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Research Article

High-Performance Thin-Layer Chromatography Chemical Fingerprinting of Salt-Tolerant Plant: An Efficient Method for Sterols and Glycosides from Various Seasonal Plant Parts of Two Halophytic *Suaeda* Species

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ABSTRACT

The study established an HPTLC fingerprinting profile of sterols and glycosides derived from the methanolic extracts of various plant parts of two halophytic *Suaeda* species, *Suaeda fruticosa* Forssk J. F. Gmel. and *Suaeda nudiflora* (Willd.) Moq. Which are having anti-inflammatory, antidiabetic and anticancer properties. The comprehensive qualitative phytochemical analyses of water, methanol, chloroform, and ethyl acetate extracts from all parts of *Suaeda* species revealed the presence of sterols and glycosides, with the methanolic extracts showing the highest amount of both compounds. The chromatographic analysis of methanol extracts was conducted using silica gel 60 F254 HPTLC aluminium sheets with a CAMAG Linomat 5 applicator. The plates were created utilizing a mobile phase composed of chloroform: ethyl acetate (8:12 v/v) for sterols and ethyl acetate: methanol: glacial acetic acid: formic acid (16:1.5:1.5:1 v/v/v/v) for glycosides. Detection of sterols and glycosides on wavelengths at 254 and 366 nm before derivatization. After using the derivatizing reagent chromatogram detected at 366 and 540 nm. Furthermore, the FTIR analysis confirms the presence of multiple functional compounds. The established fingerprinting will ultimately be invaluable for the identification and differentiation of sterols and glycosides as marker compounds in the two referenced *Suaeda* species.

Introduction

There is a halophytic genus known as *Suaeda* that belongs to the Amaranthaceae family. It is also possible to refer to them as sea-blite or seep weeds.[1,2] The Amaranthaceae family, also known as the goosefoot family, is comprised of 102 genera and 1,400 species of halophytic plants that are primarily low-growing.[3] The *Suaeda* genus is able to withstand severe saline and arid circumstances, which causes the accumulation of a number of phytoconstituents in their parts. These phytoconstituents include phenols, flavonoids, tannins, saponins, terpenoids, and steroids.[4] Owing to its diverse phytochemicals, *Suaeda* has a broad spectrum of biological properties, including anti-inflammatory, antimicrobial, antidiabetic and anticancer effects. *Suaeda* is beneficial in several herbal medicines for conditions such as hepatitis, fever, ulcers, jaundice, inflammation and dermatological issues.[5] "Lani" and "khaar" are the indigenous designations for *Suaeda fruticosa* Forssk J. F. Gmel. Certain traditional medicines utilize several parts, including the flower, fruits, leaves and seeds, administered orally in the form of the powder or decoction, juice or an emulsion conventionally employed to address gastrointestinal disorders, such as diarrhea, in addition to conjunctivitis, rheumatism, dermatological issues, cough, and influenza.[6,7] It serves as an anti-infective agent and a suitable for soap, with black seeds recognized for its cardiotonic and therapeutic properties.[8] This species is abundant in minerals, fibers, and polyunsaturated fatty acids (omega 3 and 6) while possessing a low-calorie index, making it

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suitable as a functional meal for humans.[9,10] According to[11], seed can be used to successfully extract both edible oils and unsaturated fatty acids. It has hypoglycaemic and hypolipidemic properties[12], leaf extracts have been shown to treat ophthalmia.[13] *S. fruticosa* polysaccharide (SFP) has the potential to be a special biosource of antioxidants, anti-inflammatory, and analgesic chemicals with significant therapeutic utility.[14] Methanolic extracts from the shoots of *S. fruticosa*, the isolated and the structural elucidated of a new flavonol glycoside using Liquid chromatography mass spectroscopy (LC-MS) analysis.[15] *S. fruticosa* was abundant in secondary phytoconstituents, including alkaloids, phenols, steroids, flavonoids, tannins, glycosides, which were present in the shoot portion when extracted using methanol and acetone solvents.[8,4,16] Also, *S. fruticosa* exhibited antioxidant, antimicrobial and antidiabetic potential.[17] *Suaeda nudiflora* (Willd.) Moq. commonly referred to as "Elakura," is its local designation. It is used as a leafy vegetable. Whole plant extracts with ethyl acetate, methanol, hexane, benzene, chloroform, and acetone confirmed the presence of several phytochemicals like phenol, flavonoids, steroids, cardiac glycosides, saponins, terpenoids and tannins. It's also revealed antioxidant and antibacterial properties.[18]

Planar chromatography, also known as high-performance thin-layer chromatography (HPTLC), is a contemporary method that outperforms traditional thin-layer chromatography (TLC) in terms of accuracy and separation capacity. In contrast to other analytical methods, HPTLC generates a plate image that contains detailed information about the complete extract. Since HPTLC is an offline method, the following steps are technically free and enable the study to treat several sample types at once.[19,20]

In comparison to other analytical methods, fourier transform infrared spectroscopy (FTIR) is a technique that comes with a number of advantages. It is possible to detect functional groups using this non- destructive technology, which provide information about the chemical and structural changes that occur in response to biotic or abiotic stress. [21]

Halophytes of the genus *Suaeda* are known for their rich phytochemical diversity and notable pharmacological properties. However, despite extensive studies on their biochemical composition, no HPTLC-based fingerprinting data for sterols and glycosides of *Suaeda* species have been reported so far. Establishing such chromatographic profiles is essential for chemical characterization, quality control, and comparative phytochemical evaluation of these plants. Therefore, the present study was undertaken to develop and characterize the HPTLC fingerprints of sterol and glycoside compounds from methanolic extracts of *S. fruticosa* and *S. nudiflora* collected from different plant parts. The study also integrates FTIR spectral analysis to complement and confirm the chemical profiling of these halophytic species.

Materials and Methods

Collection and Authentication of Plant Samples

Leaves, stems, roots and flowers of *S. fruticosa* and *S. nudiflora* halophytic species were collected from Northern parts of Gujarat (coordinates 23° 31′02.331′′N, 71° 28′ 31.0476′′ E) India. The Gujarat Flora was used to identify the plants. After thoroughly cleaning each component with clean water to get rid of any surrounding dirt, it was left to shade dry at room temperature for three to six days. Each sample was used to prepare the extract, and the powder was made using a grinder with a mixer for the dried parts.

Preparation of Extracts

The extraction was conducted *via* the maceration process. Samples of 1 g each were immersed in a conical flask containing 10 mL. Water, methanol, chloroform and ethyl acetate were combined and subsequently mixed on a shaker at 150 rpm for 24 hours. The extracts were filtered using Whatman filter paper No. 1, and the filtrates were collected and stored for further examination of sterols and glycosides.

Phytochemical Screening

Tests for sterols

- *Salkowski test*

0.2 g of extract was dissolved in 2 mL of chloroform and concentrated H_2SO_4 was added. The appearance of a reddish-brown coloration at the interface signifies the existence of steroids.

- *Liebermann-burchard assay*

0.2 g of each extract, 2 mL of acetic acid was added, and the solution was thoroughly chilled in ice before the careful addition of concentrated H_2SO_4. The color transition from violet to blue or bluish-green signifies the existence of a steroidal ring.

Test for glycosides

- *Keller-killani tests*

Each 1 mL extracted sample was separately treated with several drops of glacial acetic acid and ferric chloride solution, then thoroughly mixed in a test tube using a vortex mixer. Subsequently, concentrated sulfuric acid was introduced to the above combination, resulting in the creation of two distinct layers. A color change in the lower layer to reddish-brown and in the upper acetic acid layer to bluish-green showed a positive test for glycosides.

HPTLC Fingerprinting Analysis

Sample preparation and application

For all plant materials, the soxhlet apparatus was used to prepare extract (10 g powder) using methanol, a suitable

polar solvent of HPLC quality. For later analysis, all extracts were kept in a refrigerator. Plant samples (leaves, stem, and flower) were added in a 2 µL volume to the pre-activated silica gel thin-layer chromatography plate. The CAMAG Linomat-5 sample applicator was used to apply the material. The samples were loaded into the Linomat-5 with an 8 mm band width, 15 mm from the plate bottom, and 2 mm between bands using the VisionCATS Planar Chromatography Manager. They placed samples on every track. A 20×20 cm sheet of TLC plate silica gel 60 F254 (Merck) was cut with a cutter to 20 x 10 cm and used as a stationary phase.

Chromatogram development

After being dried, loaded HPTLC plates were put in a 20 x 10 cm, solvent-saturated twin-through glass chamber to develop the chromatogram for 20 minutes. About 70% of the chromatogram's development was allowed. After being taken out of the chamber, the HPTLC plate was dried with a drier.

Solvent system and derivatizing reagent

Chloroform: ethyl acetate (8:12 v/v) and ethyl acetate: methanol: glacial acetic acid: formic acid (16:1.5:1.5:1 v/v/v/v) using standard solvent system for sterol and glycoside compounds, respectively.

- Anisaldehyde sulphuric acid reagent- 0.5 mL Anisaldehyde is mixed with 10 mL glacial acetic acid, followed by 85 mL methanol and 5 mL concentrated sulphuric acid.
- Methanolic sulphuric acid reagent – 10 mL concentrated H_2SO_4 added in methanol carefully.

Chromatogram Scanning

After development, let the TLC plate dry before taking chromatogram images in fluorescent (366 nm), UV (254 nm), and white (540 nm) lighting. The same plate was scanned using different TLC Scanner-4 wavelengths. According to TLC scanner 4, the scanning speed ranged from 1 to 100 mm/s, and the slit dimension size was 6.00×0.45 mm, which regulates the area of the sample bands. After then, specific derivatizing chemicals were added to the plate. After 5 seconds, take the plate out of the reagent and heat it on a hot plate for 1 to 2 minutes at 100 °C. Then, use a TLC visualizer to look at the chromatogram. The Vision CAT software's compare mode function assessed many tracks at various detection levels.

FTIR analysis

The FTIR analysis was conducted to identify the functional groups present in the methanolic extracts. The methanolic crude extracts of the chosen plant sections were analyzed using a Shimadzu 8400S FT-IR spectrophotometer. The measurements were conducted using an automatic monitoring FTIR spectrophotometer within the range of 4000 to 400 cm^{-1}. The FTIR signal area was documented, compared with the standard peak value, and analyzed for functional groups.

Results and Discussion

Detection of Sterols

During the Salkowski test and Liebermann-Burchard test, positive results were recorded for the detection of sterols in the methanolic extracts of the leaves, stems and flowers of both species under study (Table 1). In Salkowski test, recorded positive results in water extract of leaves and root of *S. fruticosa*, the other species *S. nudiflora* showed positive results in leaves, stem and root part of summer seasons. In rainy flower part also showed presence of sterols in water extract. Positive results exhibited in chloroform extract of leaves, root and flower parts in both *Suaeda* species. Leaves parts showed positive results in ethyl acetate extracts, negative in stem and root parts of *S. fruticosa*, whereas *S. nudiflora* showed absence of sterols in all parts of ethyl acetate extract. While conducting Liebermann-Burchard test, the positive results in water extracts of stem and roots were recorded in both two species of *Suaeda*. Negative results documented in water extract of leaves and flower parts of both species. *S. fruticosa* exhibited presence of sterol in chloroform extracts of leaves and stem part, while absence in root and flower parts. *S. nudiflora* revealed presence of sterol in leaves, stem, root and flower of chloroform extracts. In extract of ethyl acetate showed positive results in leaves, stem and flower of both the *Suaeda* species.

Detection of Glycosides

Positive results were obtained during Keller-Killani's test for detecting glycosides in the methanolic extracts of leaves, stem and flower from *S. fruticosa*, while absence in root part. In *S. nudiflora* showed presence of glycosides in all the parts of methanolic extracts. Likewise, the water extracts of leaves, stems and flowers of both *Suaeda* species revealed positive response during Keller-Killani's test for glycosides. Absence in root part of water extract in summer season of *S. fruticosa* and S. *nudiflora*. whereas, exhibited presence in rainy seasons of root water extract of both *Suaeda* species. Chloroform extract of leaves showed negative response in both seasons (summer and rainy) in *S. fruticosa*. Positive results showed in stem part, negative results in root and flower parts for the glycosides. In *S. nudiflora* showed positive results in chloroform extracts leaves. But, *S. nudiflora* stem, root and flower did not show any sign of glycosides (Table 1). In *S. fruticosa*, the absence of glycosides in the ethyl acetate extracts of leaves, stems and root's part. While only presence in ethyl acetate extract of flower part. Positive response exhibited in leaves and stem of ethyl acetate extracts of *S. nudiflora*, as well as negative response in root and flower parts of ethyl acetate extracts during both seasons.

Table 1: Phytochemical analysis of sterol and glycoside from *Suaeda* species

				Leaves				*Stem*				*Root*				*Flower*			
	Season	*Phytochemical*	*Phytochemical Test*	*W*	*M*	*C*	*EA*	*W*	*M*	*C*	*EA*	*W*	*M*	*C*	*EA*	*W*	*M*	*C*	*EA*
 *S. fruticosa*	Summer	Sterol	Salkowski test													-	-	-	-
			Liebermann-Burchard test													-	-	-	-
		Glycosides	Keller-Killiani test													-	-	-	-
	Rainy	Sterol	Salkowski test																
			Liebermann-Burchard test																
		Glycosides	Keller-Killiani test																
 *S. nudiflora*	Summer	Sterol	Salkowski test													-	-	-	-
			Liebermann-Burchard test													-	-	-	-
		Glycosides	Keller-Killiani test													-	-	-	-
	Rainy	Sterol	Salkowski test																
			Liebermann-Burchard test																
		Glycosides	Keller-Killiani test																

(Note: W-Water, M- Methanol, C- Chloroform, EA- Ethyl acetate; Presence, Absent; - parts not available in that season)

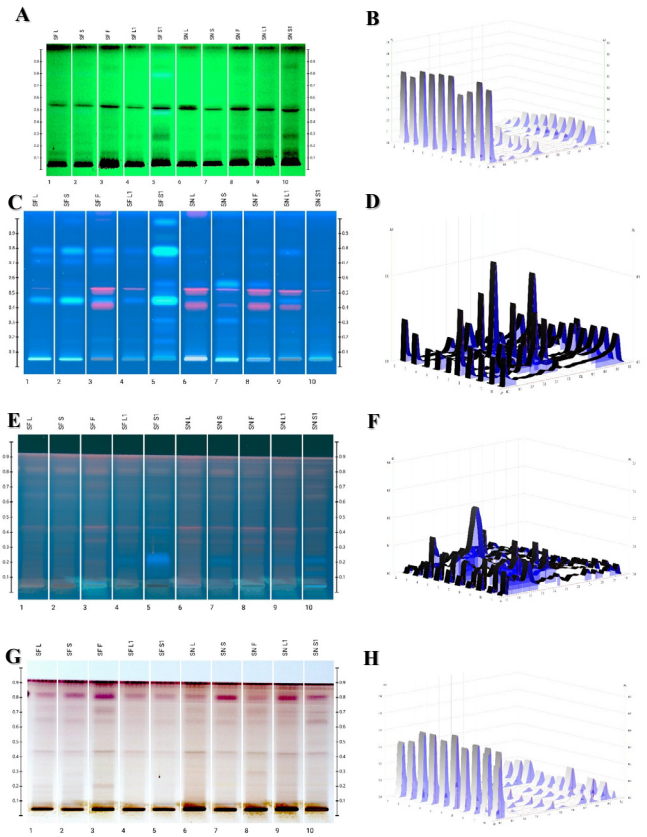

Fig. 1: HPTLC fingerprinting analysis of sterols compound. (A). Chromatogram at 254 nm (Pre derivatizing image) (B). 3D graph at 254 nm (C). Chromatogram at 366 nm (Pre derivatizing image) (D). 3D graph at 366 nm (E). Chromatogram at 540 nm (Post derivatizing image) (F). 3D graph at 540 nm (G). Chromatogram at 366 nm (Post derivatizing image) H. 3D graph at 366 nm (track-1 SFL- *S. fruticosa* leaf rainy, track-2 SFS- *S. fruticosa* stem rainy, track-3 SFF- *S. fruticosa* flower, track-4 SFL1- *S. fruticosa* leaf summer, track-5 SFS1- *S. fruticosa* stem summer, track-6 SNL- *S. nudiflora* leaf rainy, track-7 SNS- *S. nudiflora* stem rainy, track-8 SNF- *S. nudiflora* flower, track-9 SNL1- *S. nudiflora* leaf summer, track-10 SNS1- *S. . nudiflora* stem summer)

Both the *Suaeda* species explored more sterol and glycoside compounds in various parts like leaves, stems, roots and flowers across two seasons (summer and rainy), with different extracts including methanol, water, chloroform and ethyl acetate. Similar results were also obtained in a phytochemical study on *S. fruticosa* whole plant materials extracted in methanolic extracts by.[16] Their study revealed that the alkaloid, phenol, flavonoid, tannin and saponin present in various extracts like methanol, hexane, acetone and petroleum ether. The other preliminary phytochemical screening of whole plant of *S. nudiflora* was conducted by.[18] Wherein the presence of flavonoid, tannin, saponin, glycoside, alkaloid and steroid were identified in different extracts including methanol, chloroform, ethyl acetate. According to this preliminary examination both *Suaeda* species revealed presence of sterols and glycosides but summer seasons show more presence of metabolites than rainy.

HPTLC Fingerprint Analysis of Sterol

A comparative screening of *S. fruticosa* extracts exhibited multiple bands with distinct Rf values; where at 254 nm (as shown in Table 2) a sum total of 4 bands were identified in methanolic extracts of its rainy season leaves, 3 bands in its rainy stem part, 5 bands in flower as well as summer leaves extract and 6 bands in summer stem extracts (Fig. 1). In *S. nudiflora*, the methanolic extracts from leaves, stem and summer leaves displayed 4 bands of distinct Rf value ranging from 0.10 to 0.90 at 254 nm, contrastingly flower and summer stem showed 5 bands. At 366 nm, (Fig. 1C) the rainy leaf and summer stem extracts of *S. fruticosa* revealed 6 bands, while its rainy stem and summer leaves generated 4 bands. Its flower part exhibited 5 bands with ranging from 0.20 to 0.90 Rf values. In *S. nudiflora*, leaves methanolic extract showed 4 bands at different Rf values, while its stem, flower and summer stem exhibited different 5 bands. Summer leaves showed only 3 bands at distinct Rf values. For more visualization of bands, we derivatized this chromatogram with its suitable derivatizing reagent anisaldehyde sulphuric acid reagent (ASR). After derivatization at 366 nm, (Fig. 1E) the extracts of *S. fruticosa* displayed 5 bands in rainy leaves, stem and flower parts. On other hand, summer leaves and stem showed 4 and 3 bands respectively. *S. nudiflora* rainy leaves, summer leaves and stem extracts exhibited 5 bands at ranging from different Rf values at 0.35 to 0.90. While rainy stem and flower extracts displayed 3 ad 6 bands, respectively. At 540 nm, purple bands confirmed the presence of sterol compound in different Rf values. (Fig. 1G) showed 4 bands in *S. fruticosa* rainy leaves and stem methanolic extracts, 5 bands in flower extract and 3 bands in summer leaves and stem extracts with distinct Rf values at 0.30 to 0.90. However, *S. nudiflora* chromatogram separated with 4 bands in rainy leaves, rainy stem, summer leaves and summer stem. Flower extracts showed 3 bands in methanolic extracts.

HPTLC Fingerprint Analysis of Glycosides

The various Rf values generated from leaves, stem and flower extracts of both two *Suaeda* species, following their screening at 254, 366 nm and after derivatisation 366 and 540 nm is presented in Fig. 2. In the leaves of rainy and summer seasons of *S. fruticosa*, the Rf values at 254 nm ranged from 0.17 to 0.90 with maximum bands of 7 (Fig. 2A)**.** Additionally, its rainy stem extracts showed 4 bands. Flower and summer stem extracts displayed 5 bands. In *S. nudiflora* leaves and stem showed 6 and 7 bands, respectively. Summer leaves, stem and flower extracts exhibited 5 bands at different Rf values. (Fig.2C) At 366 nm, detected 5 bands in *S. fruticosa* leaves extracts of rainy and summer seasons. While 6 bands with different ranged Rf values at 0.22 to 0.89 in flower, summer leaves and stem extracts. On another side, *S. nudiflora* showed 6 bands in rainy leaves, stem, flower and summer leaves

Table 2: Comparative HPTLC fingerprinting analysis for sterols in *Suaeda* species

Wavelength	*Rf values*	*Suaeda fruticosa*					*Suaeda nudiflora*				
		SF L	*SF S*	*SF F*	*SF L1*	*SF S1*	*SN L*	*SN S*	*SN F*	*SN L1*	*SN S1*
254 nm (pre-derivatization)	*0.10*		■		■	■	■	■	■		■
	0.25	■		■	■	■			■	■	■
	0.35			■		■	■	■	■	■	
	0.45	■	■	■		■	■	■		■	
	0.65	■			■					■	■
	0.75	■		■	■	■		■	■		■
	0.90		■	■	■	■	■		■		■
366 nm (pre-derivatization)	*0.20*	■		■		■			■		
	0.30	■	■	■		■		■	■		■
	0.45			■	■	■	■	■	■	■	■
	0.55	■	■	■	■	■	■	■		■	■
	0.75	■	■		■	■	■				■
	0.80	■				■	■	■	■	■	
	0.90	■	■	■	■			■	■		■
366 nm (post-derivatization)	*0.35*			■							
	0.45	■	■	■		■	■	■	■	■	■
	0.55	■		■					■		
	0.60	■	■				■		■		
	0.65		■		■		■		■	■	■
	0.75				■					■	■
	0.80	■	■	■	■	■	■	■	■	■	■
	0.90	■	■	■	■	■	■	■	■	■	■
540 nm (post-derivatization)	*0.20*										
	0.30			■			■				
	0.45	■	■	■	■	■	■	■	■	■	■
	0.55										
	0.75	■	■	■				■		■	■
	0.80	■	■	■	■	■	■	■	■	■	■
	0.90	■	■	■	■	■	■	■	■	■	■

(Note: SFL- *S. fruticosa* leaf rainy, SFS- *S. fruticosa* stem rainy, SFF- *S. fruticosa* flower, SFL1- *S. fruticosa* leaf summer, SFS1- *S. fruticosa* stem summer, SNL- *S. nudiflora* leaf rainy, SNS- *S. nudiflora* stem rainy, SNF- *S. nudiflora* flower, SNL1- *S. nudiflora* leaf summer, SNS1- *S. nudiflora* stem summer ■ -Recorded Rf values)

extracts. Summer stem extracts displayed with only 5 bands. Screening at 366 nm after derivatized with methanolic sulphuric acid reagent (natural reagent), highlighted bands with fluorescent yellow and brown light confirmed the presence of glycosides compound in different tracks of both *Suaeda* species. Fig. 2E showed 7 bands in rainy leaves and stem extracts of *S. fruticosa*. The summer leaves, stem and flower extracts recorded the maximum number of 8 bands with Rf values ranged from 0.27 to 0.90. In *S. nudiflora* displayed maximum number of 8 bands in rainy stem, summer leaves and stem extracts. Rainy leaves and flower extracts showed 7

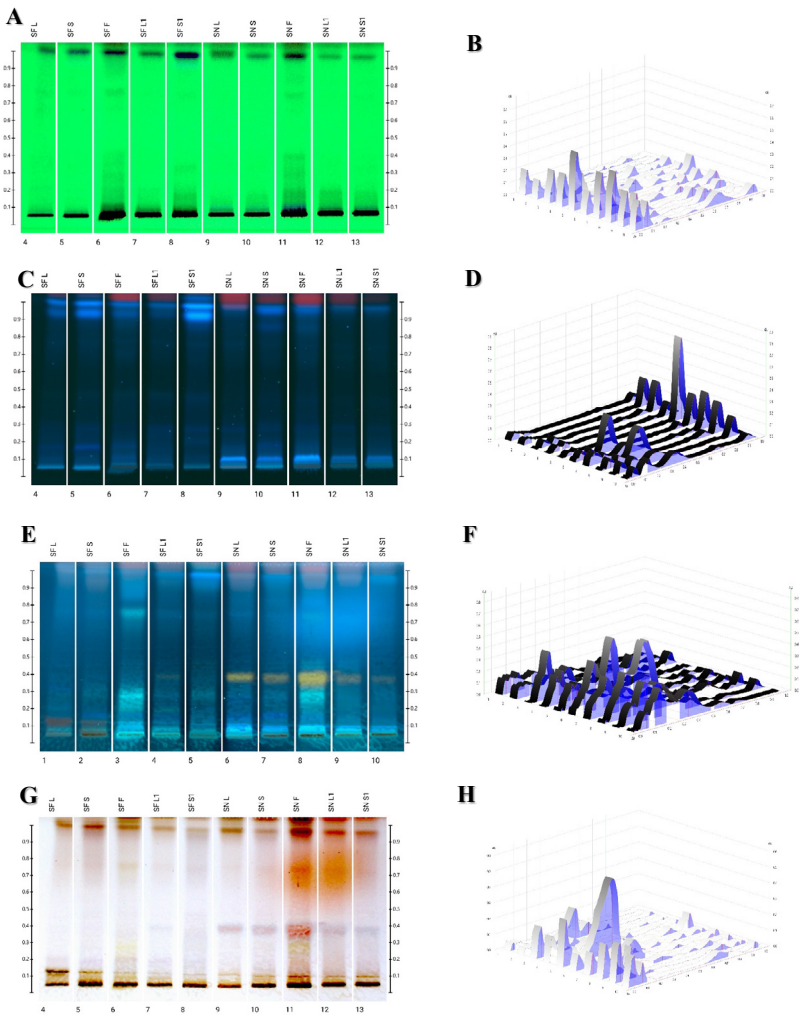

Fig. 2: HPTLC fingerprinting analysis of glycosides A. Chromatogram at 254 nm (Pre derivatizing image) B. 3D graph at 254 nm C. Chromatogram at 366 nm (Pre derivatizing image) D. 3D graph at 366 nm E. Chromatogram at 540 nm (Post derivatizing image) F. 3D graph at 540 nm G. Chromatogram at 366 nm (Post derivatizing image) H. 3D graph at 366 nm (track-1 SFL- *S. fruticosa* leaf rainy, track-2 SFS- *S. fruticosa* stem rainy, track-3 SFF- *S. fruticosa* flower, track-4 SFL1- *S. fruticosa* leaf summer, track-5 SFS1- *S. fruticosa* stem summer, track-6 SNL- *S. nudiflora* leaf rainy, track-7 SNS- *S. nudiflora* stem rainy, track-8 SNF- *S. nudiflora* flower, track-9 SNL1- *S. nudiflora* leaf summer, track-10 SNS1- *S. . nudiflora* stem summer)

bands. After 366 nm, comparison with 540 nm (Fig.2G) exhibited that banding pattern with yellow and brown color in white light. *S. fruticosa* rainy leaves and summer stem showed 6 bands, while summer leaves, rainy stem and flower extracts showed presence of 5 bands in discrete Rf values between 0.20 to 0.90. *S. nudiflora* showed maximum bands with 6 numbers in rainy leaves, stem, summer leaves and stem extracts. Minimum bands separated in flower extracts with 4 numbers. Table 2 and 3 revealed the multiple Rf values were recorded during this experiment through TLC scanner in different parts of both *Suaeda* species according to their relevant compounds. A similar type of experiment was done in Iraq with the species *S. fruticosa* with TLC analysis, which exhibited the presence of a sterol compound in aerial parts. Further, they confirmed with HPLC analysis. [22] Another literature survey also confirmed the presence of sterol compounds in 13 species of Chenopodiaceae family along with the *Suaeda linearis* (Ell.) Moq species in their leaves and stem parts. [23] From *S. fruticosa,* new flavonol glycosides reported in methanolic extracts of aerial part. [15] Based upon seasonal data fingerprinting analysis of both species showed multiple bands, in sterol compounds rainy stem part of *S. fruticosa* showed lighter bands than summer stem part. Summer stem part showed more dark and highlighted bands, its may be due to higher concentration of chemical compounds. All the data show that the comparative analysis of two parts of *Suaeda* species across two different seasons showed very little difference.

The fundamental principle of FTIR is based on the vibration of chemical bonds in the infrared region. Chemical bonds in the IR region absorb up radiation between 4000 to 400 cm^{-1}.

Table 3: Comparative HPTLC fingerprinting analysis for glycosides in *Suaeda* species

Wavelength	*Rf values*	*Suaeda fruticosa*					*Suaeda nudiflora*				
		SF L	*SF S*	*SF F*	*SF L1*	*SF S1*	*SN L*	*SN S*	*SN F*	*SN L1*	*SN S1*
254 nm (pre-derivatization)	0.17	■	■	■	■	■	■	■	■	■	■
	0.36	■	■	■	■	■	■	■	■	■	■
	0.54	■		■	■		■	■		■	■
	0.64	■			■		■	■	■		■
	0.76	■	■		■	■		■	■	■	
	0.84	■	■	■	■	■	■	■		■	■
	0.90	■			■	■	■	■	■		■
366 nm (pre-derivatization)	0.22	■	■	■	■	■	■	■	■	■	■
	0.37	■	■	■	■	■	■	■	■	■	■
	0.45	■	■	■	■	■	■	■	■	■	■
	0.55										
	0.77		■	■	■	■	■	■	■	■	
	0.82	■	■	■		■	■	■	■	■	■
	0.89	■	■	■	■	■	■	■	■	■	■
366 nm (post-derivatization)	0.27	■	■	■	■	■	■	■	■	■	■
	0.45	■	■	■	■	■	■	■	■	■	■
	0.55	■	■	■	■	■	■	■	■	■	■
	0.62	■	■	■	■	■	■	■	■	■	■
	0.68			■	■	■		■		■	■
	0.75	■	■	■	■	■	■	■	■	■	■
	0.83	■	■	■	■	■	■	■	■	■	■
	0.90	■	■	■	■	■	■	■	■	■	■
540 nm (post-derivatization)	0.20	■	■	■	■	■	■	■	■	■	■
	0.30	■	■			■	■	■		■	■
	0.45	■	■	■	■	■	■	■	■	■	■
	0.55										
	0.75	■		■	■	■	■	■	■	■	■
	0.80	■	■	■	■	■	■	■	■	■	■
	0.90	■	■	■	■	■	■	■	■	■	■

(Note: SFL- *S. fruticosa* leaf rainy, SFS- *S. fruticosa* stem rainy, SFF- *S. fruticosa* flower, SFL1- *S. fruticosa* leaf summer, SFS1- *S. fruticosa* stem summer, SNL- *S. nudiflora* leaf rainy, SNS- *S. nudiflora* stem rainy, SNF- *S. nudiflora* flower, SNL1- *S. nudiflora* leaf summer, SNS1- *S. nudiflora* stem summer, ■ -Recorded Rf values)

The presence of functional groups such as alcohol, ether, esters, carboxylic acid, alkanes, phenol, alkenes, amines, saturated aliphatic lipids, and sulfoxide was confirmed by a number of peaks in the FTIR analysis of methanolic extracts of both *Suaeda* species (Fig. 3 and Table 4). The presence of functional groups in *Suaeda* species is supported by the FTIR spectra. *Sesuvium portulacastrum* L., another halophytic species, also contains these kinds of functional groups.[24] Peak values at 503.42 and 605.65 cm^{-1} showed C = C bending with alkanes groups, 719.45 to 979.84 cm^{-1} splitted in to C-N stretch, C-H bend and C-C stretch that represents amines, benzene and

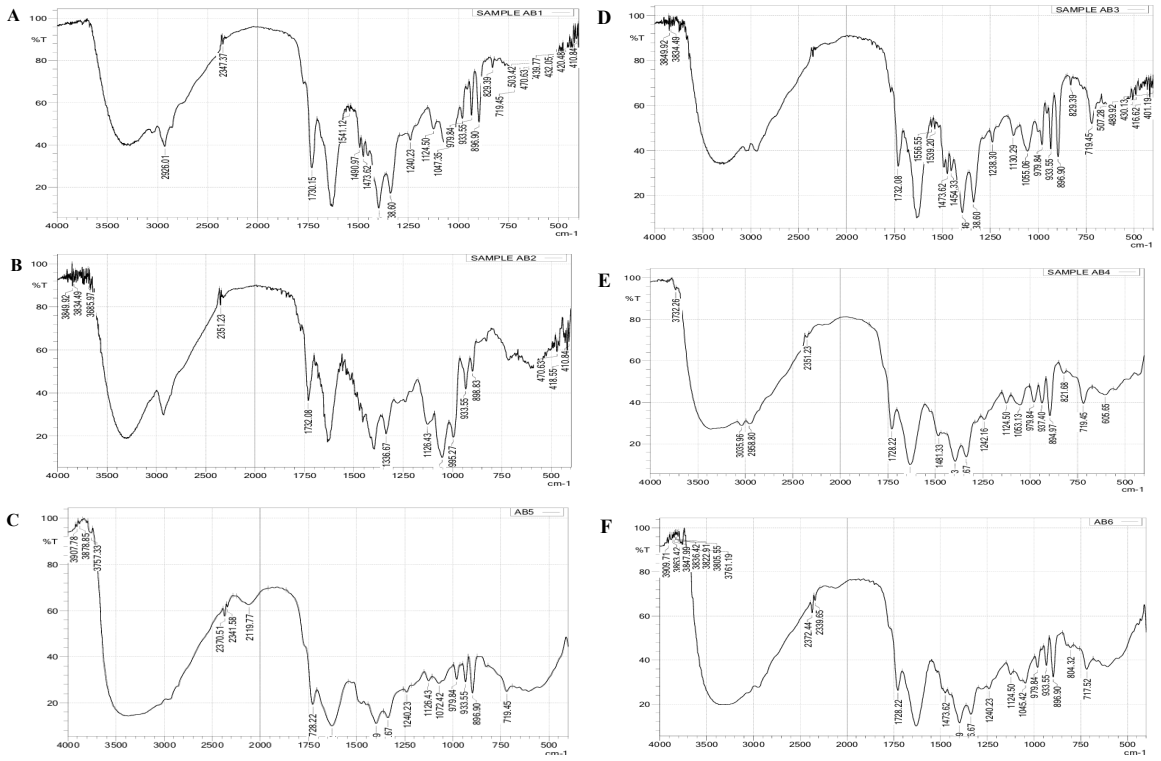

Fig. 3: FTIR analysis of various parts of *Suaeda* species (Note: A- *S. fruticosa* leaf, B- *S. fruticosa* stem, C- *S. fruticosa* flower, D- *S. nudiflora* leaf, E- *S. nudiflora* stem, F- *S. nudiflora* flower. All samples prepared in methanolic solvent)

Table 4: Representation of wavelengths and characteristic peaks of IR spectrum

Sr. No.	*Peak values (cm^{-1})*						*Stretching bonds*	*Functional groups*	*References*
	A	*B*	*C*	***D***	***E***	***F***			
1	410.84	410.84		401.19			MgO/Mg OH octahedral layer	phthalimide	[24]
2	420.48, 439.77, 470.63	418.55		416.62, 489.92					
3	503.42			507.28			C=C bending	alkanes	[25]
4					605.65				
5	669.30	669.30		669.30	669.30				
6	719.45		719.45	719.45	719.45	717.52	C-N stretch, C-H bend, C-C stretch	amines, benzene, chloride	
7	829.39			829.39		804.32			
8	896.90	898.83	896.90	896.90	894.97	896.90			
9	933.55	933.55	933.55	933.55	937.40	933.55			
10	979.84	995.27	979.84	979.84	979.84	979.84			
11	1047.35	1053.13	1072.42	1055.06	1053.13	1045.42	S=O stretch, C-N stretch, C-O stretch	sulfoxides, amines, ester, ether, alcohol, alkenes	
12	1124.50	1126.43	1126.43	1130.29	1124.50	1124.50			
13	1240.23		1240.23	1238.30	1242.16	1240.23			
14	1338.60	1336.67	1336.67	1338.60	1336.67	1336.67	C-N stretching	amine	
15	1396.46		1398.39	1396.46	1394.53	1398.39	-(CH3)3 bend	alkanes and alkyl	

Cont...

16				1454.33			C-H (CH2 deformation vibration)		
17	1473.62			1473.62		1473.62	C-C stretching	aromatics ring	
18	1490.97				1481.33				
19	1541.12			1539.20				amide I amide II	
20				1556.55					
21			1627.92		1633.71		N=O, N-H	primary amines	[24]
22	1730.15	1732.08	1728.22	1732.08	1728.22	1728.22	C=O stretching	ester carbonyl	
23	2347.37	2351.23	2119.77, 2341.58, 2370.51		2351.23	2339.65, 2372.44	C-NH stretching	amines	
24	2926.01				2958.80		Asymmetric stretching of CH(CH2) vibration	saturated aliphatic compounds lipids	
25					3035.96		S, O-H stretch, C-H stretch	carboxylic acids, alkenes	[25]
26		3685.97							
27			3757.33		3732.26	3761.19	O-H stretching	alcohol and phenol	
28		3834.49	3878.85	3834.49		3805.55, 3822.91, 3836.42, 3847.99, 3863.42	O-H stretch	alcohol	
29		3849.92	3907.78	3849.92		3909.71	N-H stretching	primary amines	

(Note: A- *S. fruticosa* leaf, B- *S. fruticosa* stem, C- *S. fruticosa* flower, D-*S. nudiflora* leaf, E- *S. nudiflora* stem, F- *S. nudiflora* flower)

chlorides. 1047.35 to 1240.23 cm^{-1} revealed functional groups like sulfoxides, amines, ester, ether, alcohol, alkenes that major components of secondary metabolites. 1338.60 to 2339.65 cm^{-1} peak showed alkanes, alkyl, aromatics ring, amide I and amide II. 2926.01 cm^{-1} peak values denote saturated aliphatic compounds lipids functional compound. Peak values at 3834.49 to 3863.42 cm^{-1} represents alcohol like major groups that formally present in many secondary metabolites. Nevertheless, the methodical characterisation of phytochemicals obtained from these plant sections greatly increased the medicinal plant species' potential as a source of pharmaceuticals. [26]

Conclusion

The present study successfully established distinct HPTLC fingerprinting profiles for sterols and glycosides in the methanolic extracts of *S. fruticosa* and *S. nudiflora*. The optimized solvent systems enabled clear resolution and reproducible identification of characteristic bands, which can serve as reliable chemical markers for both species. FTIR analysis further validated the presence of functional groups corresponding to sterol and glycoside compounds. The developed HPTLC fingerprinting provide a valuable tool for quality control, authentication, and differentiation of halophytic *Suaeda* species. In the future, isolation and structural elucidation of the major bands detected through HPTLC could reveal novel bioactive constituents. Quantitative estimation, bioassay- guided fractionation, and correlation of chemical profiles with pharmacological properties will further enhance the chemotaxonomic and therapeutic understanding of *Suaeda* species.

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References

1. Hopkins CO, Blackwell WH. Synopsis of *Suaeda* (Chenopodiaceae) in North America. SIDA, Contri. to Bot. 1977; 147–173.
2. Leuschner C, Ellenberg H. Ecology of Central European non-forest vegetation: coastal to alpine, natural to man-made habitats: vegetation ecology of Central Europe 2017; (2): Springer.
3. Evans WC. Trease and Evans. Pharma., 9th Edition Published by Saunders Elsevier. 2002; 553.

4. Oueslati S, Ksouri R, Falleh H, Pichette A, Abdelly C, Legault J. Phenolic content, antioxidant, anti-inflammatory and anticancer activities of the edible halophyte *Suaeda fruticosa* Forssk. Food Chem. 2012; 132(2): 943–947. https://doi.org/10.1016/j.foodchem.2011.11.072
5. Zaier MM, Heleno SA, Mandim F, Calhelha RC, Ferreira ICFR, Achour L, Kacem A, Dias MI, Barros L. Effects of the seasonal variation in the phytochemical composition and bioactivities of the wild halophyte *Suaeda fruticosa*. Food Bio. sci. 2022; 50: 102131. https://doi.org/10.1016/j.fbio.2022.102131
6. Ahmed N, Mahmood A, Tahir SS, Bano A, Malik RN, Hassan S, Ashraf A. Ethnomedicinal knowledge and relative importance of indigenous medicinal plants of Cholistan desert, Punjab Province, Pakistan. J. E. pharma. 2014; 155(2): 1263–1275. https://doi.org/10.1016/j.jep.2014.07.007
7. Saleh KA, Albinhassan TH, Elbehairi SEI, Alshehry MA, Alfaifi MY, Al-Ghazzawi AM, Al-Kahtani MA, Alasmari ADA. Cell cycle arrest in different cancer cell lines (Liver, Breast, and Colon) induces apoptosis under the influence of the chemical content of *Aeluropus lagopoides* Leaf extracts. Mole. 2019; 24(3): 507. https://doi.org/10.3390/molecules24030507
8. Ayaz A, Jamil Q, Hussain M, Anjum F, Sarfraz A, Alqahtani T, Hussain N, Gahtani RM, Dera AA, Alharbi HM. Antioxidant and gastroprotective activity of *Suaeda fruticosa* Forssk. Ex JF Gmel. Mole. 2022; 27(14): 4368. https://doi.org/10.3390/molecules27144368
9. Barreira L, Resek E, Rodrigues MJ, Rocha MI, Pereira H, Bandarra N, da Silva MM, Varela J, Custódio L. Halophytes: Gourmet food with nutritional health benefits? J. Food Comp. and Ana. 2017; 59: 35–42. https://doi.org/10.1016/j.jfca.2017.02.003
10. Zaier MM, Ciudad-Mulero M, Cámara M, Pereira C, Ferreira ICFR, Achour L, Kacem A, Morales P. Revalorization of Tunisian wild Amaranthaceae halophytes: Nutritional composition variation at two different phenotypes stages. J. of Food Compo. and Ana. 2020; 89: 103463. https://doi.org/10.1016/j.jfca.2020.103463
11. Weber DJ, Ansari R, Gul B, Khan MA. Potential of halophytes as source of edible oil. J. of Arid Envi. 2007; 68(2): 315–321. https://doi.org/10.1016/j.jaridenv.2006.05.010
12. Benwahhoud M, Jouad H, Eddouks M, Lyoussi B. Hypoglycemic effect of *Suaeda fruticosa* in streptozotocin-induced diabetic rats. J. E. pharma. 2001; 76(1): 35–38. https://doi.org/10.1016/S0378-8741(01)00207-0
13. Rashid S, Iftikhar Q, Arshad M, Iqbal J. Chemical composition and antibacterial activity of *Suaeda fruticosa* Forssk. from Cholistan, Pakistan. Pak. J. of Bio. Sci. (Pakistan). 2000; 3(2).
14. Mzoughi Z, Abdelhamid A, Rihouey C, Le Cerf D, Bouraoui A, Majdoub H. Optimized extraction of pectin-like polysaccharide from *Suaeda fruticosa* leaves: Characterization, antioxidant, anti-inflammatory and analgesic activities. Carbo. Poly. 2018; 185: 127–137. https://doi.org/10.1016/j.carbpol.2018.01.022
15. Oueslati S, Ksouri R, Pichette A, Lavoie S, Girard-Lalancette K, Mshvildadze V, Legault J. A new flavonol glycoside from the medicinal halophyte *Suaeda fruticosa*. Nat. prod. Res. 2014; 28(13): 960-966. https://doi.org/10.1080/14786419.2014.900771
16. Suthar R, Solanki HA. Phytochemical Screening of Halophytic Plant *Suaeda Fruticosa* (L.) Forssk. Ex JF Gmel. Int. Assoc. Biol. Compu. Dig. 2022; 1(2): 308–313. https://doi.org/10.56588/iabcd.v1i2.84
17. Ahmad I, Gul H, Noureen A, Ujjan JA, Manzoor S, Muhammad W. Antimicrobial, antioxidant and antidiabetic potential of *Suaeda fruticosa* L. Int. J. Emerg. Technol. 2021; 12: 155-60.
18. Vanga UR, Peddinti N. Phytochemical screening, antibacterial, antioxidant and anthelmintic activities of *Suaeda nudiflora* (Willd.) Moq. Int. J. Pharm. Sci. Rev. Res. 2014; 29: 320–327.
19. Lalhriatpuii T. HPTLC fingerprint in herbal drug formulations. In Herbal medicine in India: Indigenous knowledge, practice, innovation and its value. Singapore: Springer Singapore. 2019; 337-362. https://doi.org/10.1007/978-981-13-7248-3_22
20. Jain A, Parashar AK, Nema RK, Narsinghani T. High performance thin layer chromatography (HPTLC): A modern analytical tool for chemical analysis. Cur. Res. in Pharma. Sci. 2014; 8-14.
21. Yang J, Yen HE. Early salt stress effects on the changes in chemical composition in leaves of ice plant and Arabidopsis. A Fourier transform infrared spectroscopy study. Plant Physio. 2002; 130(2): 1032-1042. https://doi.org/10.1104/pp.004325
22. Al-Mohammadi SS. Detection of Cholesterol in *Suaeda Baccata* (Chenopodiaceae). Iraqi. J. Pharma. Sci. 2006; 15(2): 29-36.
23. Salt TA, Adler JH. Diversity of sterol composition in the family Chenopodiaceae. Lipids 1985; 20(9): 594-601. https://doi.org/10.1007/BF02534285
24. Devi DR, Battu GR. Qualitative phytochemical screening and FTIR spectroscopic analysis of *Grewia tilifolia* (vahl) leaf extracts. Int J of Curr Pharma Res. 2019; 11(4): 100-107.
25. Nikalje GC, Kumar J, Nikam TD, Suprasanna P. FT-IR profiling reveals differential response of roots and leaves to salt stress in a halophyte *Sesuvium portulacastrum* (L.) L. Biotech. 2019; 23: 00352. https://doi.org/10.1016/j.btre.2019.e00352
26. Devaki K. HPTLC analysis and in vitro antioxidant activity of aqueous bark extract of *Erythrina variegata* L. Isr. J. Plant Sci. 2016; 63(3): 143-157.

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