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Phytochemical profiling and bioactivity assessment of *Salsola vermiculata*, a halophyte from Southern Tunisia

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ABSTRACT

Background: Halophytes, salt-tolerant plants adapted to saline environments, represent a promising resource for sustainable feed strategies by combining agricultural resilience with their nutritional profile and bioactive content.

Objectives: The aim of this study is to assess phytochemical composition and to evaluate the antioxidant and antibacterial properties of *Salsola vermiculata* L. (*S. vermiculata*), an endemic halophyte species collected from southern Tunisia.

Methods: Decocted and ethanolic extracts were investigated. The total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC) were determined by Folin-Ciocalteau reagent, aluminum chloride, and appropriate tannin reagent methods, respectively. The antioxidant activity was evaluated by using DPPH and ABTS assays. The MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) of the potent extract were determined using eight selected food spoilage and pathogenic bacteria: 5 Gram-positive bacteria (*Staphylococcus aureus*, *Listeria monocytogenes, Enterococcus faecalis, Micrococcus luteus,* and *Bacillus cereus*) and 3 Gram-negative bacteria (*Salmonella enterica, Escherichia coli,* and *Pseudomonas aeruginosa*).

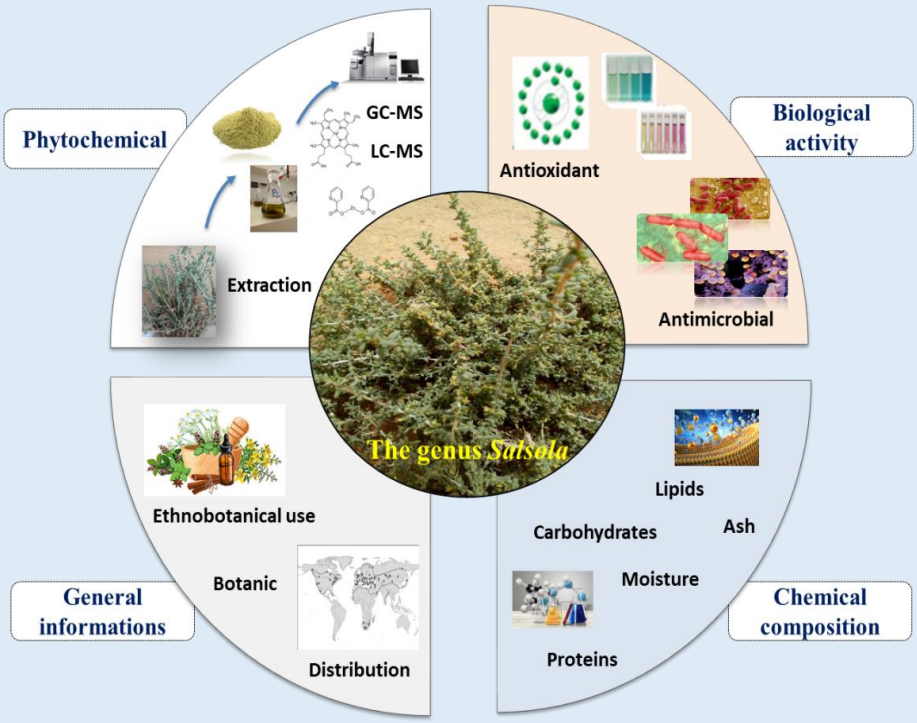
Results: *S. vermiculata* is characterized by intermediate moisture content (~48%), high carbohydrate (~33%), protein (~11%), and ash (8%) concentrations, and a low fat content (< 2%). The total phenol content of decocted and ethanolic extracts was about 1.3±0.3 and 3±0.2 g GAE/100g dry extract, respectively. Total tannin content was higher in the

ethanolic extract compared to the decocted extract, following a similar trend to total phenolics and flavonoids. The extracts exhibited DPPH radical scavenging activity of 0.5 and 3.7 g TE/100 g of dried decocted and ethanolic extracts, respectively, and ABTS radical scavenging activity of 0.3 and 1.8 g TE/100g of dried decocted and ethanolic extracts, respectively. The ethanolic extract exhibited low to moderate, strain-dependent antibacterial activity but showed an interesting phytochemical profile, including β-sitosterol, protocatechuic acid, rosmarinic acid, quercetin, and alkaloids, supporting its potential relevance for nutritional and functional applications.

Novelty: This work presents a comprehensive assessment of the nutritional value, phytochemical content, and biological activities of *S. vermiculata*. The results highlight this underexplored halophyte as a promising source of natural antioxidants and bioactive compounds.

Conclusion: *S. vermiculata* is a nutritionally valuable halophytic species with notable antioxidant and antibacterial properties. This study suggests that the plant can serve as a potential source of natural antioxidants and nutrients, supporting its potential use in functional foods and nutraceutical applications while contributing to the sustainable valorization of saline environments.

Keywords: phenolic compounds, *Salsola vermiculata*, decocted and ethanolic extract, phytochemical screening, antioxidant, antibacterial activity.



Graphical abstract: Phytochemical profiling and bioactivity assessment of Salsola vermiculata, a halophyte from Southern Tunisia.

INTRODUCTION

Soil salinization is increasingly recognized as a major constraint to agricultural productivity, a problem further exacerbated by climate change, irrigation mismanagement, and rising sea levels [1]. Current estimates indicate that more than 20% of irrigated agricultural lands worldwide are affected by salinity, posing a serious threat to global food security and sustainable agricultural development [1]. Consequently, the identification and valorization of salt-tolerant edible plant species capable of thriving in saline and marginal environments has become a strategic priority.

Halophytes, plants naturally adapted to high salinity conditions, have evolved complex physiological and biochemical mechanisms that allow them not only to survive in these conditions but also accumulate valuable nutrients and secondary metabolites under environmental stress [2]. Therefore, these plants are increasingly regarded as promising resources for functional foods, nutraceuticals, and sustainable agro-food systems [2]. Among halophytic genera, *Salsola* (*Amaranthaceae*) comprises numerous species widely distributed in arid, semi-arid, and Mediterranean regions [3]. Several *Salsola* species have been reported to contain diverse bioactive compounds, including phenolic acids, flavonoids, sterols, alkaloids, and saponins, which exhibit antioxidants, antimicrobial, and anti-inflammatory activities [3]. However, research efforts have been disproportionately distributed among species. *Salsola vermiculata* L. (*S. vermiculata*), an endemic halophyte native to North African and Mediterranean saline ecosystems, remains relatively underexplored despite its ecological abundance and traditional uses [3-4]. Recent studies have largely focused on isolated biological properties or limited phytochemical screening, often without addressing their nutritional composition or providing a comprehensive evaluation of their bioactive potential [5].

To date, no study has provided an integrated evaluation of the nutritional composition, antioxidant capacity, and antibacterial activity of *S. vermiculata*. Furthermore, traditional extraction methods such as decoction, commonly used in ethnobotanical practices, have rarely been compared with organic solvent-based extractions to assess their differential impact on phenolic recovery and antioxidant capacity. In addition, the antibacterial activity of *S. vermiculata* against foodborne and spoilage bacteria remains insufficiently documented, limiting its potential valorization as a natural antimicrobial agent.

To address these gaps, the present study provides a comprehensive assessment of *S. vermiculata* L. collected from southern Tunisia by investigating its proximate chemical composition, phenolic content, antioxidant activity, and antibacterial potential. Advanced GC–MS and LC–MS analyses were employed to characterize the phytochemical profile and identify key bioactive compounds. Overall, this work aims to support the scientific assessment of *S. vermiculata*'s potential for further sustainable exploitation as a multifunctional halophytic resource for food and nutraceutical applications in saline environments.

MATERIALS AND METHODS

Plant material and extract preparation: The aerial parts of the halophytic plant *Salsola vermiculata* were collected from southern Tunisia (Bouhedma region, Sidi Bouzid; GPS coordinates: 34°28'46,60°N. 9°40'04,47°) in March 2025 at the mature developmental stage. This region exhibits moderate to high soil salinity (ECe 2–5 dS/m) due to low rainfall, high evaporation, and saline groundwater. After collection, the fresh plant material was thoroughly washed with distilled water and used for proximate chemical composition analyses. For extraction, the plant material was oven-dried at 40 °C

until constant weight (reaching a moisture content of 9% on a wet basis), finely ground in a laboratory mixer grinder (Isolab, Laborgeraete GmbH, Germany), and stored in vacuum-sealed bags at −20°C until extraction. Ethanolic extract (EE) was prepared by extracting the plant powder with an ethanol–water mixture (80:20, v/v) for 24h at room temperature. The decocted extraction (DE) was obtained by boiling 100 g of the plant powder in 1000 mL of distilled water for 15 min. After extraction, the solutions were filtered and centrifugation at 6000 rpm for 15 min. The resulting extracts were freeze-dried (Biobase, BK-FD12P, Shandong, China) and stored at −20°C until analysis.

Proximate chemical composition of *Salsola vermiculata*: Proximate chemical composition was determined using fresh plant material of *Salsola vermiculata*. Moisture content was determined by oven drying at 105 °C, ash content by incineration at 550 °C, lipids by Soxhlet extraction with hexane, and proteins by the Kjeldahl method using a factor of 6.25. Total carbohydrate was calculated by difference using the following formula: 100 - (sum of percentages of moisture, ash, protein, and fat) [6]. All results are expressed on a fresh weight basis (wb).

Determination of total phenolic, flavonoid, and tannin contents: The total phenolic content (TPC), flavonoid content (TFC), and total tannins content (TTC) of the decocted (DE) and ethanolic (EE) extracts were determined by the method of Dbeibia et al. [7]. TPC was measured using the Folin–Ciocalteu method, in which the extract was mixed with Folin–Ciocalteu reagent and Na_2CO_3 solution, incubated in a water bath at 40 °C for 30 min, and the absorbance was measured at 765 nm using a UV–Vis spectrophotometer (PEAKII UV, C7200S, USA). Gallic acid was used as the standard (0–500 mg/L), and results were expressed as mg gallic acid equivalents (GAE) per 100 g of dry extract. TFC was determined using the aluminum chloride colorimetric method. Briefly, 0.5 mL of extract was mixed with distilled water, $NaNO_2$ (5%), and $AlCl_3$ (10%), followed by the addition of NaOH (1 M). The final volume was adjusted to 5 mL, and absorbance was measured at 510 nm. Quercetin was used as the standard (0–500 mg/L), and results were expressed as mg quercetin equivalents per 100 g of dry extract. TTC was determined using a similar colorimetric method using catechin as the standard (0–500 mg/L), and results were expressed as mg catechin equivalents per 100 g of dry extract.

Radical Scavenging Antioxidant Aactivity: The 1.1-diphenyl-2-picrylhydrazyl (DPPH) and 2′-azino bis3-ethylbenzothiazoline-6sulfonic acid (ABTS) radical scavenging activities of the decocted and ethanolic extracts of *Salsola vermiculata* were determined by the method described by Dbeibia et al. [7] using Trolox standard solutions in the range of 0–200 mg/L. For the DPPH assay, the extract solutions were incubated in the dark for 30 min, and absorbance was measured at 515 nm using a spectrophotometer (PEAKII UV, C7200S, Houston, TX, USA). For the ABTS assay, the extract solutions were incubated at room temperature for 30 min, and absorbance was measured at 734 nm. Results were expressed as g Trolox equivalent per 100 g dry extract.

GC–MS analysis: The chemical composition of the ethanolic extract of *Salsola vermiculata,* showing potent phenolic content and antioxidant activity, was analyzed by GC–MS using an Agilent 7890B system equipped with an MS detector and an HP-5MS capillary column (5% phenyl methyl polysiloxane, 30 m, 250 µM, 0.25 µM). Injector temperature was set at 280°C, and the GC oven temperature was programmed to 40°C for 2 min, and then a slope of 50°C up to 250°C was maintained for 20 min, and analysis was carried out in a full-scan mode for 60 min. Helium was used as the carrier gas at a constant

flow rate of 1 mL min^{-1}, with an electron ionization energy of 70 eV. The oven temperature was programmed from 40°C to 250°C, and analyses were performed in full-scan mode following standard conditions.

LC-MS analysis: The individual phenolic composition of the ethanolic extract, which exhibited high total phenolic content and radical scavenging activity, was performed by LC-ES-MS according to the method reported by El Hatmi et al. [8]. A quadrupole mass spectrometer with an electrospray ionization (ESI) source (Shimadzu, Kyoto, Japan) was coupled to an ultra-fast LC system comprising a binary pump, autosampler, column oven, and degasser. Separation was performed on an Inertsustain C18 column (150 mm × 3 mm, 3 µm; GL Sciences, Japan) using 0.02% acetic acid in water and acetonitrile as mobile phases with a 10-min gradient. The flow rate was 0.4 mL/min, the column temperature 40 °C, and the injection volume 20 µL. Spectra were recorded in selected-ion monitoring mode and processed using Shimadzu Lab Solutions LC–MS software. Phenolic compounds were identified by comparison of retention times and mass spectra with authentic standards (> 98% purity, Sigma, St. Louis, MO, USA) and quantified as mg per kg of dry extract (DE).

Antibacterial activity assay: The antibacterial potential of the ethanolic extract (EE) was evaluated using the broth microdilution method over a concentration range of 6.25–200 mg/mL. The assay was performed against eight reference food-related bacterial strains, including five Gram-positive species (*Staphylococcus aureus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Micrococcus luteus*, and *Bacillus cereus*) and three Gram-negative species (*Salmonella enterica*, *Escherichia coli*, and *Pseudomonas aeruginosa*) obtained from the Biotechnology Center of Sfax (Tunisia). Bacterial cultures were grown in Mueller–Hinton broth (MHB) at 37 °C, and the inoculum was standardized to approximately 10^7 CFU/mL. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined following standardized procedures [9]. Positive control (Gentamicin) and negative control (broth with inoculum alone) were included in each assay. Results were expressed in mg/mL.

Statistical Analysis: All measurements were performed in triplicate, and results were expressed as mean ± standard deviation. Differences between assays were compared by a one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered statistically significant using the IBM SPSS Statistics package version 22.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Chemical composition of *Salsola vermiculata*: The proximate composition of *Salsola vermiculata* (*S. vermiculata*) is summarized in Table 1. The plant exhibited a moisture content of approximately 48%, an interesting protein content of approximately 10%, and a low-fat content of less than 2%. This composition suggests that *S. vermiculata* may represent a complementary plant-based protein source, particularly suitable for low-energy diets. Ash content is about 8%, highlighting a substantial mineral contribution, which may be associated with halophytic plantss' capacity to accumulate inorganic elements from saline soils. Carbohydrates constituted the main macronutrient fraction (~32%), which was reflected in the calculated energy value of 1.85 ± 0.02 kcal/g wb. Overall, the nutritional profile of *S. vermiculata* is characteristic of halophytic species growing in arid and semi-arid conditions and reflects their physiological adaptation to saline environments.

Table 1. Chemical composition of *Salsola vermiculata*.

Parameters	*Salsola vermiculata*
Moisture (g/100g wb)	47.99 ± 0.46 [a]
Fat (g/100g wb)	1.84 ± 0.20 [e]
Ash (g/100g wb)	8.00 ± 0.03 [d]
Proteins (g/100g wb)	10.04 ± 0.18 [c]
Carbohydrates (g/100g wb)	32.13 ± 0.25 [b]
Energetic value (kcal/g wb)	1.85 ± 0.02

Wb: wet basis; Results are present as means ± S.D. for triplicate analysis. Values with different letters are statistically different ($p < 0.05$).

Overall, the chemical composition of *S. vermiculata* reveals a well-balanced nutritional profile, characterized by intermediate moisture content and notable mineral content. Compared to other halophytic plants, such as *Salicornia europaea*, widely recognized for its nutritional potential, *S. vermiculata* exhibits comparable levels of carbohydrates and proteins, supporting its potential incorporation into functional food formulations [10]. These findings agree with previous reports on halophytes, which have highlighted their nutritional relevance and suitability for sustainable food applications in saline environments [3, 10]. Nevertheless, further investigations focusing on mineral speciation, nutrient bioavailability, and plant safety are required to further evaluate the functional contribution of each component.

Radical scavenging activity of *Salsola vermiculata* extracts: The TPC, TFC, TTC, and antioxidant capacity (DPPH and ABTS) of the EE and DE of *Salsola vermiculata* (*S. vermiculata*) are presented in Table 2. A clear influence of the extraction method on both phytochemical yield and antioxidant performance was observed. The EE showed notably higher levels of TPC and TFC than the DE. TPC reached 3.0 ± 0.2 g GAE/100 g dry extract in the EE, which was more than twice the value recorded for the DE (1.3 ± 0.2 g Gallic Acid Equivalent (GAE)/100 g dry extract). A similar pattern was found for TFC, with values of 2.5 ± 0.3 and 0.93 ± 0.3 g Quercetin Equivalent (QE)/100 g dry extract for the EE and DE, respectively.

TTC also followed the same trend, with higher levels detected in the EE (0.90 ± 0.01 g Catechin Equivalent (CE)/100g dry extract) compared to the decocted extract (0.54 ± 0.02 g CE/100g dry extract), indicating a more efficient extraction of condensed phenolic compounds using ethanol. These differences likely reflect ethanol's higher efficiency in solubilizing phenolic compounds with different polarities compared to hot water extraction. The variations in phenolic content were correlated with the extracts' antioxidant activities. The EE exhibited substantially stronger radical scavenging activity in both DPPH and ABTS tests, reaching 3.7 ± 0.06 and 1.8 ± 0.01 g Trolox Equivalent (TE)/100 g dry extract, respectively. In contrast, the DE showed lower antioxidant capacity, with markedly lower values in both assays. The higher antioxidant performance of the EE can therefore be attributed to its greater TPC, TFC, and TTC content. This trend supports the close relationship between phenolic concentration and antioxidant capacity, as phenolic and flavonoid compounds are well recognized for their ability to neutralize free radicals through electron or hydrogen donation [11].

The reduced antioxidant activity observed in the DE may be explained by thermal degradation of heat-sensitive phenolic compounds or by their limited solubility under aqueous boiling conditions. These findings emphasize the importance of selecting appropriate extraction procedures to preserve bioactive

compounds and maximize the functional potential of plant extracts [12].

Comparable antioxidant activities have been reported for extracts of other halophytic species, reinforcing the relevance of *S. vermiculata* as a potential natural source of antioxidant compounds [3-4]. The relatively high antioxidant capacity observed, together with the balanced nutritional composition of this species, suggests possible applications in functional foods or natural health-related products. Moreover, the ability of *S. vermiculata* extracts to modulate oxidative stress, combined with their protein content, may contribute to dietary strategies aimed at improving nutritional quality and supporting metabolic health, rather than serving as a therapeutic intervention [13]. In this context, incorporating halophyte plants into food formulations has also been highlighted as a sustainable approach, allowing the valorization of saline and marginal lands while promoting healthier diets [14].

Table 2. Phytochemical (g/100g dry extract) and radical scavenging antioxidant activities (g Trolox Equivalent/100g) in ethanolic and decocted extracts of *S. vermiculata*.

Extract	TPC	TFC	TTC	DPPH-RSA	ABTS-RSA
EE	3.0±0.2^{g}	2.5±0.3^{a}	0.90±0.01^{i}	3.70±0.06^{c}	1.80±0.01^{f}
DE	1.3±0.2^{h}	0.9±0.3^{b}	0.54±0.02^{j}	0.50±0.02^{d}	0.30±0.02^{e}

EE: Ethanolic extract, DE: decocted extract, Total phenolics content: TPC (g GAE /100g Dry extract), GAE: Gallic Acid Equivalent, Total flavonoids content: TFC (g QE/100g Dry extract), QE: Quercetin Equivalent, Total Tannins content: TTC (g CE 100g Dry extract), CE: Catechin Equivalent, DPPH-Radical scavenging activity and ABTS-radical scavenging assay (g TE/100g). TE: Trolox Equivalent, DPPH-RSA: 1.1-diphenyl-2-picrylhydrazyl Radical scavenging activity, ABTS-RSA: ABTS-radical scavenging assay. Values are expressed as mean ± standard deviation (n = 3). For each parameter (TPC, TFC, TTC, DPPH-RSA, and ABTS-RSA), values followed by different letters are significantly different between extraction methods (EE vs DE) ($p < 0.05$).

GC-MS phytochemical profile of the ethanolic extract of *Salsola vermiculata*: The phytochemical profile of the ethanolic extract of *Salsola vermiculata (S. vermiculata)* using gas chromatography-mass spectrometry (GC-MS) revealed a diverse array of bioactive compounds, including sterols, fatty acids, phenols, alkaloids, and other secondary metabolites, suggesting significant therapeutic potential in managing various diseases, such as metabolic, cardiovascular, and neurodegenerative disorders (Table 3). One of the major sterols identified, β-sitosterol, is well-known for its cardiovascular health benefits. Several studies have shown that phytosterols, including β-sitosterol, can lower blood cholesterol levels and reduce the risk of heart disease by inhibiting intestinal cholesterol absorption, making it a promising natural treatment for hypercholesterolemia [3, 15]. Recent research also suggests that β-sitosterol has anti-inflammatory and antioxidant properties, which may promote cardiovascular health rather than exert direct therapeutic effects [16]. The unsaturated fatty acids identified in *S. vermiculata*, such as methyl 10,12-heptadecadiynoate and ethyl 9,12-hexadecadienoate, are known for their anti-inflammatory effects and their role in regulating lipid metabolism. These fatty acids have shown beneficial effects in animal models of obesity and insulin resistance [17]. Additionally, polyunsaturated fatty acids have been reported to reduce inflammatory markers, particularly in metabolic disorders such as type 2 diabetes [17], suggesting a possible contribution of these compounds to the extract's overall bioactivity. Phenolic compounds constituted a significant fraction of the extract and included Homovanillyl alcohol, 2-methoxy-4-vinylphenol, and 2,6-dimethoxyphenol. Phenols are widely recognized for their protective effects, antioxidant properties, and anti-inflammatory properties, helping to reduce oxidative stress, a key factor in the development of chronic diseases, including cardiovascular diseases, neurodegenerative disorders, and certain cancers [18-19]. The presence of methoxylated phenols further supports *S. vermiculata's*

antioxidant potential against oxidative damage. Oxidative stress is also implicated in the progression of insulin resistance and diabetes, suggesting that phenolic compounds may play a supportive role in metabolic health [14]. The anti-inflammatory properties of these phenols could complement their antioxidant effects, making them valuable in treating chronic inflammatory diseases. Alkaloids identified in *S. vermiculata*, have attracted attention for their potential biological effects, particularly in neuropharmacology. Numerous studies have demonstrated that alkaloids exert effects on the central nervous system, including antidepressant and anxiolytic actions, and may be useful in treating neurological disorders [20]. Recent research also suggests that certain alkaloids, such as 2-methyl-1,2,3,4-tetrahydro-β-carboline, identified as a predominant compound, exhibit anticancer properties by inducing apoptosis in cancer cells [21], warranting further investigation into their neuroprotective effects and potential applications in treating neurodegenerative diseases such as Alzheimer's. In addition, 3-methylquinoline, another nitrogen-containing heteroaromatic alkaloid, has been associated with antimicrobial and neuroactive effects, further reinforcing the pharmacological relevance of the alkaloid fraction [20-21]. Additionally, the presence of heterocyclic compounds such as indole and megastigmatrienone in the *S. vermiculata* extract is notable, as these compounds exhibit a range of biological activities, including antimicrobial and antioxidant properties, which protect cells from environmental and pathogenic damage [5]. Overall, the chemical analysis of *S. vermiculata* reveals a broad spectrum of biological activities, supporting its potential therapeutic applications in preventing and treating cardiovascular, metabolic, inflammatory, and neurodegenerative diseases. Future research should focus on isolating and identifying the active compounds, conducting *in vitro* and *in vivo* studies to evaluate their therapeutic effects in models of diabetes, obesity, and neurodegenerative diseases, and studying the molecular mechanisms underlying their biological effects. The development of therapeutic formulations or dietary supplements based on *S. vermiculata* could provide a natural approach to managing chronic diseases, and further research is essential to fully exploit its therapeutic potential.

Table 3. Phytochemical Profile of *Salsola vermiculata* analyzed by GC-MS.

Pic	RT	Compounds	Relative Area (%)
1	8.473	2-[2-Piperidino-1-hydroxy]ethanol	0.658
3	12.534	Spiro[2,4]hepta-4,6-diene	4.757
5	15.732	(1R)-Bicyclo[2.2.1]heptan-2-one	0.659
6	16.556	1-chloro-4-methoxy-Benzene	0.379
7	16.787	4-Amino-1,5-pentandioic acid	0.323
8	17.227	N,N-dimethyl-Glycine, ethyl ester	1.309
9	17.227	Doxepin	1.309
10	18.089	2,3-dihydro-Benzofuran	0.750
11	18.390	Methyl 10,12-heptadecadiynoate	0.163
12	18.494	4-Methoxycarbonyl-4-butanolide	0.984
13	19.010	Naphthalene, 1,2,3,4-tetrahydro	0.388
14	19.756	Bicyclo[3.3.1]nonan-2-one	0.316
15	20.130	Indole	1.537
16	20.686	2-Methoxy-4-vinylphenol	11.626
17	21.388	3,4-Hexanedione	3.648
18	21.686	2,6-dimethoxy-Phenol	5.030

Pic	RT	Compounds	Relative Area (%)
19	22.187	N-(phenyl)-1H-Tetrazol-5-amine	0.539
20	22.359	Methylphenidate	3.878
21	23.176	Undecyl 3-Chloropropionic acid	0.246
22	23.491	2-Butanone, 4-(2,6,6-trimethylcyclohexen-1-ylidene)	0.117
23	24.057	2,3-Dihydrobenzoxazine-2-one-3	1.238
24	24.300	1-methyl-4-(1-methylethylidene)-Cyclohexene	0.247
25	24.928	1H-Indol-4-ol	0.311
26	25.076	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	0.442
27	25.766	2,4-Di-tert-butylphenol	0.387
28	26.223	4,4'-[dithiobis(methylene)bisphenol]	0.334
29	26.403	Homovanillyl alcohol	1.710
30	26.985	3-methyl-Quinoline	7.778
31	27.372	syn-Tricyclo[5.1.0.0(2,4)]octane	0.531
32	28.344	4a-Hydroxy-4-(4-nitrophenyl)butan-2-one	0.111
33	28.441	Megastigmatrienone	0.421
34	29.137	2,5-dimethoxy-1,4-benzenediol	0.455
35	29.336	1-Oxaspiro[4.5]dec-6-ene, 2,6,10,10-tetramethyl-	0.242
36	29.792	2-n-Hexylphenol	0.687
37	29.861	1-[1-Methoxy-3,3-dimethyl-1-(1H-1,2,4-triazolyl)butan-2-one	0.251
38	30.100	(E)-2,6-Dimethoxy-4-(prop-1-en-1-yl)phenol	0.165
39	30.224	L-Phenylalanine, N-acetyl-, methyl ester	0.913
40	30.685	Thiocarbamic acid	0.161
41	30.901	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	0.650
42	31.263	Methanone, (3-methylphenyl)phenyl	0.262
43	31.540	6-Hydroxy-4,4,7a-trimethyl-5a-tetrahydrobenzofuran-2-one	0.120
44	31.627	Methyl 4-hydroxy-3,5-dimethoxybenzoate	0.483
45	32.954	Neophytadiene	1.047
46	32.954	Z-8-Methyl-9-tetradecen-1-ol	0.844
47	33.457	2-methyl-7-Octadecyne	0.115
48	33.821	Neophytadiene	0.456
49	34.657	2-Methyl-1,2,3,4-tetrahydro-β-carboline	16.303
50	35.179	25-2-Furancarboxylic acid	0.321
51	35.389	methyl 3-Indoleacrylic acid	0.648
52	35.977	trans-Sinapyl alcohol	0.183
53	36.402	1H-Indole-3-propanoic acid, α-hydroxy-, methyl ester	5.683
54	37.855	Ethyl 9,12-hexadecadienoate	0.370
55	37.974	Methyl 8,11,14-heptadecatrien	0.749
56	38.192	Neophytadiene	1.372
57	38.438	Methyl 18-fluoro-octadecanoate	0.148
58	39.158	3,3,4,5,7-Pentamethyl-1-indane	0.112
59	40.332	9H-Pyrido[3,4-b]indole-1-carbaldehyde	0.593
60	40.508	1,2,3,4-tetrahydrocarbazole	0.305
61	42.583	2-Diacetylamino-3-(1H-indol-3-yl)-propionic acid	1.618
62	42.909	Neophytadiene	0.081

Pic	RT	Compounds	Relative Area (%)
63	44.505	3-Allyl-5-(1H-indol-3-ylmethyl)-2-thioxoimidazolidin-4-one	8.855
65	45.239	1-(3-fluorobenzoyl)Piperazine	0.207
66	45.382	di(oct-3-yl) phthalic acid	0.897
67	47.218	9H-Cyclopenta[1,2-b:3,4-b]dipyridine,9-diazo	0.533
68	53.864	17-(1,5-Dimethyl-hex-2-enyl)-10,13-dimethyl	0.253
69	54.551	Cholesta-4,6-dien-3-ol (3β)	0.241
70	56.412	1,1',2,2'-tetrahydro-1,1'-dimethoxy-ψ,ψ-Carotene	0.804
71	56.917	Beta-Sitosterol	0.746

RT: retention time

Individual phenolic compounds analysis by LC–MS: The LC-MS analysis identified several key phenolic compounds in the EE of *Salsola vermiculata* (*S. vermiculata*) (Table 4), including quinic acid (95.413 mg/kg), protocatechuic acid (128.996 mg/kg), rutin, naringin, rosmarinic acid (40.868 mg/kg), and quercetin (7.819 mg/kg). These compounds are well-documented for their antioxidant, anti-inflammatory, and anticancer properties. Compared to other halophytes, *S. vermiculata* exhibits relatively high levels of protocatechuic acid and rosmarinic acid. For instance, various studies reported similar phenolic acid levels in *Salicornia bigelovii*, though at lower concentrations for rosmarinic acid [9, 22]. These compounds are especially noteworthy in pharmacology, as they reduce oxidative stress and could contribute to preventing chronic diseases, such as cardiovascular disorders and type 2 diabetes. The presence of these phenolic compounds enhances *S. vermiculata*'s potential as a source for nutraceuticals and dietary supplements. Protocatechuic and rosmarinic acids, for example, could be formulated into extracts targeting cardiovascular health due to their documented antioxidant and anti-inflammatory effects [23]. Compared with other halophytes, such as *Salicornia bigelovii* or *Atriplex halimus*, *S. vermiculata* stands out for its unique phenolic composition, including quinic acid (95.413 mg/kg) and protocatechuic acid (128.996 mg/kg), known for their anti-inflammatory and antioxidant properties. Recent studies indicate that these phenolic compounds are involved in modulating inflammatory responses and may contribute to protective effects against metabolic disorders [24]. Overall, the LC–MS phenolic profile highlights the chemical diversity of *S. vermiculata* and suggests that its bioactivity is likely due to the combined action of multiple phenolic constituents. However, further studies focusing on bioavailability, dose–response relationships, and safety are required before considering applications in nutraceutical, pharmaceutical, or cosmetic formulations.

Table 4. LC-MS individual phenolic composition of the *Salsola vermiculata* ethanolic extract

N°	RT (min)	Compounds	m/z	Concentration (mg/kg)
1	1.471	Quinic acid	191	95.40 ± 0.50
2	1.441	Protocatechuic acid	153	129 ± 5
3	1.414	Rutin	609	2.90 ± 0.6
4	1.458	Naringin	579	6.4 ± 0.5
5	2.186	Rosmarinic acid	359	41 ± 1
6	1.955	Quercetin	301	7.82 ± 0.50

RT: retention time, Values are expressed as mean ± standard deviation (n = 3).

The GC-MS and LC-MS analyses of *S. vermiculata* reveal a phytochemical profile rich in bioactive compounds, particularly sterols, hydrocarbons, and phenolic acids. These compounds are well-known for their health benefits, suggesting the potential of *Salsola vermiculata* for diverse applications in health and nutrition. Compared to other well-studied halophytes, *S. vermiculata* demonstrates a unique diversity of compounds, suggesting value for natural medicinal and pharmacological applications. Its relatively high concentrations of compounds like protocatechuic acid may even offer distinct advantages for managing inflammatory and metabolic diseases. Further studies, particularly in-depth investigation of the extract bioactivity, *in vivo* testing, and mechanism-of-action research for isolated bioactive compounds [25-26], would be beneficial in fully evaluating the therapeutic potential of *Salsola vermiculata.*

Antimicrobial activity of *Salsola vermiculata* extracts: The antibacterial activity of the ethanolic extracts (EE) of *Salsola vermiculata* (*S. vermiculata*) against eight reference bacterial strains is summarized in **Table 5**. Overall, the ethanolic extract exhibited weak and strain-dependent antibacterial activity, as indicated by MIC values generally higher than 10 mg/mL. The ethanolic extract showed weaker activity against *E. coli*, with a MIC value of 50 mg/mL and no detectable bactericidal effect within the tested range. *Salmonella enterica* and *Pseudomonas aeruginosa* were the most resistant strains, showing limited susceptibility to ethanolic extract, as evidenced by MIC and MBC values equal or exceeding 100 mg/mL. Among Gram-positive bacteria, *Micrococcus luteus* ATCC 1880 was the most sensitive strain. The ethanolic extract exhibited the lowest MIC value (6.25 mg/mL), indicating a moderate inhibitory effect compared to the other tested strains. A low antibacterial activity was observed against *Listeria monocytogenes*, with a MIC value of 100 mg/mL. Similarly, *Staphylococcus aureus* exhibited weak susceptibility (MIC = 100 mg/mL), while *Bacillus cereus* and *Enterococcus faecalis* showed very low susceptibility, with MIC values reaching 200 mg/mL, suggesting negligible antibacterial activity against these strains. Phenolic and flavonoid compounds are known to exert antimicrobial effects through membrane destabilization, enzyme inhibition, and interference with cellular metabolism. Tannins, saponins, or other polar constituents are capable of interacting with bacterial cell envelopes. The slightly higher susceptibility observed among certain Gram-positive bacteria may be attributed to the absence of an outer membrane, which facilitates the interaction of phenolic compounds and alkaloids with the bacterial cell wall [27]. However, the generally high MIC values indicate that the antibacterial effect of *S. vermiculata* ethanolic extract remains limited and strain-dependent. Overall, *S. vermiculata* extract exhibited weak to moderate and selective antibacterial activity, with *Micrococcus luteus* and *Escherichia coli* being the most sensitive strains. Nevertheless, the activity remains markedly lower than that of conventional antibiotics, as reflected by the gentamicin control. These results suggest that the antimicrobial potential of *S. vermiculata* is modest and may be enhanced through fractionation, isolation of active constituents, or optimization of extraction conditions rather than direct application of the crude extract.

Table 5. MIC (mg/mL) and MBC (mg/mL) of *Salsola vermiculata* ethanolic extract (EE) against eight bacterial strains.

Strains	**Gentamicin**		**EE**	
	MIC (µg/mL)	MBC (µg/mL)	MIC (mg/mL)	MBC(mg/mL)
Gram -				
Escherichia coli ATCC 25922	0.24 ± 0.21	0.29 ± 0.07	50±0.0	>200±0.0
Salmonella enterica ATTC 43972	0.58 ± 0.13	0.94 ± 0.78	100±0.0	>200±0.0
Pseudomonas aeruginosa ATCC 9027	0.29 ± 0.07	0.58 ± 0.27	100±0.0	>200±0.0
Gram +				
Staphylococcus aureus ATCC 25923	2.31 ± 1.14	4.66 ± 1.08	100±0.0	>200±0.0
Enterococcus faecalis ATCC 29212	0.48 ± 0.41	0.58 ± 0.13	200±0.0	>200±0.0
Micrococcus luteus ATCC 1880	1.14 ± 0.50	9.35 ± 2.22	6.25±0.0	>200±0.0
Bacillus cereus ATCC 14579	1.14 ± 0.25	4.68 ± 2.21	200±0.0	>200±0.0
Listeria monocytogenes ATCC 19117	0.58 ± 0.17	1.95 ± 1.14	100±0.0	>200±0.0

Values are expressed as mean ± standard deviation (n = 3).

CONCLUSION

Salsola vermiculata exhibited a well-balanced nutritional composition, characterized by high carbohydrate and mineral contents, moderate protein levels, and low-fat content, supporting its potential as a functional food application adapted to saline environments. Ethanolic extraction was proved more effective for recovering phenolic compounds, leading to higher antioxidant activities, alongside to moderate antibacterial activity against selected Gram-positive and Gram-negative bacteria. GC–MS and LC–MS analyses of ethanolic extract revealed a diverse and rich phytochemical profile, including β-sitosterol, protocatechuic acid, rosmarinic acid, quercetin, fatty acids and alkaloids, many of which are known for their antioxidant, anti-inflammatory, antimicrobial, and metabolic regulatory properties. Overall, the results support the potential of *S. vermiculata* as a natural source of bioactive compounds for nutraceutical and functional food applications, while highlighting the importance of halophyte valorization in sustainable agro-food systems. Further *in vivo* studies should focus on the safety of the plant and its extracts, the isolation of key bioactive compounds and evaluation of their synergistic effects in complex biological systems.

In the context of the Functional Food Development Framework (FFC) 17-step model was proposed by Martirosyan and Stratton [25], the present study addresses early-stage steps, including bioactive compound identification, extraction optimization, and *in vitro* biological evaluation, thereby providing a scientific basis for subsequent safety assessment, formulation development, and *in vivo* validation toward functional food applications.

List of Abbreviations: *Salsola vermiculata* (*S. vermiculata*), TPC: Total phenols content, GAE: Gallic Acid Equivalent, TFC: Total flavonoids content, QE: Quercetin Equivalent, TTC: Total tannins content, CE: Catechin Equivalent, DPPH-Radical scavenging activity, ABTS-radical scavenging assay.

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