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Original Article

In vitro Antibacterial Activity of Crude Extracts from *Tithonia diversifolia* (Asteraceae) and *Solanum torvum* (Solanaceae) against Selected *Shigella* Species

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Abstract

Background and objectives: Infectious diarrhea is a gastrointestinal illness that results in around 1.7 billion cases and 525,000 deaths annually, particularly among children under five, according to the World Health Organization. While some Cameroonian medicinal plants show promise for treating diarrhea, many plants are used without established scientific evidence of their efficacy. These plants include *Tithonia diversifolia* (*T. diversifolia*) and *Solanum torvum* (*S. torvum*), which are traditionally used to treat diarrheal symptoms. This study sought to investigate the anti-*Shigella* activity of leaf extracts from *T. diversifolia* and *S. torvum*.

Methods: Extracts from *T. diversifolia* and *S. torvum* were obtained by successive maceration in solvents of increasing polarity, including hexane, dichloromethane, ethyl acetate, methanol, and water. The as-prepared extracts (10) were evaluated for antibacterial activity against selected *Shigella* species using an *in vitro* experiment. The mode of action of the bioactive extracts was determined in *Shigella* through growth kinetic analysis.

Results: Hexane extract from *S. torvum* (St-HEX-F) and dichloromethane extract from *T. diversifolia* (Td-DCM-F) inhibited the growth of *Shigella flexneri* NR-518 and *Shigella boydii* NR-521 with minimum inhibitory concentration (MIC) values of 500 and 1,000 µg/mL, respectively. *Shigella flexneri* and *Shigella boydii* were the most sensitive strains, whereas *Shigella sonnei* was the most resistant strain. Bacterial growth kinetics revealed that St-HEX-F and Td-DCM-F are bacteriostatic at MIC and bactericidal at 2×MIC and 4×MIC.

Conclusions: Extracts from *T. diversifolia* and *S. torvum* possess anti-*Shigella* activity and could be used as a potential source of active ingredients for developing new treatments against diarrhea caused by multidrug-resistant *Shigella*.

Keywords: Diarrhea; *Shigella* sp.; *Tithonia diversifolia*; *Solanum torvum*; Antibacterial activity, Time-kill kinetics.

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Introduction

Diarrhea is the release of three or more loose or liquid stools per day, or more frequently than is normal for an individual.[1] Infectious diarrhea is caused by pathogens such as bacteria, viruses, and parasites, with *Shigella* being a common bacterial cause that is particularly prevalent in children six to ten years old.[1] According to the World Health Organization (WHO) statistics updated in 2022, diarrhea is the second leading cause of infant mortality after respiratory infection, with more than 525,000 deaths annually among children under five years of age.[2,3] *Shigella*-causing diarrhea, also called shigellosis, is an intestinal infection caused by *Shigella* bacteria, character-

ized by diarrhea (often bloody), fever, and abdominal pain. *Shigella* is a major cause of moderate-to-severe diarrhea and dysentery, particularly in young children in limited-resource settings.[4,5] For example, in a multicenter study in six Asian countries, the incidence of shigellosis was 2.1 episodes per 1,000 residents per year overall, but 13.2 episodes per 1,000 people per year in children under five.[6] The prevalence of *Shigella*-caused diarrhea in Cameroon varies by region and time, with studies showing rates from 3.8% in Yaounde (2008) to 4.5% in Buea (2012) and a regional pooled prevalence of 4.5% for Central Africa (2024).[7] In rural and peri-urban areas, the prevalence of diarrheal diseases is critical, accounting for more than 80% of deaths due to fragile health systems and persistent health challenges.[8] *Shigella flexneri (S. flexneri)* is the predominant species, and high levels of multidrug resistance to common antibiotics are a concern.[7,9,10] The infection is spread through contaminated food or drinking water, or from person to person as a result of poor hygiene and sanitation.[11] The treatment of diarrhea relies on oral rehydration salts, antibiotics (ciprofloxacin, azithromycin, levofloxacin, etc.), and intestinal motility inhibitors (loperamide, morphine, and its derivatives). However, the misuse and overuse of these drugs, as well as the use of substandard and counterfeit treatments, has led to bacterial drug resistance.[12,13] Moreover, diarrheal infection is exacerbated by insufficient access to safe water and proper sanitation. Although bacterial resistance to the latest generation of antibiotics, such as ceftriaxone, is scarce, high levels of resistance in *S. flexneri* have been reported, thus highlighting the need for surveillance and updated treatment guidelines to combat the growing threat of multidrug-resistant *Shigella* infections.[14,15] In addition to bacterial resistance, most conventional treatments for diarrhea show undesirable and toxic adverse effects. Because of their richness in active ingredients with various therapeutic properties, the WHO encourages the use of medicinal plants to treat various infectious diseases, including malaria.[16] While some Cameroonian medicinal plants show promise for treating diarrhea, many plants are used without established scientific evidence of their efficacy. Such plants include *Tithonia diversifolia* (Asteraceae; *T. diversifolia*) and *Solanum torvum* (Solanaceae; *S. torvum*), which are traditionally used to treat diarrheal symptoms.[17,18] Notably, other research groups have reported the antimicrobial activities of *S. torvum* and *T. diversifolia* on human and plant pathogens.[19–29] However, studies related to *T. diversifolia* and *S. torvum* extracts on the main pathogens responsible for diarrhea (*Shigella* and *Salmonella*) remain scarce. Growing evidence has shown the safety profile of *T. diversifolia* and *S. torvum*.[30–35] Therefore, the scientific validation of the use of *T. diversifolia* and *S. torvum* in treating *Shigella*-causing diarrhea is of outstanding importance. This study thus sought to investigate the anti-*Shigella* activity of leaf extracts from *T. diversifolia* (Asteraceae) and *S. torvum* (Solanaceae). Specifically, (i) the *in vitro* inhibitory effects of extracts of *T. diversifolia* and *S. torvum* were screened against three bacteria of the genus *Shigella* (*S. flexneri* NR-518, *Shigella boydii (S. boydii)* NR-521, and *Shigella sonnei (S. sonnei)* NR-519); and (ii) the bacterial kinetics of the promising extracts were evaluated on the most susceptible bacterial strains.

Materials and methods

Materials

Plant material

Leaves of *T. diversifolia* (Asteraceae) (Fig. 1a) and *S. torvum* (Solanaceae) (Fig. 1b) were collected in May 2025 in the Yaounde VI locality, Ebang district, Yaounde-Cameroon, and identified at the National Herbarium of Cameroon by comparison with specimens previously registered under reference number 10742SRF/Cam for *S. torvum* (Solanaceae) and 18591SRF/Cam for *T. diversifolia* (Asteraceae). The plant leaves were then cut into small pieces, dried at room temperature in the laboratory, and coarsely powdered using a grinder.

Microbiological material

Antibacterial activity was determined on three reference bacterial strains, including *S. flexneri* NR-518, *S. boydii* NR-521, and *S. sonnei* NR-519, kindly provided by BEI Resources (Biodefense and Emerging Infections Resources Repository). These bacterial strains were stored in tubes containing Mueller–Hinton agar by slant culture at 4°C and maintained in continuous culture at the Laboratory for Phytobiochemistry and Medicinal Plant Studies, Department of Biochemistry, University of Yaounde I.

Methods

Preparation of crude extracts

The extracts were prepared by successive maceration of the plant powder in hexane, dichloromethane, ethyl acetate, methanol, and distilled water. To this end, 40 g of each plant sample was macerated in 250 mL of each solvent for 24 h.[36] The mixtures were stirred twice a day (morning and evening), and the macerates obtained were filtered using Whatman No. 1 filter paper and then ventilated at room temperature. The crude extracts were weighed to calculate their extraction yields according to the following formula:

$$\text{Yield of extraction (\%)} = \frac{\text{Weight of crude extract}}{\text{Weight of the plant powder}} \times 100$$

The as-prepared extracts were stored in a refrigerator (4°C) for antibacterial studies.

Antibacterial activity

Preparation of the inocula

The bacterial inocula were prepared according to the 0.5 McFarland standard.[37] Colonies from 24-h cultures on Mueller–Hinton agar (Appendices 1 and 2, Table S1) were collected using a platinum loop and placed in a test tube containing 10 mL of physiological water, then calibrated to 0.5 McFarland (Appendix 3) by comparing the turbidity to obtain an inoculum with a bacterial load of 1.5×10^8 CFU/mL.

Preparation of extract solutions and the reference antibiotic ciprofloxacin

The extract stock solutions were prepared at 100 mg/mL by dissolving 100 mg of each extract in 1 mL of 100% dimethyl sulfoxide in sterile tubes, then stored at 4°C until further use. Ciprofloxacin, used as the reference antibiotic, was prepared under the same conditions at 100 µg/mL in water acidified with 0.5 N HCl.

Preliminary antibacterial screening

The antibacterial activity of the plant extracts was determined using the microdilution method, according to protocol M07-A09 described by the Clinical and Laboratory Standards Institute.[38] Preliminary screening of the extracts was performed at a single concentration (1,000 µg/mL). To this end, 98 µL of culture medium (Mueller–Hinton broth; MHB) (Appendices 1 and 2, Table S1) was added to each well of a 96-well microplate, followed by 2 µL of extract stock solution (100 mg/mL). Next, 100 µL of a bacterial suspension at a load of 10^6 CFU/mL (obtained from the 0.5 Mc-

Fig. 1. Photography of Tithonia diversifolia (a) and Solanum torvum (b) (CADN, 2025).

Farland standard) was distributed to all wells except those in the sterility control, where only MHB culture medium was added. The microplates were sealed and incubated at 37°C for 24 h. At the end of the incubation period, 20 µL of a freshly prepared resazurin solution (0.15 mg/mL) (Appendix 3) was added to all wells, and the plates were incubated under the same conditions for 30 min. The positive control consisted of culture medium, inoculum, and ciprofloxacin, whereas the negative control comprised culture medium and inoculum. Inhibition of bacterial growth was revealed by the persistence of the blue coloration (Appendix 4) of resazurin in the wells. Extracts that inhibited at least 50% of the bacterial strains tested were selected for determination of antibacterial activity parameters, such as minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs).

Determination of MICs and MBCs

Determination of MICs

The inhibitory effects of the plant extracts were determined using the microdilution method, according to protocol M07-A09 described by the Clinical and Laboratory Standards Institute.[38] Briefly, 196 µL of culture medium (MHB) was added to the first

wells in column A and 100 µL to the remaining wells of the microtiter plate. Next, 4 µL of a solution of each extract (100 mg/mL) was added to the corresponding wells, followed by a series of five geometric dilutions of order 2. Finally, 100 µL of a bacterial suspension with a load of 10^6 CFU/mL (obtained from the 0.5 McFarland standard) was distributed to all wells except those of the sterility control. The concentrations of extracts and ciprofloxacin ranged from 1,000 to 31.25 µg/mL and from 0.25 to 0.0078 µg/mL, respectively. The final bacterial load in each well was 5×10^5 CFU/mL for a final volume of 200 µL. The sterility control consisted of culture medium only. The positive control encompassed culture medium, inoculum, and ciprofloxacin, whereas the negative control comprised culture medium and bacterial suspension. The microplates were sealed and incubated at 37°C for 24 h. At the end of the incubation period, the plates were treated as described in subsection "Preliminary antibacterial screening". The tests were performed in triplicate in sterile 96-well microplates. The lowest concentration at which no color change from blue to pink (Appendix 5) was observed corresponded to the MIC and was expressed in µg/mL. To evaluate the bactericidal or bacteriostatic activity of the extracts, the MBCs were determined.

Determination of MBCs

The MBCs were determined by subculturing in liquid medium the preparations withdrawn from the plates used to determine the MICs. At the end of the incubation period for the MIC determination plates, 25 µL aliquots of the inhibitory wells (which had not yet been treated with resazurin) were aseptically removed and transferred to corresponding wells in another sterile plate containing 175 µL of culture medium (MHB). The extracts contained in these wells were then diluted eightfold to eliminate their inhibitory effects. The sterility control consisted of culture medium only, whereas the negative control encompassed inoculum and culture medium. The microplates were then incubated at 37°C for 48 h, after which 20 µL of resazurin was added to each well, followed by an additional incubation at the same conditions for 30 min. The lowest concentration of extract that showed no bacterial growth, as indicated by the persistence of the resazurin color (blue), was considered the MBC. The tests were performed in triplicate. The bactericidal or bacteriostatic effect of the extracts was estimated by calculating the ratio MBC/MIC. According to Traoré *et al*.,[39] when the MBC/MIC ratio of an antimicrobial substance is ≤4, it is classified as bactericidal; if >4, it is considered bacteriostatic.

Bacterial growth kinetics

Bacterial growth kinetics were conducted to confirm the bactericidal or bacteriostatic effect of the most promising extracts. The most active extracts were incubated with the most susceptible bacteria at sub-inhibitory, inhibitory, and supra-inhibitory concentrations (MIC/4 to 4×MIC). The evolution of bacterial mortality was studied according to the protocol of Klepser *et al*.[40] with minor modifications, using the turbidity of the cell suspension based on bacterial load rather than colony counting on agar. Briefly, the most promising extracts were diluted to obtain concentrations ranging from 4×MIC to MIC/4. Next, 100 µL of bacterial inoculum at 10^6 CFU/mL was introduced into each well, except for the sterility control wells, to obtain a final bacterial load of 5×10^5 CFU/mL. Ciprofloxacin was used as a positive control. The plates were sealed and incubated at 37°C for 24 h, during which bacterial population evolution was determined by reading optical densities at 620 nm using a microplate reader (TECAN Infinite M 200) at time intervals of 0, 2, 4, 6, 8, 10, 12, and 24 h. The tests were performed in triplicate in sterile 96-well microplates. Optical density measurements were used to plot optical density curves as a function of incubation time to evaluate bactericidal or bacteriostatic effects of the promising extracts.

Table 1. Yields of extraction obtained following the maceration of *T. diversifolia* and *S. torvum*

Plant species	Plant organs	Solvent	Yields (%)
T. diversifolia	Leaves	Hexane	0.79
		Dichloromethane	1.84
		Ethyl acetate	2.40
		Methanol	4.59
		Water	0.78
S. torvum	Leaves	Hexane	1.12
		Dichloromethane	2.08
		Ethyl acetate	1.63
		Methanol	0.56
		Water	0.61

Statistical analyses

Values were represented as mean ± standard deviation. Statistical analysis was performed using one-way analysis of variance with GraphPad Prism 8.0.1 software, followed by Dunnett's test for comparison of means with a confidence level of 95% ($P \leq 0.05$). Values of $P < 0.05$ were considered significant. The positive control consisted of culture medium, inoculum, and ciprofloxacin, whereas the negative control comprised culture medium and inoculum.

Results

Yields of extraction

The extraction yields of various plant organs are shown in Table 1. Upon maceration of *T. diversifolia* and *S. torvum* in different solvents, the extraction yields ranged from 0.78% to 4.59% and from 0.56% to 2.08%, respectively. Overall, the methanol extract of *T. diversifolia* and the dichloromethane extract of *S. torvum* afforded the highest yields compared to the other extracts. These yields varied significantly depending on the plant species and the solvent used for extraction. This highlights the variability in the content of plant secondary metabolites according to their affinity with the solvent, which might significantly influence the antibacterial activity. The as-prepared extracts were evaluated for their inhibitory effects on three *Shigella* strains, including *S. flexneri* NR-518, *S. boydii* NR-521, and *S. sonnei* NR-519.

Anti-Shigella activity

Preliminary screening

The incubation of different extracts (1,000 µg/mL) with the three bacterial strains (*S. flexneri* NR-518, *S. boydii* NR-521, and *S. sonnei* NR-519) led to significant antibacterial activity, as detailed in Table 2. Among the extracts tested, the hexane extract from *S. torvum* and the dichloromethane extract from *T. diversifolia* inhibited the growth of *S. flexneri* and *S. boydii* (Table 2). On this basis, these extracts were selected for MIC and MBC testing.

Table 2. Antibacterial activity of *S. torvum* and *T. diversifolia* extracts at 1,000 µg/mL

Extracts/Bacterial strains	Td Hex F	Td DCM F	Td EtOAc F	Td MeOH F	Td H_2O F	St Hex F	St DCM F	St EtOAc F	St MeOH F	St H_2O F	Cipro
SFNR-518	–	+	–	–	–	+	–	–	–	–	+
SO NR-519	–	–	–	–	–	–	–	–	–	–	+
SB-521	–	+	–	–	–	+	–	–	–	–	+

+, Active (MIC ≤ 1,000 µg/mL); –, Not active (>1,000 µg/mL). Cipro, Ciprofloxacin; SB, *Shigella boydii*; SF, *Shigella flexneri*; SO, *Shigella sonnei*; St-DCM-F, dichloromethane extract of *S. torvum* leaves; St-EtOAc-F, ethyl acetate extract of *S. torvum* leaves; St-H_2O-F, water extract of *S. torvum* leaves; St-Hex-F, hexane extract of *S. torvum* leaves; St-MeOH-F, methanol extract of *S. torvum* leaves; Td-DCM-F, dichloromethane extract of *T. diversifolia* leaves; Td-EtOAc-F, ethyl acetate extract of *T. diversifolia* leaves; Td-H_2O-F, water extract of *T. diversifolia* leaves; Td-Hex-F, hexane extract of *T. diversifolia* leaves; Td-MeOH-F, methanol extract of *T. diversifolia* leaves.

Table 3. Minimum inhibitory and bactericidal concentrations (µg/mL) of promising extracts

Plant extracts/ Compound	SF NR-518		SB NR-521		SO NR-519	
	MIC	MBC	MIC	MBC	MIC	MBC
St-HEX-F	500	1,000	1,000	>1,000	>1,000	>1,000
Td-DCM-F	500	1,000	1,000	>1,000	>1,000	>1,000
Ciprofloxacin	0.0625	/	0.0625	/	0.0625	/

The values represent the averages of triplicates. /, not determined. MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; SB, *Shigella boydii*; SF, *Shigella flexneri*; SO, *Shigella sonnei*; St-HEX-F, hexane extract of *S. torvum*; Td-DCM-F, dichloromethane extract of *T. diversifolia*.

Determination of MICs

According to Table 3, the MICs for *S. torvum* and *T. diversifolia* extracts ranged from 500 to 1,000 µg/mL. The hexane extract of *S. torvum* (St-HEX-F) and the dichloromethane extract of *T. diversifolia* (Td-DCM-F) were the most active extracts against *S. flexneri*, with a common MIC value of 500 µg/mL. The St-HEX-F and Td-DCM-F extracts also inhibited the growth of *S. boydii* with a common MIC value of 1,000 µg/mL. *S. flexneri* and *S. boydii* were the most sensitive strains, whereas *S. sonnei* was the most resistant strain. Moreover, ciprofloxacin, used as a positive control, showed an MIC value of 0.0625 µg/mL against all the bacterial strains.

According to the classification criteria of Nguelo Talla *et al*.[10] an extract is considered highly active if MIC < 100 µg/mL; significantly active if 100 ≤ MIC ≤ 512 µg/mL; moderately active if 512 ≤ MIC ≤ 2,048 µg/mL; weakly active if MIC > 2,048 µg/mL; and inactive if MIC > 10 mg/mL. Thus, the hexane extract of *S. torvum* leaves (St-HEX-F) and the dichloromethane extract of *T. diversifolia* (Td-DCM-F) were significantly active against *S. flexneri* NR-518. Against *S. boydii* NR-521, these extracts were moderately active. Interestingly, St-HEX-F and Td-DCM-F revealed a bactericidal trend against *S. flexneri* NR-518. However, none of the extracts (concentrations up to 1,000 µg/mL) had a bactericidal effect on *S. boydii* NR-521 and *S. sonnei* NR-519.

Effects of bioactive extracts on bacterial growth kinetics

The growth kinetics of *S. flexneri* NR-518 were studied in the presence of the most active extracts (St-HEX-F and Td-DCM-F). Figure 2 illustrates the evolution of bacterial mortality as a function of incubation time.

Upon incubation of the bioactive extracts (St-HEX-F and Td-DCM-F) at MIC, 2×MIC, 4×MIC, MIC/2, and MIC/4 with an inoculum of *S. flexneri* NR-518, and monitoring at different time intervals (0, 2, 4, 6, 8, 10, 12, and 24 h) by optical density readings, there was a concentration-dependent inhibition of bacterial

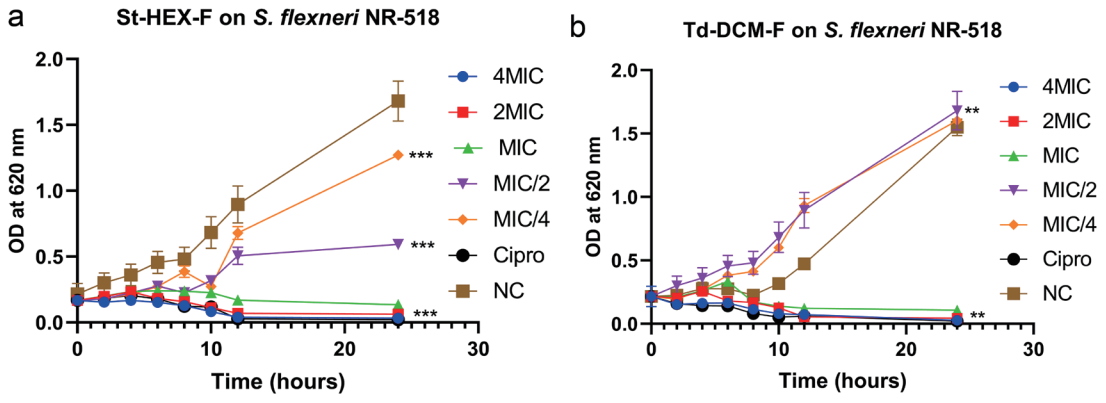

Fig. 2. Effects of hexane extract of S. torvum (a) and dichloromethane extract of T. diversifolia (b) on the growth of Shigella flexneri NR-518. Curves with the same symbols * are not significantly different (P ≤ 0.05, Dunnett's test). (***P < 0.001; **P < 0.01). Cipro, ciprofloxacin (0.125 µg/mL); MIC, minimum inhibitory concentration; NC, negative control; OD, optical density; S. flexneri NR-518, Shigella flexneri NR-518; St-HEX-F, hexane extract of S. torvum; Td-DCM-F, dichloromethane extract of T. diversifolia.

growth. The minimum time required by St-HEX-F to inhibit *S. flexneri* growth was 4 h at 4×MIC and 2×MIC, and 8 h at MIC and MIC/2. The minimum time required for bacterial inhibition by Td-DCM-F was observed after 2, 4, and 6 h at 4×MIC, 2×MIC, and MIC, respectively. At 4×MIC and 2×MIC, there was a gradual decrease in the bacterial population until 24 h of incubation, as evidenced by the slope of the curves. The activity observed with extracts at 4×MIC and 2×MIC was comparable to that of ciprofloxacin (positive control), which is well known for its bactericidal effects on *S. flexneri*.[41,42]

Thus, St-HEX-F and Td-DCM-F exerted a bactericidal effect at high concentrations (2×MIC and 4×MIC). After 10 and 12 h of incubation of *S. flexneri* NR-518 with Td-DCM-F and St-HEX-F (2×MIC and 4×MIC), respectively, the bacterial population approached zero, as evidenced by the slope of the curves intersecting the x-axis (Fig. 2a, b). The lack of bacterial growth between 10 and 24 h of incubation demonstrated the absence of bacterial growth, validating the bactericidal effect of St-HEX-F and Td-DCM-F at high concentrations (2×MIC and 4×MIC). At MIC, St-HEX-F and Td-DCM-F revealed a bacteriostatic effect because the bacterial population gradually decreased but was not completely nullified up to 24 h of incubation.

Discussion

Shigellosis is an intestinal infection caused by *Shigella* bacteria, leading to symptoms such as fever, cramps, nausea, and diarrhea that may be bloody, slimy, or watery.[43] The WHO recently classified *Shigella*-causing infections among the diseases to which priority for eradication should be attributed because of their pathogenicity and virulence.[1] In addition, the growing resistance of *Shigella* species to antimicrobials has rendered antibiotics ineffective.[44,45] To face this alarming situation, the WHO encourages the traditional use of medicinal plants to treat a number of infectious diseases, including infectious diarrhea. Notably, the validation of the use of these medicinal plants is worthwhile. *T. diversifolia* and *S. torvum* are two Cameroonian medicinal plants traditionally used to treat diarrheal symptoms. Thus, this study sought to investigate the anti-*Shigella* activity of extracts from *S. torvum* and *T. diversifolia* against three *Shigella* species, including *S. flexneri* NR-518, *S. boydii* NR-521, and *S. sonnei* NR-519.

Extracts from *S. torvum* and *T. diversifolia* leaves were obtained by successive maceration using solvents of increasing polarity, hexane, dichloromethane, ethyl acetate, methanol, and water, to afford a total of 10 crude extracts (five extracts for each plant species) (Table 1). The as-prepared extracts were then evaluated for anti-*Shigella* activity through a preliminary screening at a single concentration (1,000 µg/mL). Among these extracts, two (St-HEX-F and Td-DCM-F) inhibited the growth of at least one *Shigella* species and were selected for the determination of the MICs and MBCs. The extracts St-HEX-F and Td-DCM-F presented MIC values ranging from 500 to 1,000 µg/mL. The observed antibacterial activity might be attributed to the presence of different secondary metabolites in *S. torvum* and *T. diversifolia*.[46] Growing evidence has shown that *S. torvum* contains a variety of secondary metabolites, including alkaloids, flavonoids, saponins, anthraquinones, phenolic compounds, tannins, terpenoids, steroids, and volatile compounds.[46–48] Previous phytochemical studies have shown that *T. diversifolia* contains alkaloids, flavonoids, terpenoids, steroids, saponins, and phenolic compounds.[49,50] These metabolites are well known for their antibacterial and other medicinal properties. Flavonoids, for instance, have been reported to exert antibacterial activity through several mechanisms, including membrane disruption, inhibition of nucleic acid (DNA and RNA) and cell envelope synthesis, and inhibition of biofilm formation.[51,52] Saponins, like alkaloids, exert their antibacterial action mainly by causing the formation of pores in the bacterial membrane, leading to leakage of intracellular contents and cell death.[52,53] Thus, it is not unreasonable to speculate that *S. torvum* and *T. diversifolia* extracts might have exerted antibacterial activity through at least one of these mechanisms. The differences in antibacterial action observed on a single bacterial strain with different extracts could be due to the composition of secondary metabolites, which differs quantitatively and qualitatively depending on the plant species and the extraction solvent used.[54–57] Upon incubation of *S. flexneri* NR-518 with the bioactive extracts St-HEX-F and Td-DCM-F at sub-inhibitory, inhibitory, and supra-inhibitory concentrations (MIC/4 to 4×MIC), and monitoring optical density measurements at different time intervals (0, 2, 4, 6, 8, 10, 12, and 24 h), a bacteriostatic effect was observed at the MIC values, whereas a bactericidal inclination was revealed at 2×MIC and 4×MIC. Nonetheless, further *in vitro* and *in vivo* studies on the modes of action of the bioactive extracts (St-HEX-F and Td-DCM-F) are desirable to support this observation. Moreover, *in vitro* and *in vivo* toxicity experiments are warranted to prospect the bioactive extracts as starting points for anti-shigellosis drug discovery.

Limitations and perspectives

This research sought to investigate the antibacterial activity of extracts from *S. torvum* and *T. diversifolia* against selected *Shigella* species (*S. flexneri* NR-518, *S. boydii* NR-521, and *S. sonnei* NR-519). Among the extracts tested, the hexane extract of *S. torvum* leaves (St-HEX-F) and the dichloromethane extract of *T. diversifolia* (Td-DCM-F) significantly inhibited the growth of *S. flexneri* NR-518. Notably, St-HEX-F and Td-DCM-F showed a bactericidal inclination against *S. flexneri* NR-518. As it is important that the mechanism of action of these compounds be unveiled, kinetics of bacterial growth were used to study their effect over time. These findings validate the traditional use of *S. torvum* and *T. diversifolia* in treating infectious diarrhea and might be used as starting points for anti-diarrheal drug discovery. However, major limitations of this work include the lack of cytotoxicity tests on various human cell lines (liver, skin, kidney, and intestinal epithelial cells, among others) to identify potential toxicity risks to vital organs in humans. In addition, more antibacterial tests should be extended to a larger set of clinically relevant *Shigella* strains. Isolation and characterization of the active anti-*Shigella* compounds are warranted. Furthermore, in-depth studies on the anti-*Shigella* mechanisms of action and pharmacokinetics of extracts and compounds from *S. torvum* and *T. diversifolia* should be investigated to ensure the successful utilization of these compounds in anti-*Shigella* drug development.

Conclusions

The extracts from *S. torvum* and *T. diversifolia*, obtained by successive maceration using solvents of increasing polarity, were further screened for antibacterial effect against three *Shigella* species (*S. flexneri* NR-518, *S. boydii* NR-521, and *S. sonnei* NR-519). As a result, the hexane extract from *S. torvum* leaves (St-HEX-F) and the dichloromethane extract of *T. diversifolia* (Td-DCM-F) revealed significant antibacterial activity against *S. flexneri* NR-518, with MIC values ranging from 500 to 1,000 µg/mL.

Kinetics of bacterial mortality (*S. flexneri* NR-518) with St-HEX-F and Td-DCM-F revealed a bacteriostatic orientation at MIC and a bactericidal trend at higher extract concentrations (2×MIC and 4×MIC). Overall, extracts from *S. torvum* and *T. diversifolia* demonstrated antibacterial effects, thus confirming the traditional use of these plants in treating bacterial infections. However, the mechanistic basis of the antibacterial action, *in vitro* and *in vivo* toxicity experiments, and pharmacokinetics are warranted to support the use of these plants in ethnomedicine. Moreover, activity-guided fractionation and isolation of the antibacterial compounds should also be investigated.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization (BPK), methodology (CADN, AMM, SPT, ASML, YNN, BRP, JAK), software (CADN, AMM, SPT, ASML, YNN, BRP, JAK), validation (BPK), formal analysis (CADN, AMM, SPT, ASML, BRP, YNN, JAK), investigation (CADN, AMM, SPT, ASML, BRP, YNN, JAK), resources (BPK), data curation (CADN, AMM, SPT, ASML, BRP, YNN, JAK), writing—original draft preparation (CADN, AMM, SPT, ASML, YNN, JAK), writing—review and editing (BPK), visualization (BPK), supervision (BPK), project administration (BPK). All authors have read and agreed to the published version of the manuscript.

Ethical statement

Not applicable. This article does not involve any studies on human or animal participants.

Data sharing statement

The complete data files were submitted along with the paper and are published as corresponding Supplementary Information accessible through the electronic publication.

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