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(RESEARCH ARTICLE)



## Antimicrobial, antioxidant activities and toxicity on *Cavia porcellus* of *Dialium angolense* Welw. Ex Oliv, a traditional medicinal plant from Bagira in Eastern of DR Congo

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### Abstract

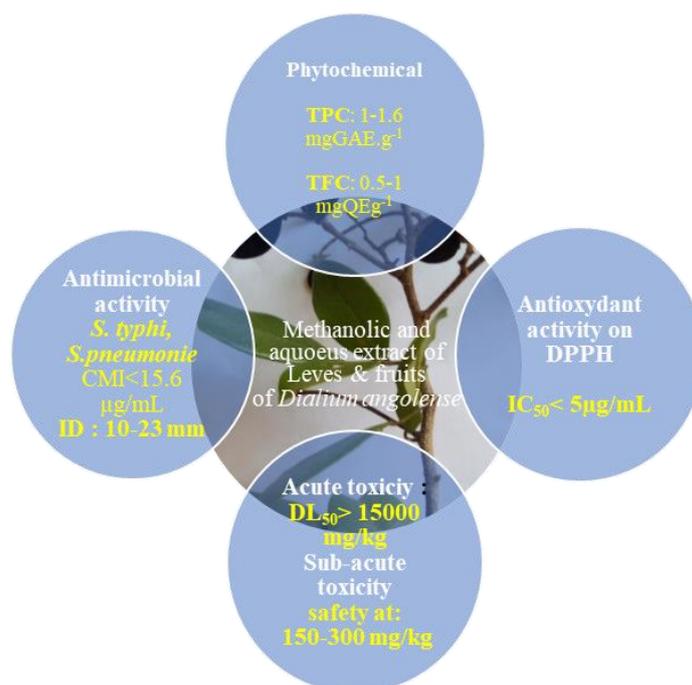
*Dialium angolense* is used in Bagira for its various medicinal properties particularly in the management of infectious diseases. In this study, the methanol and aqueous extracts of leaves and fruits were evaluated for their *in vitro* antioxidant and antimicrobial properties and their *in vivo* toxicity on *Cavia porcellus*. The major phytochemical classes of extracts were screened using standard in-tube reactions. The antimicrobial study was tested on *Candida albicans*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pneumoniae* using agar well diffusion and dilution methods, while the antioxidant activity was evaluated by a DPPH assay. For the acute toxicity study, animals (6/group) were orally given in a single dose 5000, 1000 or 15000 mg of extract/kg body weight (BW) then observed for 14 days. In sub-acute toxicity assays, 150 or 300 mg/kg BW/day were orally given, and animals observed for 28 days. Total phenolics and total flavonoids contents ranged 1.19 to 1.61 mg GAE.g<sup>-1</sup> and 0.45 to 1.01 mg QEg<sup>-1</sup>, respectively. The extracts presented antioxidant activity with IC<sub>50</sub> ranging 4.9 to 6.9 µg/mL. The minimal inhibitory concentration (MIC) on tested strains ranged from 1.9 to 500 µg/mL with the aqueous extract of fruits as a most active extract: MIC=1.9 µg/mL on *E. coli* and *C. albicans*. No signs of toxicity were noted during the acute and sub-acute toxicity assessments, suggesting a maximal tolerate doses (MDT) and LD<sub>50</sub> > 15000 mg/kg BW. This study highlights the antioxidant and antimicrobial activities of *Dialium angolense* and suggests that further studies be directed towards the isolation of active compounds.

**Keywords:** Antibacterial; Anti-free radical activity; Flavonoids; *Dialium angolense*; Phenolics; Toxicity

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## Graphical abstract



## 1. Introduction

Microbial infections are a major health concern not only because of their high prevalence but also of their high mortality in most African countries like the DR Congo [1–4]. Despite the existence of a varied therapeutic arsenal [5], the emergence of antimicrobial resistances reduces its impact and justifies the need for the research of new active therapeutic substances with various mechanisms of action [6–9]. Rich in secondary metabolites, plants used in traditional medicine have advantages that could offer humanity such expected antimicrobials [10,11]. Several screening studies, carried out in different African regions, have resulted in the isolation and characterization of antimicrobial compounds [12–14]. Several antimicrobial compounds have antioxidant potential [15–17]. That is why, there is currently a tendency to investigate antimicrobial and antioxidant activities as shown by several recent studies carried out on African medicinal plants [18–20]. Despite their reported medicinal properties, indicated by ethnobotanical studies, the pharmacology of the many plants used in traditional African medicine is quite often insufficiently investigated and their safety is mostly unknown. [21,22].

*Dialium angolense* Welw. ex Oliv., also called *Dialium evrardii* (Steyaert) belongs to the Fabaceae family, with many local names like *Kizimya* (Shi) or *Cituzo* (Havu); is one of those medicinal plants that are not well known. It is a tree of 3 to 12 meters high, endemic in tropical Africa. In DR Congo, the leaves and the fruits are used in the treatment of headaches, fever, gastritis, conjunctivitis, urethritis, amebiasis, malaria, digestive and respiratory diseases. Both organs are edible, the fruits are consumed by humans and the leaves by primates [23,24]. A recent study reported antioxidant and antiplasmodial activities *in vitro* of aqueous and methanolic extracts of *Dialium angolense* leaves [25]. Unfortunately, no information has been reported on the antimicrobial potential or less on the toxicity and phytochemical composition of the leaves and fruits of this plant. This study aims to evaluate the antimicrobial activity of aqueous and methanolic extracts of leaves and fruits of *Dialium angolense* on germs responsible for some digestive, respiratory and nosocomial infections. It also intends to establish its phytochemical profile in secondary metabolites and assess the acute and subacute toxicity of its two organs, on *Cavia porcellus*.

## 2. Material and methods

### 2.1. Plant material and experimental animals

Leaves and fruits of *Dialium angolense* were collected on June 2016 from Bagira (2°28'12.9"S; 28 ° 49'18"E; 2,883.1 m) and was identified at the herbarium of Meise in Belgium (voucher number: BR0000018879285). Healthy *Cavia porcellus* (275.5 ± 5.2 g) were obtained from animals holding unit of the zoo-technology Department of the Faculty of

Agronomic Sciences, University of Lubumbashi. They were allowed unrestricted access to food for rodents (MIDEMA-DRC) and water *ad libitum*.

## 2.2. Chemicals and reagents

Ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), ciprofloxacin, fluconazole, gallic acid, methanol, quercetin, and vanillin 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 coarsely powdered dried vegetal material in 1.5 L of methanol. 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 rotary evaporator (Büchi R-210, Switzerland) at 40 °C under reduced pressure :130-180 mbar (Yield, 12.8 %, 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, 11.9 %, m/m). For all tests, each extract was dissolved in its extraction solvent before mixed with the vehicle.

## 2.4. Screening for secondary metabolites

The plant extract was analyzed for the presence of some secondary metabolite including alkaloids, coumarins, flavonoids, saponins, steroids, tannins, terpenoids and phenols, using standard in-tube reactions [26, 27].

## 2.5. Determination of total phenolics, flavonoids and tannins contents

The total phenolics content of each sample was measured by a Folin-Ciocalteu method [28] and expressed as milligrams gallic acid equivalents per gram of dry plant extract (mg GAE/g DE) through a calibration curve gallic acid ( $y = 0.011x + 0.001$ ,  $R^2 = 0.998$ ; linearity range, 1 – 200 mg. L<sup>-1</sup>). The total flavonoids content was determined using an aluminum trichloride assay [29] and expressed as milligrams quercetin equivalents per gram of dry plant extract (mg QE.g<sup>-1</sup> DE) through the calibration curve of quercetin ( $y = 0.008x + 0.001$ ,  $R^2 = 0.996$ ; linearity range, 0.1 to 150 mg/mL). The total tannins were determined by a vanillin method [30] and expressed as milligrams gallic acid equivalents per gram of dry plant extract (mg GAE.g<sup>-1</sup> DE) through the calibration curve established for gallic acid ( $y = 0.006x + 0.0011$ ,  $R^2 = 0.997$ ; linearity range, 1 – 100 mg. L<sup>-1</sup>).

## 2.6. Antimicrobial Activity Assay

*Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 14028, *Staphylococcus aureus* ATCC 6538, and *Streptococcus pneumoniae* ATCC 49619 were supplied by the Provincial Laboratory of Lubumbashi where the tests were carried out using an agar diffusion method [31]. A broth dilution method was used in triplicates to determine the Minimum Inhibitory Concentrations (MIC) and Minimum Microbicidal Concentrations (MMC) of the extract [32]. The germs used during this study were chosen according to the anti-infectious uses of *D. angolense* in Traditional Congolese Medicine (TCM): *E. coli*, *S. typhi*, (for digestive infections), *S. aureus* (for nosocomial infections) and *S. pneumoniae* (for respiratory infections). The measurement of the diameter of inhibition (ID) made it possible to determine the sensitivity of different germs by the diffusion method and the dilution test made it possible to determine the MIC and the MMC. These various parameters made it possible to determine and characterize the antimicrobial activities of the extracts.

For the determination of the sensitivity, in sterile Petri dishes, 20 mL of Muller-Hinton agar were poured and then left to stand for 20 minutes. After solidification, on each culture medium, 1 mL of bacterial suspension of 10<sup>8</sup> CFU /mL was seeded over the entire surface. Blotting paper discs ( $\varnothing = 6$  mm) were impregnated with a volume of 5  $\mu$ L of extracts (50 and 100  $\mu$ g/mL) and placed on the surface of the solidified and infected medium. The Petri dishes were then incubated at 37 °C for 48 h in the oven. The sensitivity of the germs to the extracts was estimated by measuring the diameter (mm) of the zone of inhibition induced by the different concentrations around the discs and each experiment was repeated three times [33].

Regarding the determination of the MIC, 100  $\mu$ L of each extract at 1 mg/mL (dissolved in methanol or water depending on the extract) was mixed with 1900  $\mu$ L of the culture medium. Eight successive dilutions of order 2 (from 50  $\mu$ g / mL to 1.9  $\mu$ g / mL) were then carried out for each extract and placed in different aseptic tubes of 5 mL; 1000  $\mu$ L of the standard inoculum was then added to each tube and the mixture was incubated for 24 h at 37 °C. The growth of the microorganisms was observed visually. The MIC, considered to be the lowest concentration at which the extract prevented the visible growth of bacteria was determined [26].

Regarding the determination of the minimum microbicidal concentration (MCM), the sampling was carried out in tubes used for the determination of the MIC. The inoculation was carried out in the Petri dishes on Salmonella-shigella agar (bacteria) or Sabouraud agar (fungi) medium and the incubation was carried out at 37 °C (bacteria) or 28 °C (fungi) for 24 h. Microbial growth was checked visually and MMC, defined as the smallest concentration at which the extract prevented the visible growth of microbes (fungi or bacteria) after sub culturing was determined [32].

### 2.7. DPPH assay

Antioxidant activity was evaluated using DPPH method [25, 34]. Briefly, 50 µL of extract (or positive control) prepared at different dilutions of order 2 in methanol from a 100 µg/mL solution were interacted with 1950 µL of 0.002% DPPH in a plate 96 wells (Nunc WVR, Germany). 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 and the 0.002% DPPH solution was used as a negative control. The percentage of antioxidant activity was calculated by the formula:

$$\% \text{ AAO} = (\text{Ab} - \text{Ae}) \times 100 / \text{Ab} \quad (\text{Equation 1})$$

Where, Ab = absorbance measured in the presence of the negative control; Ae = absorbance measured in the presence of the extract, AAO (%) = Percentage of inhibition.

### 2.8. Toxicological Study

Acute toxicity was carried out as previously described [35] using, 0 (i.e. vehicle = control), 5000, 10000, 15000 mg/kg BW in single dose (oral administration; 6 animals per group, followed over 14 days). For the subacute toxicity study, *Cavia porcellus* (6 animals per group) orally received for 28 days, 0 (i.e. vehicle = control), 150, and 300 mg/kg BW/day. During blood collection and serum preparation for biochemical analysis, validated procedures were followed [35]. 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). When evaluating subacute toxicity, we used the dose of 150 mg/kg, corresponding to the dose used in traditional medicine, and double of this dose (300 mg/kg); As for the doses used for the assessment of acute toxicity, we took into account the doses from the preliminary tests following the Organization for Economic Cooperation and Development OECD procedure for which up to 3000 mg/kg no sign of toxicity was observed.

### 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 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 [36] 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, DR Congo (UNILU/FSP/DPCOL/PT/00/2014).

## 3. Results

### 3.1. Chemical screening of *Dialium angolense*

The phytochemical screening of *Dialium angolense* revealed the presence of polyphenols, tannins, terpenoids, flavonoids, coumarins, anthraquinones but the absence of alkaloids and saponins (Table 1).

The highest values in total phenols ( $1.6118 \pm 0.006 \text{ mg GAEg}^{-1}$ ), total flavonoids ( $1.0112 \pm 0.006 \text{ mg QE.g}^{-1}$ ) and total tannins ( $0.2830 \pm 0.001 \text{ GAE.g}^{-1}$ ) were observed in the methanolic extract of fruits (MEF). Overall, the total flavonoids levels are more than 3 times higher than the total of tannins and the fruit contents are higher ( $p < 0.01$ ) than leaves (Table 2).

**Table 1** Phytochemical screening of *Dialium angolense*.

Phytochemical	Leaves	Fruits
Alkaloids	-	-
Antraquinones	+	+
Coumarins	+	+
Flavonoids	+	+
Polyphenols	+	+
Saponins	-	-
Steroids	+	+
Tannins	+	+
Terpenoids	+	+

+: Positive reaction, -: Negative reaction. Screening carried out by reactions in solution from the powder of the plant drug

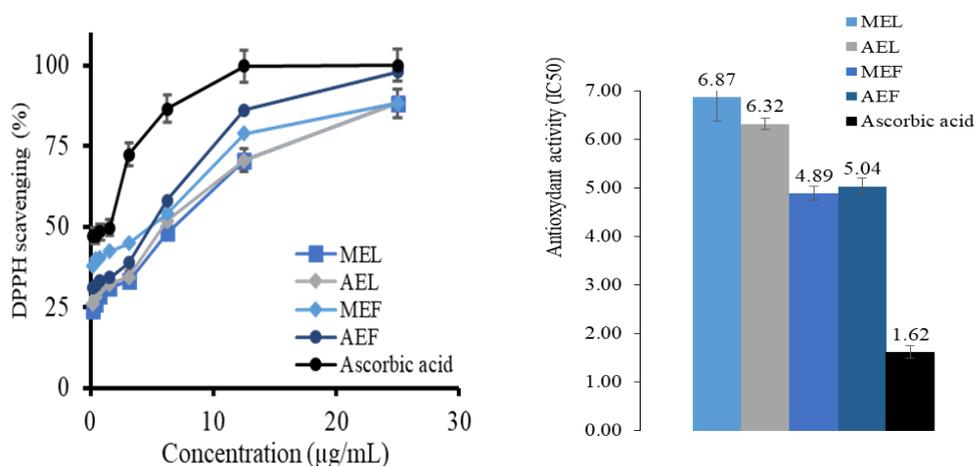
**Table 1** Total polyphenol, flavonoid and tannins contents of extract from *Dialium angolense*.

Simple	Total phenolics (mg GAEg <sup>-1</sup> )	Total flavonoids (mg QE. g <sup>-1</sup> )	Total tannins (mg GAE.g <sup>-1</sup> )
MEL	1.212 ± 0.003	0.495 ± 0.001	0.221 ± 0.002
AEL	1.191 ± 0.004	0.452 ± 0.004	0.182 ± 0.001
MEF	1.612 ± 0.006 <sup>a</sup>	1.011 ± 0.006 <sup>a</sup>	0.283 ± 0.001 <sup>a</sup>
AEF	1.583 ± 0.005 <sup>a</sup>	0.981 ± 0.008 <sup>a</sup>	0.243 ± 0.002 <sup>a</sup>

MEL: methanolic leaf extract, AEL: aqueous leaf extract, MEF: methanolic fruit extract, AEF: aqueous fruit extract. The results are expressed as the mean ± standard deviation. The comparison is made between extracts of the same nature (aqueous extract between them and methanolic extracts between them). The letter presents the level of significance of the difference: a p < 0.01, b p < 0.001

### 3.2. Antioxidant activity

Depending on their IC<sub>50</sub> values, extracts were classified as following: (i) very active if IC<sub>50</sub> ≤ 5 µg/ mL, (ii) active if 5 µg/mL ≤ IC<sub>50</sub> ≤ 15 µg/mL, (iii) moderately active if 15 µg/mL < IC<sub>50</sub> < 50 µg/mL, (iv) weakly active if IC<sub>50</sub> ≥ 50 µg/mL [25]. The scavenging ability of tested samples showed a concentration-dependent activity. The anti-free radical activity, expressed in the form of IC<sub>50</sub>, varied between 1.62 and 6.87 µg/mL suggesting that all extract were very actives. In line with their higher content in phenols, flavonoids and tannins, the fruits have an anti-radical power superior to that of the leaves (p < 0.01) and particularly AEF, although less than that of ascorbic acid, used as positive control (Figure 1).



**Figure 1** *Dialium angolense* DPPH radical scavenging activities expressed as percent reduction (a) and as IC<sub>50</sub><sup>-1</sup> (b). The results are expressed as the mean ± SD, n = 5

### 3.3. Antimicrobial activity

#### 3.3.1. Sensitivity of tested bacteria and fungus

The microbial sensibility was assessed by the measurement of inhibition diameter (ID) on agar. A microbial strain was considered "sensitive" for ID  $\geq 10$  mm [37]. The highest dose-dependent sensitivity was observed by *E. coli* (ID of AEL is  $24.0 \pm 0.2$  mm). In this study, *S. pneumoniae* and *S. typhi* are the most sensitive microbes in the study with 100% and 80% of positive tests, respectively (Table 3).

**Table 2** Sensitivity of selected microbial strains to *Dialium angolense* extracts.

Simple	Inhibition diameter (mm)				
	<i>E. coli</i>	<i>S. typhi</i>	<i>S. pneumoniae</i>	<i>S. aureus</i>	<i>C. albicans</i>
	<b>50 <math>\mu\text{g/mL}</math></b>				
FLUC	-	-	-	-	$27.3 \pm 0,1$
CIP	$28.0 \pm 0.2$	$27.0 \pm 0.1$	$18.0 \pm 0.1$	$29.0 \pm 0.1$	$8.0 \pm 0.4$
MEL	$1.2 \pm 0.1$	$9.1.0 \pm 0.2$	$10.1 \pm 0.1$	$9.0 \pm 0.1$	$10.0 \pm 0.2$
AEL	$3.0 \pm 0.2$	$10.0 \pm 0.2$	$12.0 \pm 0.2$	$8.0 \pm 0.2$	$8.0 \pm 0.1$
MEF	$8.0 \pm 0.1$	$17.0 \pm 0.2$	$14.0 \pm 0.1$	$15.0 \pm 0.1$	$14.0 \pm 0.1$
AEF	$20.0 \pm 0.2$	$19.0 \pm 0.1$	$19.0 \pm 0.2$	$18.0 \pm 0.2$	$19.0 \pm 0.1$
	<b>100 <math>\mu\text{g/mL}</math></b>				
MEL	$6.2 \pm 0.2$	$6.0 \pm 0.2$	$12.0 \pm 0.2$	$9.0 \pm 0.2$	$11.0 \pm 0.2$
AEL	$9.0 \pm 0.2$	$11.0 \pm 0.1$	$12.0 \pm 0.2$	$8.0 \pm 0.2$	$12.0 \pm 0.2$
MEF	$20.0 \pm 0.1$	$19.0 \pm 0.1$	$18.0 \pm 0.2$	$17.0 \pm 0.2$	$15.0 \pm 0.2$
AEF	$24.0 \pm 0.2$	$22.0 \pm 0.1$	$23.0 \pm 0.2$	$21.0 \pm 0.2$	$23.0 \pm 0.1$

MEL: Methanolic extract of leaves, AEL: aqueous extract of leaves, MEF: Methanolic extract of fruits, AEF: aqueous extract of fruits, Values are Mean  $\pm$  SD, n=3, ciprofloxacin (antibacterial positive control), FLUC: Fluconazole (antifungal positive control).

#### 3.3.2. MIC (Minimum Inhibitory Concentration) and Minimum Microbicidal Concentration (MMC)

The MIC allowed to categorize the activity of extracts in one of 4 following groups: i) very active extract if MIC  $\leq 5$   $\mu\text{g/mL}$ , ii) active extract if  $5 \mu\text{g/mL} \leq \text{MIC} \leq 50 \mu\text{g/mL}$ , iii) moderately active extract if  $50 \mu\text{g/mL} \leq \text{MIC} \leq 325 \mu\text{g/mL}$  and iv) low activity extract if MIC  $> 325 \mu\text{g/mL}$  [32]. All extracts were found to be at least active on *S. typhi* and *S. pneumoniae* and AEF was the most active (MIC =  $3.9 \mu\text{g/mL}$  and  $7.8 \mu\text{g/mL}$ , respectively) extract; overall the AEF extract was the most active on other strains, *E. coli* and *C. albicans* (Table 4).

**Table 4** Minimum Inhibitory Concentration (MIC;  $\mu\text{g/mL}$ ) and Minimum Microbicide Concentration (MMC;  $\mu\text{g/mL}$ ) of *Dialium angolense* extracts.

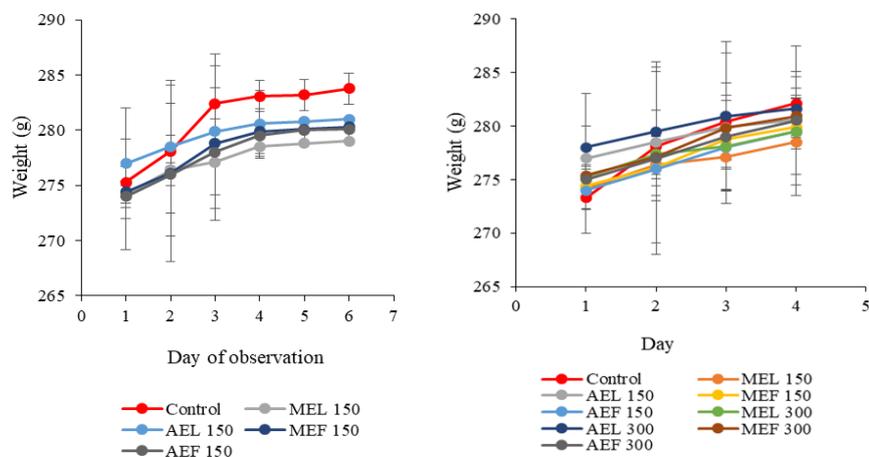
Simple		<i>E. coli</i>	<i>S. typhi</i>	<i>S. pneumoniae</i>	<i>S. aureus</i>	<i>C. albicans</i>
MEL	MIC	500	7.8	15.6	500	500
	MMC	500	7.8	15.6	500	500
	Effect	BC	BS	BC	BC	FC
AEL	MIC	500	7.8	15.6	500	125
	MMC	500	3.9	3.9	500	125
	Effect	BC	BS	BS	BC	FC
MEF	MIC	500	3.9	3.9	250	31.2
	MMC	500	15.6	3.9	250	31.2
	Effect	BC	BS	BC	BC	FC
AEF	MIC	1.9	3.9	7.8	125	1.9
	MMC	7.8	3.9	31.2	125	31.2
	Effect	BS	BC	BS	BC	FS

MEL: Methanolic extract of leaves, AEL: aqueous extract of leaves, MEF: Methanolic extract of fruits, AEF: aqueous extract of fruits, BS: bactericide, BS: bacteriostatic, FC: fungicide, FS: fungistatic.

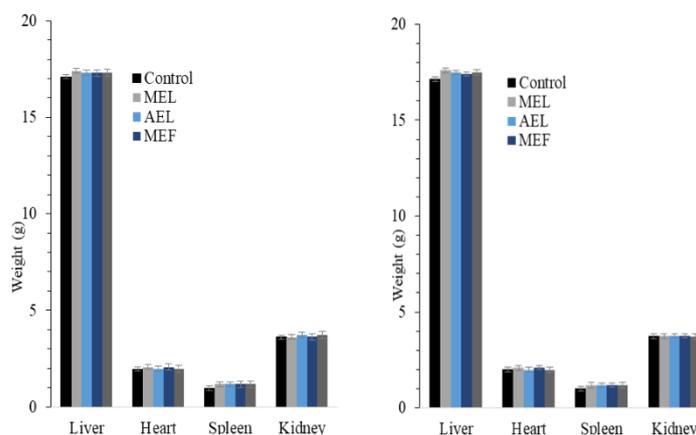
### 3.4. Acute and sub-acute toxicities

#### 3.4.1. Clinical signs, weight variation, MTD (maximal tolerate dose) and 50 % lethal dose (LD<sub>50</sub>)

As for the control group, no sign of acute or sub-acute toxicity was observed in animals following administration of *D. angolense* extracts; there were no significant variations in weight, either for body (Figure 2) or heart, liver, spleen and kidney (Figure 3a-b).



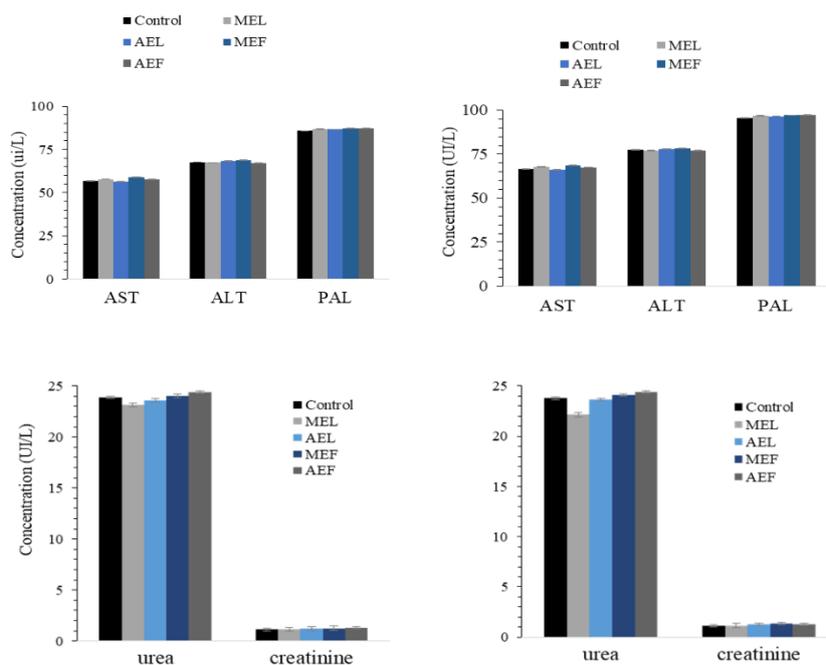
**Figure 2** Weight evolution of *Cavia porcellus* after oral administration of various extracts from the leaves and fruits of *D. angolense* during the evaluation of acute toxicity (a) with 1.5 g / kg and sub-acute (b) with 150 and 300 mg / kg. Results expressed as Mean  $\pm$  SD, n = 6. Weights were taken every 7 days from the first week before intoxication (D-7). AEL: aqueous extract of the leaves, AEF: aqueous extract of the fruits, MEL: methanolic extract of the leaves, MEF: methanolic extract of the fruits. MEL150: the methanolic extract of the leaves administered at a dose of 150 mg / kg of weight.



**Figure 1** Variation in the weight of the noble organs of *Cavia porcellus* after oral administration of doses of 150 mg / kg / d (a) and 300 mg / kg / d of extracts of leaves and fruits of *Dialium angolense* for 28 days. Results expressed as Mean  $\pm$  SD, n = 6. AEL: aqueous extract of the leaves, AEF: aqueous extract of the fruits, MEF: methanolic extracts of the leaves, MEL: methanolic extracts of the fruits.

No death was recorded during the experimentation nor any variation in serum biomarkers of the hepatic (transaminases, alkaline phosphatase) and renal (urea, creatinine) functions (Figure 4 c-f). The administration of single high doses (15000 mg/kg) or daily therapeutic doses (150 and 300 mg/kg) for 28 days of aqueous and methanolic extracts of the leaves and fruits of *Dialium angolense* does not cause toxicity on *Cavia porcellus*. The MTD and the LD<sub>50</sub> cannot be estimated from this study but are over 15000 mg extract/kg. Considering the dosages applied in traditional

medicine (300 mg of fruits/kg Day or 264 of leaves per day, corresponding to 185 mg/kg /j and 147.75 g/kg/day of aqueous extracts, respectively).



**Figure 4** Levels of hepatic (AST, ALT, PAL) and renal (Urea, Creatinine) biomarkers in *Cavia porcellus* on the 28th day of the experiment, after daily administration of the methanolic (ME) and aqueous (EA) extracts of the leaves (L) and fruits (F) of *D. angolense* at the respective doses of 150 mg/kg/day (a) and 300 mg/kg/day (b). N=6

### 3.5. Correlation between antioxidant activity and different content in phenols

The contents in total phenolics and flavonoids showed a correlation with the measured antioxidant (hydrogen-donating) activities; this was particularly the case for flavonoids (Table 5).

**Table 5** Correlation between Antioxidant (AOA) and total phenols content of different extracts from *D angolense's* leaves

		MEF	AEF	AEL	MEL	R
AAO	IC <sub>50</sub> <sup>-1</sup>	0.145	0.158	0.205	0.199	NA
TPC	Concentration	1.212	1.191	1.612	1.583	0.976
	AAO/TPC	0.120	0.133	0.127	0.125	NA
TFC	Concentration	0.495	0.452	1.011	0.981	0.971
	AAO/TFC	0.294	0.350	0.202	0.202	NA
TTC	Concentration	0.221	0.182	0.283	0.243	0.787
	AAO/TTC	0.658	0.872	0.723	0.818	NA

Data expressed as Mean (M), n=3; MEL : Methanolic extract of leaves; AEL: aqueous extracts of leaves; MEF: methanolic extract of fruits; AEF: aqueous extracts of fruits; AOA: antioxidant activity (IC<sub>50</sub>-1 in µg/mL); R : coefficient of correlation with AOA; TPC: Total polyphenol content (mg GAE.g-1); TFC: Total Flavonoids contents (mg QE. g-1); TTC: Total tannins contents (mg GAE.g-1).

### 3.6. Correlation between Antimicrobial activity and content in different phenols quantified

A positive correlation was observed between the content of phenols, especially flavonoids, and antimicrobial activity. This correlation was very significant in particular on the strains *S. typhi* (R<sub>TPC</sub> = 0.998; R<sub>TFC</sub> = 0.997) and *S. pneumonia* (R<sub>TPC</sub> = 0.845; R<sub>TFC</sub> = 0.838), suggesting that the polyphenols in particular the flavonoids contribute very significantly in the expression of the antimicrobial activity of the different extracts (table 6).

**Table 6** Correlation between Antimicrobial activity (AMA) and total phenols content of different extracts from *D. angolense's* leaves.

Strain		MEL	AEL	MEF	AEF	R (TPC)	R (TFC)
<i>E. coli</i>	TPC	1.212	1.191	1.612	1.583		
	TFC	0.495	0.452	1.011	0.981		
	AMA	0.002	0.002	0.002	0.526	0.534	0.543
	AMA/TPC	0.0017	0.0017	0.0012	0.3323		
	AMA/TFC	0.0040	0.0044	0.0020	0.5362		
<i>S. typhi</i>	AMA	0.128	0.128	0.256	0.256	0.998	0.997
	AMA/TPC	0.1056	0.1075	0.1588	0.1617		
	AMA/TFC	0.2586	0.2832	0.2532	0.2610		
<i>S. pneumoniae</i>	AMA	0.064	0.064	0.256	0.128	0.845	0.838
	AMA/TPC	0.0528	0.0537	0.1588	0.0809		
	AMA/TFC	0.1293	0.1416	0.2532	0.1305		
<i>S. aureus</i>	AMA	0.002	0.002	0.004	0.008	0.785	0.791
	AMA/TPC	0.0017	0.0017	0.0025	0.0051		
	AMA/TFC	0.0040	0.0044	0.0040	0.0082		
<i>C. albicans</i>	AMA	0.002	0.008	0.032	0.526	0.575	0.583

Data expressed as Mean (M), n=3; MEL: Methanolic extract of leaves; AEL: aqueous extracts of leaves; MEF: methanolic extract of fruits; AEF: aqueous extracts of fruits; AMA: Antimicrobial activity (MIC50-1 in µg/mL); R: coefficient of correlation with AMA; TPC: Total polyphenol content (mg GAEg-1); TFC: Total Flavonoids contents (mg QE. g-1); TTC: Total tannins contents (mg GAE.g-1).

### 3.7. Correlation between antioxidant and antimicrobial activity

Very significant correlation ( $R > 0.87$ ) between antioxidant and antibacterial activity was observed in particular with *S. typhi*, *S. pneumoniae*, *S. aureus* and *Candida albicans* and the relationship between antioxidant activity and antimicrobial (AOA / AMA) for these 4 germs ranged between 0.014 and 2.65 (Table 7).

**Table 7** Correlation between Antioxidant (AOA) and antimicrobial activity (AMA) of different extracts from *D. angolense's* leaves.

Strains		MEL	AEL	MEF	AEF	R
<i>E. coli</i>	AAO	6.87	6.32	4.89	5.04	
	MIC	500	500	500	1.9	0.510
	AOA/AMA	0.014	0.013	0.010	2.650	
<i>S. typhi</i>	MIC	7.8	7.8	3.9	3.9	0.970
	AOA/AMA	0.881	0.810	1.254	1.291	
<i>S. pneumoniae</i>	MIC	15.6	15.6	3.9	7.8	0.951
	AOA/AMA	0.441	0.405	1.254	0.646	
<i>S. aureus</i>	MIC	500	500	250	125	0.917
	AOA/AMA	0.014	0.013	0.020	0.040	
<i>C. albicans</i>	MIC	500	125	31.2	1.9	0.874
	AOA/AMA	0.014	0.051	0.157	2.650	

Data expressed as Mean (M), n=3; MEL: Methanolic extract of leaves; AEL: aqueous extracts of leaves; MEF: methanolic extract of fruits; AEF: aqueous extracts of fruits; MIC: minimum inhibitory concentration (µg/mL); R: correlation coefficient; AOA: antioxidant activity.

## 4. Discussion

Given the paucity of information on *Dialium angolense*, the present study was devoted to the phytochemical screening and evaluation of antioxidant and antibacterial activities as well as to the acute and sub-acute toxicities of leaves and fruits aqueous and methanolic extracts.

Very few studies have reported the phytochemical composition of secondary metabolites of species of the genus *Dialium*. We show in the leaves of *D. angolense* the presence of polyphenols, particularly flavonoids and tannins, as previously reported for *D. guineense* Willd and *D. indum* L.[38,39] and in the fruits flavonoids, saponins, and steroids as reported in *D. guineense* [40,41]. The fruits of *D. angolense* have higher flavonoid and tannin contents than *D. indum* [41,

42] but lower than *D. guineense* [43, 44]. There is a need to conduct an extensive phytochemical study of the thirty or so species accepted in this genus [45] to determine whether the flavonoids and/or tannins could constitute chemotaxonomic markers.

The antioxidant activity on DPPH, expressed as IC<sub>50</sub> in µg/mL, of several species of the genus *Dialium* is reported in the literature, *D. indum*, 181.6 ± 0.4 [42,46], *D. guineense*, 50.2 ± 0.2 [47], *D. corbisieri* Staner, 14.4 ± 0.1, *D. gossweileri* Baker F., > 500 [48], and *D. cochinchinense* Pierre, 65.0 ± 0.1 [49]. The results of this study suggest that *D. angolense* (IC<sub>50</sub> = 4.9 ± 0.2 µg / mL) has the highest antioxidant activity compared to the species mentioned above. This interesting antioxidant activity observed in *D. angolense* would be linked to the presence of phenolic compounds mainly flavonoids (Table 5); this is in line with previous studies that attest the antioxidant activity of flavonoids [50–54].

According to the correlation between antioxidant activity and different content in phenols. In this study, there are significant differences between extracts, indicating that the quality of polyphenols/flavonoids in the extract is certainly more important than their content [55]. This is confirmed by Vasco *et al.* [56] who states that the correlation depends on the extraction solvent, the hydrophilicity of the compounds, the sample, and the type of phenolic compounds. By evidence, not all antioxidant characteristics are assessed by the test performed here; notably, the ability to quench *in vivo* oxidative damage and lipid peroxidation largely depends on the lipophilicity of the compounds (phenols, tocopherols, carotenoids, and flavonoid aglycones) and the chelation of metals: ascorbic acid, tannins, flavonoid aglycones and glycosides [57].

The antibacterial activity of *D. angolense* fruits is higher compared *D. guineense* aqueous extract on *E. coli* (MIC = 225 mg/mL), *S. typhi* (150 mg/mL), *S. pneumoniae* (mg/mL) and *S. aureus* (225 mg/mL) [58]. It is more active than *Dialium corbisieri* Staner, aqueous extract on *E. coli* (ID = 0 and 11 mm), *S. aureus* (ID = 0 and 10 mm), *C. albicans* (ID = 0 and 10 mm) [48]. It is likely that the polyphenolic compounds, and particularly flavonoids [59–61], would be both responsible for antioxidant and antibacterial activities of leaves and fruits of *D. angolense*. The antimicrobial activity of flavonoids has previously been demonstrated [62–64] and several antimicrobial mechanisms of compounds in these groups have been demonstrated [59]. It has been established that flavonoids aggravate microorganisms in various ways including to form a complex with the cell wall components and consequently inhibit further adhesions and the microbial growth [65], adhesion to and invasion of hosts by Gram-positive bacteria [66], interaction with liposomes [67], eradication of biofilm bacteria [68] or inhibition of the DNA, RNA and proteins bacterial synthesis [69].

All studied extracts showed a particularly interesting antibacterial activity on *S. typhi*, justifying the use of *D. angolense* against a condition identified as "typhoid fever" in traditional medicine [23]. This activity is particularly interesting since co-infections *Plasmodium falciparum* – *S. typhi* are commonly reported in Bukavu city, as well as elsewhere [70]. As, in the region, the plant is also used as an antimalarial [23] its use may have various benefits.

In this study, it have been established a correlation between antioxidant and antimicrobial activity (Table 7). These results agree with the previous literature where it has been established a correlation between the antioxidant and antimicrobial activity of some plant extracts [71–73]. This correlation would probably be linked to the presence of polyphenols quantified in the plant during this study, as suggested by previous work [74, 75].

Many plants used in traditional medicine with both antioxidant and antimicrobial potential like *Punica granatum* L., Lythraceae [76], *Feijoa sellowiana* (O.Berg) O.Berg, Myrtaceae [77], *Xylopiya aethiopica* (Dunal) A.Rich., Annonaceae [78], *Zingiber officinale* Roscoe, Zingiberaceae [79] or *Fagaropsis hildebrandtii* (Engl.) Milne-Redh., Rutaceae [80], for which LD<sub>50</sub> were estimated ≥ 2000 mg / kg body weight in acute toxicity, present sub-acute toxicity in rodent animal models. By contrast, the methanolic and aqueous extracts of the leaves and fruits of *Dialium angolense* seem quite innocuous, with LD<sub>50</sub> estimated over 15000 mg extract/kg and no signs of sub-acute toxicity, hepatic or renal, observed on *Cavia porcellus* at daily doses of 150 and 300 mg/kg, thus highlighting a probably safe therapeutic use of this plant.

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## 5. Conclusion

This study highlights for the first time the antioxidant and antibacterial activities as well as an absence of sub-acute toxicity of the leaves and fruits of *D. angolense*, while pointing to the role of its polyphenols, especially flavonoids. It thus supports the traditional use of this medicinal plant and opens the way to a bio-guided-isolation of these antioxidant and antimicrobial compounds.

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## Abbreviations

**AEF**: Aqueous extracts of fruits; **AEL**: Aqueous extracts of leaves; **ALP**: Alkaline phosphatase; **ALT**: Alanine transaminase; **AMA**: Antimicrobial activity; **AOA**: Antioxidant activity; **AST**: Aspartate transaminase; **CFU**: Colony Making Unit; **CIP**: Ciprofloxacin; **D-7**: 7 day before the experimentation; **DE**: Dry extract; **DPPH**: 1,1 diphenyl-2-picrylhydrazyl radical; **FLUC**: Fluconazole; **GAE**: Gallic acid equivalents; **ID**: Diameter of inhibition ; **LD<sub>50</sub>**: 50 %Lethal dose ; **MDT**: Maximal dose tolerate; **MEF**: Methanol extract fruits; **MEF**: Methanolic extract of fruits; **MEL150**: Group who received 150 mg/kg of dose; **MIC**: Minimum inhibitory concentration; **MMC**: Minimum Microbicidal Concentrations; **OECD**: Organization for Economic Cooperation and Development; **QE**: Quercetin equivalents; **TFC** : Total flavonoid content; **TPC** : Total phenols content

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### *Author 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, Writing - review & editing: Bakari Amuri Salvius, Okusa Ndjolo Philippe, Kahumba Byanga Joh; Pierre Duez; Lumbu Simbi Jean-Baptiste.

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