



Original Article

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



Christelle Amanda Djakam Ngola¹, Aimerance Mabelle Madoung¹, Staelle Pierre Tedonzang¹, Aicha Sylvanie Magniteu Lekefack¹, Yolande Nzeulienou Noubissi¹, Jamila Aminatou Kone¹, Brice Rostan Pinlap¹ and Boniface Pone Kamdem^{1,2*} 

¹Higher Institute of Sciences and Technologies La Sapience, Faculty of Science, University of Ngaoundere, Ngaoundere, Cameroon; ²Antimicrobial and Biocontrol Agents Unit, Laboratory for Phytochemistry and Medicinal Plants Studies, Department of Biochemistry, Faculty of Science, University of Yaounde I, Yaounde, Cameroon

Received: October 21, 2025 | Revised: November 20, 2025 | Accepted: January 06, 2026 | Published online: January 30, 2026

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 anti-bacterial 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*.

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-

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

***Correspondence to:** Boniface Pone Kamdem, Higher Institute of Sciences and Technologies La Sapience, Faculty of Science, University of Ngaoundere, P.O. Box 454 Ngaoundere, Cameroon. ORCID: <https://orcid.org/0000-0002-4982-0507>. Tel: +237-680987669, E-mail: ponekamdemboniface@gmail.com

How to cite this article: Ngola CAD, Madoung AM, Tedonzang SP, Lekefack ASM, Noubissi YN, Kone JA, *et al.* *In vitro* Antibacterial Activity of Crude Extracts from *Tithonia diversifolia* (Asteraceae) and *Solanum torvum* (Solanaceae) against Selected *Shigella* Species. *J Explor Res Pharmacol* 2026;11(2):e00052. doi: 10.14218/JERP.2025.00052.

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 ceftazidime, 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 Her-

barium 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 Phytochemistry 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 per-

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

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

formed 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.

Statistical analyses

Values were represented as mean \pm 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 ₂ O F	St Hex F	St DCM F	St EtOAc F	St MeOH F	St H ₂ O 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-H₂O-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-H₂O-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

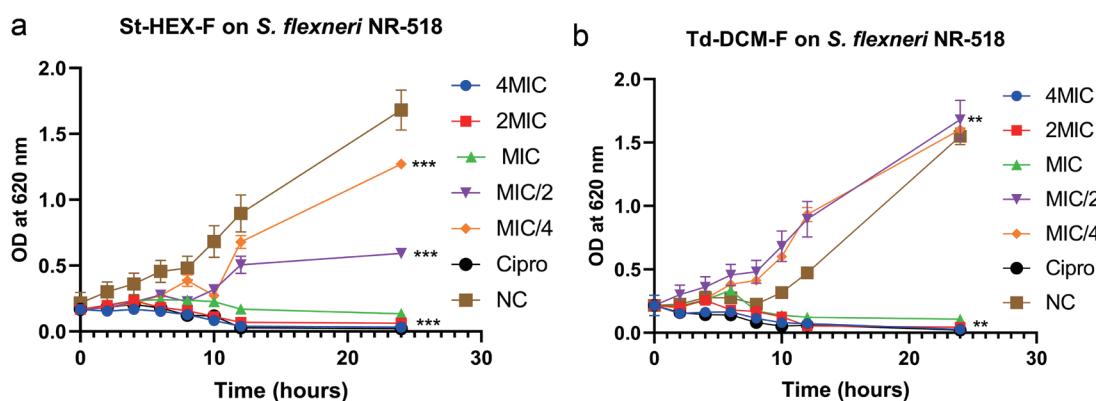


Fig. 2. Effects of hexane extract of *S. torvum* (a) and dichloromethane extract of *T. diversifolia* (b) on the growth of *Shigella flexneri* NR-518. Curves with the same symbols * are not significantly different ($P \leq 0.05$, Dunnett's test). (**P < 0.001; **P < 0.01). Cipro, ciprofloxacin (0.125 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.

Acknowledgments

The authors are thankful to the National Herbarium of Cameroon (Yaounde, Cameroon) for the identification of the plants. The authors also thank BEI Resources (Biodefense and Emerging Infections Resources Repository) for providing the bacterial strains.

Funding

This research received no external funding.

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.

References

- World Health Organization (WHO). Diarrhoeal disease. Available from: <https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease>. 2024. Accessed September 26, 2025.
- Hartman RM, Cohen AL, Antoni S, Mwenda J, Weldegebriel G, Biey J, et al. Risk Factors for Mortality Among Children Younger Than Age 5 Years With Severe Diarrhea in Low- and Middle-income Countries: Findings From the World Health Organization-coordinated Global Rotavirus and Pediatric Diarrhea Surveillance Networks. *Clin Infect Dis* 2023;76(3):e1047–e1053. doi:10.1093/cid/ciac561, PMID:35797157.
- Unicef Data. Diarrhoea. 2024. Available from: <https://data.unicef.org/topic/child-health/diarrhoeal-disease/>. Accessed October 17, 2025.
- Mamishi S, Arab Yazdi Z, Mahmoudi S, Moradzadeh M, Taghi Hagh Ashtiani M, Pourakbari B. Antimicrobial-resistance pattern of *Shigella* species in children: a six-year study in an Iranian referral Hospital. *Ann Ig* 2019;31(4):356–364. doi:10.7416/ai.2019.2297, PMID:31268120.
- Libby TE, Delawalla MLM, Al-Shimari F, MacLennan CA, Vannice KS, Pavlinac PB. Consequences of *Shigella* infection in young children: a systematic review. *Int J Infect Dis* 2023;129:78–95. doi:10.1016/j.ijid.2023.01.034, PMID:36736579.
- von Seidlein L, Kim DR, Ali M, Lee H, Wang X, Thiem VD, et al. A multicentre study of *Shigella diarrhoea* in six Asian countries: disease burden, clinical manifestations, and microbiology. *PLoS Med* 2006;3(9):e353. doi:10.1371/journal.pmed.0030353, PMID:16968124.
- Nyarkoh R, Odoom A, Donkor ES. Prevalence of *Shigella* species and antimicrobial resistance patterns in Africa: systematic review and meta-analysis. *BMC Infect Dis* 2024;24(1):1217. doi:10.1186/s12879-024-09945-2, PMID:39472797.
- Djoukouo Kouontang S. Persistance des maladies hydriques en zone rurale au Cameroun: étude qualitative à Bansoa-Chefferie. Louvain-la-Neuve: Université catholique de Louvain, Louvain-la-Neuve, Belgium; 2020.
- Njunda AL, Assob JC, Nsagha DS, Kamga HL, Awafong MP, Weledji EP. Epidemiological, clinical features and susceptibility pattern of shigellosis in the buea health district, Cameroon. *BMC Res Notes* 2012;5:54. doi:10.1186/1756-0500-5-54, PMID:22264300.
- Nguelo Talla AC, Madesse Kemgne EA, Ngouana V, Noumboue Kouamou BL, Nzeye Ngameni LM, Pinlap BR, et al. Anti-Shigellosis Activity and Mechanisms of Action of Extracts from *Diospyros gilletii* Stem Bark. *Drugs Drug Candidates* 2024;3(1):256–274. doi:10.3390/ddc3010015.
- The World Health Organization (WHO). Guidelines for the control of shigellosis, including epidemics due to *Shigella dysenteriae* type 1. 2005. Available from: <https://www.who.int/publications/item/9241592330>. Accessed September 29, 2025.
- Ferraz MP. Antimicrobial resistance: the impact from and on society according to one health approach. *Societies (Basel)* 2024;14(9):187. doi:10.3390/soc14090187.
- Ahmed SK, Hussein S, Qurbani K, Ibrahim RH, Fareeq A, Mahmood KA, et al. Antimicrobial resistance: Impacts, challenges, and future prospects. *J Med Surg Public Health* 2024;2:100081. doi:10.1016/j.gimedi.2024.100081.
- Salleh MZ, Nik Zuraina NMN, Hajissa K, Ilias MI, Banga Singh KK, Deiris ZZ. Prevalence of Multidrug-Resistant and Extended-Spectrum Beta-Lactamase-Producing *Shigella* Species in Asia: A Systematic Review and Meta-Analysis. *Antibiotics (Basel)* 2022;11(11):1653. doi:10.3390/antibiotics11111653, PMID:36421297.
- Baharvand A, Molaeipour L, Alesaeidi S, Shaddel R, Mashatan N, Amiriani T, et al. The increasing antimicrobial resistance of *Shigella* species among Iranian pediatrics: a systematic review and meta-analysis. *Pathog Glob Health* 2023;117(7):611–622. doi:10.1080/2047724.2023.2179451, PMID:36794800.
- Onukansi FO, Anokwuru CC, Eneh SC, Odey GO, Onah DO, Sokunbi T, et al. Investing in traditional medicine: leveraging evidence and innovative research to strengthen the fight against malaria in Nigeria. *Malar J* 2025;24(1):232. doi:10.1186/s12936-025-05359-w, PMID:40665320.
- Di Giacomo C, Vanella L, Sorrenti V, Santangelo R, Barbagallo I, Calabrese G, et al. Effects of *Tithonia diversifolia* (Hemsl.) A. Gray extract on adipocyte differentiation of human mesenchymal stem cells. *PLoS One* 2015;10(4):e0122320. doi:10.1371/journal.pone.0122320, PMID:25848759.
- Joseph NK, Severin IBJ, Parfait KBG, Jean-Claude AK. Propriétés antidiarrhéiques de l'extrait aqueux de *Solanum torvum* (Solanaceae) chez le rat de souche Wistar. *Eur Sci J* 2020;16(6):112–125. doi:10.19044/esj.2020.v16n6p112.
- Khunbutstri D, Naimon N, Satchasataporn K, Inthong N, Kaewmongkol S, Sutjarit S, et al. Antibacterial Activity of *Solanum torvum* Leaf

Extract and Its Synergistic Effect with Oxacillin against Methicillin-Resistant *Staphylococci* Isolated from Dogs. *Antibiotics (Basel)* 2022;11(3):302. doi:10.3390/antibiotics11030302, PMID:35326765.

[20] Ngoule CC, Ngene JP, Ladoh-Yemedé CF, Yinyang J, Kidik Pouka C, Heudeu Ngofanki EL, et al. Evaluation of the antibacterial activity and cytotoxicity of extracts of endophytic bacteria isolated from *Solanum torvum* Sw (Solanaceae). *Sch Int J Tradit Complement Med* 2022;5(2):33–37. doi:10.36348/sijtcm.2022.v05i02.003.

[21] Claude-Charlène KM, Konan KF, Okou OC, Kra AKM, Guessennnd N, et al. Evaluation of the antibacterial activity of *Solanum torvum* fruit extracts on hospital strains of Enterobacteria. *Microbiol Res J Int* 2024;34(12):214–223. doi:10.9734/mrji/2024/v34i121522.

[22] Claude-Charlène KM, Tiekoura B, Totoy AA, Okou OC, Kra AKM, Guessennnd N, et al. Antibacterial activity of *Solanum torvum* fruit extracts by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *J Adv Microbiol* 2025;25(8):86–94. doi:10.9734/jamb/2025/v25i8977.

[23] Nguta JM. Antimicrobial Activity of *Solanum torvum* Crude Extracts against Important Mycobacterial Strains. *Int J Mycobacteriol* 2025;14(3):246–252. doi:10.4103/ijmy.ijmy_69_25, PMID:40953202.

[24] Masih D. 2025. Antifungal, antibacterial and nematicidal properties of *Solanum* species. *Int J Adv Multidisc Res Stud* 2025;5(3):1165–1171. doi:10.62225/2583049X.2025.5.3.4431.

[25] Ferreira Farias AL, Lobato Rodrigues AB, Lopes Martins R, de Menezes Rabelo É, Ferreira Farias CW, Moreira da Silva de Almeida SS. Chemical Characterization, Antioxidant, Cytotoxic and Microbiological Activities of the Essential Oil of Leaf of *Tithonia Diversifolia* (Hemsl) A. Gray (Asteraceae). *Pharmaceuticals (Basel)* 2019;12(1):34. doi:10.3390/ph12010034, PMID:30836657.

[26] Merciline O, Dominic M. Phytochemical screening and antimicrobial activity of crude extract of *Tithonia diversifolia*. *Open Biol Sci J* 2020;5(1):30–33. doi:10.17352/ojbs.000021.

[27] Opondo FA, K'Owino IO, Chepkwony SC, Kosgei VJ. In vitro antibacterial activity of essential oils From *Tithonia diversifolia* leaves and flowers against *Ralstonia solanacearum*. *Eur J Appl Sci* 2022;10(2):523–539. doi:10.14738/ajvp.102.12172.

[28] Opondo FA, K'Owino IO, Chepkwony SC, Kosgei VJ, Pili NJ. In vivo antibacterial activity of extracts of *Tithonia diversifolia* against *Ralstonia solanacearum* in tomato. *Sci Afr* 2023;22:e01962. doi:10.1016/j.sci.2023.e01962.

[29] Linthoingambi W, Singh MS. Antimicrobial activities of different solvent extracts of *Tithonia diversifolia* (Hemsl) A. Gray. *Asian J Plant Sci Res* 2013;3(5):50–54.

[30] Goffin E, Ziemons E, De Mol P, de Madureira Mdo C, Martins AP, da Cunha AP, et al. In vitro antiplasmodial activity of *Tithonia diversifolia* and identification of its main active constituent: tagitinin C. *Planta Med* 2002;68(6):543–545. doi:10.1055/s-2002-32552, PMID:12094301.

[31] Elufioye TO, Alatise OI, Fakoya FA, Agbedahunsi JM, Houghton PJ. Toxicity studies of *Tithonia diversifolia* A. Gray (Asteraceae) in rats. *J Ethnopharmacol* 2009;122(2):410–415. doi:10.1016/j.jep.2008.12.007, PMID:19121378.

[32] Ezeonwumelu JOC, Omolo RG, Ajayi AM, Agwu E, Tanayen JK, Adiukwu CP, et al. Studies of phytochemical screening, acute toxicity and anti-diarrhoeal effect of aqueous extract of Kenyan *Tithonia diversifolia* leaves in rats. *Br J Pharmacol Toxicol* 2012;3(3):127–134.

[33] Devi TB, Jena S, Patra B, Singh KD, Chawla S, Raina V, et al. Acute and sub-acute toxicity evaluation of dihydro-p-coumaric acid isolated from leaves of *Tithonia diversifolia* Hemsl. A. Gray in BALB/c mice. *Front Pharmacol* 2022;13:1055765. doi:10.3389/fphar.2022.1055765, PMID:36506583.

[34] Pérez JL, Castillo AA, Salas MH, Zapata EP, Hernández JB, Horruitiner EJ, et al. Oral toxicity of *Solanum torvum* Sw. (prendejera) (in Spanish). *Rev Cubana Plant Med* 2011;16(4):390–395.

[35] Balamurugan K. Repeated dose oral toxicity study of ethanolic extract of *Solanum torvum* in rats using-OECD 407 guidelines. *J Cardiovasc Dis Res* 2017;8(3):176–186.

[36] Mouanji MCF, Ngouana V, Madiisse Kemgne EA, Pone Kamdem B, Tchuentem D, Wendji Monkam DS, et al. Phytochemical screening, anti-*Salmonella* and antioxidant activities of extracts from *Calistemon rigidus* R. Br. (Myrtaceae). *S Afr J Bot* 2025;184:263–279. doi:10.1016/j.sajb.2025.06.004.

[37] Balouiri M, Sadiki M, Ibsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal* 2016;6(2):71–79. doi:10.1016/j.jpha.2015.11.005, PMID:29403965.

[38] Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—ninth edition. CLSI document M07-A9. Wayne (PA): Clinical and Laboratory Standards Institute; 2012.

[39] Traoré Y, Ouattara K, Yé D, Doumbia I, Coulibaly A. Recherche des activités antifongique et antibactérienne (extrait aqueux) des feuilles d'*Annona senegalensis* Pers. (Annonaceae) (in French). *J Appl Biosci* 2012;58:4234–4242.

[40] Klepser ME, Ernst EJ, Lewis RE, Ernst ME, Pfaller MA. Influence of test conditions on antifungal time-kill curve results: proposal for standardized methods. *Antimicrob Agents Chemother* 1998;42(5):1207–1212. doi:10.1128/AAC.42.5.1207, PMID:9593151.

[41] Liu Y, Li H, Zhang Y, Ye Y, Gao Y, Li J. In vitro and in vivo activity of ciprofloxacin/fosfomycin combination therapy against ciprofloxacin-resistant *Shigella flexneri* isolates. *Infect Drug Resist* 2019;12:1619–1628. doi:10.2147/IDR.S208071, PMID:31354311.

[42] Zhang Y, Zhang Y, Ma R, Sun W, Ji Z. Antibacterial Activity of Epigallocatechin Gallate (EGCG) against *Shigella flexneri*. *Int J Environ Res Public Health* 2023;20(6):4676. doi:10.3390/ijerph20064676, PMID:36981585.

[43] Salimiyan Rizi K, Farsiani H, Sasan MS. High rate of resistance to ceftriaxone and azithromycin among *Shigella* spp. isolates at three children's referral hospitals in Northeast Iran. *J Infect Chemother* 2020;26(9):955–958. doi:10.1016/j.jiac.2020.04.022.

[44] Puzari M, Sharma M, Chetia P. Emergence of antibiotic resistant *Shigella* species: A matter of concern. *J Infect Public Health* 2018;11(4):451–454. doi:10.1016/j.jiph.2017.09.025, PMID:29066021.

[45] Ranjbar R, Farahani A. *Shigella*: Antibiotic-Resistance Mechanisms And New Horizons For Treatment. *Infect Drug Resist* 2019;12:3137–3167. doi:10.2147/IDR.S219755, PMID:31632102.

[46] Yapo SES, Kouadio OKS, Kouakou TH. Phytochemistry and biological potential of wild eggplant *Solanum torvum* Sw. 1788 (Solanaceae). In: Ramawat KG, Mérillon JM (eds). *Natural Products*. Berlin, Heidelberg: Springer; 2025:1–17. doi:10.1007/978-3-642-36202-6_264-1.

[47] Djoueudam FG, Fowa AB, Fodouop SPC, Kodjio N, Gatsing D. *Solanum torvum* Sw. (Solanaceae): Phytochemical screening, antisalmonelal and antioxidant properties of leaves extracts. *J Med Plants Stud* 2019;7(1):5–12.

[48] Darkwah WK, Koomson DA, Miwonunyue N, Nkoom M, Puplampu JB. Review: phytochemistry and medicinal properties of *Solanum torvum* fruits. *All Life* 2020;13(1):498–506. doi:10.1080/26895293.2020.1817799.

[49] Herrera RS, Verdecia DM, Ramírez JL. Chemical composition, secondary and primary metabolites of *Tithonia diversifolia* related to climate. *Cuban J Agric Sci* 2020;54(3):425–433.

[50] Sabaris SB, Muhammad S, Aswathy KA, Sireesha R, Munuswamy-Ramanujam G. Comparative bioactivity evaluation of secondary metabolites from the leaves, stem and flowers of *Tithonia diversifolia*. *Mater Today Proc* 2023;93(Pt 2):28–39. doi:10.1016/j.mtpr.2023.09.042.

[51] Zhou H, Chen L, Ouyang K, Zhang Q, Wang W. Antibacterial activity and mechanism of flavonoids from *Chimonanthus salicifolius* S. Y. Hu. and its transcriptome analysis against *Staphylococcus aureus*. *Front Microbiol* 2022;13:1103476. doi:10.3389/fmicb.2022.1103476, PMID:36704556.

[52] Liu Y, Zhu J, Liu Z, Zhi Y, Mei C, Wang H. Flavonoids as Promising Natural Compounds for Combating Bacterial Infections. *Int J Mol Sci* 2025;26(6):2455. doi:10.3390/ijms26062455, PMID:40141099.

[53] Dong S, Yang X, Zhao L, Zhang F, Hou Z, Xue P. Antibacterial activity and mechanism of action of saponins from *Chenopodium quinoa* Willd. husks against foodborne pathogenic bacteria. *Ind Crops Prod* 2020;149:112350. doi:10.1016/j.indcrop.2020.112350.

[54] Ayalew Tiruneh T, Ayalew Tiruneh G, Chekol Abebe E, Mengie Ayele T. Phytochemical investigation and determination of antibacterial activity of solvent leave extracts of *Carissa spinarum*. *Infect Drug Resist* 2022;15:807–819. doi:10.2147/IDR.S352049, PMID:35281572.

[55] Lee JE, Jayakody JTM, Kim JI, Jeong JW, Choi KM, Kim TS, et al. The

influence of solvent choice on the extraction of bioactive compounds from Asteraceae: A comparative review. *Foods* 2024;13(19):3151. doi:10.3390/foods13193151.

[56] Dirar Al, Alsaadi DHM, Wada M, Mohamed MA, Watanabe T, Devkota HP. Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. *S Afr J Bot* 2019;120:261–267. doi:10.1016/j.sajb.2018.07.003.

[57] Arya P, Vaidya D, Kaushal M, Devi S, Gupta A, Chand S. Effects of different solvents on phytochemical constituents, in-vitro antimicrobial activity, and volatile components of *Boehmeria rugulosa* Wedd. wood extract. *Sci Rep* 2025;15:9135. doi:10.1038/s41598-025-14506-x.