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(RESEARCH ARTICLE)

Antiplasmodial, antioxidant and toxicological study of leaves extracts of *Dalbergia katangensis* Lecheneaud (Fabaceae) from Eastern DR Congo

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Abstract

Dalbergia katangensis is used in traditional medicine in Bukavu in management of malaria. In this study, antiplasmodial, antioxidant activities, and 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 plants used traditionally to treat malaria. Extrac's 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, twenty animals (5/group) were given orally singular 2000 mg of extract/kg body weight (BW) then observed for 14 days. In sub-acute toxicity assay, 200, 400 mg/kg BW/Day were given orally, and animals were observed for 28 days. The total phenolic (0.58-0.61 mg $GAEg^{-1}$), total flavonoid (0.14-0.15 mg QEg^{-1}) and total terpenoids contents (0.32-0.55 %w/w) were in the same rate in the two extracts as well as the antioxidant activity with IC_{50} value 1.55 ± 0.012 and 1.71 ± 0.014 µg/mL. At the highest oral dose,400 mg/kg body weight, all extracts produced 73–75% chemo-suppression against *P. berghei* ANKA and 28 survival days. No deaths were recorded during the acute toxicity assay suggesting the LD_{50} >2000 mg/kg and no abnormal behavior or variation in toxicity biomarkers were observed during the subacute toxicity assessment. *Dalbergia katangensis* 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: Bukavu; antimalarial; DPPH; *Plasmodium berghei; Mus norvegicus; Mus musculus*

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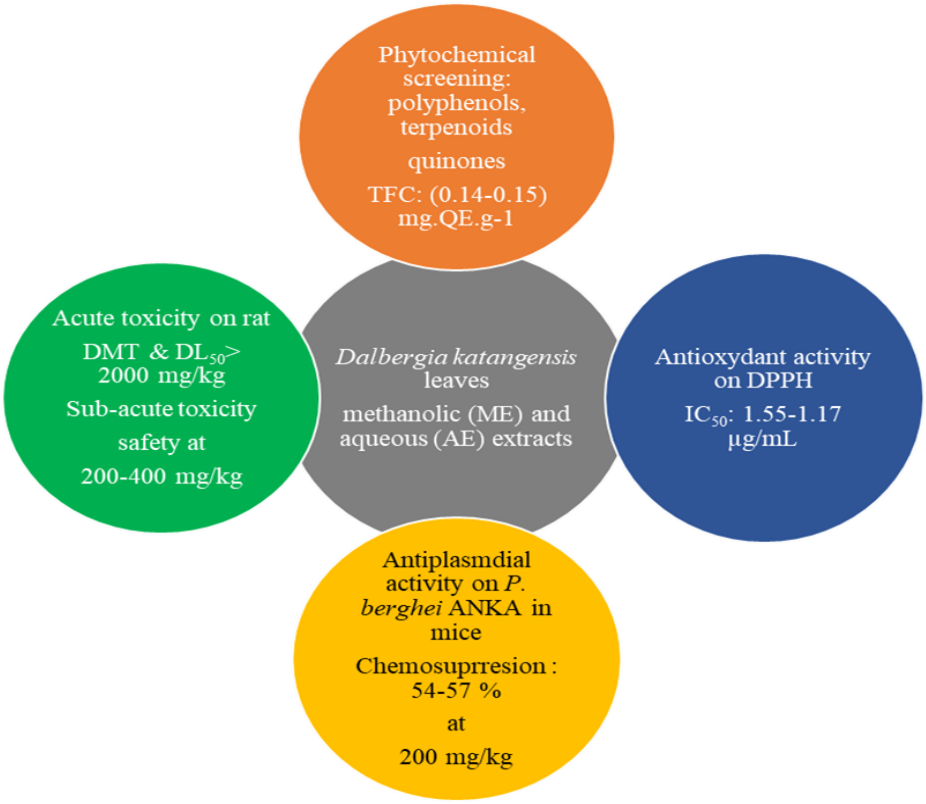
Graphical Abstract



1. Introduction

Malaria is one of the most prevalent and serious protozoan tropical diseases which causes millions of clinical cases worldwide each year, and approximatively 1 million of death annually [1]. The World Health Organization (WHO) African Region accounted for 93% of all cases in 2018 and more than half of all cases were in six countries: Nigeria (25% of cases), Democratic Republic of Congo, DRC (12%), Uganda (5%), Ivory Coast, Mozambica and Niger, 4% each [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 America [6,7], in Asia [8,9] as well as in Africa [10,11] and particularly in DRC [12,13].

In Bukavu, among the many plants reported as antimalarials [5,14] we have selected *Dalbergia katangensis* Lecheneaud (Fabaceae) [4]. It is a tree from which there is not much information in the literature. The plant is locally named *Mungobole, Nfuma* (shi), *Munyereza* (fulero), and it's used in the treatment of malaria and other infectious diseases such vaginitis, bacillary dysentery (root bark), cholera, tuberculosis (stem bark) and conjunctivitis (leaves). 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 antiplasmodial activity *in vivo* and antioxidant *in vitro* of methanolic, and aqueous extracts of the leaves of *Dialium angolense*. On this same occasion, we evaluate the acute and subacute toxicity on rats, and we look for large phytochemical groups mainly secondary metabolites with antimalarial potentiality.

2. Material and methods

2.1. Plant material and experimental animals

Leaves of *Dalbergia katangensis* were collected from Bagira (2 ° 28'12.9''S; 28 ° 49'18''E; 2,883.1 m) in june 2015, and was identified at the herbarium of Meise in Belgium with the following voucher number: BR0000018879285. Healthy *Mus musculus* (21.6 ± 2.1 g) and *Mus norvegicus* (265.4 ± 0.73 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 12h 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, have been provided from (Sigma-Aldrich (USA) and all chemicals 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, 12.1 %, W/W). Aqueous extracts (AE) were prepared according to the protocol used in traditional medicine by decocting 340 g of the sample in 1.5 L for 25 minutes of local tap water (boiling for 0.5 h in a close recipient and filtration on paper). The extract was lyophilized (yield, 10.5%, W/W) and for alltest, 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 [15,16].

2.5. Total phenolic, flavonoids and terpenoids contents

The total phenolics content of each sample was measured by a Folin–Ciocalteu method [17] and expressed as milligrams gallic acid equivalents per gram of dry plant extract (mg GAE/g DE) through a calibration curve gallic acid ($y = 0.014x + 0.003$, $R^2 = 0.997$; linearity range, 0.5 – 200 mg. mL^{-1}). The total flavonoids content was determined using an aluminum trichloride assay [18] and expressed as milligrams quercetin equivalents per gram of dry plant extract (mg QE/g DE) through the calibration curve of quercetin ($y = 0.006 x + 0.004$, $R^2 = 0.998$; linearity range, 0.1 to 150 mg/mL). Regarding the determination of total terpenoids contents, dried plant extract 100mg (A) was taken and soaked in 9mL of ethanol (Sigma-Aldrich, USA) for 24 hours. The extract after filtration was taken up by 10mL of petroleum ether (Sigma-Aldrich, USA) using separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (B). Ether was evaporated and the yield (%) of total terpenoids contents (TTC) was measured by the formula:

$\%TTC = A - B$ [19]. (Equation 1).

2.6. Antioxidant activity-DPPH Assay

DPPH radical scavenging activity of the plant extracts at varying concentrations (1.95-125 µg/mL) were measured *in vitro* via the DPPH assay [20]. 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:

$$\% \text{ AAO } = \frac{(Ab - Ae)x100}{Ab} \text{ (Equation 2)}$$

Ab = absorbance measured in the presence of the negative control, Ae = absorbance measured in the presence of the extract, and % AAO = Percentage of inhibition. Depending on their IC_{50} values, extracts were classified as following: (i) very active if $IC_{50} \leq 5$ µg/ mL, (ii) active if 5 µg / mL ≤ $IC_{50} \leq 15$ µg / mL, (iii) moderately active if 15 µg / mL < IC_{50} < 50 µg / mL, (iv) weakly active if $IC_{50} \geq 50$ µg / mL[20].

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 [21]. Briefly, donor *Mus musculus* previously infected with *Plasmodium berghei* and having parasitemia level of 20-30% were used to infect the experimental mice intra-peritoneally with 0.2 mL of their infected blood. The infected mice were randomly divided into six groups of 5 each by weight. Three hours after inoculation, each *Mus musculus* was orally treated with 200 µL of oral dose of the sample (200, 400 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 4, 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:

$$\% \text{ P} = \frac{\text{Number of Parasitized RBCx100}}{\text{Total Number of RBC Count}}$$ (Equation 3). (RBC: Red Blood Cells).

From day 4 to day 7, thin blood smears were made from the tail of each *Mus musculus*, Giemsa stained and examined microscopically for assessment of parasitemia. On day 7, the level of parasitemia in each group of mice was determined so that the percentage chemo-suppression (TSP) were calculated as:

$$\%\text{TSP} = \frac{(\text{A}-\text{B})\text{x}100}{\text{A}}$$ (Equation 4)

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) [22,23]. *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 [24].

2.8. Toxicological study

Acute toxicity was carried out as described previously [25] using 2000 mg/kg by Weight (BW) in single dose (oral administration; 5 animals per group, followed over 14 days). In subacute toxicity, *Mus norvegicus* (5 each group) received orally for 28 days, 0 (negative control), 200 or 400 mg/kg BW/day. During blood collection and serum preparation for biochemical analysis, validated procedures were followed [25]. 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 Co-operation 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 [26] were duly observed. 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).

3. Results

3.1. Phytochemical screening of *Dalbergia katangensis* extracts

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

Table 1 Phytochemical composition of extract of *Dalbergia katangensis.*

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 0.58-0.61 mg $GAEg^{-1}$ and are four times higher than the Total flavonoids content: TFC values (0.14-0.15 mg QEg^{-1}). Total terpenoids content: TTC, vary between 0.3-0.5 (% W / W) and, a difference between aqueous and methanolic extracts is only observed with TTC with $p < 0.01$ (Table 2).

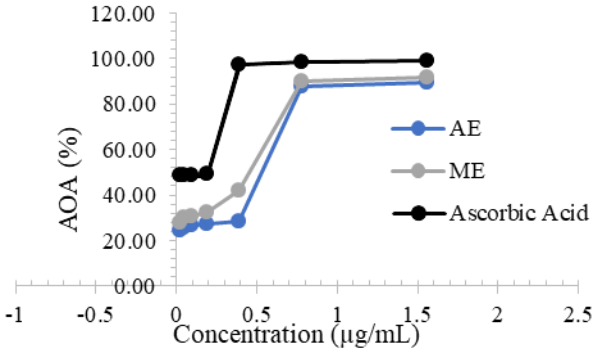
Table 2 Total polyphenol, flavonoid and terpenoids contents of extract of *D. katangensis*

Simple	Total phenolics content (TPC)	Total flavonoids content (TFC)	Total terpenoids content (TTC)
	(mg GAEg-1)	(mg QEg-1)	(% w/w)
ME	0.6120 ± 0.011	0.1530 ± 0.014	0.551 ± 0.011
AE	0.5814 ± 0.012	0.1453 ± 0.015	0.323 ± 0.03[a]

ME: Methanolic extract, **AE**: aqueous extract; Data are expressed as mean ± SD (n = 3) and compared to ME; [a] if $p < 0.01$.

3.2. Antioxidant activity

The scavenging ability of the samples tested showed a dependent concentration activity profile. The anti-free radical activity expressed in the form of IC50-1 is respectively 1.55 ± 0.012 (AE) and 1.71 ± 0.014 µg/mL suggesting a very good antioxidant activity of studied extracts according to the previously proposed classification [20]. No statistically significant difference was observed between the two extracts. In contrast, their antioxidant activity is lower ($p < 0.001$) than ascorbic acid used as a positive control (figure 1).



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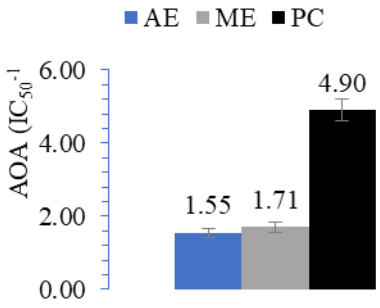


1b

Figure 1 DPPH radical scavenging activities of *Dalbergia katangensis* expressed as a percentage (1a) and as the value of IC_{50}^{-1} in µg/mL(1b). Positive control (PC): ascorbic acid. Data expressed as Mean ± SD, n=5. AOA: antioxidant activity. 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 400 mg/kg WB/day (ME 400) exhibited maximal suppression (74.81 ± 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. katangensis* during established infection (Mean ± SD, n=5).

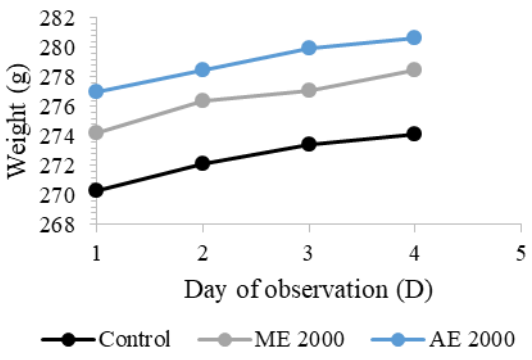
Group	Dose (mg/kg WB)	P (%): Parasitemia	Suppression Rate: PSR (%)	TS(D): Survival Time
ME 200	200	4.86 ± 0.05[a]	57.02 ± 0.25	28
AE 200	200	5.12 ± 0.02[a]	54.73 ± 0.03	28
ME 400	400	2.85 ± 0.18[b]	74.81 ± 0.05	28
AE 400	400	3.01 ± 0.23[b]	73.38 ± 0.15	28
Quinine	10	1.01 ± 0.03[c]	91.06 ± 0.11	28
NaCl 0,9%	-	11.31 ± 0.02	NA	10

D: day. ME 200: Group treated with 200 mg / kg body weight methanolic extracts from leaves of *Dalbergia katangensis*. 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; [a] if $p < 0.01$, [b] if $p < 0.001$, [c] if $p < 0.0001$.

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_{50})

When assessing acute toxicity, signs of asthenia, hair straightening and anorexia were observed in the test groups. On the other hand, during the evaluation of the subacute toxicity, no signs of toxicity were observed. No significant variation in weight was observed either during the assessment of acute toxicity or during the assessment of sub-acute toxicity (figure 2). Likewise, no variation in the weight of certain organs was observed during the evaluation of the subacute toxicity (figure 3). No death was recorded during the toxicological experimentation nor any serum variation of the biomarkers of the hepatic function (ASAT, ALAT, PAL) nor renal (urea, creatinine), in the treated groups (ME200, AE200, ME400, AE400) compared to the control group (0.9% NaCl) (figure 3. c-d). Administration of therapeutic doses (200 and 400 mg/kg) for 28 days of aqueous and methanolic extracts of the leaves of *Dalbergia katangensis* does not cause toxicity in *Mus norvegicus*. The DMT is thus estimated > 2000 mg/kg and the LD_{50} > 2000 mg/kg.



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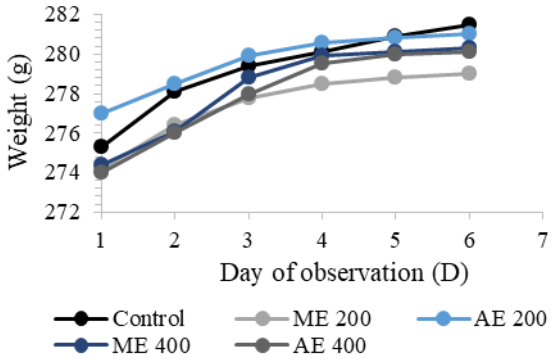

2b

Figure 2 Ponderal evolution of *Mus norvegicus* during the experimental (a): in acute toxicity, (b): in subacute toxicity). Data are expressed as mean (n=5). 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.

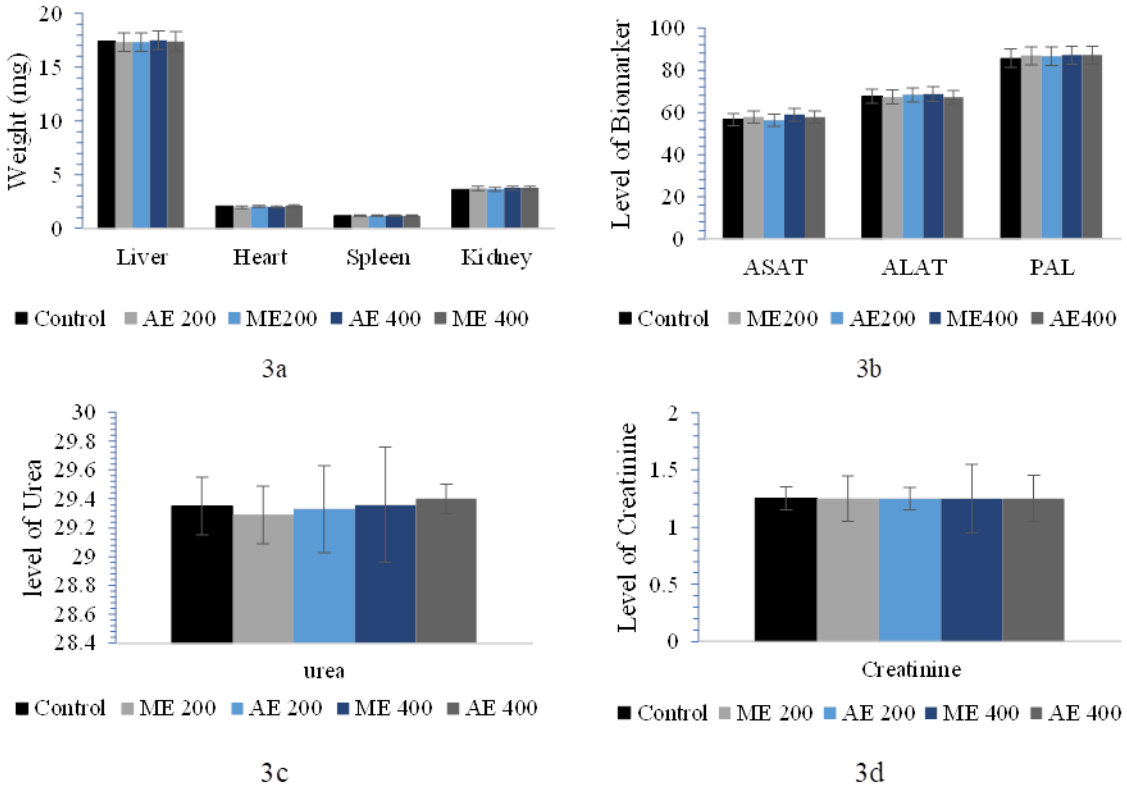

Figure 3 *Mus norvegicus* organs weight (a), hepatic (b) and renal (c, d) variation level of some biomarkers when exposed to *Dalbergia katangensis* extract, 200 and 400 mg/kg BW. ME200: Methanolic extract given at 200 mg/kg BW/day, AE400: Aqueous extract given at 400 mg/kg BW/day. Data expressed as Mean ± SD, n=5.

4. Discussion

In this study, we provide knowledge on the aqueous and methanolic extracts of the leaves of *Dalbergia katangensis* regarding the phytochemical composition on secondary metabolites, the pharmacological activity (antiplasmodial *in vivo* on *Plasmodium berghei* and antioxidant *in vitro* on DPPH), the acute, and subacute toxicities in *Mus norvegicus* model. Water is the solvent used in traditional medicine during the preparation of antimalarial recipe based on *D. katangensis* leaves [4]. On the other hand, methanol, is the solvent most used during the biological screening of plants because it is easily handled, thanks to its great evaporative power. A concomitant test with the aqueous extract and the methanolic extract consists in varying the possibility of switching from the solvent used in traditional medicine to that used in the experimental laboratory.

This study reports a chemical similarity composition in secondary metabolites between methanolic and aqueous extract of leaves of *D. katangensis* by the concomitant presence of flavonoids, quinones, terpenoids and overall polyphenols (Table 1) suggesting the possible similarity in pharmacological activities. Several compounds with antioxidant [27–32] and antiplasmodial [33–37] activity from different groups identified within the leaves of *Dalbergia katangensis* during this study were isolated from many natural product, so that these groups are increasingly considered as groups with antimalarial and antioxidant potential.

No phytochemical study has been reported regarding *D. katangensis;* Nevertheless, other species of the same genus Dalbergia have been studied for phytochemical composition. During this study, we encountered in the two aqueous, and methanolic extracts, polyphenols, particularly flavonoids, quinones, steroids and terpenoids. Several phytochemical groups, including flavonoids, phenols, quinones, steroids, and terpenoids have been isolated from various species of the Dalbergia genus but mainly in the heartwood part. In Leaves theses phytochemical group have been found in *D. sissoo* DC, *D. odorifera* T.C.Chen, and *D. lanceolaria* L.f.; most compound isolated were polyphenols particularly flavonoids [38]. Isoflavones particularly apioglucoside has been proposed as a chemotaxonomic marker of the genus Dalbergia [39].

The methanolic extract from the leaves of *D. katangensis* (Table 2) contains the TPC tree times higher than that of *D. sissoo* (0.182 ± 0.007 mg $GAEg^{-1}$) [40]. In contrast, *D. sissoo* with TFC = 5.5 $mgQEg^{-1}$ of methanolic extract [41], contains

a TFC, 50 times greater than that of *D. katangensis.* The polyphenols, however abundant they may be in the leaves of *D katangensis*, are mostly non-flavonoid.

This study (figure 1) confirms the interesting antioxidant activity of aqueous and methanolic extracts of *Dalbergia katangensis* leaves previously reported [20]. At the genus level, The antioxidant activity at DPPH, of the leaves of some species of the genus Dalbergia has been previously reported, in particular for *D. paniculata* Roxb, IC_{50} = 70.6 µg/mL [42], *D. odorifera* T.C.Chen, IC_{50} = 5.1 ± 0.12 µg/mL [43], *D. brasiliensis* Vogel, IC_{50} = 74.5 ± 1.3 µg/mL [44] and *D. sissoo*, IC_{50} = 3.2 µg/mL [45]. Obviously, *D. katangensis* (ME IC_{50} = 0.58 µg/mL) exhibits an antioxidant activity greater than that of the above-mentioned plant species. According to the classification proposed previously [20], this antioxidant activity of *D. katangensis* is very good and would very probably be linked to the presence of the polyphenolic compounds identified (Table 1) and quantified (Table 2) during this study, especially since phenolic compounds are known as antioxidants [46–48].

D. katangensis extracts sufficiently reduced parasitemia in the animals on day 7 of observation (Table 3) and these reductions in parasitemia were dose dependent. This situation is observed in plants with antimalarial activity in the rodent model and constitutes one pledge of their activities [49–51]. According to the classification previously proposed [52], all extract presented a good antiplasmodial activity *in vivo*. Some species of the genus Dalbergia have previously led to the isolation of antimalarial compounds. The antimalarial activity of the stem of *D. parviflora* Roxb. has been attributed to dalparvone, a flavonoid [53], that of *D. louvelii* R.Vig., was attributed to 4 compounds: (R)-4′′-methoxydalbergione(quinone),7,4'Dihydroxy-3-methoxyisoflavone(flavonoid), Obtusafuran (phenol) and isoliquiritigenin (Phenol) [54]. Isoliquiritigenin has also been reported in *D. odorifera* T.C.Chen [55] and *D. cochinchinensis* Laness [56]. It is therefore probable to discover within *D. katangensis*, antimalarial compounds resulting from these phytochemical groups identified in the plant during the present study (Table 1).

The leaves of *D. katangensis* exhibited an antiplasmodial activity positioned variously in relation to some antimalarial species from the DRC studied previously. Its activity is lower than the activity of the leaves of *Senna occidentalis* (L.) Link (Fabaceae*)*: ME, TSP 73% at 200 mg/kg BW [57] and the bark of the roots of *Alstonia congensis* Engl. (Apocynaceae): AE, TSP: 80.43 ± 0.12 mg/kg BW [1] but it is superior to the leaves of *Physalis angulata* L (Solanaceae): ME, TSP = 60% at 300 mg/kg BW and *Anisopappus chinensis* Hook (Asteraceae): AE, TSP: 46.6% at 300 mg/kg [23].

At experimental doses of 200, 400 mg/kg (subacute toxicity) and 2000 mg/kg (acute toxicity), the toxicity of *D. katangensis* has not been sufficiently established. We can assume, as for other previous studies on other species of the same genus, in particular *D. sissoo* DC. [58], *D. saxatilis* Hook.f. [59], *D. monetaria* L.f. [60], *D. candenatensis* (Dennst.) Prain [61], that the toxicity of *D. katangensis* leaves would only occur at higher doses. This information further supports the use of *D. katangensis* in traditional medicine. However, it should be noted that unlike these aforementioned species, *D. katangensis* showed some signs of toxicity during the acute toxicity assessment, suggesting that at doses higher than those used during this study, the plant would present some toxicity *in vivo*. This aspect should be considered in subsequent studies to establish the toxicological profile of this plant.

5. Conclusion

For the first time, a promising antiplasmodial activity *in vivo* against *P. berghei* on *Mus musculus* model with an interesting toxicological profile on *Mus norvegicus* is demonstrated for the leaves of *Dalbergia katangensis* and its antioxidant activity *in vitro* previously known is confirmed. This plant is particularly interesting for a further investigation as very few is known about its phytochemical composition.

Compliance with ethical standards

Acknowledgments

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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.

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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