



Phytochemical profile, antibacterial activity and acute toxicity of *Rhoicissus tridentata* used to manage dog bites

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ABSTRACT

Dog bites often result into polymicrobial wound contamination, which pose several health risks including bacterial infections. In Uganda, *Rhoicissus tridentata* is traditionally used to manage dog bites, yet its secondary metabolite profile, antibacterial efficacy, and *in-vivo* toxicity had remained unexplored. Thus, the metabolites and the scientific evidence to validate the antibacterial activity and safety of the plant was limited. Identification of potent antibacterial agents could be crucial to manage dog-bite-related bacterial infections. The root extracts were analyzed using UHPLC–HRMS/MS–qTOF, followed by MZmine processing, and the metabolites characterized with GNPS Feature-Based Molecular Networking. For the first time, the high-resolution metabolomic approach resulted into annotation of 15 bioactive polyphenols like flavonoids, tannins. The antibacterial activity of the extracts was evaluated against standard strains of the zoonotic oral bacteria commonly associated with dog bites, namely: *Enterococcus faecalis* (ATCC 29,212), *Streptococcus aureus* (ATCC 25,932), *Streptococcus mutans* (ATCC 25,175), *Proteus mirabilis* (ATCC 25,933), *Klebsiella pneumoniae* (ATCC 700,603), and *Escherichia coli* (ATCC 25,922). The extracts exhibited moderate antibacterial activity against all the strains. The MIC and MBC ranged from (0.78 to 6.25) and (1.56 to 12.5) mg/mL respectively. The MBC/MIC ratios were between 1.9 and 2, signifying bactericidal extracts. *In vivo* acute toxicity testing, the extract showed no adverse signs of toxicity at doses up to 5000 mg/kg (LD₅₀ > 5000 mg/kg), suggesting a favorable safety margin. These findings support the ethnopharmacological use of *R. tridentata* in managing dog-bite-related bacterial infections and merit further investigations on its bioactive constituents identified for future antibacterial discovery.

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Introduction

Dog bite poses a significant global health challenge particularly in developing countries like in Africa, where it accounts for 91.9 % of the animal bite cases [22]. Many of the dog bite cases in Africa go undetermined with certainty [12], leaving communities vulnerable to polymicrobial infections. This is because, dog bites are a source of zoonotic bacterial infections in humans [27], as pathogenic bacteria from the dog's oral cavity are introduced into the bite wound(s). The pathogens such as *Pasteurella* species [7], *Staphylococcus aureus*, *Streptococcus mutans*, *Klebsiella pneumoniae* and *Escherichia coli* [11,15,17] associated with dog bites, can result in severe infections if not managed appropriately. The management of the bacterial infections is complicated further by the rise of antibiotic-resistant strains, which render the current antibiotic therapeutic agents less effective [38]. Medicinal plants are widely used in communities as alternative or complementary therapies for dog bites [14,39].

Rhoicissus tridentata is traditionally used in Uganda to manage dog bites [18]. Previous studies have reported a wide range of bioactive constituents in *R. tridentata*, including flavonoids, alkaloids, tannins, phlobatannins and organic acids [8,19]. Steenkamp *et al.*, [29] identified organic acids such as malic, succinic, funaric, gallic, vanillic, and ferulic from methanolic extracts of *R. tridentata* tubers using GC–MS. Brookes and Katsoulis [4] isolated proanthocyanidin monomers: fisetinidol, galocatechin, mollisacacin, catechin hydrate, epigallocatechin, epicatechin, and epicatechin-3-O-gallate, then dimers: procyanidin B3, procyanidin B4, fisetinidol-(4 α –8)catechin and fisetinidol-(4 β –8)catechin from the root methanolic extracts of *R. tridentata*. Other compounds isolated include: oleanolic acid, 20(29)-lupen-3-one, 20-epi- ψ -taraxastanol, and γ -sitosterol by comparing chromatographic chromatograms. Methanol-water root and lignotuber extracts of *R. tridentata* yielded utero-active compounds of: *trans*-resveratrol-3-O- β -glucopyranoside, quercetin-3-O-rhamnopyranoside, morin-3-O- α -L-rhamnopyranoside, catechin, β -sitosterol, linoleic acid, asiatic acid, and arjunolic acid [20]. The identification of the constituents reported previously largely relied on qualitative screening and or low resolution phytochemical analytical techniques hence providing a limited array of the bioactive compounds. Nonetheless, the presence of these phytoconstituents reported may be responsible for the pharmacological activities of *R. tridentata* reported [8,24,33].

The *in vitro* studies on the extracts of *R. tridentata* have shown varying degrees of antibacterial activities against different bacteria strains, where basically MIC values have been reported [13,31]. Additionally, *in vitro* cytotoxicity evaluations have shown that the extracts of *R. tridentata* are non-toxic to moderately toxic in Vero cells [33]. The antibacterial potency and safety reported on *R. tridentata* could not integrate MBC values, the MBC to MIC ratios, and *in vivo* toxicity assessments respectively. Despite the previous findings, scientific data supporting the traditional use of *R. tridentata* to manage dog bite-related bacterial infections were scarce, and the *in vivo* toxicity assessments were also limited. This study therefore aimed at characterizing the phytoconstituents of *R. tridentata*, evaluate its antibacterial activity against dog bite-related pathogens, and assess its *in vivo* acute toxicity, thus validating its traditional use and safety profile.

Materials and methods

Plant material and sample preparation

The representative samples of *R. tridentata* root tubers (voucher specimen, PM/03/2023) were collected from its savannah habitat in Luuka district (00° 42'S 33° 18'E), Eastern Uganda. The plant material was authenticated by a botanist, shade-dried, ground into fine powder, and extracted using dichloromethane/methanol (1:1) analytical grade solvent, Sigma-Aldrich (Germany). Extraction was performed in an ultrasound bath (GTsonic-D9) for 45 min at 35 °C. The resulting extract was filtered and concentrated under reduced pressure using a rotary evaporator (DLAB RE100-Pro) at 40 °C. The concentrated extract was then air-dried to a constant weight at room temperature and stored for further analysis.

Secondary metabolite profiling

Untargeted metabolomics was performed using Ultra-High-Performance Liquid Resolution Mass Spectrometry coupled with high-resolution tandem mass spectrometry (UHPLC–HRMS/MS), followed by Feature-Based Molecular Networking (FBMN) on the GNPS2 platform [36], to characterize the phytochemicals in *R. tridentata* root extracts. For this purpose, chromatographic separation was performed on a Dionex Ultimate 3000 rapid separation liquid chromatography (RSLC) system coupled to a Bruker max 4 G ultra-high-resolution quadrupole time-of-flight (UHR-qTOF) MS equipped with a high-resolution electrospray ionization (HRESI) source. The separation of 5 μ L sample was achieved with a linear 5–95 % gradient of acetonitrile with 0.1 % formic acid in ddH₂O with 0.1 % formic acid on an ACQUITY BEH C18 column equipped with a Waters VanGuard BEH C18 1.7 μ m guard column at a flow rate of 0.6 mL/min and 45 °C for 18 min with detection by a diode array detector at 200–600 nm. The acquired tandem MS spectrograms were exported as an MZML file using Data Analysis 5.3 and via MZmine, the .mgf file was submitted to the GNPS2 FBMN online workflow [36]. Compound annotation was achieved by spectral matching against the GNPS2 spectral library. The molecular network job obtained can be accessed at: <https://gnps2.org/status?task=01dc18a018274b39b5cf3eabcfea4bbc>. Molecular networks were visualized using the cystoscape application.

Antibacterial activity assay

The antibacterial activity of *R. tridentata* root extracts was evaluated against standard bacterial model strains commonly found in

dog oral cavities [17,27]. The standard bacterial strains used included: *Enterococcus faecalis* (ATCC 29,212), *Streptococcus aureus* (ATCC 25,932), *Streptococcus mutans* (ATCC 25,175), *Proteus mirabilis* (ATCC 25,933), *Klebsiella pneumoniae* (ATCC 700,603), and *E. coli* (ATCC 25,922). The antimicrobial activity was determined using agar well diffusion and microbroth dilution techniques. The antibacterial activity was assessed in terms of zones of inhibition diameter (mm), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and the MBC/MIC ratio. Chloramphenicol served as the positive control, while sterile normal saline was the negative control.

In vivo acute toxicity studies

To assess the safety of the extract, *in vivo* acute toxicity studies were conducted in Swiss albino mice following Lorke's method [6]. The animals were observed for clinical signs of toxicity and mortality over a 14-day period to capture both early and delayed toxic effects. For detailed procedures for the toxicity experiments and further methodology, see the experimental procedures provided in the Supplementary material.

Statistical analysis

The data obtained were presented as mean $\pm$ standard deviation or standard error of mean, SEM. Statistical analyses were performed using Real Statistics version 1 (Release 9.5.5). One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was carried out. The post hoc test was utilized to assess differences between the control and treatment groups. Statistically significant differences were defined by $p < 0.05$.

Results and discussion

Ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) coupled with a quadrupole time-of-flight (qTOF) mass analyzer used has the potential to generate accurate MS¹ measurements and precise corresponding MS² fragment patterns of the molecular ion [5] from the plant extract. This in turn offered both sensitivity and selectivity potentials to identify natural products in the plant extract and the total ion chromatograms (TIC) was obtained (Fig. 1). The MZmine dereplication of the LC-MS/MS data set yielded three flavonoid derivatives: isokaempferide (12) isomers, 3-O-methylquercetin (13), and odoratin (15), with similarity scores of 0.87, 0.88, and 0.72 respectively. The GNPS2 feature-based molecular network obtained as visualized in Cystoscape v 3.10.3 (Fig. 2) comprised of 135 nodes and 34 edges containing fifteen clusters and ninety-two (92) singletons. Spectral matching to the GNPS2 libraries led to the annotation of fourteen secondary metabolites (Figs. 3) consisting of primarily polyphenolic compounds such as flavonoids, tannins, phenols with cosine scores ranging between 0.71 and 0.97 (Table 1).

Three spectral features (nodes) in cluster **a** at m/z 359.113, 329.102, and 331.082 were putatively identified as flavone derivatives, annotated as Quercetin-3,7,3',4'-tetramethylether/retusin (4), Kaempferol-3,7,4'-trimethylether (5), and 3,4'-dimethoxy-5,7,3'-trihydroxyflavone/quercetin-3,4'-dimethylether (6). The four nodes at m/z 287.055, 287.056, 303.050, and 301.071 in cluster **b** were

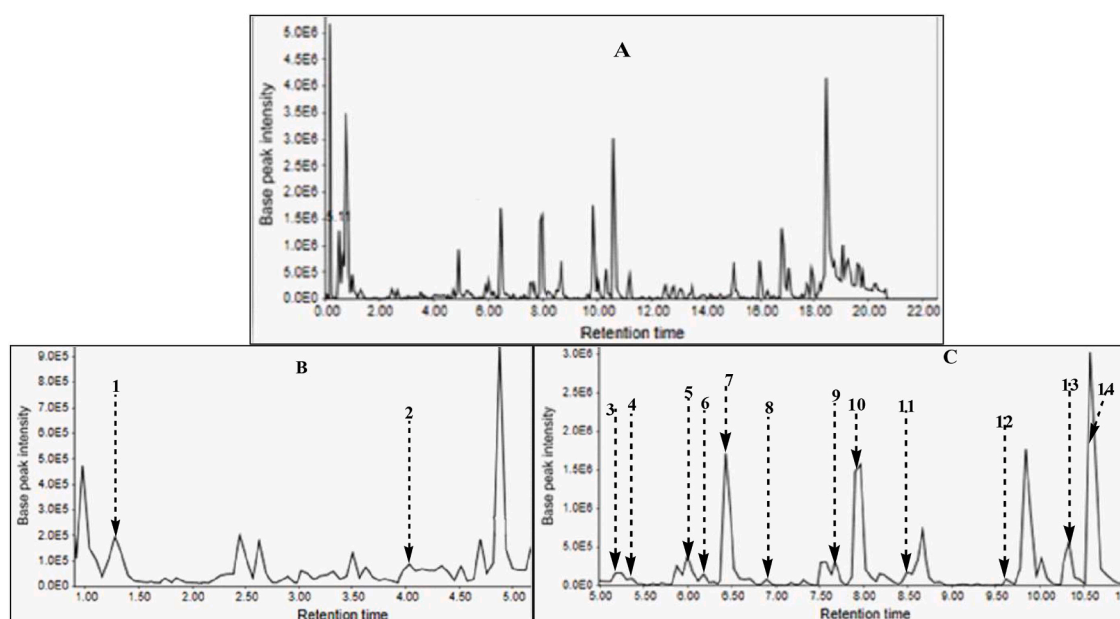


Fig. 1. Zoom sections of the UHPLC-MS/MS total ion chromatograms (TIC) in the positive mode for the root extract of *R. tridentata* (A), for retention time (rt) 0 to 5 min (B), and 5 to 11 min (C). Dotted lines show the retention time for the identified compounds.

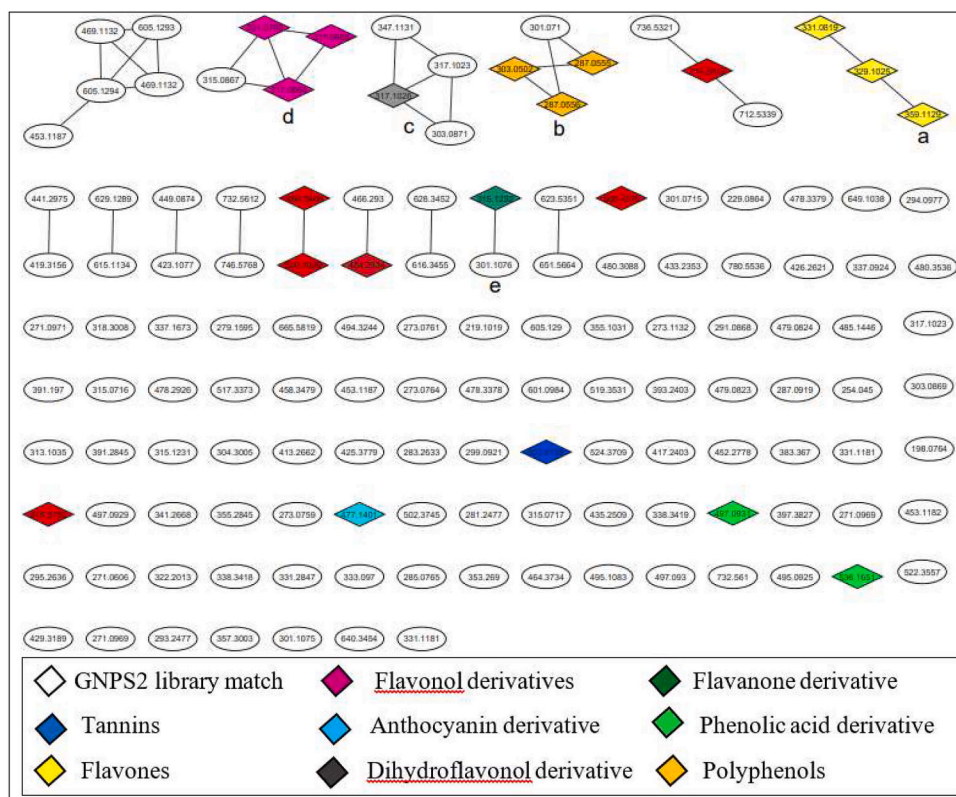


Fig. 2. GNPS2 Feature based molecular network, FBMN analysis of *R. tridentata* root extract highlighting the classes of secondary metabolites identified. Clusters (a to e) are annotated secondary metabolites. Nodes shaded red were annotated as lipids/fatty acyls from GNPS2 FBMN spectral library match.

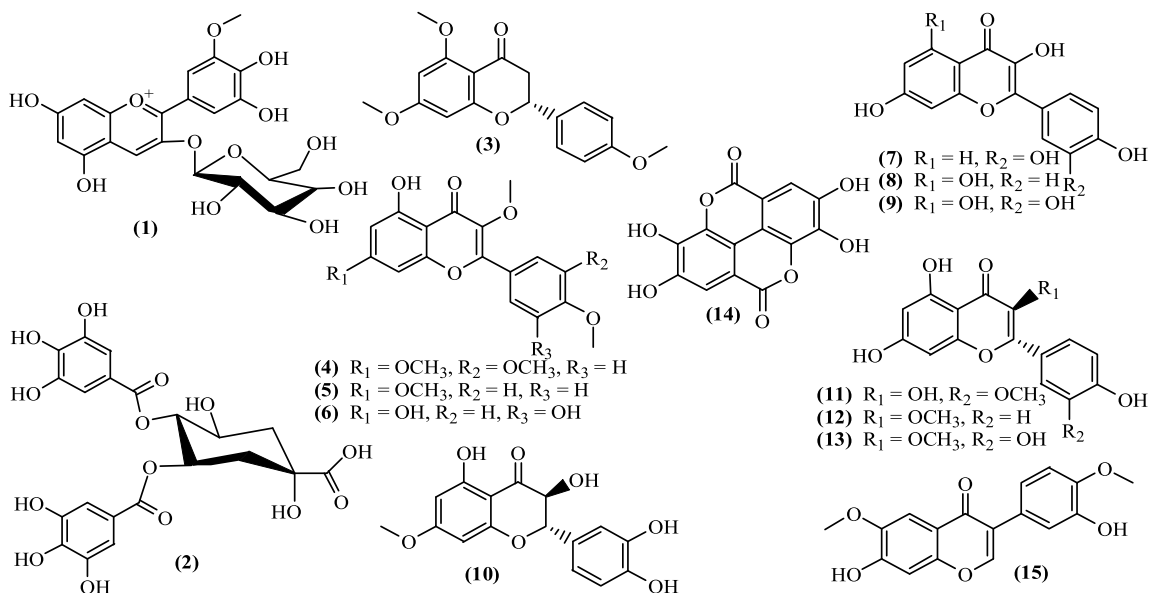


Fig. 3. Chemical structures of the annotated compounds (1 – 15) from the root extracts of *R. tridentata*.

Table 1Annotated compounds from the root extracts of *R. tridentata* by GNPS2 FBMN and MZmine.

Peak	RT	Compound name (No.)	Molecular formula	[M + H] ⁺ accurate m/z	Cosine	MS2 fragments (m/z)	Spectrum reference
1.	1.3	3,4-di-O-galloylquinic acid (2)	C ₂₁ H ₂₀ O ₁₄	497.093	0.97	153	CCMSLIB00004692123
2.	4.1	Ellagic acid (14)	C ₁₄ H ₆ O ₈	303.014	0.87	285, 275, 257, 247, 229, 219, 201, 167	CCMSLIB00006370204
3.	5.1	Fisetin (7)	C ₁₅ H ₁₀ O ₆	287.055	0.71	241, 231, 213, 185, 153	CCMSLIB00000478050
4.	5.2	Isokaempferide (12)	C ₁₆ H ₁₂ O ₆	301.071	0.79	286, 258, 229, 213	CCMSLIB00006404916
5.	6.1	Quercetin (9)	C ₁₅ H ₁₀ O ₇	303.053	0.86	257, 229, 153	CCMSLIB00000578413
6.	6.4	3-O-methylquercetin (13)	C ₁₆ H ₁₂ O ₇	317.065	0.87	302, 274, 228	CCMSLIB00000078868
7.	6.5	Petunidin 3-galactoside (1)	C ₂₂ H ₂₃ O ₁₂	479.118	0.97	315	CCMSLIB00005741327
8.	6.9	Kaempferol (8)	C ₁₅ H ₁₀ O ₆	287.056	0.91	213, 153	CCMSLIB00000222220
9.	7.6	3,4'-dimethoxy-5,7,3'-trihydroxyflavone (6)	C ₁₇ H ₁₄ O ₇	331.079	0.94	316, 301, 273, 245, 217	CCMSLIB000003138441
10.	7.9	Padmatin (10)	C ₁₆ H ₁₄ O ₇	319.081	0.73	228, 211, 167	CCMSLIB00000848383
11.	8.5	Isorhamnetin (11)	C ₁₆ H ₁₂ O ₇	317.066	0.73	300, 272	CCMSLIB00004718194
12.	8.6	Odoratin (15)	C ₁₇ H ₁₄ O ₆	315.087	0.72	-	-
13.	9.6	Quercetin-3,7,3',4'-tetramethyl ether (4)	C ₁₉ H ₁₈ O ₇	359.113	0.80	359, 344, 329, 301, 283, 273	CCMSLIB000003139066
14.	10.3	Kaempferol-3,7,4'-trimethyl ether (5)	C ₁₈ H ₁₆ O ₆	329.102	0.89	314, 299, 271, 243	CCMSLIB00000424753
15.	10.6	Naringenin trimethyl ether (3)	C ₁₈ H ₁₈ O ₅	315.122	0.91	181, 161	CCMSLIB000003139835

RT = retention time.

identified to belong to the flavonol class of polyphenols and putatively annotated as Fisetin (7), Kaempferol (8), Quercetin (9), but the node at *m/z* 301.071 remained unannotated. Relatedly, the four nodes at *m/z* 303.087, 317.102, 317.103, and 347.113 in cluster **c** were recognized as members of dihydroxyflavonols, and only the node at *m/z* 317.103 was putatively annotated as padmatin (10). Cluster **d** consisted of four spectral features at *m/z* 301.071, 315.067, 317.066, and 315.087 which were identified as methoxylated derivatives of flavonol. The node at *m/z* 315.087 remained unannotated while nodes at *m/z* 301.071, 315.067, and 317.066 were putatively annotated as 3'-methoxyquercetin/Isorhamnetin (11), Isokaempferide (12), and 3-O-methylquercetin (13) respectively. The two spectral features in cluster **e** with *m/z* 315.123, and 301.107 were flavanone derivatives of which the node at *m/z* 301.107 was not annotated while the one at *m/z* 315.123 was putatively annotated as Naringenin trimethyl ether or 4',5,7-Trimethoxy flavanone (3). The singletons at *m/z* 477.140 and 303.013 were identified as derivatives of anthocyanins and tannins, which were respectively annotated as Petunidin 3-galactoside (1) and ellagic acid (14). Two more singletons which seemed to be isomeric at *m/z* 497, were identified as polyphenolic ester derivatives, that were putatively annotated as 3,4-di-O-galloylquinic acid (2).

Except quercetin (9) which has been isolated from *R. tridentata*, and *R. tomentosa* [8], the rest of the annotated compounds represent the first putative report from genus *Rhoicissus*. The other annotated compounds have been previously isolated from other natural sources such as *Spiranthes vernalis*, *Boesenbergia rotunda*, *Siparuna gigantotepala* [1], *Hydrangea serrata*, *Aloe vera* (L.), *Euphorbia* spp [2], *Inula viscosa* [3], *Hymenoxys odorata* [23]. Quercetin has been reported to exhibit antibacterial, anticancer, antioxidant, anti-inflammatory, antiviral potential [8]. These other annotated metabolites are associated with diverse pharmacological effects which include antibacterial, antiviral, anti-inflammatory, antioxidant, antineoplastic neuroprotective, antineoplastic, antimicrobial, anticancer, antipyretic [26,35,40], molluscicidal, antimutagenic [37], healing potential, gastroprotective, and antidiarrheal [30].

The UHPLC-MS/MS-qTOF supported by MZmine and GNPS2 FBMN, enabled the annotation of the 15 metabolites. The approach offered a high level of chemical resolution and molecular annotation of the secondary metabolites from the root extracts of *R. tridentata* with confidence due to the Qtof analyzer used [5]. This is the first high-resolution, mass-spectrometry-based metabolomic profile of the *R. tridentata* root extracts to be reported. The study has established a foundational untargeted chemical map useful for the future bioactivity evaluation of the plant, as opposed to the prior studies which were basically using conventional and targeted analytical approaches [4,8,19].

Table 2Mean zones of inhibition diameter, MIC, MBC, and the ratio of MBC/MIC of *R. tridentata* crude extracts against the test bacteria strains, *n* = 3.

Bacterial species	Crude extract				Chloramphenicol			
	Zones (mm)	MIC (mg/mL)	MBC (mg/mL)	$\frac{MBC}{MIC}$	Zones (mm)	MIC (μg/mL)	MBC (μg/mL)	$\frac{MBC}{MIC}$
<i>E. faecalis</i>	12.25 ± 0.35	1.56	3.13	2.00	19.50 ± 0.35	0.023	0.023	1.00
<i>S. aureus</i>	22.00 ± 0.71	3.13	6.25	1.99	25.50 ± 0.71	0.012	0.012	1.00
<i>S. mutans</i>	18.50 ± 0.71	0.78	1.56	2.00	20.00 ± 0.00	0.012	0.012	1.00
<i>P. mirabilis</i>	14.00 ± 0.00	6.25	12.5	2.00	19.50 ± 0.71	0.023	0.023	1.00
<i>K. pneumoniae</i>	12.00 ± 0.71	6.25	12.5	2.00	25.50 ± 0.71	0.012	0.012	1.00
<i>E. coli</i>	11.20 ± 0.11	6.25	12.5	2.00	20.50 ± 0.71	0.023	0.023	1.00

Mean ± standard deviation.

In vivo acute toxicity evaluation.

The methanolic root extract of *R. tridentata* was assessed for its antibacterial effects against both gram-positive and gram-negative bacteria strains. The extract exhibited moderate antibacterial activity against the two forms of bacteria. The zones of inhibition ranged from 11.20 ± 0.11 to 22.00 ± 0.71 mm, the minimum inhibitory concentrations (MICs) were from 0.78 mg/mL to 6.25 mg/mL, and the minimum bactericidal concentrations (MBCs) were between 1.56 mg/mL and 12.5 mg/mL (Table 2). *S. aureus* strain was the most susceptible strain with 22.00 ± 0.71 mm zone of inhibition, MIC of 3.13 mg/mL, and MBC of 6.25 mg/mL. *E. coli* was the least susceptible to the extracts with a zone of inhibition of 11.20 ± 0.11 mm, MIC of 6.25 mg/mL, and MBC of 12.5 mg/mL. The other bacteria strains tested (*E. faecalis*, *P. mirabilis*, *K. pneumoniae*) showed intermediate responses in terms of zones of inhibition, MIC and MBC. Notably, *S. mutans* showed a low MBC value (1.56 mg/mL) indicating that the extract could be bactericidal at relatively lower concentrations of the plant extract. Across all the tested bacteria strains, the MBC/MIC ratios obtained ranged from 1.99 to 2. Such ratios signified that the *R. tridentata* root extract mode of action was bactericidal ($\text{MBC/MIC} \leq 4$) [16]. The integrated antibacterial evaluation, incorporating MIC, MBC, and MBC/MIC ratios in addition to the growth zones of inhibition, gave a deeper understanding into the antibacterial potency of *R. tridentata* than the previous reports [8] which relied particularly on the zones of inhibition and MIC values.

The observed antibacterial potential is likely attributable to the annotated phytochemical compounds such as kaempferol, quercetin, ellagic acid, and 3,4-di-O-galloylquinic acid, each of which is known to exhibit antibacterial properties [2,8,30,37]. Fisetin, padmatin, isorhamnetin, isokaempferide, and 3-O-methylquercetin have also been documented with antimicrobial effects [26,32,40]. These findings support the traditional use of *R. tridentata* in treating bacterial infections and henceforth could justify its application in therapeutic management of bacterial infections related to dog bites.

The *in vivo* acute toxicity evaluation of the root extracts of *R. tridentata* in mice model revealed no significant adverse deviation of the toxicity signs from the normal at the tested doses from (10 to 1600) mg/kg. However, at oral doses from 2900 mg/kg, mild transient toxicity signs: tremors, itching, increased urination, and ventilation were observed within the first two to four hours (Supplementary material Table S1). The overall clinical signs such as lethargy, defecation, salivation, reflex responses, and food consumption observed remained normal in all the mice groups, and there was no mortality recorded. Therefore, the LD₅₀ exceeded 5000 mg/kg and thus the extract was categorized as practically non-toxic and relatively harmless [10]. On the contrary, *in-vitro* studies by Tshikalange et al., [33] described the plant as moderately toxic. The observed significant ($p < 0.05$) loss in body weight of mice at 1600 mg/kg to 5000 mg/kg (Supplementary material Fig. S3), could suggest low metabolism leading to low growth [9]. On the other hand, the decrease in mean organ weigh (MOW) and relative organ weight (ROW) observed was non-significant (Supplementary material Table S2). This suggest that the animal's organ physiology was possibly not affected, which could have resulted into abnormal atrophy or hypertrophy [25] to alter the body physiology. Thus, the extracts might have sustained a healthy body condition of the mice [21], hence not toxic.

The kidney function indicators (urea, creatinine) were elevated ($p < 0.05$) at all the doses of the extracts (Supplementary Table S4), accompanied by increased urine output (Supplementary Table S1), but without significantly observable lesions or anomalies (Supplementary Fig. S5), suggesting no significant nephrotoxicity caused. Additionally, hematological findings showed higher RBCs and HCT, normal MCV, and reduced RDW (Supplementary Table S3), which may indicate that the extract contained erythropoietin-like agent(s). The decreased WBCs and platelet indices hint at immunomodulation without bleeding risks [28,34]. The elevated AST levels without change in the corresponding ALT (Supplementary Table S4), suggested no significant hepatocellular injury. These findings indicated that *R. tridentata* root extract were relatively safe for oral administration in mice model, showing minimal organ-specific toxicity. It is equally important to note that this is the first validated *in vivo* safety data for *R. tridentata* plant root extracts since the previous studies were *in vitro* cytotoxicity assays in nature [33].

Conclusion

The current study provided the first ever high-resolution, mass-spectrometry-based metabolomic profile, comprehensive antibacterial potency, and validated *in vivo* safety evaluation of *R. tridentata* root extracts. The integration of UHPLC-MS/MS—qTOF, followed by the MZmine deconvolution, and then GNPS2 FBMN guided metabolomics profiling approach revealed that the extracts contain diverse flavonoids (flavones, flavonols, dihydroflavonols, anthocyanins, and flavanones), tannins, phenolic acids, and polyphenols. The extensive antibacterial evaluation, incorporating MIC, MBC, and MBC/MIC ratios, provided deeper insights into antibacterial activity of the plant than prior reports that relied only of zones of inhibition and MICs. The extracts demonstrated potent antibacterial activity due to the moderate zones of inhibition observed, with MBC/MIC ratios of up to 2. Further still, with the oral LD₅₀ exceeding 5000 mg/kg and absence of acute toxicity evidence suggested that the extracts are of a moderate safety in mice. The findings support the therapeutic use of *R. tridentata* in the traditional management of dog bites and the associated bacterial infections. The study findings further posit that *R. tridentata* could be an alternative natural source used for potential leads in the development of relatively safe and efficacious antibacterial agents. Future research should focus on isolating and structure characterization of the active antibacterial phytochemicals using spectroscopic techniques. Advanced *in silico* modeling and pharmacodynamic studies are also recommended to elucidate mechanisms of action and optimize lead compounds for drug development. This will boost the rational strategy of identification of future phytochemical leads to discovery of antibacterial agents.

Ethics approval

The protocol for the current study was reviewed and approved by Mbarara University of Science and Technology Ethics Committee (MUST-2021-76), and clearance was obtained from the Uganda National Council of Science and Technology (UNCST, NS290ES).

CREDIT authorship contribution statement

Paul Mukasa: Writing–original draft, Methodology, Data curation, Conceptualization. **Patrick Engeu Ogwang:** Supervision. **Moses Andima:** Writing – review & editing, Methodology. **Christopher Adaku:** Writing – review & editing, Supervision. **Samuel Baker Obakiro:** Methodology, Supervision. **Julius Bunny Lejju:** Supervision. **Ibrahim Ntulume:** Writing – review & editing, Methodology. **Denis Byamugisha:** Writing – review & editing, Supervision. **Emmanuel Ntambi:** Writing – review & editing, Supervision. **Richard Oriko Owor:** Writing – review & editing, Conceptualization, Supervision.

Data availability

All the other data associated to this article are provided as supplementary materials.

Declaration of interest statement

No potential conflict of interest was reported by the authors.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.sciaf.2025.e03113](https://doi.org/10.1016/j.sciaf.2025.e03113).

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