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Biochemical composition, antimicrobial and antibiofilm activities of *Syntrichia ruralis* (Hedw.) F.Weber & D.Moh extracts against some resistant bacteria

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ABSTRACT: This study investigates the biochemical composition, antimicrobial, and antibiofilm properties of *Syntrichia ruralis* (Hedw.) F.Weber & D.Moh extracts obtained using ethanol, methanol, *n*-hexane, and distilled water as solvents. Disk diffusion assays demonstrated selective antimicrobial activity against specific bacterial strains, including *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Proteus vulgaris*. The MIC value for *S. epidermidis* was notably low (155 µg/mL) with the methanol extract, while other strains exhibited MIC values exceeding 10,000 µg/mL. Antibiofilm assays revealed significant inhibitory effects, particularly against foodborne *Listeria innocua* and clinical *Escherichia coli* isolates, with inhibition rates ranging from 57% to 87%, comparable to the commercial disinfectant Halamid. In contrast, biofilm formation was enhanced in *Listeria monocytogenes* upon exposure to the extracts. The ethanol extract showed limited antioxidant activity in the DPPH assay (30.69% at 1000 µg/mL), with an EC_{50} of 1743.47 µg/mL. GC-MS analysis identified a total of 50 compounds across all extracts. Palmitic acid, neophytadiene, vitamin E, and triterpenoids such as β-amyrin acetate were predominant in the ethanol extract, while the methanol and *n*-hexane extracts contained long-chain alkanes, sterols, and fatty acid derivatives. The presence of these compounds may underlie the observed biological effects. This study represents the first comprehensive report on the antimicrobial, antibiofilm, and antioxidant activities of *S. ruralis* and highlights its potential as a source of bioactive metabolites, especially for targeting biofilm-associated infections.

KEYWORDS: *Syntrichia ruralis* (Hedw.) F.Weber & D.Moh; antimicrobal activity; antibiyofilm activity; resistant bacteria; GC/MS.

1. INTRODUCTION

Bryophytes are considered the closest living relatives of the earliest plants that successfully adapted to terrestrial life approximately 470 to 515 million years ago [1]. This group represents a heterogeneous assemblage of plants comprising three major clades: mosses (Bryophyta), liverworts (Marchantiophyta), and hornworts (Anthocerotophyta) [2,3]. A defining feature of bryophytes is the dominance of the haploid gametophyte phase in their life cycle. Currently, there are approximately 14,000 species of mosses, 6,000 species of liverworts, and around 300 species of hornworts known worldwide [4].

Mosses are among the earliest land plants to evolve, characterized by simple morphology, low cellular differentiation, and a relatively high potential for cell growth. They thrive on a wide variety of substrates including soil, stones, rocks, tree bark, decaying wood, rooftops, and the banks of lakes and rivers [5,6]. Although mosses are not consumed by humans, they have been employed in traditional medicine for centuries in regions such as China, Europe, North America, and India [7]. Due to their antimicrobial properties, mosses have been used in the treatment of various pathological conditions, including bronchitis, skin infections, and the healing of wounds and burns [8]. Despite these beneficial effects, the biological activities of mosses remain

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underexplored. However, in recent years, increasing attention has been given to these plants due to their potential therapeutic applications [9].

In this study, the biological activities and biochemical composition of the moss species *Syntrichia ruralis* (Hedw.) F.Weber & D.Moh were investigated. The genus *Syntrichia* is a monophyletic group comprising more than 90 species distributed across temperate regions, ranging from deserts to alpine habitats [10,11]. *S. ruralis* typically forms bright golden-green, loose cushions or denser turfs that may reach 1–2 cm in height. The leaves, usually 4 to 6 mm long, strongly curve away from the stem when moist, giving the shoots a star-like appearance, and become twisted upon drying [12].

Mosses share fundamental organic constituents with other land plants, although their relative proportions vary. The majority of their cell wall content consists of hemicellulose and pectin (30–60%), followed by cellulose (15–25%), proteins (5–10%), polyphenols (5–10%), and inorganic compounds (3–10%). Literature data indicate that various extraction and analytical methods have been employed to study the chemical profiles of mosses. Given the diversity of polarities among active compounds, the selection of appropriate solvents and extraction techniques is particularly important. Commonly used solvents include water, methanol, ethanol, ethyl acetate, hexane, chloroform, and dichloromethane [13]. This study aimed to evaluate the potential *in vitro* bioactivities of extracts obtained from *S. ruralis* using various solvents.

2. RESULTS

2.1. Disc diffusion results

As a result of the disk diffusion analyses, it was determined that the ethanol, methanol, and *n*-hexane extracts of *S. ruralis* exhibited inhibitory activity against specific microorganisms. Among the reference strains, inhibition zones with a diameter of 7.00 mm were observed for all three extracts only against *Enterobacter aerogenes* ATCC 13048 and *Staphylococcus epidermidis* DSM 20044. For all other reference strains, no inhibition zones were detected (zone diameter = 0 mm). The results are presented in detail in Table 1.

Table 1. Disk diffusion results of *Syntrichia ruralis* extracts against reference strains (mm).

Microorganisms	***S. ruralis* Extracts[a]**			**Antibiotic Dics[b]**	
	Ethanol	Methanol	*n*-hexane	Gentamicin	Clindamycin
***Bacillus subtilis* DSM 1971**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	30	34
***Enterobacter aerogenes* ATCC 13048**	7.33 ± 0.57	7.00 ± 0.00	7.33 ± 0.57	24	0
***Enterococcus faecalis* ATCC 29212**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	12	0
***Escherichia coli* ATCC 25922**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	22	0
***Listeria monocytogenes* ATCC 7644**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	28	11
***Pseudomonas aeruginosa* DSM 50071**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	15	0
***Pseudomonas fluorescens* P1**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	13	8
***Salmonella enteritidis* ATCC 13076**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	21	0
***Salmonella typhimurium* SL 1344**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	24	0
***Staphylococcus aureus* ATCC 25923**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	21	24
***Staphylococcus epidermidis* DSM 20044**	7.33 ± 0.57	7.00 ± 0.00	7.00 ± 0.00	22	35
***Staphylococcus hominis* ATCC 27844**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	18	24
***Staphylococcus warneri* ATCC 27836**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	23	26
***Bacillus cereus* RSKK 863**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	24	25
***Shigella flexneri* RSKK 184**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	18	17
***Acinetobacter baumannii* CECT 9111**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	13	22

[a] The discs were loaded with 150 µL of extracts.
[b] The discs were loaded with 10 µg of antibiotic.
Values are reported as mean ± standard deviation (n = 3)

In tests against isolate strains, the *Klebsiella pneumoniae* (FI) isolate showed inhibition zones of 8.00 mm with the *n*-hexane extract and 7.66 mm with both the ethanol and methanol extracts. For *Staphylococcus lugdunensis* (CI), inhibition zones of 7.00 mm were observed with the ethanol and methanol extracts, while the *n*-hexane extract showed no activity. *Shigella flexneri* (CI) exhibited a 7.00 mm inhibition zone only with ethanol extract. The *Klebsiella pneumoniae* (CI) isolate demonstrated inhibition only with ethanol and methanol extracts, with zones of 7.00 mm. Among the multidrug-resistant (MDR) strains, only *Proteus vulgaris* (MDR) demonstrated inhibition zones of 7.33 mm with all three extracts. No inhibition was observed in the other

MDR strains. When compared with antibiotics, gentamicin and clindamycin discs produced significantly larger inhibition zones in both reference and isolate strains. Detailed results are presented in Table 2.

Table 2. Disk diffusion results of *Syntrichia ruralis* extracts against isolate strains (mm).

Microorganisms	*S. ruralis* Extracts[a]			Antibiotic Discs[b]	
	Ethanol	Methanol	*n*-hexane	Gentamicin	Clindamycin
***Enterococcus durans* (FI)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	11	30
***Enterococcus faecium* (FI)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	28	30
***Klebsiella pneumoniae* (FI)**	7.66 ± 0.57	7.66 ± 0.57	8.00 ± 0.00	19	0
***Listeria innocua* (FI)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	13	0
***Salmonella infantis* (FI)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	17	0
***Salmonella kentucky* (FI)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	12	0
***Escherichia coli* (FI)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0	0
***Staphylococcus aureus* (CI)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	30	18
***Streptococcus mutans* (CI)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	12	24
***Staphylococcus hominis* (CI)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	24	9
***Staphylococcus haemolyticus* (CI)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	12	0
***Staphylococcus lugdunensis* (CI)**	7.00 ± 0.00	7.00 ± 0.00	0.00 ± 0.00	22	0
***Shigella boydi* (CI)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	28	0
***Acinetobacter baumannii* (CI)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	15	0
***Shigella flexneri* (CI)**	7.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	13	0
***Staphylococcus aureus* 2 (CI)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	21	0
***Enterococcus faecalis* (CI)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	24	9
***Klebsiella pneumoniae* (CI)**	7.00 ± 0.00	7.00 ± 0.00	0.00 ± 0.00	21	8
***Escherichia coli* (MDR)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	8	0
***Klebsiella pneumoniae* (MDR)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	15	0
***Acinetobacter baumannii* (MDR)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0	0
***Enterobacter aerogenes* (MDR)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	16	0
***Serratia odorifera* (MDR)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	7	0
***Proteus vulgaris* (MDR)**	7.33 ± 0.57	7.33 ± 0.57	7.33 ± 0.57	11	9
***Streptococcus pneumoniae* (MDR)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	10	9
***Staphylococcus aureus* MRSA (MDR)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0	45
***Staphylococcus aureus* MRSA 2 (MDR)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	22	38
***Providencia rustigianii* (MDR)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	16	25
***Achromobacter* sp. (MDR)**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	9	0

[a] The discs were loaded with 150 µL of extracts.
[b] The discs were loaded with 10 µg of antibiotic.
Values are reported as mean ± standard deviation (n = 3); CI: Clinical isolate; FI: foodborne; MDR: Multi drug resistance.

According to the results of the one-way ANOVA, the effects of *S. ruralis* extracts on eight different microorganisms were statistically evaluated. For the *E. aerogenes* ATCC 13048 strain, no significant difference was observed among the extracts ($p = 0.216$). Similarly, the p-values for *S. epidermidis* DSM 20044, *K. pneumoniae* (FI), and *P. vulgaris* (MDR) were calculated as 0.078, 0.629, and 1.000, respectively, indicating no statistically significant differences among the extract groups for these strains. In the cases of *K. pneumoniae* (CI), and *S. lugdunensis* (CI), identical values were recorded across all extract groups; therefore, ANOVA could not be applied.

2.2. Minimum inhibitory concentration results of *S. ruralis*

The MIC and MBC analyses performed using ethanol, methanol, *n*-hexane, and distilled water extracts of *S. ruralis* revealed that the extracts were generally effective only at high concentrations. Detailed results are presented in Table 3.

Table 3. MIC and MBC results of *Syntrichia ruralis* extracts (µg/mL).

Microorganisms	Ethanol[a]		Methanol[a]		*n*-hexane[a]		Distilled Water	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
***Bacillus subtilis* DSM 1971**	>10086	>10086	>13510	>13510	>1243	>1243	>15353	>15353
***Escherichia coli* ATCC 25922**	10086	>10086	>13510	>13510	>1243	>1243	15353	>15353
***Listeria monocytogenes* ATCC 7644**	>10086	>10086	>13510	>13510	>1243	>1243	>15353	>15353
***Enterobacter aerogenes* ATCC 13048**	>10086	>10086	>13510	>13510	>1243	>1243	-	-
***Staphylococcus epidermidis* DSM 20044**	>10086	>10086	>13510	>13510	155	>155	-	-
***Listeria innocua* (FI)**	2521	>2521	3377	>3377	>1243	>1243	>15353	>15353
***Klebsiella pneumoniae* (FI)**	>10086	>10086	>13510	>13510	>1243	>1243	–	–
***Proteus vulgaris* (MDR)**	10086	>10086	13510	>13510	>1243	>1243	–	–
***Staphylococcus lugdunensis* (CI)**	5043	>5043	6755	>6755	–	–	–	–
***Shigella flexneri* (CI)**	>10086	>10086	-	-	-	-	–	–
***Klebsiella pneumoniae* (CI)**	>10086	>10086	>13510	>13510	-	-	–	–
***Escherichia coli* (CI)**	>10086	>10086	>13510	>13510	>1243	>1243	>15353	>15353

[a] The solvent was evaporated, and the extracts were prepared using DMSO.
A dash (-) indicates that the test was not performed; CI: Clinical isolate; FI: foodborne; MDR: Multi drug resistance.

The ethanol extract exhibited the MIC value of 10086 µg/mL only against *Escherichia coli* ATCC 25922 and *Proteus vulgaris* (MDR), but no bactericidal effect was observed. For *Staphylococcus lugdunensis* (CI), the MIC was determined as 5043 µg/mL, although the MBC was not determined. The MIC value of 2521 µg/mL was recorded against *Listeria innocua* (FI).

In tests with the methanol extract, the MIC value of 155 µg/mL was determined against *Staphylococcus epidermidis* DSM 20044; however, the MBC value was not measurable. Moderate MIC values of 3377 µg/mL and 6755 µg/mL were recorded for *Listeria innocua* (FI) and *Staphylococcus lugdunensis* (CI), respectively.

The n-hexane extract demonstrated MIC values below 1243 µg/mL only against *Staphylococcus epidermidis* DSM 20044, but no bactericidal or fungicidal effects were observed. In the case of the distilled water extract, all MIC values were recorded as >15353 µg/mL. For all other microorganisms, the MIC and MBC values exceeded the maximum tested concentrations.

2.3. Antibiofilm activity results of *S. ruralis*

The highest biofilm formation by *Bacillus subtilis* DSM 1971 was observed in 0% glucose medium after 48 hours of incubation (0.598 ± 0.18). *Escherichia coli* ATCC 25922 exhibited the highest biofilm production in %0 glucose medium after 24 hours of incubation (0.844 ± 0.22). *Listeria monocytogenes* ATCC 7644 showed the most intense biofilm formation in 0% glucose medium after 48 hours of incubation (0.968 ± 0.09). *Listeria innocua* (FI) reached peak biofilm formation after 48 hours with 0% glucose medium (0.309 ± 0.11), while clinical isolate *Escherichia coli* (CI) showed maximum biofilm production under the same 0% glucose medium conditions after 24 hours (0.393 ± 0.11). As a result of the analysis conducted under the established biofilm detection conditions, the biofilm inhibition levels of *S. ruralis* extracts at MIC/2 concentration varied depending on the microbial strains. Detailed results are presented in Table 4.

Table 4. Biofilm inhibition results of *Syntrichia ruralis* extracts and Halamid (%).

Microorganisms	Ethanol[a]	Methanol[a]	*n*-hexane[a]	Distilled Water	Halamid
	MIC/2	**MIC/2**	**MIC/2**	**MIC/2**	**1000 µg/ml**
***Bacillus subtilis* DSM 1971**	0.62 ± 2.36	21.56 ± 3.16	19.85 ± 5.70	23.72 ± 3.85	1.35 ± 5.70
***Escherichia coli* ATCC 25922**	64.36 ± 3.25	35.23 ± 3.71	18.02 ± 9.31	12.44 ± 4.47	79.16 ± 2.58
***Listeria monocytogenes* ATCC 7644**	-42.49 ± 6.86	-41.54 ± 4.62	-45.73 ± 5.10	6.34 ± 7.61	-3.57 ± 3.66
***Listeria innocua* (FI)**	85.58 ± 1.69	87.25 ± 3.76	86.41 ± 2.62	86.85 ± 2.36	88.01 ± 1.77
***Escherichia coli* (CI)**	73.63 ± 4.59	69.13 ± 7.25	57.48 ± 9.46	61.29 ± 7.02	87.20 ± 0.12

[a] The solvent was evaporated, and the extracts were prepared using DMSO.
Negative values indicate an increase in biofilm formation at that concentration; CI: Clinical isolate; FI: foodborne.

One-way ANOVA was performed to evaluate the biofilm inhibition effects of *S. ruralis* extracts and Halamid on five different microorganisms. Additionally, the absorbance values (OD_{550}) are presented graphically in Figure 1.

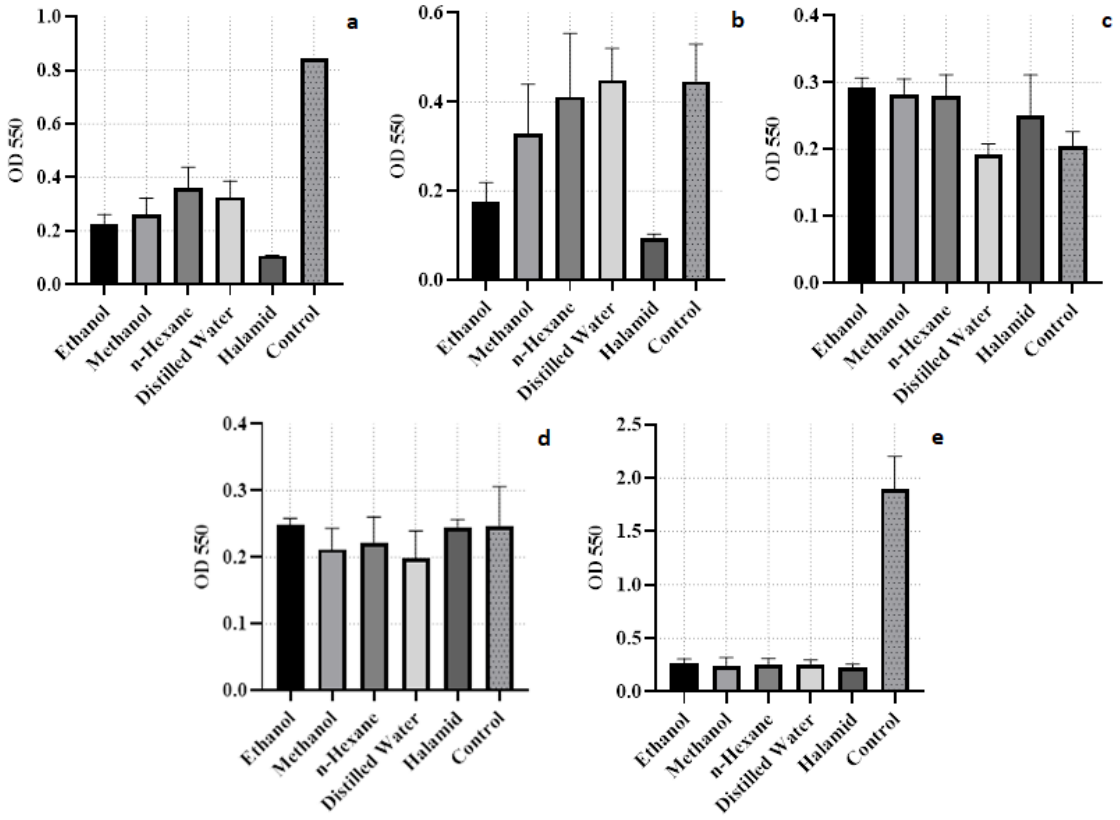

Figure 1. Absorbance readings (OD) of the extracts and Halamid tested for antibiofilm activity. a: *E. coli* (CI), b: *E. coli* ATCC 25922; c: *L. monocytogenes* ATCC 7644; d: *B. subtilis* DSM 1971; e: *L. innocua* (FI).

Among the Gram-negative strains, the highest biofilm inhibition in *E. coli* ATCC 25922 was achieved with the ethanol extract 64.36%, followed by the methanol and *n*-hexane extracts. Halamid exhibited a higher inhibition rate of 79.16%. In *E. coli* (CI), ethanol extract exhibited 73.63% inhibition, followed by methanol 69.13% and *n*-hexane 57.48%. The distilled water extract showed 61.29% inhibition, while Halamid achieved 87.20%. In contrast, for *L. monocytogenes* ATCC 7644, all extracts—except for the distilled water—enhanced biofilm formation. The extracts showed negative inhibition values, indicating that they promoted biofilm development. Similar trends were observed in the distilled water and Halamid groups, with either negative or very low positive effects. Among the Gram-positive strains, *B. subtilis* DSM 1971 exhibited significant biofilm inhibition with methanol and *n*-hexane extracts. The ethanol extract was ineffective, showing only 0.62% inhibition, whereas the distilled water extract showed a comparatively higher inhibition rate of 23.72%. In *L. innocua* (FI), all extracts exhibited strong inhibition levels above 80%. These values were found to be comparable to the inhibition achieved by Halamid.

The results revealed among treatment groups for *B. subtilis* DSM 1971 ($p = 0.000081$), *E. coli* ATCC 25922 ($p < 0.000001$), *L. monocytogenes* ATCC 7644 ($p < 0.000001$), and *E. coli* (CI) ($p = 0.0019$), indicating that the inhibition levels varied significantly depending on the extract used. In contrast, no statistically significant difference was observed among the treatments for *L. innocua* (FI), with a p-value of 0.817, suggesting a uniform inhibition across all groups.

2.4. DPPH radical scavenging activity of *S. ruralis* ethanol extract

The ethanol extract of *S. ruralis* exhibited a concentration-dependent, though limited DPPH radical scavenging activity. At the highest tested concentration of 1000 µg/mL, the extract achieved a maximum scavenging effect of 30.69%. As the concentration decreased, the antioxidant activity declined

correspondingly, with the lowest activity observed at 7.81 µg/mL, where it reached only 1.40%. A detailed summary of the results is provided in Table 5.

Table 5. DPPH radical scavenging activity (%) of *S. ruralis* ethanol extract and ascorbic acid.

Concentration (µg/ml)	*S. ruralis*	Ascorbic acid
1000.00	30.69 ± 0.86	94.71 ± 0.00
500.00	14.92 ± 2.48	94.33 ± 0.06
250.00	7.57 ± 2.38	92.43 ± 0.01
125.00	7.32 ± 5.28	91.01 ±0.02
62.50	2.65 ± 0.78	73.04 ± 0.04
32.25	2.40 ± 1.81	40.23 ± 0.07
15.62	1.81 ± 1.96	23.21 ± 0.27
7.81	1.40 ± 0.99	11.77 ± 0.04

In contrast, ascorbic acid, used as a reference antioxidant, exhibited consistently high DPPH scavenging activity at all tested concentrations. Inhibition rates of 94.71% at 1000 µg/mL, 94.33% at 500 µg/mL, and 92.43% at 250 µg/mL were observed. Even at the lowest concentration of 7.81 µg/mL, ascorbic acid maintained a notable scavenging activity of 11.77%. These findings highlight that relatively low antioxidant potential of the ethanol extract of *S. ruralis* against DPPH radicals.

A strong and statistically significant positive correlation was found between the concentration of *S. ruralis* ethanol extract and its antioxidant activity ($r = 0.994$, $p < 0.001$), indicating a concentration-dependent increase in DPPH radical scavenging. In contrast, ascorbic acid exhibited a weaker and statistically non-significant correlation ($r = 0.630$, $p = 0.094$). The EC_{50} of *S. ruralis* was estimated at 1,743.47 µg/mL, with a wide confidence interval, suggesting a poor model fit and limited antioxidant efficacy. Conversely, the EC_{50} value of ascorbic acid was calculated as 41.17 µg/mL.

2.5. Biochemical analyses results of *S. ruralis* extracts

As a result of the GC-MS analysis, various chemical compounds were identified in the ethanol, methanol, and *n*-hexane extracts of *S. ruralis*. A total of 50 compounds were detected, with most being specific to individual solvents. Detailed information on the identified compounds is provided in Table 6.

Table 6. GC-MS analysis results of *Syntrichia ruralis* extracts.

Compound Name	Retention Time	Formula	Molecular Weight	Ethanol	Methanol	*n*-hexane
	Minute		**g/mol**	**Area (%)**	**Area (%)**	**Area (%)**
Furfuryl alcohol	7.904	$C_5H_6O_2$	98.10	-	0.29	-
Furfural	7.927	$C_5H_4O_2$	96.08	0.39	2.60	-
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone	10.120	$C_6H_8O_4$	144.12	-	0.44	-
5-Hydroxymethylfurfural	17.922	$C_6H_6O_3$	126.11	5.04	40.36	-
Methyl 3-methylorsellinate	30.395	$C_{10}H_{12}O_4$	196.20	-	0.56	-
Ethyl atrarate	31.107	$C_{11}H_{14}O_4$	210.23	0.68	-	-
Neophytadiene	32.007	$C_{20}H_{38}$	278.50	12.51	2.27	-
Isophytol, acetate	33.036	$C_{20}H_{42}O_2$	338.60	-	0.44	-
Methyl palmitate	34.886	$C_{17}H_{34}O_2$	270.50	-	2.09	-
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	35.408	$C_{17}H_{24}O_3$	276.40	-	-	0.31
Palmitic Acid	36.157	$C_{16}H_{32}O_2$	256.42	10.13	6.18	28.25
Methyl eicosa-5,8,11,14,17-pentaenoate	37.488	$C_{21}H_{32}O_2$	316.50	1.36	-	-
Cyclododecatriene	38.057	$C_{12}H_{18}$	162.27	-	0.52	-
Methyl linoleate	39.529	$C_{19}H_{34}O_2$	294.50	-	3.00	-
Methyl vaccenate	39.850	$C_{19}H_{36}O_2$	296.50	-	0.21	-
Ethyl Linoleate	40.681	$C_{20}H_{36}O_2$	308.50	5.33	-	-
9,12,15-Octadecatrienoic acid ethyl ester	40.883	$C_{20}H_{34}O_2$	306.50	7.03	-	-
Linolenyl alcohol	41.002	$C_{18}H_{32}O$	264.40	2.91	0.24	-
Stearic acid	41.446	$C_{18}H_{36}O_2$	284.50	1.21	0.76	2.59
cis,cis,cis-7,10,13-Hexadecatrienal	42.092	$C_{16}H_{26}O$	234.38	-	6.90	-
Oleic Acid	42.658	$C_{18}H_{34}O_2$	282.50	-	-	9.69
Icosapent	44.002	$C_{20}H_{30}O_2$	302.50	-	0.85	-
Arachidonic acid	44.954	$C_{20}H_{32}O_2$	304.50	2.18	-	-
Icosapent methyl	45.260	$C_{21}H_{32}O_2$	316.50	0.87	-	-
Tricosane	45.453	$C_{23}H_{48}$	324.60	-	-	1.14
2,5,8-Triazatricyclo[7.5.0.0,2,7]tetradeca-1(9),7-diene	47.246	$C_{11}H_{17}N_3$	191.27	-	-	0.34
Tricyclo[20.8.0.0(7,16)]triaconta-1(22),7(16),9,13,24,28-hexaene	47.801	$C_{30}H_{44}$	404.70	-	-	0.21
Tetracosane	48.230	$C_{24}H_{50}$	338.70	-	-	3.44
Hexacosane	54.057	$C_{26}H_{50}$	366.70	-	-	5.90
Heptacosane	55.329	$C_{27}H_{56}$	380.70	0.97	-	15.96
Octadecyl chloride	58.648	$C_{18}H_{37}Cl$	288.90	-	-	0.23
Squalene	59.037	$C_{30}H_{50}$	410.70	2.23	0.66	-
Octacosane	60.124	$C_{28}H_{58}$	394.80	-	-	16.35
E-sesqui-lavandulol	60.538	$C_{15}H_{26}O$	222.37	-	-	0.11
Bisabolol	62.358	$C_{15}H_{26}O$	222.37	1.99	-	-
Stigmasta-3,5-diene	66.053	$C_{29}H_{48}$	396.70	1.45	1.63	3.92
Vitamin E	68.227	$C_{29}H_{50}O_2$	430.71	8.58	5.99	1.14
Cholest-5-en-3-ol	68.551	$C_{27}H_{46}O$	386.70	-	0.67	-
Nonacosane	70.789	$C_{29}H_{60}$	408.80	-	-	0.30
Hydroxyisohopane	71.874	$C_{30}H_{50}O$	442.76	2.08	-	-
1-Chloroheptacosane	73.190	$C_{27}H_{55}Cl$	415.20	3.78	-	1.71
Beta-Sitosterol	74.622	$C_{29}H_{50}O$	414.70	-	4.28	-
Lanosterol	74.795	$C_{30}H_{50}O$	426.70	2.18	-	-
5.alpha.-Cholestane-3.beta.,6.alpha.,7.alpha.,8.beta.,15.alpha.,16.beta.,26-heptaol	74.933	$C_{27}H_{48}O_7$	484.70	-	0.87	-
Stigmasta-3,5-dien-7-one	75.366	$C_{29}H_{46}O$	410.70	1.79	0.06	-

Ergosta-14,22-dien-3-ol, acetate, (3.beta.,5.alpha.,22E)-	75.557	$C_{30}H_{48}O_2$	440.70	-	1.52	-
Beta-Amyrin Acetate	76.274	$C_{32}H_{52}O_2$	468.80	2.08	-	-
Cycloartenol	76.357	$C_{30}H_{50}O$	426.70	-	1.46	-
Ergost-25-ene-3,5,6,12-tetrol, (3. beta.,5.alpha.,6.beta.,12.beta.)-	76.340	$C_{28}H_{48}O_4$	448.70	-	-	1.03
Stigmast-4-en-3-one	76.521	$C_{29}H_{48}O$	412.70	4.12	-	-

In the ethanol extract, the major constituents were palmitic acid (10.13%), neophytadiene (12.51%), vitamin E (8.58%), 9,12,15-octadecatrienoic acid ethyl ester (7.03%), and 1-chloroheptacosane (3.78%). Additionally, several triterpenoids, including squalene (2.23%), bisabolol (1.99%), beta-amyrin acetate (2.08%), and lanosterol (2.18%) were also identified.

In the methanol extract, 5-hydroxymethylfurfural (40.36%) was the dominant compound. Significant levels of palmitic acid (6.18%), vitamin E (5.99%), beta-sitosterol (4.28%), and oleic acid (9.69%) were also detected. Furthermore, sterol and steroid compounds such as cycloartenol, ergosta derivatives, and stigmasta-3,5-dien-7-one were identified in lower concentrations.

In the *n*-hexane extract, the predominant compounds included octacosane (16.35%), heptacosane (15.96%), and palmitic acid (28.25%). Long-chain aliphatic compounds and phytosterols such as stigmasterol derivatives (3.92%), stearic acid (2.59%), and tricosane (1.14%) were also present.

Certain compounds were detected exclusively in specific extracts. For example, compounds such as ethyl linoleate, arachidonic acid, and beta-amyrin acetate were found only in the ethanol extract; sterols such as beta-sitosterol, ergosta-14,22-dien-3-ol acetate, and cycloartenol were unique to the methanol extract; and long-chain aliphatic hydrocarbons and chlorinated fatty acid derivatives (e.g., 1-chloroheptacosane and octadecyl chloride) were detected only in the *n*-hexane extract. Palmitic acid and vitamin E were found in all three extracts, though their concentrations varied depending on the solvent used.

3. DISCUSSION

In this study, the antimicrobial activity of ethanol, methanol, and *n*-hexane extracts of *S. ruralis* were evaluated against both reference strains and clinical isolates. Disk diffusion analysis revealed that the *S. ruralis* extracts generally exhibited limited antimicrobial activity. The ethanol, methanol, and *n*-hexane extracts showed weak inhibition against a few reference strains, such as *E. aerogenes* ATCC 13048 and *S. epidermidis* DSM 20044. Among the isolates, inhibitory effects were noted against *K. pneumoniae* (FI and CI), *S. lugdunensis* (CI), and *P. vulgaris* (MDR). However, MIC analyses indicated that these effects were mostly observed at concentrations exceeding 10,000 µg/mL for most strains. Notably, only the methanol extract exhibited a MIC value of 155 µg/mL against the *S. epidermidis* strain, suggesting that the bacteriostatic activity of the extracts may be limited and species-specific, particularly toward certain Gram-positive bacteria.

These findings suggest that *S. ruralis* exhibits weaker antimicrobial activity compared to other species within the genus *Syntrichia*. For instance, in a study conducted by Jiménez et al. [14] on *Syntrichia laevipila*, the ethanol/water extract demonstrated significant antibacterial activity against four *S. aureus* strains. In comparison with *S. ruralis*, although solvent-dependent selective activity appears to be a common feature within the genus within the genus, *S. laevipila* appears to be more effective against Gram-positive bacteria.

On the other hand, an indirect reference to *Syntrichia caninervis* in a study [16] suggested that the growth-promoting effect of the cyanobacterium *Microcoleus vaginatus* on this moss species might be attributed to its ability to reduce microbial contamination. This finding implies that certain moss species may provide indirect antimicrobial effects.

In this context, *S. ruralis* exhibited limited antimicrobial activity in the present study but produced notable results. This can be interpreted in two ways. First, the high antimicrobial activity observed in species such as *S. laevipila* and *S. intermedia* is likely due to differences in metabolite composition. While the literature reports that these species are rich in flavonoids, triterpenoids, and brassinosteroids, the presence of such compounds in *S. ruralis* extracts has not yet been thoroughly investigated. Second, technical variables such as the type of solvent and extraction method should be taken into account as they may significantly influence the biological activity of the extract. Notably, in *S. intermedia*, fractions tested using a 10 mg/mL DMSO solution demonstrated activity, suggesting that higher activity may also be achieved in *S. ruralis* through alternative solvent systems or fractionation techniques [15]. A more detailed characterization of the phenolic content and chemical profile of this genus may help guide future bioactivity-directed fractionation studies to address this gap.

A review of the literature revealed no prior studies investigating the antibiofilm activity of *Syntrichia* species. The present study is the first to report the antibiofilm potential of the moss *S. ruralis*. The extracts of *S. ruralis* demonstrated notable antibiofilm effects, particularly against certain foodborne and clinical isolates. In isolates such as *Listeria innocua* (FI) and *Escherichia coli* (CI), extracts prepared with all solvents exhibited inhibition rates ranging from 57% to 87%. These values are comparable to those of Halamid, a commercial disinfectant. In particular, the effects of the polar extracts (ethanol and methanol) on these two microorganisms suggest that natural compounds may offer effective alternatives for inhibiting biofilm formation.

On the other hand, in the case of *L. monocytogenes*, the extracts were found to increase biofilm formation by up to 45%. This finding supports the hypothesis that certain compounds, at low concentrations, may stimulate quorum sensing mechanisms and act as a stress signal for the microorganism [17].

In the DPPH radical scavenging assay, the ethanol extract of *S. ruralis* demonstrated 30.69% antioxidant activity at a concentration of 1000 µg/mL. In contrast, the DPPH free radical scavenging capacity of the *S. laevipila* extract was reported as 89.65%, along with high flavonoid (13.13 ± 0.33 µg QE/mL) and phenolic compound content (19.05 ± 0.21 µg GAE/mL) [14]. This comparison suggests that *S. ruralis* possesses a lower phenolic content, which may directly contribute to its weaker antimicrobial and antioxidant effects. To improve the biological activity of this species, studies explore alternative extraction methods, fractionation approaches, or analyses of environmental influences would be beneficial.

GC-MS analyses identified the major compound groups in the extracts and clarified their potential associations with biological effects. The ethanol extract was found to contain neophytadiene (12.51%), palmitic acid (10.13%), vitamin E (8.58%), phytosterols (e.g., beta-sitosterol, stigmast-4-en-3-one), and triterpenoids such as beta-amyrin acetate. These compounds have been previously reported to exhibit their antioxidant, membrane-stabilizing, and antibiofilm properties [18-20].

The methanol extract was rich in 5-hydroxymethylfurfural (40.36%), a compound whose membrane-disruptive effects have been demonstrated in other plant species with similar composition [21]. The *n*-hexane extract, on the other hand, was notable for its content of long-chain alkanes (e.g. heptacosane, octacosane), oleic acid, and stigmasta-3,5-diene. These lipophilic compounds may contribute to antibiofilm activity, particularly through their interactions with microbial membrane structure [22, 23]. The GC-MS profile supports the observed biological activities. In the phenolic profile of a congeneric moss species, secondary metabolites with well-documented antimicrobial effects—such as maslinic acid, oleanolic acid, apigenin, and chalcone derivatives—have also been identified [14].

4. CONCLUSION

This study demonstrated that the extracts of *S. ruralis* exhibit selective effects on specific microorganisms and biological activities. Notably, the ethanol and methanol extracts exhibited pronounced effects in terms of biofilm inhibition against certain Gram-negative strains. The compound 5-hydroxymethylfurfural, found in high concentration in the methanol extract, stands out due to its potential for interacting with microbial membranes. In terms of antioxidant activity, the ethanol extract showed a concentration-dependent increase, although the overall activity level remained relatively low. GC-MS analyses confirmed the presence of biologically active constituents such as fatty acids, phytosterols, and triterpenoids. Overall, the data indicates that *S. ruralis* possesses promising biological potential, which varies depending on the solvent used and the type of microorganism tested.

5. MATERIALS AND METHODS

5.1. Materials

Ethanol, methanol, *n*-hexane and dimethyl sulfoxide (DMSO) were obtained from Merck (Darmstadt, Germany). Mueller-Hinton (MHB) broth and Luria-Bertani (LB) broth were purchased from Difco (Detroit, Michigan, USA).

5.2 Plant sample

S. ruralis was collected at the coordinates 41°03'15.8"N, 32°28'06.1"E and taxonomically identified by Dr. Ayşe Dilek Unan. A voucher specimen was deposited in the herbarium of the Fauna and Flora Research and Application Center (FAMER), Dokuz Eylül University, under the accession number FFDEU-MEB1.

5.3 Extraction method

The dried *S. ruralis* sample was ground into a fine powder. Bioactive compounds were extracted using ethanol, methanol, and *n*-hexane by shaking the powdered material at room temperature for 48 hours. Following the extraction process, the mixtures were filtered through Whatman No. 1 filter paper into glass flasks, and the filtrates were concentrated under reduced pressure at 45 °C using a rotary evaporator [24].

The resulting residues were weighed, and stock solutions were prepared by dissolving them in appropriate volumes of ethanol, methanol, or *n*-hexane. Additionally, the concentrated extracts were dissolved in DMSO and subsequently diluted with ultrapure water obtained from a distillation system (Thermo Fisher Scientific, USA) to achieve a final concentration of 1% DMSO [25].

5.4 Antimicrobial activity

5.4.1 Disc diffusion method

To evaluate the antimicrobial activity of the ethanol, methanol, and *n*-hexane extracts of *S. ruralis*, the disk diffusion method previously described in detail by Turu et al. [26] was employed. From the stock solutions of the respective extracts of ethanol, methanol, and *n*-hexane extracts, 150 µL aliquots were applied onto blank sterile antibiotic discs, corresponding to extract loadings of 5.02 mg (ethanol), 5.23 mg (methanol), and 1.49 mg (*n*-hexane) per disc, respectively. The assay was conducted against a total of 44 microbial strains, including 16 standard strains (ST), 7 foodborne isolates (FI), 11 clinical isolates (CI) , and 11 multidrug-resistant (MDR) bacterial strains.

Incubation was performed at 37 °C for 24 hours for all strains. The inocula for each microorganism were prepared in 0.9% sterile saline solution, and the turbidity of each suspension was adjusted to the 0.5 McFarland standard [27].

Petri dishes containing discs loaded with ethanol extracts were incubated under the respective time and temperature conditions described above. Following incubation, the diameters of the inhibition zones were measured in millimeters (mm) and recorded. Blank antibiotic discs and discs containing only ethanol were used as negative controls. A wide range of antibiotics was used as positive controls.

5.4.2 Minimum inhibitory concentration (MIC) assay

The Minimum Inhibitory Concentration (MIC) method was employed to determine the antimicrobial potential of the plant extracts against selected bacterial strains. The assay was conducted using U-bottom 96-well microplates, with each well initially filled 100 µL of MHB. DMSO-dissolved extracts were added to wells A1 to F1 (100 µL) and subjected to two-fold serial dilutions across columns 1 to 10. Bacterial suspensions adjusted to the 0.5 McFarland standard were added (50 µL) to all wells, except for column 12, which served as the negative control and contained only MHB and extract. Wells in row H, containing MHB and bacterial suspension, served as positive controls to confirm bacterial viability. All tests were performed in triplicate, and results were evaluated based on visual observation of microbial growth [28].

5.4.3 Minimum bactericidal concentration (MBC) assays

To determine the lowest concentration of an antimicrobial agent required to kill the pathogenic microorganism, the Minimum Bactericidal Concentration (MBC) test was conducted following the MIC assay. From the wells showing no visible growth, 10 µL aliquots were streaked onto Mueller Hinton agar media. The plates were incubated at 37°C for 24 hours, and the lowest concentration with which no colony formation occurred was defined as the MBC [29].

5.5. Determination of antibiofilm activity of plant extracts

The antibiofilm activity of plant extracts was assessed in two stages: (i) Optimization of biofilm formation conditions, and (ii) Determination of antibiofilm effects.

5.5.1 Optimization of biofilm formation conditions

Luria-Bertani (LB) broth was prepared and dispensed into sterile glass test tubes. To each tube, D-(+)-glucose monohydrate was added at six different final concentrations: 0%, 0.5%, 1%, 1.5%, 2%, and 2.5%, followed by a second sterilization step via autoclaving. Strong biofilm producing bacterial cultures were incubated at 37°C for 24 and 48 hours, respectively. After incubation, wells were gently washed with distilled water and allowed to dry. Wells were then filled with 0.1% crystal violet and incubated for 15 minutes. After removal of excess dye and another washing step, a solvent mixture of 30% acetone and 70% ethanol was utilized [30]. The resulting solution was transferred to a new microplate for optical density measurement.

Optimal glucose concentration and incubation duration for biofilm production were determined based on OD measurement at 550 nm. All experiments were performed in triplicate, and LB broth without glucose was used as the negative control [31].

5.5.2 Evaluation of antibiofilm activity of extracts

Using the optimized parameters, the assay was repeated with a key modification: plant extracts were added at ½ MIC concentration prior to the inoculation of microbial strains. After incubation, the optical densities (OD) of the alcohol–acetone eluates were measured at 550 nm were measured to quantify biofilm inhibition. All tests were conducted in triplicate. Halamid served as the positive control, and LB broth without additives was used as the negative control.

5.6 DPPH assay for antioxidant activity of ethanol extract

The antioxidant activity of the plant extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. For this purpose, 3.9432 mg of DPPH was dissolved in 50 mL of ethanol [32]. The microplate assay consisted of three steps: (i) addition of ethanol, (ii) addition of extract, and (iii) addition of DPPH solution. After loading, the plates were incubated in the dark for 30 minutes, and absorbance was measured at 517 nm. Ascorbic acid was used as the positive control, while a mixture of ethanol and DPPH served as the negative control.

5.7 Gas chromatography-Mass spectrometry (GC-MS) analysis

The biochemical composition of the bryophyte sample was analyzed following the protocols described by Benek et al. [29]. GC-MS analysis was performed using the Agilent GC 8890 system combined with the MSD 5977B detector. Helium served as the carrier gas during the entire analysis. Compounds were identified by comparing the obtained mass spectra with reference data from the NIST and Wiley libraries. Compounds with concentrations higher than 0.5% were considered major constituents.

5.8 Statistical analysis

To determine significant differences between groups and evaluate interaction effects of independent variables, one-way and two-way analysis of variance (ANOVA) were applied. Additionally, Pearson's correlation coefficient was calculated to assess linear relationships between variables. All analyses were performed in triplicate, and p-values ≤ 0.05 were considered statistically significant. Statistical analyses were conducted using R Studio software (RStudio-2024.12.0-467). Pearson correlation analysis was used to assess the direction and strength of the relationship between the concentration of the bryophyte extract and its antioxidant activity. Antioxidant assay results are expressed as mean ± standard deviation (SD) from three independent replicates. Following statistical evaluation, EC_{50} values were calculated using a four-parameter logistic regression model (4PL) with a 95% confidence interval.

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