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EFFECTS OF AQUEOUS AND ETHANOL EXTRACTION ON *IN VITRO* ANTIBACTERIAL ACTIVITY OF *SMILAX GLABRA* ROXB. RHIZOME EXTRACTS

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Abstract

Growing concerns about antimicrobial resistance have renewed interest in plant-derived compounds as potential sources of antibacterial agents. This study compared *in vitro* antibacterial activity of aqueous and ethanolic extracts of *Smilax glabra* Roxb. rhizomes and determined their total phenolic content (TPC) and total flavonoid content (TFC). Radical-scavenging activity was also assessed using DPPH and ABTS assays. The extracts were prepared by aqueous extraction or maceration with 50%, 70%, and 96% ethanol (E50, E70, and E96), then tested against *Escherichia coli*, *Salmonella paratyphi* A, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis* using agar well diffusion and broth microdilution assays. The ethanolic extracts generally showed greater antibacterial activity than the aqueous extract, with activity varying by concentration and bacterial strain. E96 had a minimum inhibitory concentration (MIC) of 6.25-12.5 mg/mL against *S. paratyphi* A, and its MIC values across the five strains ranged from 6.25 to 50 mg/mL. The minimum bactericidal concentration (MBC) values exceeded the highest concentration tested (100 mg/mL) for all extract-strain combinations. E96 had the highest TPC (284.85 ± 0.66 mg GAE/g dry weight), whereas E50 had the highest TFC (750.56 ± 1.43 mg QE/g dry weight). At 100 µg/mL, E96 also showed the highest radical-scavenging activity in the DPPH (84.04 ± 0.17%) and ABTS (68.09 ± 2.36%) assays. Overall, these findings identify E96 as the most active preparation among those tested and provide a comparative *in vitro* profile of the antibacterial activity of aqueous and ethanolic *S. glabra* rhizome extracts.

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Keywords: Smilax glabra Roxb.; rhizome extracts; aqueous and ethanolic extraction; antibacterial agent; plant-derived compound

1. Introduction

Plants produce a diverse range of secondary metabolites, some of which contribute to plant defense against microbial attack and have been investigated as potential antimicrobial agents [1], [2]. The continued rise in antimicrobial resistance has intensified interest in these compounds as potential leads for controlling pathogenic microorganisms [3]. Ellward et al. screened 664 extracts from 132 traditional Chinese medicinal plant species and identified activity in extracts from 17 species, mainly against Gram-positive bacteria [4]. In another study, Assefa et al. examined extracts from three Ethiopian medicinal plants against five bacterial pathogens and found that activity varied with the plant species and extraction solvent [5]. However, antibacterial activity varies among plant preparations and is influenced by factors such as the plant material, extraction solvent and procedure, concentration tested, and susceptibility of the target microorganism [6]. Accordingly, the antibacterial activity of each preparation should be established under defined assay conditions.

The rhizome of *Smilax glabra* Roxb. has been investigated as a source of antibacterial constituents. Xu et al. extracted the rhizomes with 50% and 95% ethanol under reflux, then examined the crude extracts, solvent fractions, and isolated compounds against several Gram-positive and Gram-negative bacteria [7]. Jeeno et al. compared water, ethanol, and methanol extracts prepared by soaking the plant material at room temperature; the ethanol and methanol extracts inhibited two oral bacterial isolates, whereas the water extract showed no detectable activity under the assay conditions used [8]. These studies support the antibacterial potential of *S. glabra* rhizome, while illustrating that reported activity depends on the extract preparation and the bacterial targets examined. Although assisted techniques such as ultrasound-assisted extraction have also been applied to plant materials, conventional maceration remains a practical option for comparative studies [9]. Nevertheless, comparative data remain limited on the antibacterial profiles of aqueous and graded-ethanol preparations of *S. glabra* rhizome across a multi-species panel spanning both Gram-positive and Gram-negative bacteria.

In this study, we compared the *in vitro* antibacterial activity of an aqueous decoction and ethanol macerates (50%, 70%, and 96%) prepared from *S. glabra* rhizome. The five test organisms comprised three Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella paratyphi* A) and two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). The panel includes enteric bacteria and opportunistic pathogens, with *B. subtilis* serving as an additional Gram-positive test organism. The extracts were assessed by agar diffusion and determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Total phenolic content (TPC) and total flavonoid content (TFC) were also measured to characterize the preparations alongside their antibacterial profiles.

2. Materials and methods

2.1. Chemicals and reagents

Ethanol, resazurin sodium salt, ABTS, and quercetin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid, aluminum chloride hexahydrate, sodium nitrite, sodium

hydroxide, potassium persulfate, and sodium carbonate were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Folin-Ciocalteu reagent and DPPH were obtained from Merck (Darmstadt, Germany) and Tokyo Chemical Industry (TCI, Tokyo, Japan), respectively. All chemicals and solvents were used as received without further purification.

2.2. Plant material and extraction

Rhizome material designated as *Smilax glabra* Roxb. was purchased in Kon Tum, Vietnam. The rhizomes were washed, sliced, sun-dried, and ground into powder before extraction.

For aqueous extraction, powdered rhizome (100 g) was mixed with 600 mL of distilled water and soaked for 8 h. The mixture was filtered through cotton, and the filtrate was frozen and lyophilized. The dried extract was stored at 4 °C until analysis.

For ethanol extraction, powdered rhizome (300 g) was divided into three 100 g portions and macerated separately in 600 mL of 50%, 70%, or 96% ethanol. Each extraction consisted of three successive 24 h cycles, using fresh solvent for each cycle. The filtrates were pooled by ethanol concentration and concentrated by rotary evaporation (60 °C, 60 rpm). The concentrates were dried at room temperature, with residual solvent removed by lyophilization, and the dried extracts were stored at 4 °C until analysis.



Figure 1. Rhizome material used in this study: (A) sliced rhizomes and (B) ground rhizome powder.

2.3. Bacterial strains and culture conditions

Antibacterial activity was evaluated against five ATCC reference strains: *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 9027), and *Salmonella paratyphi* A (ATCC 9150). *E. coli, S. aureus*, and *B. subtilis* were cultured on Luria Bertani (LB) agar and in LB broth. *S. Paratyphi* A was cultured on Mueller-Hinton agar (MHA) and in LB broth, whereas *P. aeruginosa* was cultured on MHA and in Brain Heart Infusion (BHI) broth. Stock cultures were maintained on agar slants at 4 °C and subcultured monthly.

2.4. Determination of total phenolic and flavonoid contents

2.4.1. Total phenolic content

Total phenolic content was determined using a modified Folin-Ciocalteu colorimetric assay [10]. Each extract was diluted tenfold with distilled water before analysis. An aliquot of the diluted extract (0.25 mL) was mixed with distilled water (2.25 mL) and Folin-Ciocalteu reagent (0.25 mL). After vigorous mixing for 5 s, the mixture was allowed to stand for 5 min, followed by the addition of 2.5 mL of 20% (w/v) sodium carbonate. The reaction mixture was incubated in the dark at room temperature for 90 min, and its absorbance was measured at 765 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan). Gallic acid was used to generate a calibration curve (10–100 µg/mL), and the results were displayed as gallic acid equivalents

(GAE). Measurements were performed in triplicate, and TPC was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g dry weight). TPC was calculated using the following general formula:

$$\text{TPC (mg GAE/g dry weight)} = \frac{C \times k \times V}{m}$$

C (mg/mL): Concentration obtained from the standard calibration curve

k: dilution factor

V (mL): volume of extract used

m (mg): mass of extract analyzed

2.4.2. Total flavonoid content

Total flavonoid content was determined using the aluminum chloride colorimetric method with modifications [11]. The extract solution (0.5 mL; 100 µg/mL) was mixed with distilled water (2 mL), followed sequentially by 0.15 mL of 5% (w/v) sodium nitrite and 0.15 mL of 10% (w/v) aluminum chloride. After 1 min, 1 mL of 1 M sodium hydroxide was added, and the mixture was brought to a final volume of 5 mL with distilled water. After incubation for 10 min, absorbance was measured at 510 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan). Quercetin was used to generate the calibration curve, and the results were displayed as quercetin equivalents (QE). Measurements were performed in triplicate, and TFC was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g dry weight). TFC was calculated using the following general formula:

$$\text{TFC (mg QE/g dry weight)} = \frac{C \times k \times V}{m}$$

C (mg/mL): Concentration obtained from the standard calibration curve

k: dilution factor

V (mL): volume of extract used

m (mg): mass of extract analyzed

2.5. Determination of radical-scavenging activity

2.5.1. DPPH radical-scavenging assay

DPPH radical-scavenging activity was determined using a 0.5 mM DPPH solution prepared in ethanol [12]. For each extract, the reaction mixture contained 0.3 mL of DPPH solution, 0.5 mL of extract solution, and 3.0 mL of ethanol. The sample blank contained 0.5 mL of extract solution and 3.3 mL of ethanol, while the control contained 0.3 mL of DPPH solution and 3.5 mL of ethanol. The mixtures were incubated in the dark at room temperature for 100 min. Absorbance was then measured at 517 nm using a UV-Vis spectrophotometer. Radical-scavenging activity was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = \left[1 - \frac{A_s - A_b}{A_c}\right] \times 100$$

A_s: absorbances of sample reaction mixture

A_b: absorbances of sample blank

A_c: absorbances of control

2.5.2. ABTS radical-scavenging assay

The ABTS radical cation (ABTS•$^+$) solution was prepared by mixing equal volumes of 7 mM ABTS and 2.45 mM potassium persulfate solutions [13]. The mixture was incubated in the dark at room temperature for 12-16 h and then diluted with distilled water to an absorbance of 0.68-0.72 at 734 nm. In a 96-well plate, 20 µL of extract solution was mixed with 180 µL of ABTS•$^+$ solution. After incubation in the dark at room temperature for 6 min, absorbance was measured at 734 nm using a microplate reader. The control contained 20 µL of distilled water and 180 µL of ABTS•$^+$ solution. Radical-scavenging activity was calculated as follows:

$$\text{ABTS scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

A_s: absorbances of sample reaction mixture

A_c: absorbances of control

2.6. Antibacterial activity

The antibacterial activity of *S. glabra* rhizome extracts was evaluated using agar well-diffusion and broth microdilution assays to determine inhibition zones, minimum inhibitory concentration (MIC), and minimum bactericidal concentrations (MBC) [14].

2.6.1. Agar well-diffusion assay

Bacterial suspensions were spread evenly over the surface of the appropriate agar plates. Six wells (5 mm in diameter) were made in each plate and filled with the extract concentrations (100, 50, 25, and 12.5 mg/mL), sterile distilled water as the negative control, or amoxicillin (200 µg/mL) or gentamicin (400 µg/mL) as the positive control. The plates were incubated at 37 ^{o}C for 24 h. Inhibition zones were measured as follows:

$$\text{Zone of inhibition } (\Delta) = D - d$$

Δ (mm): Difference in inhibition zone diameter

D (mm): total diameter of the well and surrounding inhibition zone

d (mm): well diameter (5 mm)

2.6.2. MIC and MBC determination

MICs were determined in 96-well microplates. Each well contained 50 µL of extract at the test concentration and 50 µL of bacterial suspension (3×10^8 cells/mL)[15]. Amoxicillin was used as the positive control for *E. coli, S. aureus, B. subtilis*, and *Salmonella paratyphi* A; gentamicin was used for *P. aeruginosa*. LB medium served as the negative control. After incubation at 37 ^{o}C for 24 h, 30 µL of 0.015% resazurin solution was added to each well. A blue-to-pink color change indicated bacterial growth. The MIC was defined as the lowest extract concentration that prevented this color change.

For MBC determination, 10 µL from each well without a color change was inoculated onto the appropriate agar medium [16]. The plates were incubated at 37 ^{o}C for 24 h. The MBC was defined as the lowest extract concentration that yielded no visible colonies.

2.7. Statistical analysis

Experimental data were expressed as mean ± standard deviation (SD) from three independent replicates, as specified for each assay. Inhibition-zone data were analyzed by two-way analysis of variance (ANOVA), with extract type and concentration as factors. Total phenolic content, total flavonoid content, DPPH and ABTS radical-scavenging activities were analyzed by one-way ANOVA, followed by Tukey's honestly significant difference (HSD) test for pairwise comparisons. Statistical significance was set at $p < 0.05$. Data were processed using Microsoft Excel 2016 and IBM SPSS Statistics version 20.

3. Results and discussion

3.1. Total phenolic and flavonoid contents

Phenolic and flavonoid contents are commonly measured to characterize plant extracts and to examine how extraction solvents affect the recovery of these broad classes of secondary metabolites. Because these compounds differ in polarity and chemical structure, changing the ethanol concentration may alter their extraction profiles in different ways. The TPC and TFC results for the aqueous and ethanolic *S. glabra* rhizome extracts are summarized in Table 1.

Table 1. Total phenolic and flavonoid contents of aqueous and ethanolic extracts of *Smilax glabra* Roxb. rhizome.

Extraction solvent	TPC (mg GAE/g dry weight)	TFC (mg QE/g dry weight)
Aqueous	103.40 ± 0.02^a	177.06 ± 0.95^a
E50	224.06 ± 0.02^b	750.56 ± 1.43^b
E70	239.13 ± 1.49^c	413.88 ± 0.00^c
E96	284.85 ± 0.66^d	639.38 ± 0.12^d

Different lowercase letters within the same column indicate significant differences ($p < 0.05$)

The extracts differed significantly in both TPC and TFC ($p < 0.05$). TPC increased progressively across the ethanol series, from 224.06 ± 0.02 mg GAE/g extract in E50 to 239.13 ± 1.49 mg GAE/g extract in E70 and 284.85 ± 0.66 mg GAE/g extract in E96. The aqueous extract had a substantially lower TPC (103.40 ± 0.02 mg GAE/g extract). Thus, within the extraction conditions examined, E96 yielded the highest Folin-Ciocalteu response, while all three ethanolic extracts exceeded the aqueous extract. Lu et al. measured 208.45 ± 3.74 mg GAE/g in a crude 70% ethanol extract and cited a previously published value of 262.6 ± 12.7 mg GAE/g for a 60% ethanol extract [17]. In the present study, E70 had a numerically higher TPC than the crude 70% ethanol extract, while E96 had the highest TPC among the values compared.

The different rankings for TPC and TFC are plausible because these colorimetric assays provide aggregate estimates and do not quantify an identical set of compounds. Solvent composition may therefore favor recovery of compounds contributing differently to each assay; the current data do not identify which individual compounds account for the observed pattern. Overall, E96 produced the highest TPC, whereas E50 produced the highest TFC, showing that the ethanol concentration associated with the greatest recovery differed between these two measured indices.

3.2. Radical-scavenging activity

DPPH and ABTS assays were used to compare the radical-scavenging responses of the extracts at 100 µg/mL. The extracts differed significantly in both assays ($p < 0.05$; Table 2). E96 showed the highest activity in both assays, while the relative activities of E50 and E70 varied between DPPH and ABTS.

Table 2. DPPH and ABTS radical-scavenging activities of aqueous and ethanolic *Smilax glabra* Roxb. rhizome extracts at 100 µg/mL.

Extraction solvent	DPPH radical-scavenging activity (%)	ABTS radical-scavenging activity (%)
Aqueous	32.27 ± 0.17^a	30.03 ± 1.72^a
E50	79.55 ± 0.17^b	62.57 ± 0.48^c
E70	82.51 ± 0.33^c	48.28 ± 1.78^b
E96	84.04 ± 0.17^d	68.09 ± 2.36^d

Different lowercase letters within the same column indicate significant differences ($p < 0.05$)

In the DPPH assay, E96 showed the highest scavenging activity (84.04 ± 0.17%), followed by E70 (82.51 ± 0.33%) and E50 (79.55 ± 0.17%). The aqueous extract showed substantially lower activity (32.27 ± 0.17%). The ABTS results followed a similar pattern for E96 and the aqueous extract, which showed the highest (68.09 ± 2.36%) and lowest (30.03 ± 1.72%) activities, respectively. E70 showed greater DPPH scavenging activity than E50, whereas E50 showed greater ABTS scavenging activity than E70. Thus, the ranking of E50 and E70 depended on the radical-scavenging assay.

The observed activity is consistent with previous reports on *S. glabra*. Jeeno et al. reported DPPH and ABTS scavenging activities of 71.94 ± 1.46% and 59.84 ± 4.80%, respectively, for an ethanolic extract [8]. This study evaluated a concentration series, so these values provide context for the activity observed here but cannot be directly ranked against measurements at a single concentration. Lu et al. reported 91.91% DPPH scavenging at 100 µg/mL for a phenolic-enriched *S. glabra* fraction [17]. E96 showed 84.04% activity at the same nominal concentration. The higher value reported for the enriched fraction is consistent with its additional purification step, although the assay procedures also differed between the studies.

The antioxidant activity of *S. glabra* flavonoids provides further context for these findings. Zhao et al. showed that several flavonoids isolated from the species, including epicatechin and astilbin-related compounds, scavenged DPPH and ABTS radicals [17]. These findings support the potential contribution of flavonoid constituents to the activity of *S. glabra* preparations, but the compounds responsible for the responses in the present extracts were not identified.

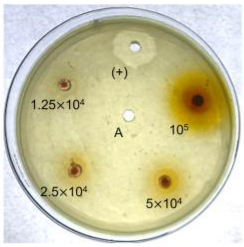
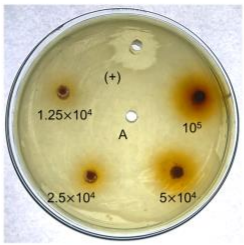
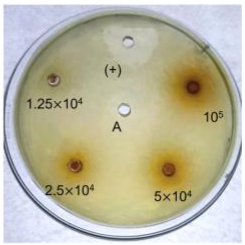
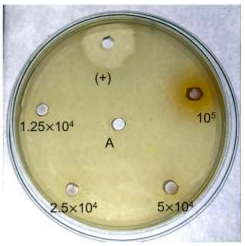
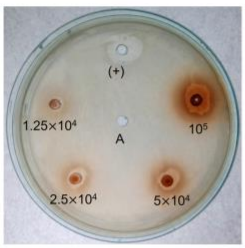
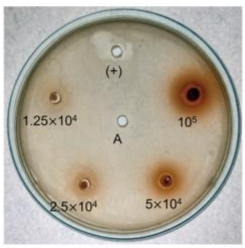
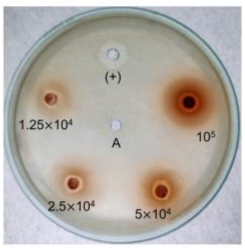
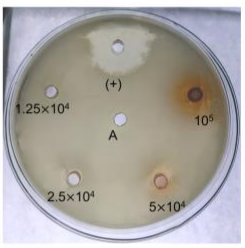
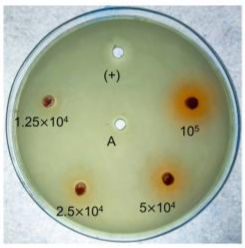
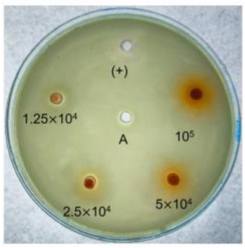
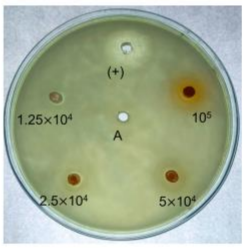
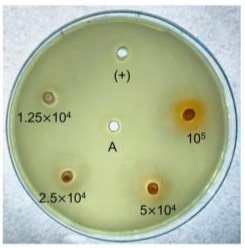
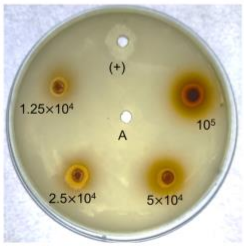
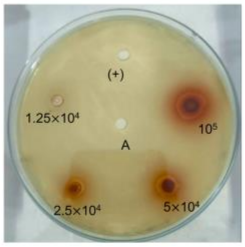
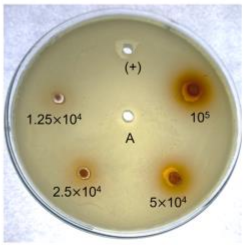
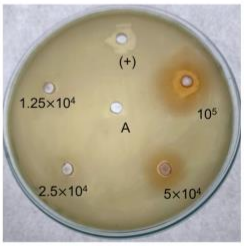
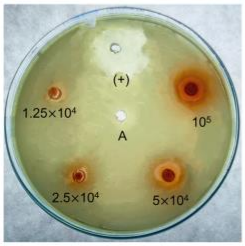
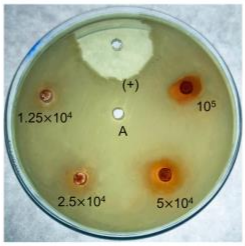
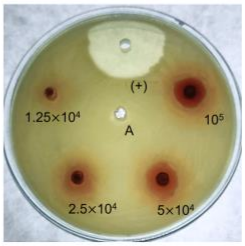
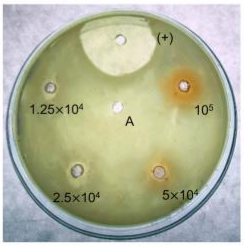
3.3. Antibacterial activity

3.3.1. Agar well-diffusion assay

Table 3. Inhibition zone diameters (mm) produced by *Smilax glabra* Roxb. rhizome extracts against the tested bacterial strains at different extract concentrations.

Bacterial strains	Extraction solvent	Extract concentration (mg/mL)			
		100	50	25	12.5
E. coli	E96	3.20 ± 0.30^{a1}	2.00 ± 0.50^{a2}	1.20 ± 0.30^{a3}	0.70 ± 0.30^{a4}
	E70	3.00 ± 0.50^{a1}	2.00 ± 0.50^{a2}	1.30 ± 0.60^{a3}	0.50 ± 0.50^{a4}
	E50	2.20 ± 0.30^{ab1}	1.50 ± 0.50^{ab2}	1.20 ± 0.30^{ab3}	0.50 ± 0.00^{ab4}
	Aqueous	1.70 ± 0.30^{b1}	1.20 ± 0.30^{b2}	0.80 ± 0.30^{b3}	0.60 ± 0.40^{b4}
S. paratyphi A	E96	5.00 ± 0.50^{a1}	2.80 ± 0.30^{a2}	1.80 ± 0.30^{a3}	1.20 ± 0.30^{a4}
	E70	4.30 ± 0.60^{a1}	3.20 ± 0.30^{a2}	2.00 ± 0.50^{a3}	1.20 ± 0.30^{a4}
	E50	4.00 ± 0.00^{a1}	2.80 ± 0.30^{a2}	2.00 ± 0.00^{a3}	1.70 ± 0.60^{a4}
	Aqueous	3.30 ± 0.60^{b1}	2.20 ± 0.80^{b2}	1.30 ± 0.60^{b3}	0.30 ± 0.60^{b4}
P. aeruginosa	E96	3.30 ± 0.60^{a1}	2.30 ± 0.60^{a2}	1.30 ± 0.60^{a3}	0.70 ± 0.30^{a4}
	E70	1.80 ± 0.60^{b1}	1.20 ± 0.30^{b2}	0.80 ± 0.30^{b3}	0.70 ± 0.30^{b4}
	E50	3.00 ± 0.00^{a1}	1.70 ± 0.30^{a2}	1.20 ± 0.30^{a3}	0.40 ± 0.20^{a4}
	Aqueous	1.30 ± 0.30^{b1}	0.80 ± 0.30^{b2}	0.50 ± 0.00^{b3}	0.30 ± 0.20^{b4}
S. aureus	E96	4.80 ± 0.80^{b1}	3.80 ± 0.80^{b2}	2.80 ± 0.80^{b3}	1.30 ± 0.60^{b4}
	E70	3.80 ± 0.30^{a1}	2.70 ± 0.60^{a2}	1.70 ± 0.60^{a3}	1.00 ± 0.00^{a4}
	E50	3.70 ± 0.60^{a1}	2.30 ± 0.60^{a2}	1.50 ± 0.50^{a3}	0.80 ± 0.30^{a4}
	Aqueous	2.50 ± 0.50^{c1}	1.20 ± 0.30^{c2}	0.50 ± 0.50^{c3}	0.20 ± 0.30^{c4}
B. subtilis	E96	4.50 ± 0.50^{b1}	3.30 ± 0.60^{b2}	2.30 ± 0.60^{b3}	1.30 ± 0.60^{b4}
	E70	2.80 ± 0.30^{a1}	2.50 ± 0.50^{a2}	2.20 ± 0.80^{a3}	0.80 ± 0.30^{a4}
	E50	3.30 ± 0.60^{a1}	2.30 ± 0.60^{a2}	1.50 ± 0.50^{a3}	0.80 ± 0.30^{a4}
	Aqueous	1.20 ± 0.30^{c1}	0.30 ± 0.60^{c2}	0.20 ± 0.20^{c3}	0.00 ± 0.00^{c4}

Different lowercase letters (a, b, c) indicate significant differences among columns, whereas different superscript numerals (1, 2, 3, 4) indicate significant differences among rows within the same bacterial strain ($p < 0.05$)

A	E96	E70	E50	Aqueous
E. coli				
S. paratyphi A				
P. aeruginosa				
S. aureus				
B. subtilis				

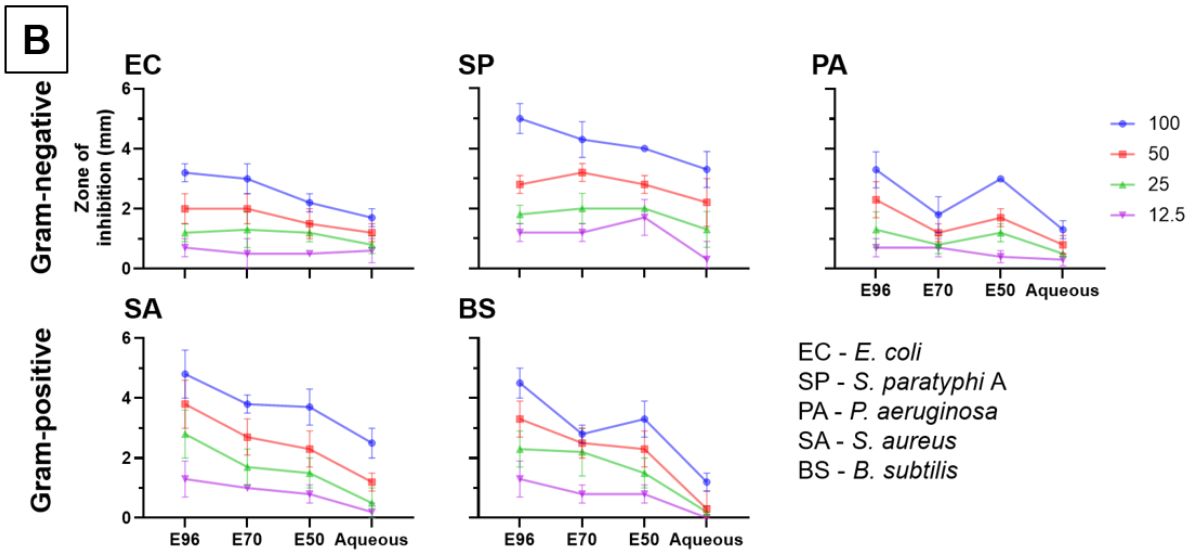


Figure 2. Antibacterial activity of E96, E70, E50, and aqueous extracts against Gram-negative and Gram-positive bacteria at different extract concentrations. (A) Agar plates showing inhibition zones against five bacterial strains at different extract concentrations (ppm). *Note: (+) - Antibiotic; A - Aqueous*. (B) Comparison of antibacterial activity against five bacterial strains.

The inhibition zones produced by the *S. glabra* rhizome extracts varied with extract type, concentration, and bacterial strain (Table 3). At 100 mg/mL, E96 produced the largest mean inhibition zone for each strain, measuring 5.00 ± 0.50 mm against *S. paratyphi* A, 4.80 ± 0.80 mm against *S. aureus*, 4.50 ± 0.50 mm against *B. subtilis*, 3.30 ± 0.60 mm against *P. aeruginosa*, and 3.20 ± 0.30 mm against *E. coli*. Inhibition zones generally became smaller as extract concentration decreased. This trend was evident for the ethanolic extracts across most strains, although its magnitude differed among the extract-strain combinations. The aqueous extract produced smaller zones than the ethanolic extracts in most comparisons. No inhibition zone was detected for the aqueous extract against *B. subtilis* at 12.5 mg/mL. Two-way ANOVA detected a significant interaction between extract type and concentration for *B. subtilis* and *P. aeruginosa* ($p < 0.05$). The interaction was not significant for *E. coli, S. paratyphi* A, or *S. aureus* ($p > 0.05$).

Previous studies have reported different antibacterial profiles for *S. glabra* preparations. Shah et al. observed activity against the Gram-positive bacteria *S. aureus* and *B. subtilis*, whereas *E. coli* and *S. typhi* were resistant to the extracts examined [16]. In the present study, measurable inhibition was observed against both *E. coli* and *S. paratyphi* A. Xu et al. also reported antibacterial activity among compounds isolated from *S. glabra* rhizomes against Gram-positive and Gram-negative bacteria [7]. These studies provide context for the range of bacterial responses reported for *S. glabra*. Differences in extract preparation, bacterial strains, and assay conditions may contribute to the variation among studies.

3.3.2. Minimum inhibitory and bactericidal concentrations

The MIC profiles differed among the extracts and bacterial strains (Table 4). E96 showed the lowest MIC interval against *S. paratyphi* A, at 6.25-12.5 mg/mL. Its MIC intervals against *S. aureus* and *B. subtilis* were 12.5-25 mg/mL, while those against *E. coli* and *P. aeruginosa*

were 25-50 mg/mL. The aqueous extract generally had higher MICs. No inhibitory endpoint was reached for this extract against *B. subtilis* within the tested concentration range.

The strain-specific results showed that E96 had a lower MIC interval than the other extracts against *S. paratyphi* A, *S. aureus*, and *B. subtilis*. Against *E. coli*, its MIC interval was similar to that of E70, while against *P. aeruginosa* it was similar to that of E50. The relatively low MIC of E96 against *S. paratyphi* A is consistent with the large inhibition zone observed for this strain in the agar well-diffusion assay.

The MBC exceeded 100 mg/mL for every extract-strain combination, so a bactericidal endpoint was not reached within the concentration range tested. Thus, the extracts inhibited bacterial growth at the MIC levels reported in Table 4, but the present assay did not establish bactericidal activity at or below 100 mg/mL.

Table 4. MIC intervals and MBC of aqueous and ethanolic *Smilax glabra* Roxb. rhizome extracts against the tested bacterial strains.

Extraction solven	***Bacterial strains***									
	E. coli		***S. paratyphi* A**		***P. aeruginosa***		***S. aureus***		***B. subtilis***	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
E96	25 - 50	>100	6.25 - 12.5	>100	25 - 50	>100	12.5 - 25	>100	12.5 - 25	>100
E70	25 - 50		12.5 - 25		50 - 100		25 - 50		50 - 100	
E50	50 - 100		12.5 - 25		25 - 50		25 - 50		25 - 50	
Aqueous	50 - 100		25 - 50		50 - 100		50 - 100		>100	

4. Conclusion

Aqueous and ethanolic extracts of *Smilax glabra* Roxb. rhizome showed distinct antibacterial profiles against the five bacterial strains examined. Among the preparations tested, E96 generally showed the greatest antibacterial activity and had the lowest MIC interval against *S. paratyphi* A. However, the MBC of all extracts exceeded 100 mg/mL, indicating that bactericidal activity was not demonstrated within the tested concentration range. E96 also had the highest total phenolic content and the greatest radical-scavenging activity in both assays, whereas E50 had the highest total flavonoid content. This comparative assessment shows that extraction solvent influences the measured properties of *Smilax glabra* Roxb. rhizome extracts and identifies E96 as the most promising preparation among those tested. The findings provide a basis for selecting extraction conditions and highlight the *in vitro* potential of *Smilax glabra* Roxb. rhizome extracts as sources of antibacterial and radical-scavenging activity.

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Conflicts of Interest

The authors declare no conflict of interest

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