



Phytochemical composition of the halophyte *Suaeda vera* grown under natural abiotic stress conditions

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Abstract

Suaeda vera (S. vera) Forssk. ex J.F. Gmel is a halophytic perennial subshrub of the Amaranthaceae family naturally thriving in saline and arid environments of North Africa and the Middle East. Despite its ecological resilience and ethnomedicinal relevance, the phytochemical composition of wild populations remains poorly characterized under natural salinity and aridity stress. This study aimed to investigate the metabolite profile of wild-growing *S. vera* collected from a saline coastal area in northwestern Algeria during March–April 2024, under conditions of elevated temperature and limited precipitation. The hydromethanolic crude extract of *S. vera* was characterized through a multi-analytical approach combining qualitative phytochemical screening, thin-layer chromatography, spectrophotometric quantification of major phytochemicals, ATR-FTIR spectroscopic fingerprinting, and UPLC-ESI-MS/MS-based metabolite profiling. Qualitative phytochemical screening revealed the presence of phenolics, flavonoids, tannins, alkaloids, and saponins, with hydromethanolic extracts exhibiting the richest profiles. Phenolic compounds, flavonoids, and tannins were confirmed as the dominant secondary metabolite classes, with total phenolic, flavonoid, and condensed tannin contents of 48.57 ± 0.71 mg GAE/g, 39.88 ± 0.26 mg QE/g, and 3.70 ± 0.13 mg CE/g, respectively. ATR-ATR-FTIR analysis identified characteristic functional groups related to hydroxyl, aromatic, and carbonyl structures, supporting the presence of phenolic constituents. UPLC-ESI-MS/MS profiling identified 30 compounds from diverse chemical classes, with the stress-related amino acid L-proline being the predominant constituent, followed by catechin, salicylic acid, resveratrol, and ferulic acid. The predominance of L-proline and salicylic acid reflects activation of conserved defense pathways in response to combined salt and drought stress conditions. These findings provide a comprehensive chemical characterization of *S. vera* and highlight its potential as a promising source of bioactive metabolites and as a candidate for further investigation into stress-induced secondary metabolism in halophytes.

Keywords: *S. vera*, bioactive compounds, natural abiotic stress, phytochemical profile, stress-related metabolites.

1. Introduction

In recent years, there has been growing interest in plant-derived bioactive compounds, particularly phenolic constituents, due to their well-documented antioxidant, anti-inflammatory, and antimicrobial properties (Ainazzahra *et al.*, 2025). These naturally occurring molecules have attracted attention as potential alternatives to synthetic additives in the development of health-promoting foods, nutraceuticals, and pharmaceutical products. As a result, research has increasingly focused on resilient and underutilized plant species growing under extreme environmental conditions and producing distinctive phytochemical profiles (Wang *et al.*, 2022; Barba-Ostria *et al.*, 2025).

Suaeda vera (Forssk. ex J.F. Gmel., formerly known as *S. fruticosa*) is a halophytic perennial subshrub of the Amaranthaceae family, naturally thriving in highly saline and arid environments such as salt marshes, coastal areas, and desert regions of North Africa and the Middle East (Asghar *et al.*, 2025). Its

succulent leaves and reduced floral structures reflect specialized adaptations to high salinity and water-limited conditions (Asghar *et al.*, 2023), making it a suitable model for studying stress-related metabolite accumulation in halophytes. Beyond its ecological adaptation, *S. vera* possesses considerable environmental, nutritional, socioeconomic, and ethnomedicinal value (Öztürk *et al.*, 2019; Saleem *et al.*, 2021; Wang *et al.*, 2022). In the present study, *S. vera* is used as the accepted taxonomic name, whereas *S. fruticosa* (used in some earlier studies) is treated as a synonym.

From an environmental perspective, it contributes to the phytoremediation of saline and contaminated soils (Asghar *et al.*, 2025), given its capacity to accumulate heavy metals at concentrations exceeding background soil values (Wang *et al.*, 2022). Additionally, *Suaeda* seeds contain approximately 20% oil rich in unsaturated fatty acids, along with proteins and micronutrients, giving them nutritional and economic value (Zhao *et al.*, 2019; Yu *et al.*, 2022). Its seeds have been proposed as an alternative edible oil source, and the genus is also used as green food and livestock fodder in native regions (Weber *et al.*, 2001; Weber *et al.*, 2007; Öztürk *et al.*, 2019).

In addition to its environmental and nutritional importance, this plant species has long been used in traditional medicine and local practices (Ayaz *et al.*, 2022). It is traditionally used to alleviate inflammation, skin diseases, and gastrointestinal disturbances (Ayaz *et al.*, 2022). The biological activities of this plant are closely linked to its phytochemical constituents (Mohammed *et al.*, 2014). Previous studies on related *Suaeda* species have reported the presence of phenolic acids, flavonoids, and other bioactive metabolites exhibiting antioxidant and antimicrobial activities, highlighting the genus's potential as a natural source of therapeutically relevant compounds (Ayaz *et al.*, 2022).

Environmental factors play a key role in shaping the phytochemical composition of plants (Khan *et al.*, 2025). Abiotic stresses such as salinity and temperature fluctuations stimulate the synthesis of secondary metabolites, particularly phenolics and flavonoids, which contribute to oxidative stress protection and cellular adaptation (Salam *et al.*, 2023; Khan *et al.*, 2025). Seasonal drought and high sunlight exposure also enhance phenolic accumulation and antioxidant capacity in plant systems (Brahmi *et al.*, 2022; Mansinhos *et al.*, 2024). Similarly, plants naturally exposed to water-deficit conditions produce elevated levels of stress-related metabolites that support physiological resilience and environmental tolerance (Yadav *et al.*, 2021). A widely reported adaptive response to environmental stress is the accumulation of low-molecular-weight soluble metabolites, such as soluble sugars and free amino acids, which function as compatible solutes involved in osmotic regulation, cellular stabilization, and reactive oxygen species detoxification under salinity- and drought-induced stress conditions (Bao *et al.*, 2023). Concurrently, stress exposure has been shown to strongly activate secondary metabolic pathways, especially flavonoid biosynthesis, in halophytic plants, indicating that these pathways play a central role in strengthening antioxidant defenses and maintaining cellular integrity under adverse environmental conditions (Feng *et al.*, 2023; Joshi *et al.*, 2023).

Most current knowledge of stress-induced metabolite accumulation in halophytes derives from controlled greenhouse experiments, which establish relationships between individual stress factors and metabolic responses but do not fully reflect the complexity of natural environments, where salinity, aridity, thermal extremes, and nutrient limitations often occur simultaneously (Bao *et al.*, 2023; Feng *et al.*, 2023; Joshi *et al.*, 2023; Khan *et al.*, 2025). Field-based studies are therefore essential for capturing integrated metabolic responses under multifactorial stress and providing ecologically relevant insights into plant adaptation (Yadav *et al.*, 2021; Mansinhos *et al.*, 2024). The exceptional ability of *S. vera* to withstand high salinity and water limitation suggests that it may serve as a reservoir of stress-associated bioactive metabolites. However, previous studies have focused mainly on bioactivity screening and general phenolic and flavonoid composition (Ayaz *et al.*, 2022), while the metabolite profile of wild *S. vera* under naturally occurring combined salinity and aridity remains largely unexplored. Most available studies have also relied on controlled conditions or cultivated material, leaving a significant gap in understanding the metabolic responses of wild populations exposed to real-world environmental

stressors. This study therefore aimed to characterize, for the first time, the metabolite profile of wild-growing *S. vera* using an integrated multi-analytical approach, with a focus on compounds linked to stress tolerance and pharmacological relevance.

2. Materials and methods

Plant materials

Plant collection was carried out in Northwestern Algeria during the peak flowering period from March to April 2024. The halophytic species *S. vera* was collected from the saline coastal area of El Mactaa, Oran province (35°46'49.1"N, 0°07'47.4"W). Voucher specimens were deposited at the herbarium of the Faculty of Natural and Life Sciences, University of Relizane, Algeria, under the accession number RE-2024-02. According to meteorological data from the Oran Port station (JMA climate data, 2024), the sampling period was characterized by mean temperatures of 17.1 °C in March and 18.1 °C in April 2024, exceeding the 1991–2020 long-term averages (15.5 °C and 16.9 °C, respectively). Precipitation during this period was notably low, with only 25 mm recorded in March and 17 mm in April, compared to the monthly normals of 23.2 mm and 27.5 mm (JMA climate data, 2024). The standardized precipitation index (SPI) values for March and April 2024 indicated moderately dry to severely dry conditions, consistent with recent drought assessments in northwestern Algeria (Boukhari *et al.*, 2025). These climatic data confirm that the sampling period was characterized by anomalously warm and dry conditions, constituting a clear departure from long-term patterns and imposing substantial environmental stress on plant system (JMA climate data, 2024). Under these conditions, *S. vera* exhibited typical xeromorphic adaptations, including succulence and a more compact leaf structure, which are commonly observed in halophytes exposed to combined salt and drought stress. Only leaves were collected in this study. The collected plant material was thoroughly washed, shade-dried at room temperature, and finely ground using a laboratory mill. The resulting powders were stored in opaque, hermetically sealed containers to protect the bioactive metabolites from light, humidity, and thermal degradation.

Preparation of plant extracts

To ensure the extraction of a wide range of chemical compounds, three different solvents were used: hydro-alcoholic solvents (75% methanol and 75% ethanol), selected for their efficiency in extracting phenolic and flavonoid compounds (Dai and Mumper, 2010), and distilled water, used as a green solvent to recover highly hydrophilic metabolites (Sultana *et al.*, 2007). *S. vera* crude extracts were obtained as described by Pérez-Jiménez *et al.* (2008), with slight modifications. Specifically, the extraction time was extended to 24 h instead of 2 h, and the solvent-to-sample ratio was adjusted to 40:1 (v/w) instead of 10:1 (v/w) to improve extraction efficiency. For each extraction, 1 g of shade-dried plant powder was immersed in 40 mL of either 75% methanol, 75% ethanol, or distilled water (Pérez-Jiménez *et al.*, 2008). The extraction process was conducted by maceration at room temperature (20–25 °C) for 24 h under continuous mild agitation. After filtration through Whatman No. 1 paper, the organic solvents were removed using a rotary evaporator (Heidolph Laborota 4000, Germany), while aqueous extracts were further dried in an oven at 45 °C to yield the final crude extracts. The drying temperature of 45 °C was selected as a mild and commonly used temperature for aqueous extract concentration, allowing rapid solvent removal while minimizing degradation of thermolabile compounds (Sultana *et al.*, 2007; Do *et al.*, 2014).

Phytochemical characterization

Qualitative phytochemical screening

A qualitative phytochemical screening was performed to identify the main classes of secondary metabolites in the plant extracts as previously described for alkaloids, carbohydrates, reducing sugars, proteins and amino acids, phenolic compounds, tannins, quinones, resins, cholesterol, cardiac glycosides, and anthocyanins (Shaikh and Patil, 2020), saponins, anthraquinones, gums and mucilages, flavonoids, phlobatannins, and glycosides (Jothi-Muniyandi and Lakshman, 2018), terpenoids (Dubale *et al.*, 2023), coumarins (Saradhi Settaluri *et al.*, 2024), and steroids (Dhaniala *et al.*, 2023). The extracts were prepared at a concentration of 25 mg/mL in their respective solvents, and 1 mL of each extract was

used for each test. The detection of bioactive compounds was based on the formation of precipitates or characteristic color changes following the addition of specific reagents. In this study, the extract that exhibited the richer and more diverse bioactive profiles was selected for subsequent phytochemical analyses.

Thin layer chromatography

Thin-layer chromatography (TLC) was used for the qualitative detection of polyphenols, flavonoids, and tannins in the selected crude extract. Samples were applied onto silica gel plates (60 F254, Merck, Germany; 0.2 mm thickness). Appropriate mobile phases were selected to ensure efficient separation of the different compound classes. For polyphenols, a butanol-acetic acid-water mixture (6:1:2, v/v/v) was employed, and spots were visualized with 10% Folin-Ciocalteu reagent. Flavonoids were separated using ethyl acetate-formic acid-acetic acid-water (100:11:11:27, v/v/v/v) and detected with 1% vanillin in absolute ethanol, followed by sulfuric acid treatment and heating. Tannins were analyzed using ethyl acetate-methanol-water (40:8:5, v/v/v), and revealed with 2% ferric chloride solution. Retention factor (Rf) values were calculated for each spot according to the equation (1) and compared with reference standards to support compound identification using the following formula:

$$Rf = \left(\frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}} \right) \quad (2)$$

Quantitative determination of major phytochemicals

The hydromethanolic extract of *S. vera* was also subjected to spectrophotometric analysis for the determination of its total phenolic, flavonoid, and condensed tannin contents. For each assay, a blank control (75% methanol) was included to zero the spectrophotometer and correct for any background absorbance. All assays were performed in three technical replicates ($n = 3$) to ensure reliability of the measurements.

Total phenolic content

Total phenolic content was determined using the method described by Singleton and Rossi (1965), with minor modifications. Briefly, 20 μL of the extract was mixed with 1.58 mL of distilled water and 100 μL of Folin-Ciocalteu reagent. After pre-incubation of 5 min, 300 μL of sodium carbonate solution (20%) was added. The mixture was incubated at room temperature for 2h, and absorbance was measured at 765 nm using a UV-visible spectrophotometer. Gallic acid was used as the reference standard for quantification, and results were expressed as mg gallic acid equivalents per gram of extract (mg GAE g^{-1}). All quantitative phytochemical contents are expressed as mg per gram of dry weight (DW) of plant material.

Total flavonoid content

Total flavonoid content was determined according to the method described by Lianda *et al.* (2012). Briefly, 1 mL of the extract was mixed with an equal volume of 2% methanolic aluminium chloride (AlCl_3) and incubated at room temperature for 30 min. Absorbance was measured at 415 nm. Quercetin served as the reference standard, and the results were expressed as mg quercetin equivalents per gram of extract (mg QE g^{-1}).

Condensed tannins content

Condensed tannins were determined using the vanillin-HCl method as described by Broadhurst and Jones (1978), with minor modifications. Briefly, 250 μL of the extract was mixed with 1 mL of vanillin solution (4% in methanol) and 750 μL of concentrated hydrochloric acid (37%). The reaction mixture was incubated in the dark at 20 °C for 15 min. Absorbance was measured at 500 nm. Catechin was used as the reference standard, and the results were expressed as mg catechin equivalents per gram of extract (mg CE g^{-1}).

ATR-FTIR analysis

Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) analysis was performed using a Bruker FT-IR spectrometer (INVENIO model, Bruker, Germany) according to the method described by Pebotuwa *et al.* (2020) to identify the main chemical functionalities present in the extract. A small amount of sample was placed on an ATR crystal and dried using the cool setting of a blow-dryer. ATR-FTIR spectra were recorded in the mid-infrared region ($400\text{--}4000\text{ cm}^{-1}$) as recommended by Liu and Kazarian (2022). The resulting absorption bands were analyzed to determine the predominant functional groups.

UPLC-ESI-MS/MS analysis

The characterization of secondary metabolites from *S. vera* was carried out using a UPLC-ESI-MS/MS (Shimadzu LCMS-8040, Kyoto, Japan) equipped with Ultra-Fast Mass Spectrometry (UFMS) technology and Nexera XR LC-20AD binary pump, as described by Alileche *et al.* (2025), with slight modifications. Chromatographic separation was achieved on a Restek Ultra C18 column ($150 \times 4.6\text{ mm}$; $3\text{ }\mu\text{m}$), using a binary mobile phase composed of acidified water (0.1% formic acid) (A) and methanol (B) at a mobile phase flow rate of 0.2 mL/min . The gradient program was as follows: 0–0.2 min, 98% A; 0.2–2.5 min, 25% A; 2.5–4.0 min, 0% A; 4.0–10.0 min, 0% A; 10.0–10.01 min, 98% A; and 10.01–15.0 min, 98% A. The injection volume of $5\text{ }\mu\text{L}$. Mass spectrometric detection was performed using electrospray ionization in positive mode with the following parameters: heating block temperature at $400\text{ }^{\circ}\text{C}$, desolvation line temperature at $250\text{ }^{\circ}\text{C}$, CID gas pressure of 230 kPa , conversion dynode voltage of -6 kV , nebulizing gas flow at 3.0 L/min , drying gas flow at 10.0 L/min , and Q1 pre-rod voltage (cone voltage) of 20 V . Identification of compounds was based on Multiple Reaction Monitoring (MRM) transitions and retention times. For five key compounds (L-proline, catechin, salicylic acid, resveratrol, and ferulic acid) identification was confirmed using authentic reference standards analyzed under identical conditions. For the remaining 25 compounds, identification was tentatively performed by comparing MRM transitions with those reported in the literature.

Data analysis

Data are expressed as mean $\pm$ standard deviation (SD). The coefficient of variation (CV) was calculated as $(\text{SD}/\text{mean}) \times 100$ to assess the precision of the analytical measurements. All statistical calculations were performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). CV values lower than 4% indicate good precision of the analytical method.

3. Results and discussion

Phytochemical Characterization

Qualitative phytochemical screening

The qualitative phytochemical screening of *S. vera* crude extracts identified a diversity of secondary metabolites, with the compound classes varying considerably depending on the extraction solvent used (Table 1). Phenolic compounds were strongly detected in both hydromethanolic and hydroethanolic crude extracts, while flavonoids were more abundant in the aqueous extract. This selective enrichment of the aqueous extract in hydrophilic macromolecules is consistent with their hydrogen-bonding solubility properties (Shiam *et al.*, 2023). In Addition, tannins were found in the hydromethanolic and aqueous extracts, with only traces in the hydroethanolic extract. Regarding alkaloids, they were exclusively detected in the hydroethanolic and aqueous extracts, while terpenoids, quinones, and saponins were restricted to the organic extracts. Furthermore, cholesterol and cardiac glycosides were detected in both hydromethanolic and aqueous extracts. Anthraquinones, coumarins, carbohydrates, anthocyanins, and steroids were not detected in any of the tested extracts, suggesting either their natural absence in this species or presence below detectable thresholds. Unlike the organic extracts, the aqueous extract was further characterized by selective enrichment in gums and mucilages, along with a comparatively reduced overall diversity of secondary metabolites. These finding aligns with previous reports on *S. vera* (Oueslati *et al.*, 2012; Saleem *et al.*, 2021; Ayaz *et al.*, 2022) and other *Suaeda* species (Rahman Neamah *et al.*, 2021; Pavithra *et al.*, 2025).

Table 1. Phytochemical screening results of *Suaeda vera* crude extracts.

Tests	Methanol (75%)	Ethanol (75%)	Distilled water
Alkaloids	-	+++	+
Carbohydrates	-	-	-
Reducing sugars	+	-	Traces
Proteins and amino acids	++	+++	+
Phenolic compounds	+++	+++	+
Tannins	+	Traces	++
Saponins	++	+	-
Terpenoids	+	++	-
Quinones	++	++	-
Anthraquinones	-	-	-
Coumarins	-	-	-
Gums and mucilages	++(CP)	++(WP)	+++ (CP)
Resins	Traces	-	++
Cholesterol	+	Traces	+
Cardiac glycosides	+	Traces	+
Flavonoids	++	Traces	+++
Phlobatanins	-	-	Traces
Anthocyanins	-	-	-
Glycosides	-	+	-
Steroids	-	-	-

-: absent (no reaction observed); +: weak positive reaction (faint color change or precipitate); ++: moderate positive reaction (clearly visible color change or precipitate); +++: strong positive reaction (intense color change or abundant precipitate); CP: cloudy precipitate; WP: white precipitate.

Although the aqueous extract showed higher flavonoid content, the hydromethanolic extract was selected for subsequent analyses due to its broader chemical diversity, encompassing phenolic, flavonoid, tannin, alkaloid, and terpenoid classes, while also being more compatible with UPLC-MS/MS and ATR-FTIR analyses. In contrast, the aqueous extract contained high amounts of gums and mucilages that could interfere with chromatographic separation and spectral interpretation. Therefore, the hydromethanolic extract was selected for further phytochemical and biological analyses.

TLC results

TLC analysis confirmed the presence of the three major classes of bioactive compounds through characteristic color reactions (Table 2), with different R_f values, consistent with standard chromogenic responses (Wagner and Bladt, 1996; Ayaz *et al.*, 2022).

Table 2. Thin-layer chromatography (TLC) profile of the hydromethanolic crude extract of *Suaeda vera*.

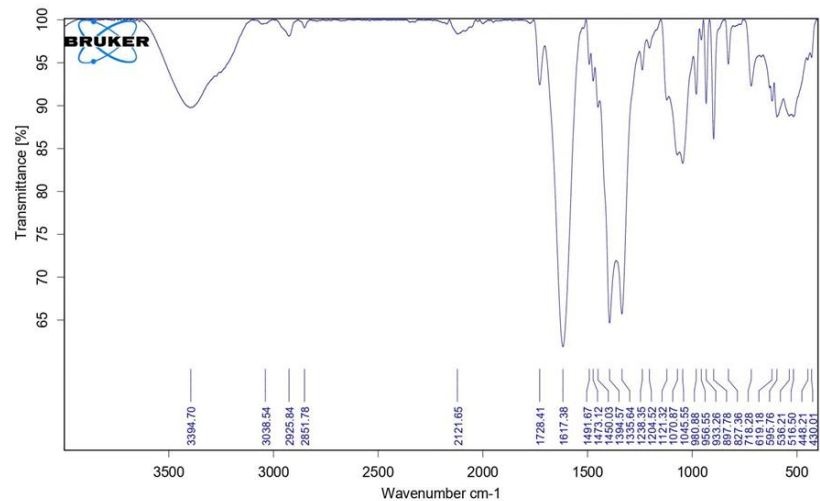
Compound class	R_f value	Spot color
Polyphenols	0.507	Blue-gray
Flavonoids	0.452	Yellow
Tannins	0.610	Dark blue

Quantitative determination of major phytochemicals

Quantitative evaluation of phenolic compounds revealed total phenolic, flavonoid, and tannin contents of 48.57 ± 0.71 mg GAE g^{-1} (CV = 1.46%), 39.88 ± 0.26 mg QE g^{-1} (CV = 0.65%), and 3.70 ± 0.13 mg CE g^{-1} (CV = 3.51%), respectively. The calibration curves for gallic acid (phenolics), quercetin (flavonoids), and catechin (tannins) showed excellent linearity with R^2 values of 0.999, 0.998, and 0.995, respectively. The low coefficients of variation (CV < 4%) reported in this study indicate good precision of the analytical method. The levels of total phenolic, flavonoid, and tannin contents are

comparable to those reported for halophytic species, which typically produce moderate phenolic compounds as part of their adaptive stress response (Gheraissa *et al.*, 2023). Compared to Oueslati *et al.* (2012), who reported lower contents (31.76 mg GAE g⁻¹, 26.2 mg QE g⁻¹, and 1.5 mg CE g⁻¹) for acetonetic shoot extracts of *S. vera*, our findings show higher phenolic and flavonoid levels. In contrast, Ayaz *et al.* (2022) reported considerably higher values (205 ± 23.52 mg GAE g⁻¹ and 90.02 ± 6.88 mg QE g⁻¹) for methanolic whole-plant extract. These discrepancies likely reflect differences in extraction conditions, plant parts used, geographical origin, and environmental factors, as flavonoid accumulation in halophytes is known to be upregulated under salt stress (Feng *et al.*, 2023).

Figure 1. ATR-FTIR spectrum of the hydromethanolic leaf extract of *Suaeda vera*.



ATR-FTIR results

ATR-FTIR spectroscopy provided complementary structural information. The main absorption bands are presented in Table 3 and resulting spectrum illustrated in Figure 1. The broad band observed around 3390 cm⁻¹ indicated O--H stretching vibrations of phenolic and alcoholic groups, confirming the presence of polyphenols (Wongsa *et al.*, 2022). The band at 3038 cm⁻¹ corresponded to aromatic C--H stretching vibrations (Akwuba *et al.*, 2025), while the carbonyl band at 1728 cm⁻¹ suggested esters or carboxylic derivatives (Beattie *et al.*, 2004; Maeda *et al.*, 2007; Vukoje *et al.*, 2018), potentially linked to lipid-associated metabolites involved in halophyte stress adaptation (Vaziriyeganeh *et al.*, 2021). The region between 1617 and 1450 cm⁻¹ reflects aromatic skeletal vibrations, suggesting a structurally diverse phenolic composition, as previously reported (Abumhdi *et al.*, 2025), while the band at 1450 cm⁻¹ reflects C--H deformation of --CH₃ and --CH₂ groups commonly found in alkanes, fatty acids, and terpenoid structures (Xie *et al.*, 2006; Athukorala *et al.*, 2009; Inarkar and Lele, 2012). The multiple peaks recorded in the C--O stretching region (1250--1040 cm⁻¹) indicate the presence of alcohols, ethers, esters, and glycosidic bonds within a phenolic fraction, as reported by Hussein *et al.* (2016), suggesting that some phenolic compounds may occur as glycosylated flavonoids or complex oxygenated metabolites. The bands at 1238--1204 cm⁻¹ and 1121--1045 cm⁻¹ are consistent with alkyl/aryl ether and secondary alcohol vibrations, as previously reported (Collier *et al.*, 1992; Parusnath *et al.*, 2023). In the fingerprint region (<1000 cm⁻¹), the multiple bands corresponding to aromatic substitution patterns highlight structural diversity, while the additional low-frequency bands (below 600 cm⁻¹) may be associated with mineral or inorganic components typical of halophyte species, as suggested by Calone *et al.* (2022). This spectral variety confirmed the structural diversity of the extract and supported the phytochemical screening results.

Table 3. Main ATR-FTIR absorption bands and their assignments for the hydromethanolic leaf extract of *Suaeda vera*.

Frequency (cm ⁻¹)	Peak position (cm ⁻¹)	Functional group	Probable compound
3500-3200	3394.70 (~3400)	O–H stretch, H–bonded	Alcohols, phenols
3100-3000	3038.54	C–H stretch, few bands	Aromatics
3000-2850	2925.84–2851.78	C–H stretch	Alkanes
2260-2100	2121.65	C≡C stretch	Alkynes
1760-1665	1728.41	C=O stretch	Carbonyls (general)
1650-1600	1617.38	C=C Stretch	Conjugated alkene
1500-1450	1491.67–1473.12	C=C stretch (aromatic ring) and/or C–H bending (CH ₂ , CH ₃)	Phenolic compounds, flavonoids, terpenoids
1450	1450.03	C–H bending (-CH ₃ , -CH ₂ - groups)	Methyl and methylene groups in alkanes, fatty acids, or terpenoids.
1420-1330	1394.57–1335.64	O–H bending	Alcohol
1275-1200	1238.35–1204.52	C–O stretching	Alkyl aryl ether
1124-1087 1075-1020	1121.32 1070.87–1045.55	C–O, C–O–C stretching	Secondary alcohol, vinyl ether
	980.88–956.55– 933.26–897.78	C–H Stretching	Aromatic substitution on phenols
900-700	827.36–718.28	C–H bending	Type of substitution of aromatic rings
< 700	619.18–595.76– 536.21–516.50– 448.21–430.01	Fingerprint (aromatic skeleton and mineral salts)	Inorganic groups and specific fingerprint of halophilic metabolites

UPLC-ESI-MS/MS based phytochemical profiling

The phytochemical profile of the hydromethanolic crude extract of *S. vera*, determined by UPLC-ESI-MS/MS (Table 4), identified a total of 30 compounds belonging to diverse chemical classes, with L-proline as the most abundant (67.10%). This predominance is ecophysiologicaly significant, as proline functions as a primary organic osmolyte, molecular chaperone, and signaling molecule essential for maintaining cellular turgor and protecting protein integrity under osmotic stress (Ghosh et al., 2022). The exceptionally high proline levels observed in this study likely reflect a cumulative stress response to the combination of high salinity and extreme aridity during the sampling period, consistent with reports of proline upregulation in *S. salsa* (Bao et al., 2023), *S. nudiflora* (Joshi et al., 2023), and *S. vera* (Hidri et al., 2025) under salt and drought stress.

Table 4. Phytochemical profile of *Suaeda vera* methanolic leaf extract determined by UPLC–ESI–MS/MS.

ID#	Name	Formula	ESI charge	CE(v)	transition m/z	Ret. Time	Area (%)	Height
1	(-) Catechin	C ₁₅ H ₁₄ O ₆	+	-13	291.1000>161.1000	6.525	6.46	228652
2	Chrysin 6-C-glucoside	C ₂₁ H ₂₀ O ₉	+	-25	417.0000>267.0000	5.678	0.03	543
3	Myricetin	C ₁₅ H ₁₀ O ₈	+	-20	318.5000>256.0500	5.626	0.68	16219
4	Myricetin-3-O-rhamnoside	C ₂₁ H ₂₀ O ₁₂	+	-44	465.0000>319.1000	5.026	0.01	239
5	Orxylin A	C ₁₆ H ₁₂ O ₅	+	-22	285.1000>269.9000	5.967	0.09	2077
6	Quercetin 3-glucoside	C ₂₁ H ₂₀ O ₁₂	+	-18	465.1000>303.0500	5.029	0.75	14543
7	Apigenin	C ₁₅ H ₁₀ O ₅	+	-33	271.1000>152.9000	5.653	0.72	16568
8	2-methoxybenzoic acid	C ₈ H ₈ O ₃	+	-14	153.0500>77.0000	3.480	0.10	1775

9	Curcumin	C ₂₁ H ₂₀ O ₆	+	-20	368.9000>177.0500	4.757	0.19	1672
10	Epicatechin	C ₁₅ H ₁₄ O ₆	+	-15	290.8000>139.1500	4.498	0.11	1242
11	Ferulic acid	C ₁₀ H ₁₀ O ₄	+	-16	195.0000>44.7000	5.308	4.67	63794
12	Luteolin	C ₁₅ H ₁₀ O ₆	+	-32	286.7500>69.0000	5.496	0.41	7033
13	Oleanolic acid	C ₃₀ H ₄₈ O ₃	+	-15	457.3000>411.4000	6.128	0.61	8163
14	Oleuropein	C ₂₅ H ₃₂ O ₁₃	+	-15	540.5000>523.4000	6.462	0.16	3658
15	Quercetin	C ₁₅ H ₁₀ O ₇	+	-52	303.0500>153.1000	4.960	0.20	2505
16	Resveratrol	C ₁₄ H ₁₂ O ₃	+	-22	229.0500>107.1000	5.117	5.71	117210
17	Riboflavin	C ₁₇ H ₂₀ N ₄ O ₆	+	-7	377.1000>360.3000	5.806	0.12	1660
18	Rutin	C ₂₇ H ₃₀ O ₁₆	+	-10	611.0000>303.1000	4.947	0.84	17292
19	Beta-carotene	C ₄₀ H ₅₆	+	-19	537.1000>505.0000	10.126	1.52	48570
20	Kojic acid	C ₆ H ₆ O ₄	+	-25	142.7500>40.9500	4.502	0.44	1720
21	Naringenin	C ₁₅ H ₁₂ O ₅	+	-43	273.0500>153.0000	5.448	0.19	2270
22	Vanillin	C ₈ H ₈ O ₃	+	-15	153.0500>64.7500	4.959	0.33	4925
23	Caffeic acid	C ₉ H ₈ O ₄	-	34	179.1500>135.0000	4.779	0.35	6877
24	Gallic acid	C ₇ H ₆ O ₅	-	15	169.1000>125.1000	5.635	0.12	2573
25	Vanillic acid	C ₈ H ₈ O ₄	-	17	166.9500>151.9000	4.803	<0.01	73
26	p-coumaric acid	C ₉ H ₈ O ₃	-	28	163.0500>118.9500	5.062	0.21	3975
27	Salicylic acid	C ₇ H ₆ O ₃	-	37	137.2000>92.9500	5.408	6.20	140646
28	L-proline	C ₅ H ₉ NO ₂	+	-17	116.1000>69.9000	1.618	67.10	1350430
29	Nicotinic acid	C ₆ H ₅ NO ₂	+	-23	123.9000>79.8500	2.275	1.63	26617
30	Sudan III	C ₂₂ H ₁₆ N ₄ O	+	-31	353.0500>77.1500	6.134	0.03	744

ESI: Electrospray ionization; CE: collision energy; Ret. Time: retention time.

Beyond proline, the extract contained several stress-related metabolites with recognized roles in abiotic stress tolerance. Salicylic acid, a key stress-signaling phytohormone, regulates defense gene expression and antioxidant enzyme activity (SOD, CAT) under salinity and heat stress (Decsi *et al.*, 2025). Ferulic acid, a cell wall-associated phenolic, mitigates oxidative damage through free radical scavenging and enzyme stimulation under water deficit (Khan *et al.*, 2024). Catechin, a flavan-3-ol, accumulates in response to multiple stressors and enhances antioxidant defenses (Ahammed *et al.*, 2023), while resveratrol contributes to cellular redox regulation as a free radical scavenger (Khan *et al.*, 2024). The identified flavonoids, including rutin, quercetin-3-glucoside, apigenin, myricetin, luteolin, and naringenin, possess well-documented pharmacological properties, including antioxidant, anti-inflammatory, antibacterial, anticancer, and neuroprotective activities (Calderon-Montano *et al.*, 2011; Ullah *et al.*, 2020; Batiha *et al.*, 2020; Shikha *et al.*, 2024).

The qualitative consistency of the phenolic profile under environmental stress suggests the involvement of conserved stress-responsive pathways, while the quantitative variations in stress-responsive metabolites (proline, salicylic acid, and catechin) indicate dynamic metabolic adjustment. This interpretation aligns with the documented role of stress in enhancing secondary metabolite production in halophytes (Feng *et al.*, 2023; Joshi *et al.*, 2023). While UPLC-ESI-MS/MS profiling allowed the identification of 30 compounds, authentic standards were available only for the five major compounds (L-proline, catechin, salicylic acid, resveratrol, and ferulic acid). Future targeted quantification using authenticated standards would strengthen the structural assignment of the remaining metabolites.

4. Conclusion

The hydromethanolic extract of *S. vera* growing under natural abiotic stress exhibits a phytochemical

profile dominated by stress-related metabolites. The high amount of L-proline, accompanied by salicylic acid, catechin, resveratrol, and ferulic acid, indicates the activation of conserved defense pathways under combined salinity and aridity. These findings provide a chemical basis for the pharmacological potential of *S. vera* and highlight its value for studying stress-induced secondary metabolism in halophytes.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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