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Antibacterial and antioxidant activities, toxicity and physicochemical properties of *Tetradenia discolor* Phillipson and *Tetradenia riparia* (Hochst) Cood (Lamiaceae)

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Abstract

In Democratic Republic of Congo, traditional healers employ *Tetradenia discolor*, and *T. riparia* to manage bacterial gastroenteritis. We systematically evaluated methanolic leaf and stem-bark extracts of both taxa for antibacterial and antioxidant activities, acute and subacute oral toxicity, physicochemical parameters, and total phenolic, flavonoid, and tannin contents. Antibacterial activity was assessed by disc diffusion and tube macrodilution assays; radical-scavenging capacity by the DPPH assay. Physicochemical profiles were determined by gravimetric, spectrophotometric, and classical tests. Acute and subacute oral toxicity followed OECD protocols in *Cavia porcellus* models. All extracts inhibited *Staphylococcus aureus* and *Salmonella typhi*, yielding inhibition zones of 17–22.5 mm and MICs of 1.95–62.5 µg/mL. The *T. discolor* stem-bark extract exhibited the strongest bacteriostatic effect (zones 19.1–21.1 mm; MICs 1.95–15.63 µg/mL). DPPH IC_{50} values ranged from 11.6 to 21.8 µg/mL, with the *T. riparia* stem-bark extract demonstrating the greatest radical-scavenging potency. Phytochemical screening identified coumarins, quinones, flavonoids, phenols, saponins, tannins, and terpenoids in all extracts. Notably, the *T. riparia* leaf extract contained the highest total phenolics (300 mg GAE/g), flavonoids (57 mg QE/g), and tannins (151 mg GAE/g). Both taxa displayed an LD_{50} > 5000 mg/kg and produced no adverse effects at 200 mg/kg over 30 days. Total ash and acid-insoluble ash values ranged from 11.2–15.8% and 9.33–9.83%, respectively. These findings substantiate the safety and bioactivity of both extracts, highlight phenolics as the principal bioactive constituents, validate their ethnomedical use against gastroenteritis, and support the development of TD- and TR-based phytomedicines.

Keywords: *Cavia porcellus*; Gastroenteritis; DPPH; Lamiaceae; *S. typhi; S. aureus.*

1. Introduction

Tetradenia discolor Phillipson (TD) and *Tetradenia riparia* (Hochst) Cood (TR) are two species of the Lamiaceae family endemic to Sub-Saharan Africa. Both species are used in traditional Congolese medicine to treat chronic gastroenteritis.

TR, locally known as *Mutuzo* (Swahili) or *Muravumba* (Hutu), has gained scientific attention for its ethnobotanical, phytochemical, and pharmacological profile. Traditional healers employ leaf and stem infusions to soothe infections, gastroenteritis, fevers, and inflammation. Phytochemical analyses reveal abundant diterpenoid lactones, sesquiterpenes, flavonoids, and phenolic acids, which underpin its antimicrobial, antioxidant, anti-inflammatory, and analgesic activities. *In vivo* studies suggest low acute toxicity, validating traditional uses and guiding novel drug

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discovery. This synergy between ancestral knowledge and modern research invigorates efforts toward sustainable cultivation, and bioactive discovery [1,2].

In contrast to TR, TD, locally known as Mutuza (Bemba) or Mukuza (Mashi), needs to be better documented in the available literature. In DRC the leaf infusions is used to treat stomach pain, and diarrhea while, the decoction of stem bark is used against coughs, colds, and bronchial irritation. Both plant organs are employed in the treatment of gastroenteritis.

While antibiotic therapy remains a cornerstone of modern medicine, the accelerating emergence of resistant pathogens underscores the urgent need for new molecular entities. In this context, traditional medicine stands out as a credible and efficacious reservoir of bioactive compounds, guiding the discovery of next-generation antimicrobials [3,4]. The utilization of ethnomedicinal knowledge is constrained by the paucity of knowledge regarding the efficacy and safety of these natural resources. This study is situated within the broader context of previous research that has screened natural resources, particularly medicinal plants, for the development of new antimicrobial [5–9], and antioxydant [10–12] recipes, as well as the assessment of their toxicities [13–15]. In a series of investigations, key species from Lushois traditional medicine were systematically screened for antimicrobial activity. These included *Dialium angolense* Welw. ex Oliv (Fabaceae)*, Ochna schweinfurthiana* F. Hoffm. (Ochnaceae), *Rothmannia engleriana* (K. Schum) Keay (Rubiaceae), *Crassocephalum montuosum* (S Moore) Milne-Redh (Asteraceae), and *Crassocephalum picridifolium* (DC) S Moore [5,6,13,16–19].

The objective of this study was to evaluate the i*n vitro* antimicrobial effectiveness of methanolic extracts of the leaves, and the stem bark of TD and TR against bacterial species frequently associated with bacterial gastroenteritis. Moreover, the objective was to assess their potential antioxidant activity, and acute, and subacute toxicity. In addition, the physicochemical parameters were determined, and the total phenols, flavonoids, and tannins were investigated and assayed.

2. Material and methods

2.1. Plant material and animal model

The plant material used in this study consisted of leaves and stem bark of TD (S 11°33’43’’; E 27°21’58’’; 1239 m) and TR (S 11°34’21’’E 27°22’32''; 1239 m). Leaves and Stem bark are used in traditional medicine to treat gastro-enteritis. The taxa were collected in May 2023 in Lubumbashi, Democratic Republic of Congo, and identified at the Kipopo herbarium (herbarium code: TD, KIP533620379; TR, KIP 533620374).

The *Cavia porcellus* specimens (672.5 ± 5.4 g) utilized as animal models were procured from the animal holding unit of the Zoo-Technology Department, Faculty of Agronomic Sciences, University of Lubumbashi. Throughout the experiments, the animals were provided with unlimited access to local rodent food (MIDEMA-RDC), and water *ad libitum*.

2.2. Reagents, substrates and solvents

The reagents, substrates, and solvents utilized in the experiments included ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), ciprofloxacin, gallic acid, methanol, quercetin, and vanillin, which were obtained from Sigma-Aldrich (USA). All chemicals and solvents employed were of analytical quality.

2.3. Extract preparations

Methanolic extracts were obtained through the maceration of 350 grams of coarsely powdered, dried plant material in 1.5 liters of methanol for a period of 72 hours. Following the filtration and recovery of extractable matter on three occasions, each with the same solvent (extractable matter: 30 % - 35%, m/m). The filtrates of the same solvents were combined, concentrated, and dried using a rotary evaporator (Büchi R-210, Switzerland) at 40°C under reduced pressure (130-180 mbar). For all tests, each extract was dissolved in its extraction solvent prior to combination with the vehicle [19].

2.4. Anti-bacterial activity test

Antibacterial activity was assessed by the macrodilution test in a tube, by determining MIC, and by macrodiffusion on a disk, by determining DZI [13,18–20].

The bacterial strains included in this study were *Escherichia coli* ATCC 25922 (*E. coli*), *Campylobacter jejuni* ATCC 33291 (*C. jejuni*), *Staphylococcus aureus* ATCC 6538 (*S. aureus*), *Shigella sonnei* ATCC 25931 (*S. sonnei*), and *Salmonella typhi* ATCC 14028 (*S. typhi*). The bacterial strains were obtained from the Lubumbashi Provincial Laboratory, where tests were conducted using an agar diffusion method to ascertain the diameter of the zone of inhibition (DZI). Additionally, the broth dilution method was employed in triplicate to ascertain the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC) of the extract [19].

These distinct parameters (DZI, MIC, and MBC) were utilized to delineate the antimicrobial activity of the extracts. The microorganisms selected for this study were based on their prevalence in locally observed instances of bacterial gastroenteritis.

For susceptibility testing, 20 mL of Muller-Hinton agar was poured into sterile Petri dishes and allowed to stand for 20 minutes. Subsequently, 1 mL of a bacterial suspension containing 10^8 CFU (colony-forming units) per mL was evenly distributed over the entire surface of each culture medium. Blotting paper discs (with a diameter of 6 mm) were impregnated with 5 µL of extracts (with a concentration of 50 µg. mL^{-1}), and placed on the surface of the solidified, infected medium. Subsequently, the Petri dishes were incubated at 37°C for 48 hours in an oven. The susceptibility of the germs to the extracts was estimated by measuring the diameter (in millimeters) of the zone of inhibition induced by different concentrations of plant extracts around the discs. Each experiment was repeated three times. The Extracts were categorized as follows according to their MIC values: very active (MIC < 100 µg/mL), moderately active (100 ≤ MIC < 500 µg/mL), weakly active (500 ≤ MIC ≤ 1000 µg/mL), and inactive (MIC > 1000 µg/mL) [13,18–20].

To determine MIC, 100 µL of each extract at a concentration of 1 mg/mL, dissolved in methanol , was combined with 1900 µL of culture medium. Subsequently, eight successive dilutions of order 2 (ranging from 500 µg. mL^{-1} to 1.9 µg. mL^{-1}) were prepared for each extract and placed in different 5 mL aseptic tubes. After that, 1000 µL of standard inoculum was added to each tube, and the mixture was incubated for 24 h at 37°C. The growth of microorganisms was observed visually. The minimum inhibitory concentration (MIC) was determined as the lowest concentration at which the extract prevented visible bacterial growth [5]. Extracts were categorized as follows according to their MIC values: very active (MIC < 100 µg. mL^{-1}), moderately active (100 ≤ MIC < 500 µg. mL^{-1}), weakly active (500 ≤ MIC ≤ 1000 µg . mL^{-1}), and inactive (MIC > 1000 µg.mL^{-1}).

To determine the minimum bactericidal concentration (MBC), samples were taken from tubes that exhibited no visible growth, as had been used to determine the minimum inhibitory concentration (MIC). Inoculation was conducted in Petri dishes on Mueller Hinton agar medium, and incubation was performed at 37°C for 24 hours. The growth of microorganisms was monitored visually, and the minimum bactericidal concentration (MBC), defined as the lowest concentration at which the extract prevented visible bacterial growth after transplantation, was determined [21]. The effect of the extract was determined according to the ratio between the minimum bactericidal concentration (MBC), and the minimum inhibitory concentration (MIC). A bactericidal effect was considered present when the ratio was ≤ 2, while a bacteriostatic effect was observed when the ratio was >2 [13]. The experiment was performed in triplicate.

2.5. DPPH assay

The antioxidant activity of the samples was evaluated using the DPPH assay, as previously described [22]. Briefly, 50 µL of extracts (or positive control) were prepared at different dilutions of order 2 in methanol from 100 g. mL^{-1} solution, was interacted with 1950 µL of 0.002% DPPH in a 96-well plate (Nunc WVR, Germany). Following a 30-minute incubation period in the dark, the solution was read at 492 nm (Thermo Fisher Scientific Inc., Waltham, USA). The assays were conducted in triplicate, with the 0.002% DPPH solution as the negative control. The percentage of antioxidant activity was calculated using the following formula :

$$\text{AAO (\%)} = \frac{(\text{Ab}-\text{Ae})}{Ab} x\ 100\ \% \ldots\ldots\ldots\ldots (1)$$

Where, (Ab) is the absorbance measured in the presence of the negative control, (Ae) is the absorbance measured in the presence of the extract, and (AAO) is the calculated percentage of inhibition. The experiment was performed in triplicate.

In this study, the antioxidant activity of the extracts was categorized as follows: very strong when IC_{50} < 50 µg/mL, strong when 50 µg/mL ≤ IC_{50} ≤ 100 µg/mL, moderate when 100 µg/mL < IC_{50} < 250 µg/mL, weak: 250 µg/mL < IC_{50} < 500 µg/mL, and absent when IC_{50} ≥ 500 µg/mL [19,22].

2.6. Toxicity tests

Acute toxicity was assessed as previously described [15] by administering a single oral dose of 5000 mg.kg^{-1} of extracts by gavage (test groups). Animals (5 per group) were observed for 28 days, clinical signs were observed daily, and body weights were measured every seven days. On the last day, blood was collected from the jugular vein and serum was prepared to analyze biochemical parameters (AST, ALT, ALP, urea, creatinine). To determine the dose of 5000 mg.Kg^{-1}, we considered the preliminary studies of acute toxicity assessment according to the Organization for Economic Cooperation and Development (OECD) procedure, in which no signs of toxicity were observed up to 3000 mg.Kg^{-1}.

In the subacute assay, *Cavia porcellus* (5 animals per group) were given 200 mg. Kg^{-1} bw per day (test group). In this subacute toxicity study, in addition to the biochemical parameters examined in the acute toxicity evaluation, weights of the valuable organs were weighed after the animals were sacrificed on day 28.

Validated procedures were followed for blood collection and serum preparation for biochemical analysis [23]. Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities, and urea and creatinine levels were determined by colorimetric assays using Labtest® kits (Minas Gerais, Brazil).

2.7. Preliminary phytochemical screening

The whole plant powders were analyzed for the presence of alkaloids, coumarins, flavonoids, saponins, steroids, tannins, terpenoids, and phenols. This was conducted using standard tube reactions, as previously reported [6,24,25].

2.8. Determination of total phenol, flavonoid and tannin content

The total phenolic content of each sample was quantified using the Folin-Ciocalteu method [26]. The procedure involved the preparation of a solution of extract (100 µg . mL^{-1}) or gallic acid (100-600 µg . mL^{-1}) in 1 mL of 10% (w/v) Folin-Ciocalteu reagent, which was then mixed with 2.5 mL of the solution. Subsequently, 2.0 mL of Na_2CO_3 (75%) was added to the mixture, which was then incubated at 50 ºC for 10 minutes with intermittent stirring. Subsequently, the sample was cooled, and the absorbance was measured using a UV spectrophotometer (Shimazu, UV-1800) at 765 nm against a blank. The results were expressed in milligrams of gallic acid equivalents per gram of dry extract (mg GAE.g^{-1}) using a calibration curve ($y = 0.0698x + 0.0126$, $R^2 = 0.998$; linearity range, 1–1200 mg. mL^{-1}).

As previously described, the total flavonoid content was quantified using an aluminum trichloride assay [27]. Briefly, An aliquot of 1 mL extract solution (100 µg . mL^{-1}) or quercetin (25-250 µg. mL^{-1}) was combined with 0.2 mL of a 10% (w/v) $AlCl_3$ solution in methanol, 0.2 mL of a 1 M potassium acetate solution, and 5.6 mL of distilled water. The mixture was incubated for 30 minutes at room temperature, after which the absorbance was measured at 415 nm against the blank. The results were expressed in milligrams of quercetin equivalents per gram of dry extract (mg QE.g^{-1}) using a quercetin calibration curve ($y = 0.006 x + 0.004$, $R^2 = 0.996$; linearity range, 0.1 to 150 mg. mL^{-1}).

The total condensed tannin content was determined using a vanillin method, as previously described [28]. Briefly, 1 mL of a solution of the extract (100 µg/mL) or gallic acid (100-600 µg/mL) was mixed with 5 mL of a vanillin solution (0.5 g reagent and 200 mL 4% HCl in methanol). The absorbance at 500 nm, following a 20-minute reaction time, was quantified using a spectrophotometer (SP 2000 UV, Bel Photonics®, Piracicaba, SP, Brazil) in comparison to a blank. The results were expressed in milligrams of gallic acid equivalents per gram of plant dry extract (mg GAE.g^{-1}ED) using a calibration curve based on gallic acid ($y = 0.004 x + 0.0013$, $R^2 = 0.997$; linearity range, 1 - 100 mg. L^{-1}).

2.9. Determination of physicochemical parameters

2.9.1. Loss on drying (LOD)

The loss on drying was quantified according to the following protocol: A flat-bottomed capsule with a diameter of approximately 50 mm and a height of approximately 30 mm was used to weigh 0.50 g of finely pulverized dry drug. The capsule was then oven-dried at 105°C for three hours. After drying, the capsule was cooled in a desiccator, and the weight was recorded. The result was expressed as a percentage:

$$\text{LOD (\%)} = \frac{a}{b} \text{ x } 100 \text{ \% } \ldots\ldots\ldots\ldots \text{ (2)}$$

a: weight after proofing, and b is the weight of the sample before proofing.

2.9.2. Total ash (TA)

One gram of the pulverized drug was placed in a porcelain crucible. The test sample was distributed evenly within the crucible. The sample was subjected to an oven drying process for one hour at a temperature range of 100-105°C. After that, it was incinerated in a muffle furnace at 600 ± 25°C. It is important that the sample does not ignite at any point during this process. Once the sample had been incinerated, it was allowed to cool in a desiccator before being weighed using an analytical balance. Subsequently, the crucible was permitted to cool in a desiccator before being weighed on an analytical balance. The total ash content was calculated as follows [19]:

$$\text{TA (\%)} = \frac{\text{m}}{\text{n}} \text{ x } 100 \text{ \%} \quad \ldots\ldots\ldots\ldots \quad (3)$$

m is the weight after incineration, **n** is the test sample.

2.9.3. Hydrochloric acid soluble ash (HAA)

To ascertain the ash content, 15 mL of distilled water and 10 mL of hydrochloric acid were added to the residue obtained during the total ash determination. Subsequently, the crucible was covered with a watch glass and boiled gently for ten minutes. Following this, the crucible was allowed to cool. Subsequently, the residue was filtered through an ash-free filter paper, with the filtrate subsequently washed with hot water until it reached a neutral pH. Subsequently, the filtrate was transferred to a dry, tared crucible. Subsequently, the crucible containing the filter paper was subjected to an oven firing for 24 hours. Subsequently, the crucible was subjected to a further calcination process for six hours at a temperature of 600°C. Following cooling in a desiccator, the crucible containing the ash was reweighed, and the hydrochloric ash was calculated as follows [19,29]:

$$\text{HAA (\%)} = \frac{\text{P1}}{\text{P2}} \text{ x } 100 \text{ \%} \quad \ldots\ldots\ldots\ldots \quad (4)$$

P1 is the weight after calcination and P2 the weight before calcination.

2.9.4. Swelling index

The swelling index was defined as the volume (mL) occupied by 1 g of the drug. A 25 mL graduated cylinder (0.5 mL graduations; height 125 ± 5 mm; diameter 1.5 cm) was employed. One gram of the drug, either intact or finely powdered, was introduced into the cylinder, moistened with 1.0 mL of 90 % ethanol, and then 25 mL of distilled water was added. The cylinder was stoppered and its contents were shaken vigorously every 10 minutes for 1 hour, after which it was allowed to stand undisturbed for 3 hours. The final volume occupied by the drug was recorded. Three determinations were performed simultaneously, and the swelling index was calculated as the mean of the three measured values [19].

2.10. Statistical analysis

The data were analyzed using GraphPad Prism 6 (GraphPad Software, La Jolla, USA). Comparisons between the different groups were made by analysis of variance (ANOVA), with a probability level of $p < 0.05$ considered significant.

2.11. Ethical considerations

The principles governing the use of laboratory animals as set out by the OECD, the University of Montreal's Animal Use Ethics Committee, and the Canadian Council on Animal Care protocol were duly observed. The project proposal and procedures were reviewed, and approved by the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Lubumbashi, Democratic Republic of the Congo (FSP-UNILU-DP-BD-032223).

3. Results

3.1. Sensitivity of bacteria to different extracts

The extracts showed a diameter of inhibition zone (DZI) ranging from 10.0 ± 0.2 mm (TDLV on *S. sonnei*) to 22.5 ± 0.3 mm (TRSB on *S. aureus*) on the different germs studied in solid medium. According to the classification system previously proposed [19,30], all the extracts showed activity (DZI > 10 mm). It is noteworthy that *Staphylococcus aureus* exhibited susceptibility to all tested extracts, with inhibition zone diameters (IZD) exceeding 20 mm. Additionally, the TDSB preparation demonstrated broad-spectrum activity, effectively inhibiting all evaluated microbial strains with IZDs > 19 mm (Table 1).

Table 1 Inhibition zone diameter of organic extracts of *T discolor* and *T riparia* at 50 µg. mL^{-1}.

Simple	*E. coli*	*C. jujeni*	*S. aureus*	*S. sonnei*	*S. typhi*
CIP	28.0 ± 1.2	26.1 ± 0.4	18.1 ± 0.2	25.0 ± 0.1	25.1 ± 0.4
TDLV	20.2 ± 0.2	12.7 ± 0.1	21.4 ± 0.2	10.0 ± 0.2	19.2 ± 1.2
TDSB	21.1 ± 0.2	19.3 ± 0.2	20.1 ± 0.4	20.1 ± 0.2	19.1 ± 0.2
TRLV	19.7 ± 0.1	19.1 ± 0.1	20.0 ± 0.2	11.2 ± 0.2	18.4 ± 0.2
TRSB	20.1 ± 0.2	21.8 ± 0.2	22.5 ± 0.3	12.1 ± 0.2	17.1 ± 0.2

Legend - TDLV: *Tetradenia discolor* leaves, TDSB : *Tetradenia discolor* Stem Bark, TRLV: *Tetradenia riparia* leaves, TRSB: *Tetradenia riparia* Stem bark; Results expressed as mean ± standard deviation with n= 3; Ciprofloxacin (CIP) was used as a positive control (DZI: 18-28 mm) and the dilution solvent (5%) from each extract was used as negative control. No zone of inhibition was observed in the negative control.

3.2. Minimum inhibitory concentration, minimum bactericidal concentration and effect of extracts

The minimum inhibitory concentrations (MICs) of all evaluated extracts spanned a wide range (1.95–62.50 µg/mL), underscoring their diverse antibacterial potencies. Each extract suppressed the growth of *Staphylococcus aureus* at concentrations of 7.82 µg/mL or below, highlighting a uniformly strong antistaphylococcal effect. The TDSB extract proved especially compelling, inhibiting every tested bacterial strain at MICs no greater than 15.625 µg/mL, and thus demonstrating true broad-spectrum efficacy. Within the *Tetradenia discolor* extracts, stem bark extracts emerged as the most potent fraction (MIC 1.5–16.6 µg/mL), whereas for *Tetradenia riparia*, leaf extracts outperformed stem bark (MIC: 7.8–15.6 µg/mL). Complementary bactericidal assays confirmed that these formulations not only arrest bacterial proliferation but actively kill pathogens at their respective MICs (Table 2).

Table 2 Minimum inhibitory concentration (MIC in µg. mL^{-1}) and minimum bactericidal concentration (MBC in µg/mL) of organic extracts of *T discolor* and *T riparia***.**

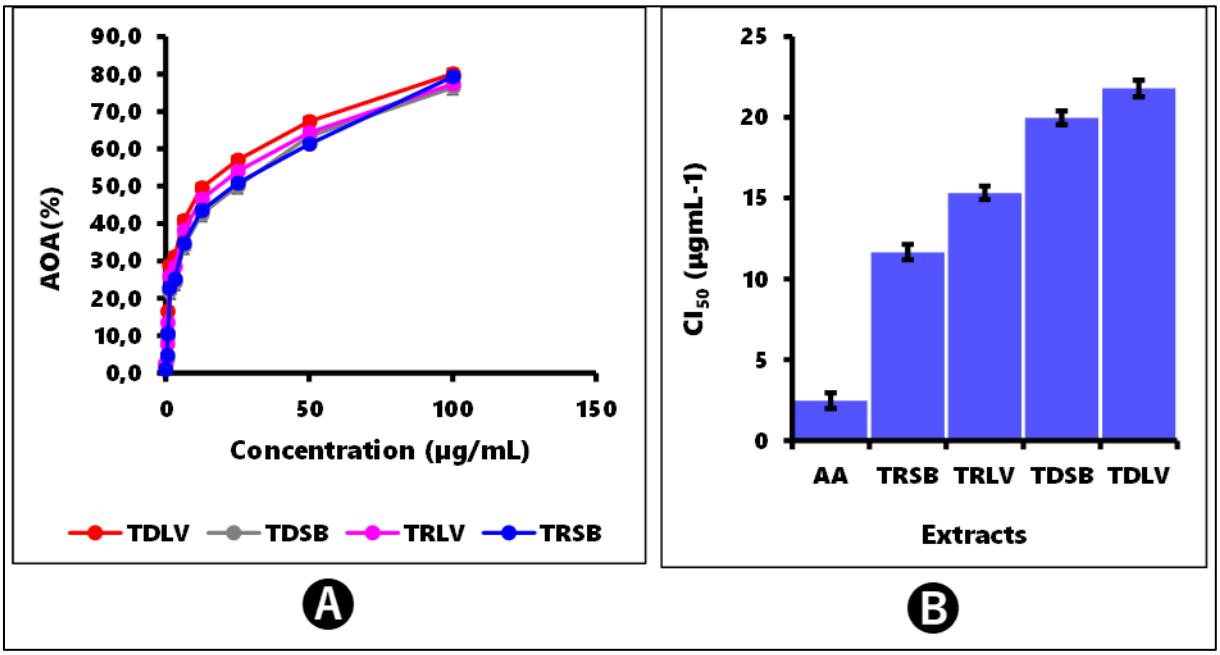
Extracts	Bacteria	MIC	MBC	MBC/MIC	Effects
CIP	*E. coli*	1.95	1.95	1	BC
	C. jujeni	3.90	3.90	1	BC
	S. aureus	3.90	3.90	1	BC
	S. sonnei	3.90	3.90	1	BC
	S. typhi	1.95	1.95	1	BC
TDLV	*E. coli*	15.63	15.63	1	BC
	C. jujeni	125	125	1	BC
	S. aureus	1.95	1.95	1	BC
	S. sonnei	62.50	62.50	1	BC
	S. typhi	15.63	15.63	1	BC
TDSB	*E. coli*	7.82	7.82	1	BC
	C. jujeni	15.63	15.63	1	BC
	S. aureus	1.95	1.95	1	BC
	S. sonnei	15.63	15.63	1	BC
	S. typhi	7.82	7.82	1	BC
TRLV	*E. coli*	15.63	15.63	1	BC
	C. jujeni	15.63	15.63	1	BC
	S. aureus	7.82	7.82	1	BC
	S. sonnei	31.25	31.25	1	BC

Extracts	Bacteria	MIC	MBC	MBC/MIC	Effects
	S. typhi	15.625	15.625	1	BC
TRSB	*E. coli*	15.63	15.63	1	BC
	C. jujeni	31.25	31.25	1	BC
	S. aureus	15.63	15.63	1	BC
	S sonnei	62.50	62.50	1	BC
	S typhi	31.25	31.25	1	BC

Legend **-** CIP **:** Ciprofloxacin, TDLV: *Tetradenia discolor* leaves, TDSB : *Tetradenia discolor* Stem Bark, TRLV: *Tetradenia riparia* leaves, TRSB: *Tetradenia riparia* Stem bark; BC: Bactericide; BS: Bacteriostatic. Ciprofloxacin was employed as a positive control, demonstrating MIC values below 5 µg/mL, whereas the uninoculated culture medium and the 5% methanol vehicle served as negative controls and showed no detectable antibacterial activity.

3.3. Antioxidant activity

The antioxidant activity of the extracts was found to vary significantly, with values ranging from 1% to 80% in accordance with the concentration, and exponential model employed. In accordance with the aforementioned classification (2.5), the extracts demonstrated high antioxidant activity, as evidenced by their IC_{50} values (11.6–21.8 µg. mL^{-1}). However, this activity was observed to be at a lower level than that of ascorbic acid (IC_{50} = 2.5 µg. mL^{-1}), which served as a positive control. The methanolic extract of TRSB exhibited the highest antioxidant activity (Figure 1).



Legend – TDLV: *Tetradenia discolor* leaves, TDSB : *Tetradenia discolor* Stem Bark, TRLV: *Tetradenia riparia* leaves, TRSB: *Tetradenia riparia* Stem bark AA: Ascorbic acid used as positive control; AOA: Antioxidant activity.

Figure 1 Antioxidant activity of organic extracts of *Tetradenia discolor* and *Tetradenia riparia* expressed as percentage DPPH inhibition **A** and as IC_{50} **B**; ((X)¯± S, n=3).

3.4. Phytochemical groups identified

Excluding alkaloids, every other class of secondary metabolites—including anthraquinones, coumarins, flavonoids, polyphenols, saponins, steroids, tannins, and terpenoids—was detected in both leaf and stem bark extracts of *Tetradenia discolor,* and *Tetradenia riparia*. Notably, coumarins, flavonoids, polyphenols, saponins, and tannins appeared in every extract analyzed, and seven out of the eight targeted metabolite groups were present in the stem bark of *T. riparia*. These results highlight the rich and varied phytochemical profiles of these species, laying a strong foundation for future investigations into their biological activities (Table 3).

Table 3 Phytochemical groups of antibacterial interest identified in the leaves and Stem bark parts of *T discolor* and *T riparia.*

Phytochemicals group	TDLV	TDSB	TRLV	TRSB
Alkaloids	-	-	-	-
Anthraquinones	-	+	-	+
Coumarins	+	+	+	+
Flavonoids	+	+	+	+
Polyphenols	+	+	+	+
Saponins	+	+	+	+
Steroids	-	+	+	+
Tannins	+	+	+	+
Terpenoids	+	+	-	+

Legend - Present: + ; Absent: - ; TDLV: *Tetradenia discolor* leaves, TDSB : *Tetradenia discolor* Stem Bark, TRLV: *Tetradenia riparia* leaves, TRSB: *Tetradenia riparia* Stem bark. Screening performed on powders of leaves and stem bark.

The total phenol and tannin contents were expressed as gallic acid equivalents per gram (mg GAE.g^{-1}), and the range was observed to be from 251.3 ± 3.1 to 300.2 ± 1.2 for phenols, as well as from 105.0 ± 5.0 to 106.7 ± 7.6 for tannins, respectively. The total flavonoid content, expressed as quercetin equivalents per gram (mg QE. g^{-1}), was found to be 50.48 ± 0.44 to 57.34 ± 0.35. In general, the highest values were observed with TRLV (Table 4).

Table 4 Content of total phenols, total flavonoids and total tannins in extracts from the leaves and Stem bark of *T discolor* and *T riparia*.

Extract	Total phenols (mg GAE.g^{-1})	Total flavonoids (mg QE. g^{-1})	Total tannins (mg GAE.g^{-1})
TDLV	223.3 ± 1.9	50.48 ± 0.44	105.0 ± 5.0
TDSB	275.8 ± 1.9^{a}	53.33 ± 0.43	106.7 ± 7.6^{a}
TRLV	300.2 ± 1.2^{a}	57.34 ± 0.35^{a}	151.0 ± 5.0^{a}
TRSB	251.3 ± 3.1	51.43 ± 0.44	134.1 ± 7.6^{a}

Legend - TDLV: *Tetradenia discolor* leaves, TDSB : *Tetradenia discolor* Stem Bark, TRLV: *Tetradenia riparia* leaves, TRSB: *Tetradenia riparia* Stem bark; GAE: gallic acid equivalent, QE: quercetin equivalent. a if $p < 0.01$; ANOVA test comparison with TDLV ; ($\bar{X}$ ± S, n=3).

3.5. Correlations between total phenol, total flavonoid, and antioxidant and antibacterial activity.

We centered our investigation on *Staphylococcus aureus* because it proved the most vulnerable pathogen across the extract panel, a finding that underscores the clinical relevance of our botanical preparations. In parallel, the TDSB and TRLV fractions emerged as the most potent antibacterial agents, directing our focus toward these extracts.

A positive linear correlation (R: 0.9993-0.9995) was observed between the antibacterial activity of TDSB on *S. aureus,* and the total phenolic content (y = 10,235x - 2,3909), total flavonoids content (y = 2,0084x + 0,2208), and total tannins content (y = 5,5481x - 1,3049). Similarly, the antibacterial activity of TRLV on *S. aureus* exhibited a positive linear relationship (R: 0.97-0.98) with the content of total phenolics, flavonoids, and tannins (Figure 2).

Our analysis revealed a robust positive linear correlation (R = 0.96–0.99) between the antioxidant capacity, and antibacterial efficacy of both TDSB and TRLV against *Staphylococcus aureus*, and *Salmonella typhi*. This finding suggests that the radical-scavenging constituents within these extracts play a pivotal role in mediating their antibacterial activity (Figure 3).

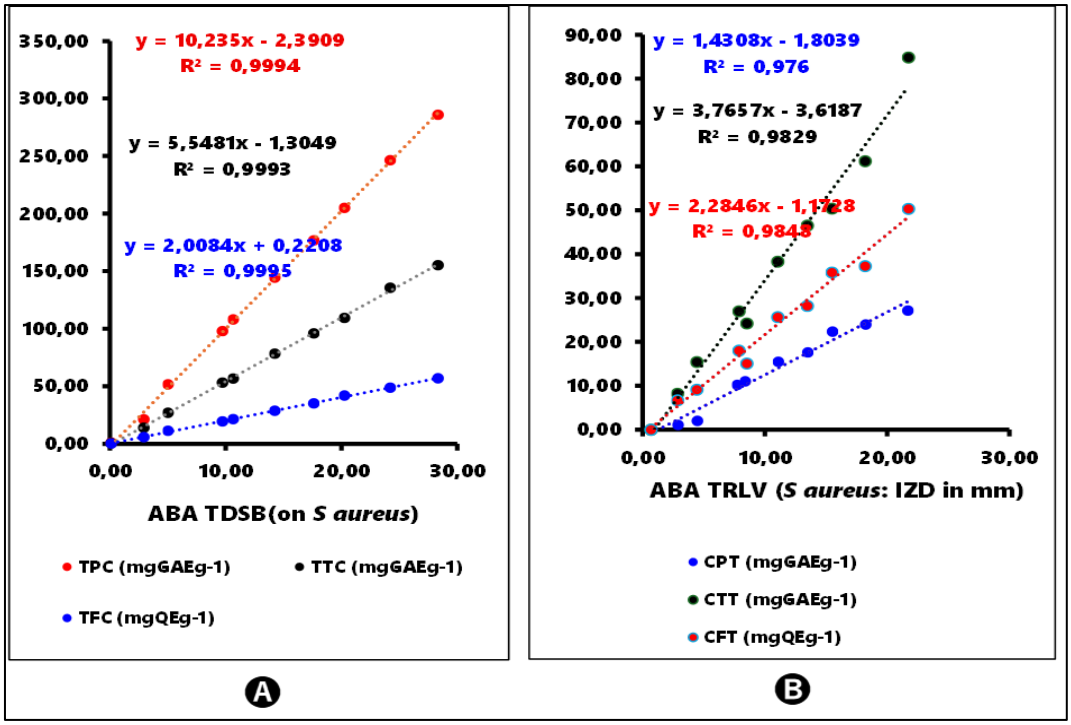


Figure 2 Linear correlation between antibacterial activity of TDLV on *S. aureus* (a) and antibacterial activity of TRLV on *E. coli* (b) with total phenolic content (red), total condensed tannin content (black) and total flavonoid content (blue), respectively

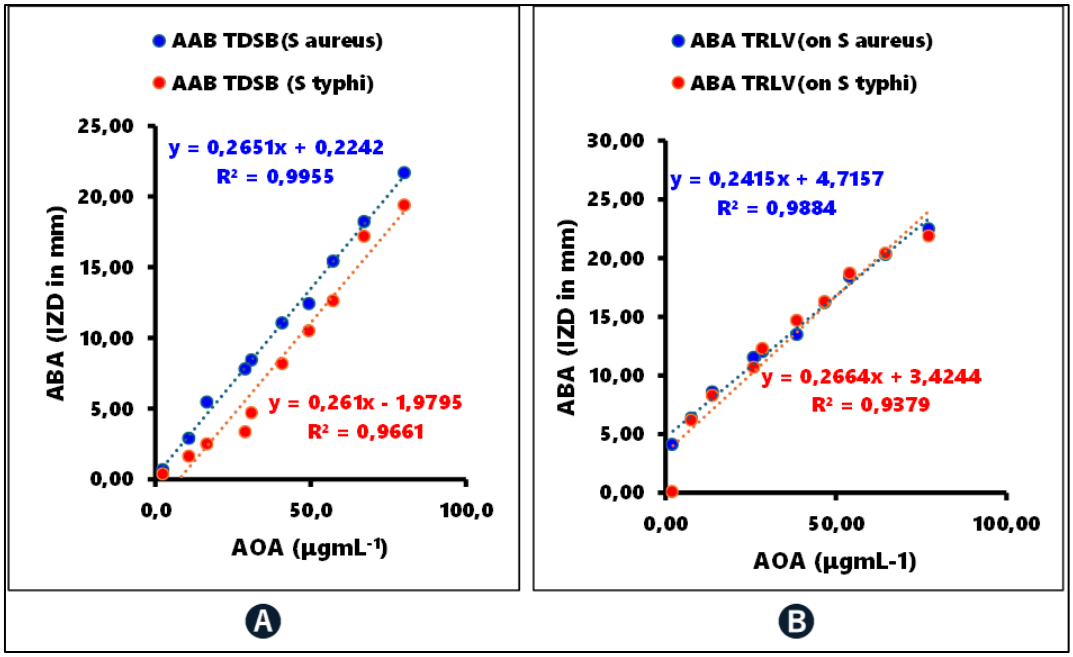


Figure 3 Linear correlation between antioxidant and antibacterial activities of *Tetradenia discolor* leaves (a) and *Tetradenia riparia* Stem bark (b) methanolic extracts.

3.6. Toxicity *in vivo*

To assess acute toxicity, guinea pigs received a single oral dose of 5 000 mg/kg body weight of the extract. No mortality occurred, nor were there significant differences in body weight or any observable motor, respiratory, or cardiovascular disturbances compared with the control group (Figure 4a). Subacute toxicity was evaluated by daily administration of 200 mg/kg over the study period; this regimen produced no significant changes in body weight (Figure 4b), organ weights (Figure 5), or in standard serum biomarkers of hepatic, cardiac, and renal function (Table 5).

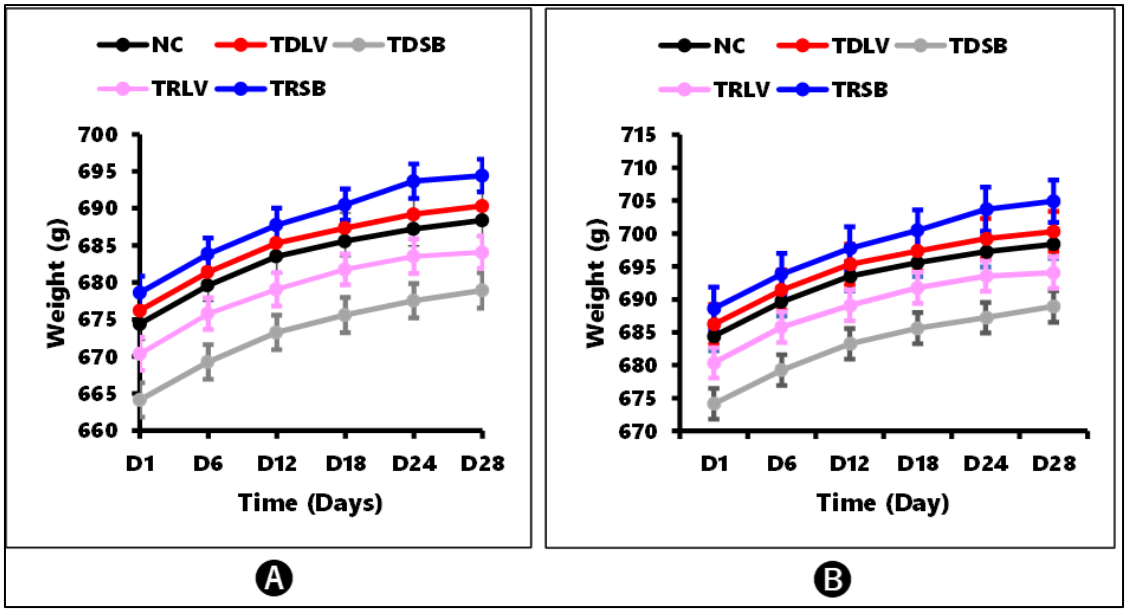


Figure 4 Weight evolution of *Cavia porcellus* during acute (a) and subacute (b) toxicity evaluation after oral administration of 5000 mg.kg^{-1} (a) and 200 mg.kg^{-1} (b) methanolic extracts of TDLV: *Tetradenia discolor* leaves, TDSB : *Tetradenia discolor* Stem Bark, TRLV: *Tetradenia riparia* leaves, TRSB: *Tetradenia riparia* Stem bark ((X)¯± S, n=3).

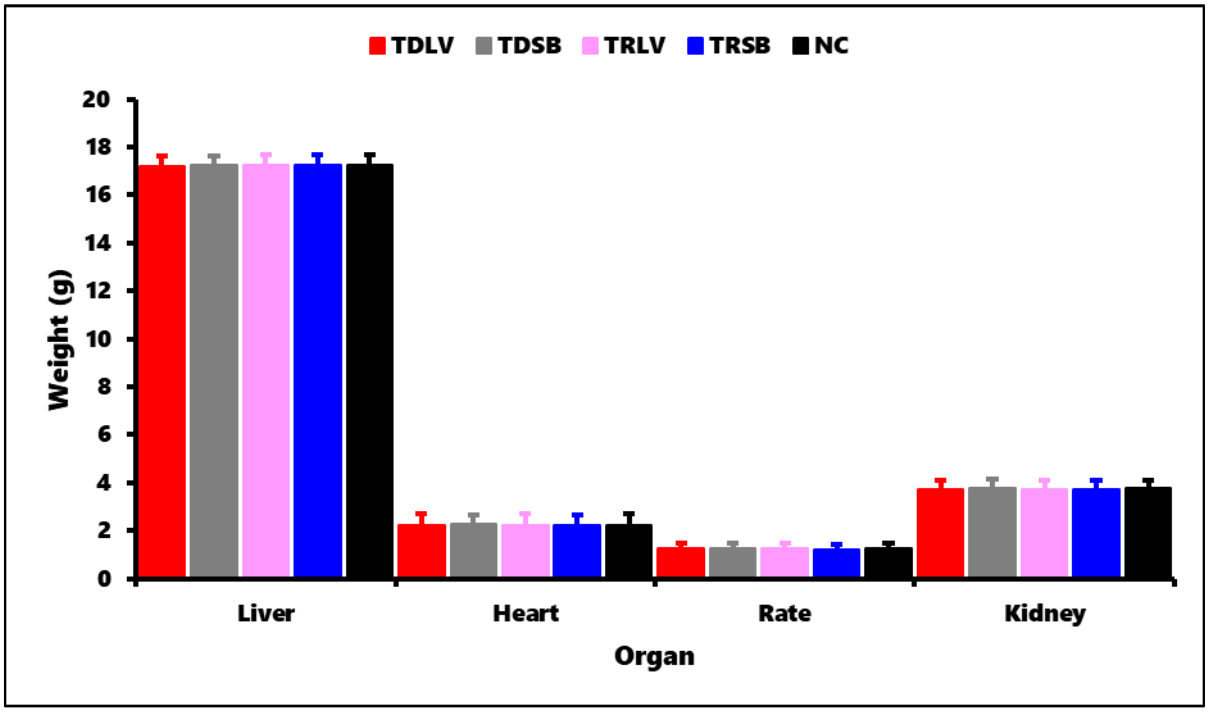


Figure 5 Variation in organ weights of *Cavia porcellus* when assessing the subacute toxicity of TDLV: *Tetradenia discolor* leaves, TDSB : *Tetradenia discolor* Stem Bark, TRLV: *Tetradenia riparia* leaves, TRSB: *Tetradenia riparia* Stem bark; ((X)¯ ± S, n=3)

Table 5 Biochemical and hematological parameters assessed in the evaluation of the subacute toxicity of methanolic extracts of *T discolor* and *T riparia*.

	Control	TDLV	TDSB	TRLV	TRSB
AST(UI/L)	31.7 ± 0.11	31.8 ± 0.12	31.3 ± 0.13	31.9 ± 0.10	31.1 ± 0.14
ALT(UI/L)	32.5 ± 0.21	32.6 ± 0.19	32.2 ± 0.13	32.7 ± 0.13	32.6 ± 0.24
ALP (UI/L)	55.4 ± 0.41	55.8 ± 0.31	55.5 ± 0.22	55.6 ± 0.28	55.7 ± 0.21
Urea (mg/dL)	24.1 ± 0.12	24.3 ± 0.13	24.1 ± 0.14	24.2 ± 0.12	24.2 ± 0.18
Creatinine (mg/dL)	1.31 ± 0.13	1.32 ± 0.10	1.31 ± 0.12	1.31 ± 0.15	1.31 ± 0.14
WBC ($\times 10^3/mm^3$)	7.8 ± 0.12	7.8 ± 0.15	7.8 ± 0.13	7.8 ± 0.15	7.8 ± 0.16
RBC ($\times 106/mm^3$)	5.7 ± 0.51	5.7 ± 0.52	5.7 ± 0.53	5.7 ± 0.54	5.7 ± 0.57
HTC (%)	45.4 ± 0.53	45.6 ± 0.54	45.3 ± 0.56	45.7 ± 0.56	45.7 ± 0.54
SV (mm/h)	3.6 ± 0.52	3.6 ± 0.54	3.6 ± 0.52	3.6 ± 0.53	3.6 ± 0.55

Legend - TDLV: *Tetradenia discolor* leaves, TDSB : *Tetradenia discolor* Stem Bark, TRLV: *Tetradenia riparia* leaves, TRSB: *Tetradenia riparia* Stem bark; SV: sedimentation rate, HTC: hematocrit, RBC: red blood cell, WBC: white blood cell, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase; ($\bar{X}$ ± S, n=3).

3.7. Physicochemical characteristics

Total ash content in both taxa was found to range from 11 to 15 %, while the fraction insoluble in hydrochloric acid accounted for approximately 9.3–9.8 %. Methanol-soluble extractives were notably abundant, varying between 29.9 and 35.1 %, with leaf material yielding the highest proportion—a reflection of its rich phytochemical reservoir. Moisture loss on drying remained below 1 %, indicative of minimal residual water, and the swelling index did not exceed 6.5 mL, suggesting a restrained expansion capacity. Finally, a 10 % (w/v) aqueous suspension exhibited a mildly acidic pH between 5.4 and 6.1, compatible with gentle biological interactions (Table 6).

Table 6 Physicochemical parameters of powders from *T discolor* and *T riparia*

Parameter	Unit	TDLV	TDSB	TRLV	TRSB
Total ash	%	15.78 ± 1.92	14.24 ± 0.73	11.78 ± 1.92	11.24 ± 0.73
Hydrochloric ash	%	09.74 ± 0.37	09.83 ± 0.24	09.44 ± 0.37	09.33 ± 0.24
Loss on drying	%	0.91 ± 0.16	00.94 ± 0.21	0.96 ± 0.16	00.93 ± 0.21
Extractible matter	%	35.1 ± 0.15	33.94 ± 0.21	32.93 ± 0.16	29.97 ± 0.21
Swelling index	mL	05.83 ± 0.55	05.62 ± 0.52	06.2 ± 0.55	04.97 ± 0.52
pH S	$mol.L^{-1}$	05.4 ± 0.50	05.71 ± 0.51	05.4 ± 0.42	06.1 ± 0.50

Legend - pH S: pH of a 10% aqueous solution (w/v). TDLV: *Tetradenia discolor* leaves, TDSB : *Tetradenia discolor* Stem Bark, TRLV: *Tetradenia riparia* leaves, TRSB: *Tetradenia riparia* Stem bark.

4. Discussion

The inhabitants of Lubumbashi often resort to traditional remedies or self-medication using plants to treat various ailments, particularly bacterial gastroenteritis [19]. Despite their widespread use, rigorous data on the efficacy, and safety of these treatments remain scarce. This investigation therefore examines two locally employed Tetradenia species—*T. discolor* and *T. riparia*—traditionally used to alleviate gastroenteric symptoms. *In vitro* antibacterial activity was assessed concurrently with acute and subacute toxicity *in vivo*. Special emphasis was placed on correlating antimicrobial efficacy with the concentrations of antioxidant-active phytochemical classes (polyphenols, flavonoids, and tannins). Furthermore, critical physicochemical parameters of the plant materials—such as moisture content, pH, ash value, and extractive yield—were measured to inform future standardization and quality-control protocols.

4.1. Antibacterial activity of plant extracts

This study is the first to demonstrate antibacterial activity of methanolic extracts from *Tetradenia discolor* leaves and Stem bark against all tested strains, filling a gap in the literature. It also corroborates the potent efficacy of *Tetradenia riparia* against Enterobacteriaceae: ethyl acetate leaf fractions produced 30–32 mm inhibition zones against *Salmonella typhi*, and *Escherichia coli*—exceeding penicillin—while methanolic root extracts achieved MICs of 1.25–5.00 mg/mL against *E. coli*, *Staphylococcus aureus*, and *Enterococcus faecalis* [31,32].

Several potent antibacterial agents have been isolated from *Tetradenia riparia*. A bioassay-guided approach yielded three labdane diterpenes—8(14),15-sandaracopimaradiene-7α,18-diol, deacetylumuravumbolide, and umuravumbolide—with IC_{50} values in the low- to mid-μg/mL range against a panel of foodborne pathogens, and these compounds also demonstrated bactericidal, and antibiofilm activities [33]. Further fractionation of the methanolic extract uncovered abietane diterpenes (abieta-7,9(11)-dien-13-β-ol and ibozol) alongside a flavonoid/α-pyrone mixture (astragalin, boronolide, luteolin), all exhibiting sub-μg/mL MICs against *Staphylococcus aureus,* and related Gram-positive bacteria [34]. Finally, the essential oil—rich in oxygenated sesquiterpenes, and monoterpenes such as fenchone, τ-cadinol, 14-hydroxy-9-epi-caryophyllene, and (E,E)-farnesol—showed broad-spectrum MICs of 31.2–500 μg/mL against cariogenic, and food-spoiling organisms, lending scientific support to its traditional uses in food preservation, and oral hygiene [32,35]. The phytochemicals previously documented in the literature on *Tetradenia riparia* align closely with our own screening results, and substantiate the positive correlation we observed between antioxidant capacity, and the levels of total phenolics, flavonoids, and tannins—an association that also underpins the extracts' antibacterial efficacy.

Extensive studies have shown that the antibacterial effectiveness of these extracts stems from multiple concurrent actions: increased membrane permeability, leakage of intracellular contents, local acidification, and hyperpolarization of the bacterial cytoplasm. Disruption of the cellular redox balance further impairs essential oxygen-dependent processes, ultimately leading to a bactericidal outcome. This redox-driven mechanism may also explain the positive association between antioxidant, and antibacterial activities reported in several investigations [29,36] [37–39]. Such a link is especially evident in extracts whose antimicrobial potency is largely attributed to phenolic compounds. However, it remains clear that neither antioxidant nor antibacterial effects can be solely ascribed to phenolics.

4.2. Antioxidant activity of plant extracts

This study presents the initial evidence of the antioxidant activity of methanolic extracts derived from the *Tetradenia discolor* leaves and stem bark (Figure 1). Unlike *T. discolor*, *T. riparia* is consistently recognized for its strong antioxidant properties. *In vitro* evaluation of its leaf, flower-bud, and stem extracts reveals substantial reactive oxygen species inhibition (45–82 %), DPPH radical-scavenging IC_{50}s between 0.51, and 8.47 mg/mL, ferric-reducing antioxidant power (FRAP) of 0.35–0.81 μM $FeSO_4$ equivalents per mg, and β-carotene–linoleic acid oxidation inhibition of 76–102 %. Total phenolics range from 10.70 to 111.68 μg gallic acid equivalents per mg, while a phenolic-rich fraction (FR-IV) peaks at 181.67 μg GAE/mg, with a DPPH IC_{50} of 0.61 μg/mL, 55.6 % protection in the β-carotene assay, and a FRAP value of 4.59 μM $FeSO_4$/mg. The strong positive correlation between phenolic content—especially flavonoids—and antioxidant efficacy also helps explain the extract's antimicrobial activity [34,35].

Indeed, the mechanisms of action of phenolic compounds on bacterial cells have been partially attributed to the following: bacterial membrane damage, modification of cell cytoplasm pH, inhibition of virulence factors such as enzymes and toxins, and suppression of bacterial biofilm formation [40,41]. Specifically, the antibacterial activity of flavonoids has been associated with their capacity to inhibit nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism [42,43]. Furthermore, flavonoids have been demonstrated to inhibit virulence factors, and other forms of bacterial threat, including biofilm formation [44,45]. With regard to tannins, it has been demonstrated that they inhibit bacterial growth through a number of mechanisms, including iron chelation, inhibition of cell wall synthesis, disruption of cell membranes, and inhibition of fatty acid biosynthetic pathways. Furthermore, they can act as quorum-sensing inhibitors, and attenuate the gene expression of several virulence factors, including biofilms, enzymes, adhesins, motility, and toxins [43,46,47].

4.3. Correlation between antioxidant activity, antibacterial activity and total phenols and flavonoids in *T discolor* and *T riparia*.

In this study, a positive linear correlation was observed between the antibacterial activity against *S aureus,* and *Salmonella typhi* of the methanolic extract of *T riparia*, and *T discolor*, and the content of total phenols, flavonoids, and tannins ($R > 0.9$; Figure 2). This suggests that increasing the content of phenols, flavonoids, and total tannins enhances the antibacterial effect of the plants studied, indicating their involvement in the expression of antibacterial activity

against these germs. The contribution of phenols, particularly flavonoids and, tannins, to the expression of antibacterial activity has been previously reported for *T riparia* [35]. This study therefore confirms the results previously reported for *T. riparia*.

4.4. Acute and subacute oral toxicity of methanolic extracts of *T discolor* and *T riparia*.

In addition to the absence of clinically and biologically observable toxicity, no deaths were recorded in the animals following the oral administration of methanolic extracts of *T discolor* and *T riparia* at a dose of 5000 $mg.kg^{-1}$ in *Cavia porcellus*. This finding suggests that the lethal dose 50 (LD_{50}) of the extracts examined is higher than the administered dose. It was subsequently reported that, by the LD_{50}, the extract can be considered highly toxic if the LD_{50} is equal to or less than 1 mg/kg, very harmful if the LD_{50} is between 1 mg/kg and 50 mg/kg, moderately toxic if the LD_{50} is between 50 mg/kg and 500 mg/kg, and practically non-toxic if the LD_{50} is greater than or equal to 1500 mg/kg. The LD_{50} is equal to or less than 1 mg/kg, indicating that the extracts are weakly toxic. If the LD_{50} is between 500 mg/kg and 5000 mg/kg, the extracts are considered to be moderately toxic. However, if the LD_{50} is between 5 g/kg and 15 g/kg, the extracts are practically non-toxic. Finally, if the LD_{50} is greater than or equal to 15 g/kg, the extracts are safe [19,48]. Therefore, the classification of *T discolor* and *T riparia* as practically non-toxic is supported by these findings.

This study provides the first *in vivo* acute, and subacute toxicity data for *Tetradenia discolor*, closing a major knowledge gap. In contrast, *T. riparia's* safety profile is well established: single oral doses of its hydroalcoholic leaf extract up to 5 g/kg in Wistar rats produced no mortality, behavioral changes, or shifts in body or organ weights, and 28-day repeated dosing left liver, kidney, and spleen histology intact with stable serum markers. Minor increases in total leukocytes, lymphocytes, and basophils—alongside slight decreases in urea and creatinine—and an LD_{50} above 5 g/kg underscore the extract's excellent tolerability, and support its ethnopharmacological use [2,49].

This constitutes the initial evidence regarding the safety of *T discolor*, which encourages further studies to validate their therapeutic properties *in vivo*. Additional studies are required to determine the effects of extracts of these two plants on female animals, chronic administration, fetal animals, pregnant animals, and their reproductive capacity, neurotoxicity, and genotoxicity. These studies are necessary to complete the safety profile of these two plants.

Abbreviations

AA: Ascorbic Acid; **AAB**: Antibacterial activity; **AOA:** antioxidant activity; Absent: **-**; **ALP**: Alkaline phosphatase; **ALT**: alanine aminotransferase; **AST**: aspartate aminotransferase; **BC**: Bactericide; **BS**: Bacteriostatic; **CFU** : colony-forming units; **CIP**: Ciprofloxacin; **CN**: negative control; **TDLV:** *Tetradenia discolor* leaves**, TDSB :** *Tetradenia discolor* Stem Bark**, TRLV:** *Tetradenia riparia* leaves**, TRSB:** *Tetradenia riparia* Stem bark; **DPPH**: 1,1-diphenyl-2-picrylhydrazyl radical; **DZI**: diameter of the zone of inhibition; **EE** : Petroleum ether extracts; **GAE**: gallic acid equivalents; **HAA**: Hydrochloric acid soluble ash; **HTC**: Hematocrit; **IC_{50}**: concentration of an inhibitor where the response (or binding) is reduced by half; **LOD**: Loss on drying; **MBC**: minimum bactericidal concentration; **ME**: methanol extracts; **MIC**: minimum inhibitory concentration; Present: **+** ; **QE**: quercetin equivalents; **RBC**: Red blood cells; **SV**: Sedimentation velocity; **TA**: Total ash; **TFC**: Content Flavonoid content; **TPC**: total phenol content; **TTC**; Total tannins content; **WBC**: White Blood Cells.

5. Conclusion

Our results show that methanolic extracts of *Tetradenia discolor,* and *T. riparia* leaves and stem bark exhibit potent antibacterial activity while remaining non-toxic in both acute and subacute oral assays. Phytochemical profiling highlights phenolic compounds—especially flavonoids and tannins—as key drivers of these antimicrobial effects. These findings provide scientific validation for the traditional Congolese use of these plants against bacterial gastroenteritis and establish a solid groundwork for future bioassay-guided isolation of antibacterial agents, particularly from the as-yet underexplored *T. discolor*.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

Data Availability

All data are available upon request to the corresponding author.

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No funding was received for this research.

Authors' contributions

BCV designed, coordinated this research, drafted the manuscript, carried out experiments and data analysis. BCV, IKM, BAS,MMH, and ONP conceived of the study, and participated in research coordination. The all authors read and approved the final manuscript.

Highlight

- *Tetradenia discolor* (TD), and *Tetradenia riparia* (TR) are used in traditional medicine in Lubumbashi for the treatment of gastroenteritis without any evidence of efficacy in the accessible literature.
- TDSB has the best antibacterial activity on all germs tested, and TRSB has the best antioxidant activity.
- TRLV exhibits the highest levels of total phenols, flavonoids, and tannins. Nevertheless, both taxa demonstrate an excellent linear correlation between antibacterial and antioxidant activities and total phenols, flavonoids, and tannins.
- Both taxa were practically non-toxic *in vivo* to *Cavia porcellus* L with LD_{50} > 5000 $mg.kg^{-1}$.

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