditional medicine in Bagira-Bukavu in the management of malaria. In this study, *in vivo* antiplasmodial and *in vitro* antioxidant activities, phytochemical screening of secondary metabolic and *in vivo* toxicological studies where carried out on aqueous and methanolic extracts of its leaves. The plant was selected following an ethnobotanical survey conducted in DR Congo and focusing on antimalarial plants. Extracts' phytochemical secondary metabolites were determined using standard procedures and the antiplasmodial activity was evaluated using 4-day suppressive test, while antioxidant activity was evaluated by DPPH assay. In acute toxicity, eighteen animal (6/group) were given orally singular 2000 mg of extract/kg body weight (BW) then observed for 14 days. In sub-acute toxicity assay, 150 or 300 mg/kg BW/Day were given orally, and animals (6/group) were observed for 28 days. The total phenolic (0.89 - 0.98 mg GAEg⁻¹), total flavonoid (0.42 - 0.44 mg QEg⁻¹) and total tannin contents (0.080 – 0.098 mg GAEg⁻¹) were in the same rate in the two extracts as well as the antioxidant activity with IC₅₀ value 6.1 and 6.8 μg/mL.

At the highest oral dose, 300 mg/kg body weight, all extracts produced 70.4 – 70.8% chemo-suppression against *P. berghei* ANKA and 28 survival days. No deaths were recorded during the acute toxicity assay suggesting the LD₅₀ > 2000 mg/kg and no abnormal behavior or variation in toxicity biomarkers were observed during the subacute toxicity assessment. *D. angolense* leaves extracts showed a great antiplasmodial and a very good antioxidant activity. It can be used to prepare antimalarial recipe or isolate antimalarial compounds in the future.

**Keywords:** Bagira; *Dialium angolense*; DPPH; *Plasmodium berghei*; *Mus norvegicus*; *Mus musculus*
1. Introduction

Malaria is one of the most prevalent and serious protozoan tropical diseases which causes millions of clinical cases worldwide each year, and approximately 1 million of death annually [1]. The World Health Organization (WHO) African Region accounted for 93% of all cases in 2018 and 40% of all world cases were in two countries: Nigeria (25% of cases), and Democratic Republic of Congo, DRC (15%) [2]. DRC is one of the central African country where malaria with *Plasmodium falciparum* is highly endemic with 97% of prevalence [3], being one of the most important health problems in the country [4].

Many Congolese people do not have access to modern health care and use medical plants but, many plants used in traditional medicine have not been studied [5]. Another element of the antimalarial control strategy would consist in validating the use of antimalarial plants with the hope to discover new compounds or produce improved traditional drugs. Studies have been conducted to search for antimalarial plants both across the world, in Asia [6,7] as well as in Africa [8,9] and particularly in DRC [10,11].

In Bukavu, among the many plants reported as antimalarial [4,5,12], we have selected *Dialium angolense* Welw. ex Oliv. (Fabaceae). It is a small to medium-sized tree up to 20 (~30) m tall; bole branchless for up to 15 m, normally straight and cylindrical or slightly fluted at the base, up to 90 cm in diameter, with small to fairly large buttresses; bark surface scaly with small irregular scales, yellowish brown to reddish brown, inner bark thin, brittle, yellowish white to pinkish, with sticky, reddish exudate; crown rounded, dense, with sinuous branches; twigs with many lenticels, quickly glabrous.

Leaves alternate, imparipinnately compound with 3–5 leaflets; stipules linear, caducous; petiole and rachis together 8–17 cm long; petiolules about 0.5 cm long; leaflets alternate or nearly opposite, oblong-elliptical, 8–23 cm × 3–8 cm, wedge-shaped to rounded at base, acuminate at apex, leathery, glabrous, pinnately veined with about 10 pairs of lateral veins. Inflorescence a terminal or axillary panicle up to 20 cm long, yellowish-brown hairy. Flowers bisexual, zygomorphic, fragrant; short pedicel; sepals 5, free, triangular, c. 4 mm long, pubescent; petal 1, spatulate, about 4 mm long, yellowish; pentagonal disc, about 2 mm in diameter, dark brown pubescent; stamens 2; ovary superior, ovoid, sessile, pubescent, 1-celled, style arched. Fruit a slightly flattened, globose to obvoid pod, c. 2.5 cm × 1.5 cm, densely dark brown hairs, with greenish-white pulp, sepals persistent at base, indehiscent, 1 (~2) -seed. Seeds flattened ellipsoid, about 1 cm long, dark brown to black. The plant is locally named *Kizimya* (Shi), *Kabalala* (Bemba) or *Cituzo* (Havu), and is used in the treatment of malaria and other infectious diseases such headaches, fever, gastritis, conjunctivitis, urethritis, amebiasis. The fruits, beyond being edible, are used in the treatment of pneumonia [4,13]. This plant has already been investigated for *in vitro* antiplasmodial activity, study which revealed a great activity [4]. However, no information is reported on its phytochemical composition nor on the *in vivo* antimalarial activity, and toxicity.
This study aims to evaluate the in vivo antiplasmodial and in vitro antioxidant activities of methanolic, and aqueous extracts of the leaves of Dialium angolense. On this same occasion, we evaluate the acute and subacute toxicities on rats, and we screen phytochemical group mainly secondary metabolites with antimalarial potentiality.

2. Material and methods

2.1. Plant material and experimental animals

Leaves of Dialium angolense were collected in Bagira (2 ° 28'11.9"S; 28 ° 49'19"E; 2,884.1 m) in April 2015, and was identified at the herbarium of Meise in Belgium with the following voucher number: BR00000188792866. Healthy Mus musculus (21.5 ± 1.1 g) and Mus norvegicus (262.41 ± 0.71 g) male were procured from animals holding unit of Institut National de Recherches Biomédicales (INRB) Kinshasa-DRC. The animals were acclimatized to 28 °C one week before the experiment by being subjected to a 12 h light-dark cycle, consuming a standard rodent food (MIDEMA/DRC) and drinking ad libitum.

2.2. Chemicals and reagents

Quinine HCl, Ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), Gallic acid, Quercetin, were obtained from (Sigma-Aldrich, USA) and all chemicals and solvents were of analytical grade.

2.3. Preparation of extracts

Methanolic extracts (ME) were obtained by macerating 350 g of dried leaves powder in 1.5 L of methanol (Sigma-Aldrich, USA). After 72 h, the extract was filtered on paper (Whatman, USA) and the residue was macerated twice in a similar manner. The filtrates were combined, concentrated, and dried using a rotavapor (Büchi R-210, Switzerland) at 40 °C under reduced pressure, 130-180 mbar (yield, 12.9%, W/W). Aqueous extracts (AE) were prepared according to the protocol used in traditional medicine by decocting 320 g of the sample in 2 L of local tap water (boiling for 1 h in a close recipient and filtration on paper). The extract was lyophilized (yield, 11.5%, W/W) and for the all test, the extract was dissolve in NaCl 0.9%.

2.4. Phytochemical screening

The plant extract was analyzed for the presence of some secondary metabolite including alkaloids, coumarins, flavonoids, saponins, steroids, tannins, terpenoids and phenols, using standard procedures in tube reaction [14,15].

2.5. Total phenolic, flavonoids and tannin contents

The total phenolics content of each sample was measured by a Folin–Ciocalteu method [16] and expressed as milligrams gallic acid equivalents per gram of dry plant extract (mg GAE/g DE) through a calibration curve gallic acid (y = 0.015x + 0.002, R² = 0.996; linearity range, 0.5 – 200 mg/mL). The total flavonoids content was determined using an aluminum trichloride assay [17] and expressed as milligrams quercetin equivalents per gram of dry plant extract (mg QE/g DE) through the calibration curve of quercetin (y = 0.006 x + 0.005, R² = 0.996; linearity range, 0.1 to 150 mg/mL). Total tannin content was expressed as milligrams gallic acid equivalents per gram of dry plant extract (mg GAE/g DE) through the calibration curve of gallic acid (y = 0.005 x + 0.0014, r² = 0.997); its linearity range was from 1.0–100.0 mg/L⁻¹

2.6. Antioxidant activity-DPPH assay

DPPH radical scavenging activity of the plant extracts at varying concentrations were measured in vitro via the DPPH assay [18]. Briefly, 50 μL of extract prepared at different concentrations were interacted with 1950 μL of 0.002% DPPH in a plate 96 wells (Nunc WVR, Germany) giving concentrations of extracts ranging from 0.048 to 3.125 μg/mL. After mixing and incubating in the dark for 30 minutes, the solution was read at 492 nm (Thermo Fisher Scientific Inc., Waltham, USA). The tests were carried out in triplicate. The percentage of antioxidant activity (AOA) was calculated by the formula:

% AOO = \frac{(Ab–Ae)\times 100}{Ab} \quad \text{(Equation 1)}

Ab = absorbance measured in the presence of the negative control, Ae = absorbance measured in the presence of the extract, and % AOO = Percentage of inhibition. Depending on their IC₅₀ values, extracts were classified as following: (i) very active if IC₅₀ ≤ 5 μg/mL, (ii) active if 5 μg/mL ≤ IC₅₀ ≤ 15 μg/mL, (iii) moderately active if 15 μg/mL < IC₅₀ < 50 μg/mL, (iv) weakly active if IC₅₀ ≥ 50 μg/mL [18].
2.7. Antiplasmodial activity-4-day suppressive test

The in vivo antiplasmodial activity of the extracts were evaluated using the 4-day suppressive test against *Plasmodium berghei* (ANKA MRA 311 supplied by the INRB) infections in mice [19]. Briefly, donor *Mus musculus* previously infected with *Plasmodium berghei* and having parasitemia level of 20-30% were used to infect the experimental mice intraperitoneally with 0.2 mL of their infected blood. The infected mice were randomly divided into six groups of 5 each according to their weight. Three hours after inoculation, each *Mus musculus* was orally treated with 200 µL of oral dose of the simple (150 and 300 mg kg$^{-1}$ weight) daily for 4 days. A positive control-group received 10 mg/kg BW of quinine per day, while the negative-control group animals were administered 200 µL of the vehicle (NaCl 0.9%). On day 7, thin blood smear was made and stained with 10% Giemsa and examined under the light microscope with 100 times magnification to determine parasitemia level. Percentage of parasitemia was counted based on infected erythrocytes calculated per 1000 erythrocytes:

$$\% P = \frac{\text{Number of Parasitized RBCs} \times 100}{\text{Total Number of RBC Count}} \quad \text{(Equation 2). (RBC: Red Blood Cells)}.$$

On day 7, the level of parasitemia in each group of mice was determined so that the percentage chemo-suppression (TSP) were calculated as:

$$\% \text{TSP} = \left(\frac{A - B}{A}\right) \times 100 \quad \text{(Equation 3)}$$

Where A is the parasitemia in the negative-control group and B the parasitemia in the test group. All the mice were kept alive until the 28th day to assess the survival time (TS) [20,21]. In vivo antiplasmodial activity of extracts were classified as moderate, good, and very good if an extract displayed respective percent parasite suppression equal to or greater than 50% at doses of 500, 250, and 100 mg/kg body weight per day, respectively [22].

2.8. Toxicological study

Acute toxicity was carried out as described previously [23] using 2000 mg/kg by Weight (BW) in single dose (oral administration; 6 animals per group, followed over 14 days). In subacute toxicity, *Mus norvegicus* (6 each group) received orally for 28 days, 0 (negative control), 150 or 300 mg/kg BW/day. During blood collection and serum preparation for biochemical analysis, validated procedure were followed [23]. The activities of alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), and the levels of urea and creatinine were determined by colorimetric assays with Labtest® kits (Minas Gerais, Brazil).

2.9. Statistical analysis

Values were analyzed using GraphPad Prism 6 (GraphPad Software, La Jolla, USA). Comparisons between different groups were carried out by analysis of variance, ANOVA; a probability level $p < 0.05$ was considered significant.

2.10. Ethical approval

The principles governing the use of laboratory animals as laid out by Organization for Economic Cooperation and Development: OECD, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review [24] were duly observed.

3. Results

3.1. Phytochemical screening of *Dialium angolense* leaves extracts

The phytochemical screening of *D. angolense* leaves extracts revealed the presence of quinones, flavonoids, polyphenols, terpenoids, but the absence of alkaloids, coumarins, steroids, and saponins (Table 1).
Table 1 Phytochemical composition of extract of Dialium angolense.

| Phytochemical class | Aqueous extract (AE) | Methanolic extract (ME) |
|---------------------|----------------------|-------------------------|
| Alkaloids           | -                    | -                       |
| Anthraquinones      | +                    | +                       |
| Coumarins           | -                    | -                       |
| Flavonoids          | +                    | +                       |
| Polyphenols         | +                    | +                       |
| Saponins            | -                    | -                       |
| Steroids            | -                    | -                       |
| Terpenoids          | +                    | +                       |

+: positive reaction, -: negative reaction

The different total phenolics content (TPC) values vary between 0.89-0.982 mg GAEG⁻¹ and are two times higher than the total flavonoids content (TFC values: 0.44 - 0.42 mg QE⁻¹). Total tannins content (TTC) vary between 0.090 - 0.098 mg GAEG⁻¹ and a difference between aqueous and methanolic extracts is only observed with TTC with p < 0.01 (Table 2).

Table 2 Total polyphenol, flavonoid and tannins contents of extract of D. angolense

| Simple | Total phenolics content (TPC) (mg GAEG⁻¹) | Total flavonoids content (TFC) (mg QE⁻¹) | Total tannins content (TTC) (mg GAEG⁻¹) |
|--------|------------------------------------------|----------------------------------------|----------------------------------------|
| ME     | 0.894 ± 0.042                            | 0.424 ± 0.012                          | 0.098 ± 0.011                          |
| AE     | 0.982 ± 0.012                            | 0.441 ± 0.013                          | 0.080 ± 0.003a                         |

ME: Methanolic extract, AE: aqueous extract; Data are expressed as mean ± SD (n = 5) and compared to ME; a p < 0.01.

3.2. Antioxidant activity

The scavenging ability of the samples tested showed a dependent concentration activity profile. The anti-free radical activity expressed as IC₅₀ was respectively 6.1 ± 0.02 (AE) and 6.8 ± 0.9 µg/mL (ME) suggesting a good antioxidant activity of studied extracts according to the previously proposed classification [18]. No statistically significant difference was observed between the two extracts. In contrast, their antioxidant activity was lower (p < 0.001) than ascorbic acid used as a positive control (figure 1).

Figure 1 DPPH Radical Scavenging Activities of D. Angolense Expressed as A Percentage and as the value of IC₅₀ in µg/mL. Positive Control (Pc): Ascorbic Acid. Data Expressed as Mean ± SD, N=5. Aoa: Antioxidant Activity. Pc: Ascorbic Acid, Ae: Aqueous Extract, Me: Methanolic Extract.
3.3. Antiplasmodial activity

The percentage suppression analysis of the extracts showed decrease (p <0.01) in parasitemia at all dose levels as compared to the negative control group. The group received 300 mg/kg WB/day (ME 300) exhibited maximal suppression (70.81 ± 0.05%); the effect was significantly lower than the group which received quinine (p<0.001). All doses of the extract significantly enhanced the survival time (TS) of the mice in a “not dose dependent manner” as compared to the negative control group (Table 3).

Table 3 Antiplasmodial activity of the different doses of the extracts of D. angolense during established infection (Mean ± SD, n=5).

| Group       | Dose (mg/kg WB) | Parasitemia P (%): | Suppression Rate: PSR (%) | Survival Time : TS(D) |
|-------------|-----------------|---------------------|---------------------------|-----------------------|
| ME 150      | 150             | 4.86 ± 0.05<sup>a</sup> | 52.02 ± 0.21              | 28                    |
| AE 150      | 150             | 5.12 ± 0.02<sup>a</sup> | 51.73 ± 0.04              | 28                    |
| ME 300      | 300             | 2.85 ± 0.18<sup>b</sup> | 70.81 ± 0.06              | 28                    |
| AE 300      | 300             | 3.01 ± 0.23<sup>b</sup> | 70.38 ± 0.14              | 28                    |
| Quinine     | 10              | 1.01 ± 0.03<sup>c</sup> | 91.06 ± 0.12              | 28                    |
| NaCl 0.9%   | -               | 11.31 ± 0.02         | NA                        | 8                     |

D: day. ME 150: Group treated with 150 mg / kg body weight methanolic extracts from leaves of D. angolense. The results (P and PSR) express the values obtained on the 7th day. TS is obtained on the 28th day of observation, NA: Not applicable. All extracts are compared to negative control (NaCl 0.9%); the level of significance of difference is expressed by letters a, b, c; <sup>a</sup>p < 0.01, <sup>b</sup>p < 0.001, <sup>c</sup>p < 0.0001.

3.4. Acute and sub-acute toxicities

3.4.1. Clinical signs, weight variation, maximum tolerated dose (DMT) and 50% lethal dose of animal (LD<sub>50</sub>)

With the acute toxicity test at the test dose of 2000 mg/kg, neither mortality nor changes related to behavioral, neurological, and physical profile were observed within the first 24 h and during the 14 days’ followup period. The DMT and the LD<sub>50</sub> are thus estimated > 2000 mg/kg. No significant variation in weight was observed either during the assessment of acute toxicity or during the assessment of sub-acute toxicity (figure 2).

Figure 2 Ponderal evolution of Mus norvegicus during the experimental (a): in acute toxicity, (b): in subacute toxicity. Data are expressed as mean (n=6). Weights were taken every 7 days from the week, (D-7) preceding the day of the poisoning (D0). ME 2000: group received 2000 mg of methanolic extract /kg BW in single dose. AE2000: group received 200mg/kg BW of aqueous extracts.
3.4.2. Variation in rat organ weights as well as biomarkers of hepatic and renal functions

No variation in the weight of some organs was observed during the subacute toxicity test. No death was recorded during the toxicological experimentation nor any serum variation of the biomarkers of the hepatic function (AST, ALT, PAL) nor renal (urea, creatinine), in the treated groups (ME 150, AE 150, ME 300, AE 300) compared to the control group (0.9% NaCl) (figure 3. c-d). Administration of therapeutic doses (150 and 300 mg/kg) for 28 days of aqueous and methanolic extracts of the leaves of D. angolense does not cause toxicity in Mus norvegicus.

![Graphs showing organ weights and biomarker levels](image)

Figure 3 Mus norvegicus organs weight (a), hepatic (b) and renal (c, d) variation level of some biomarkers when exposed to D. angolense extract, 150 and 300 mg/kg BW. ME150: Methanolic extract given at 150 mg/kg BW/day, AE300: Aqueous extract given at 300 mg/kg BW/day. Data expressed as Mean ± SD, n=6.

4. Discussion

In this study, we evaluated the in vivo antimalarial activity, safety profile, and chemical constituents of the crude leaf extract to validate the traditional claim of D. angolense for its use in the treatment of malaria in folk medicine in Bukavu.

In the in vivo antimalarial study, we found a dose-dependent chemo suppressive effect (51-71%) by crude leaf extract of D. angolense against Plasmodium berghei ANKA respectively at the dose of 150 and 300 mg/kg (Table 3). According to the classification previously proposed [25], all extract presented a good antiplasmodial activity in vivo. This activity makes the two extracts of the leaves of D. angolense belong to a category lower than that to which their antiplasmodial activity in vitro carried out previously made them belong: substances with a very strong in vitro activity [18]. This difference in activity can be justified by the fact that in vivo, the extract has encountered barriers capable of constituting a constraint in the expression of the activity on the plasmodia strain. In addition, the nature of the strain tested in vitro is different from that used in vivo during this study. This reality shows the need to perform an in vivo screening in addition to in vitro studies during studies of interesting antimalarial plants likely to lead to bioguided fractionation in order to discover antimalarial compounds.

In comparison to some antimalarial drugs used in traditional medicine in the DRC, the aqueous and methanolic extracts of the leaves of D. angolense are less active than the leaves of Senna occidentalis (L.) Link (Fabaceae): EM, TSP from 73%
to 200 mg / kg on P. berghei ANKA [26]. Phyllanthus niruri L., Phyllanthaceae: Ethanolic extract TSP from 73% to 200 mg / kg [27] and the bark of roots of Alstonia congensis Engl. (Apocynaceae): EA, TSP of 80.43 ± 0.12 mg / kg on P. berghei ANKA [1]. Their activities were nevertheless more pronounced than leaves of Physalis angulata L (Solanaceae): EM, TSP = 60% at 300 mg / kg and Anisopappus chinensis Hook (Asteraceae): EA, TSP: 46.6% at 300 mg / kg [21] as well as the leaves of Morinda morindoides (Baker) Milne-Redh., Rubiaceae: Ethanolic extract, TSP: 31.3% at 200 mg / kg [27] and, Lantana camara L. (Verbenaceae): EM, TSP: 72.6% at 500 mg / kg [28].

This study reports a chemical similarity composition in secondary metabolites between aqueous and methanolic extracts of D. angolense by the concomitant presence of quinones, flavonoids, terpenoids, tannins and overall polyphenols (Table 1) suggesting that there are chances of finding similar pharmacological properties between these two extracts. In addition, the presence of these phytochemical groups previously reported as groups with antimalarial potential [29–31] could constitute a first explanation of this interesting antiplasmodial activity observed during this study.

In this study we provide a good antioxidant activity of extract leaves of D. angolense (6.1- 6.8 μg/mL) as in previous studies [18,32]. In comparison with some species of the genus Dialium, this activity appears to be much greater. Indeed, the antioxidant activity on DPPH, expressed as IC50 in μg / mL, of several species of the genus Dialium is reported in the literature, D. indum, 181.6 ± 0.4 [33,34], D. guineense, 50.23 ± 0.15 [35], D. corbisieri, 14.44 ± 0.12 and D. gossweileri, > 500 [36], D. cochinchinesis, 65.01 ± 0.12 [37]. This interesting antioxidant activity observed in D. angolense would be linked to the presence of phenolic compounds mainly flavonoids, identified and quantified within the plant during this study (table 2), as well as several previous studies which testify to the antioxidant activity of flavonoids [38–42]. Besides flavonoids, other antimalarial [43,44] and antioxidant [45–47] compounds belonging from other phytochemical groups identified in the leaves of D. angolense during this study has been isolate. These suggest the possibility of isolating in the future within D. angolense, molecules with both antimalarial and antioxidant activity, following the example of previous studies [48,49]. The concomitant presence of antiplasmodial and antioxidant activity in a plant is particularly interesting insofar as during infection with plasmodium, an oxidative stress responsible for the evolution of the disease towards anemia and neuronal malaria is observed [32].

At the experimental doses of 150, 300 mg/kg (subacute toxicity) and 2000 mg/kg (acute toxicity), the toxicity of D. angolense has not been sufficiently established. D. angolense presented an estimated LD50 greater than 2000 mg / kg, as some antimalarial remedies. This is the case of the root bark of Dialium guineense [50], or the case of some Congolese antimalarial medicinal plant lake Nauclea pobeguinii, Rubiaceae [51], Dalbergia katangensis Lecheneaud, Fabaceae [52] or a Congolese antimalarial phytomedicine that combines the plants Enantia olivacea Robyns & Ghesq., Annonaceae, Garcinia punctata Oliv., Gusiaceae and Massularia acuminata (G.Don) Bullock ex Hoyle, Rubiaceae [53].

5. Conclusion

For the first time, a promising in vivo antiplasmodial activity against P. berghei on Mus musculus model with an interesting toxicological profile on Mus norvegicus is demonstrated for the leaves of D. angolense and its antioxidant activity in vitro previously known is confirm. These results can partially support the use of this plant part for the treatment of malaria in Congolese traditional medicine. This plant is particularly interesting for a further investigation as very few is known about its phytochemical composition.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have not known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors contributions

Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, software and writing - original draft: Bashige Chiribagula valentin, Supervision: Bakari Amuri Salvius, Okusa Ndjolo Philippe, Lumbu Simbi
Jean-Baptiste; Writing - review & editing: Manya Mboni Henry, Bakari Amuri Salvius, Okusa Ndjolo Philippe, Kahumba Byanga Joseph, Lumbu Simbi Jean-Baptiste.

Statement of ethical approval
The project proposal and procedures were reviewed and approved by the Department of Pharmacology in the faculty of Pharmaceutical Sciences from the University of Lubumbashi, DRC (UNILU/FSP/DPCOL/PT/002/2014).

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