ASSESSMENT OF THE HALOPHYTE PLANT (*SALSOLA BARYOSMA*) SECONDARY METABOLITE AND THEIR ANTIMICEOBIAL ACTIVITIES

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Abstract. *Salsola baryosma* is a valuable food source for livestock in Saudi Arabia, notably for camels. *Staphylococcus aureus* is one of the most common agents of infection-related mortality and morbidity worldwide. Therefore, this study aimed to investigate the activity of the aqueous ethanolic extract of *Salsola baryosma* (AEESB) against *Staphylococcus aureus* and to identify the key polyphenol and volatile components using high-performance liquid chromatography (HPLC) and gas chromatography–mass spectroscopy (GC-MS), respectively. The results indicated that AEESB displayed a remarkable activity against *Staphylococcus aureus*, as evidenced by a disc diffusion assay and DNA fragmentation assay. The minimum inhibitory concentration (MIC) was 43 µg/mL. The HPLC analysis of AEESB resulted in the identification of fourteen polyphenolic bioactive compounds, where ferulic acid (3055.87 µg/g), gallic acid (1749.35 µg/g) and caffeic acid (421.89 µg/g) were the major phenolic acids in *Salsola baryosma*. While rutin (911.41 µg/g), naringenin (539.40 µg/g) and daidzein (265.42 µg/g) were the major flavonoids. Subsequent analysis of *Salsola baryosma* by GC-MS revealed thirty volatile components; the major percentages were oleic acid (34.86%), l-(+)-ascorbic acid 2,6-dihexadecanoate (13.25%), and butylated hydroxytoluene (8.24%). In addition, extracts from the aerial parts of *S. baryosma* demonstrated anti-microbial activity against *Staphylococcus aureus*.
Keywords: *Amaranthaceae, polyphenols, volatile compounds, HPLC, GC-MS*

Introduction

Halophytes are a group of plants that thrive in extremely salty environments and are common in desert oases and around the Mediterranean coast. They display remarkable potential to cope with such drastic conditions, partly via the production of a wide array of secondary metabolites such as phenolic and flavonoid compounds, which are used as antioxidants and antimicrobials (Al-Omar et al.,2020). Among halophytic plants are the species of the genus Salsola, a member within the Amaranthaceae family (formerly Chenopodiaceae). Salsola is the most numerous and widespread genus in this family. Plants belonging to the genus Salsola are commonly found in the dry regions of the Middle East, Africa, and Europe. There are many species of this genus that are still employed in the practice of conventional medicine (Murshid et al., 2022). The shrub known as Salsola encrusted In Saudi Arabia, Forssk. (also known as Chenopodium baryosmum, Salsola foetida, Caroxylon imbricata, and Salsola baryosma) is big,

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gloomy, pale, excessively branched, and fast-growing. Salsola comprises 64 species, including Salsola baryosma. Harm, the feed of camels, which has its origins in the Arabic language (Osman et al., 2016). This plant has been shown to offer a wide range of medical and biological properties, including the ability to act as an oral contraceptive, as well as having antioxidant, anti-inflammatory, antidiabetic, diuretic, tyrosinase inhibitory, and central nervous system (CNS)-depressive properties (Osman et al., 2016). Biphenylpropanoids (Oueslati et al., 2017), flavonoids and flavonoid glycosides (Osman et al., 2016), coumarins and coumarin glycosides (Ahmad et al., 2006), and phenolic compounds have all been isolated from this plant as a result of earlier phytochemical investigations (Osman et al., 2016; Shehab and Abu-Gharbieh, 2014). Ahmad et al. (2006) isolated six compounds from Salsola baryosma: scopoletin, bergaptol, daphnoretin, bergaptol 5-O-β-D-glucopyranoside, daphnorin, and chrysoeriol 7-O-β-D-glucopyranoside. S. baryosma was used in folk medicine for treating indigestion, diarrhea, dysentery, itching, sores, colds, improving maleness, asthma, migraines, headaches, and inflammation (Turki, 1999; Klopper and Van Wyk, 2001). It has recently been reported that the ethanolic leaf extract of Salsola imbricata growing in Taif city showed a significant ameliorative role against oxidative stress and hepatic injury induced by acrylamide in animal models (Soliman et al., 2022). Additionally, a number of different compounds including β-sitosterol-3-O-β-D-glucoside, phytolacca cerebroside (4), 1,2-di-O-palmitoyl-3-O-(6-sulfoquinovopyranosyl)-glycerol (5), isorhamnetin-3-robinobioside (6), and isorhamnetin-3-rutinoside have been reported for the first time from the whole plant extract of Salsola imbricate as evidenced by various chemical analyses, including nuclear magnetic resonance (NMR), gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS), chemical analyses including acid hydrolysis and the anisaldehyde solution-detecting reagent (Suleiman et al., 2022). As one of the most prominent bacterial pathogens, Staphylococcus aureus causes millions of severe infections (e.g., pneumonia, cardiovascular, and nosocomial bacteremia) worldwide (Kranjec et al., 2021). Staphylococcus aureus resists most antibiotics, even when applied in combination with other drugs. Excessive use of antibiotics to combat microbial pathogens including Staphylococcus aureus could lead to the appearance of antibiotic resistance. In order to avoid the potential consequences of the spread of antibiotic resistance, it is of a premium importance to focus on the extracts of halophytic wild plants as an alternative strategy to combat pathogenic microbes. Furthermore, little is known about the antimicrobial activities of Salsola baryosma against the potential pathogenic bacterial species, Staphylococcus aureus. Therefore, the aim of the current study was to evaluate the antibacterial activity of AEESB against Staphylococcus aureus and identify the polyphenolic and volatile compounds present in the extract using HPLC and GC–MS methods. The use of AEESB to reduce biofilm development would consequently minimize the emergence of antibiotic resistance and pave the way for the future application of plant extract ingredients for potentially beneficial purposes.

Materials and methods

Plant material

Salsola baryosma Schult. (syn. *Chenopodium baryosmon*, *Salsola foetida* and *Salsola imbricata*) (Migahid, 1978) was collected from Al-Ahsa, Saudi Arabia, in March 2021. The plant specimens were authenticated by Prof. Adel Kamel Youssef

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(Department of Medicinal and Aromatic Plants, Desert Research Center (DRC)), and a voucher specimen was deposited in the herbarium of DRC (CAIH-1098-R). The fresh aerial parts of the plant were cleaned, dried in an oven at 50°C till constant weight (complete dryness) (Müller and Heindl, 2006), then ground to a fine powder and prepared for use in the investigations.

Preparation of the S. baryosma extract (AEESB)

First, 950 g of dried aerial parts of *Salsola baryosma* was extracted with 2600 ml of 70% ethyl alcohol (AEESB) using a Soxhlet apparatus at 80°C. The extraction procedure was continued until the solution became clear. The alcoholic extract was filtered and evaporated under reduced pressure until dry and subsequently weighed (14.1 g) (Lala, 1993).

Disc diffusion assay

The King Faisal University College of Medicine provided the *S. aureus* and *E. coli* bacterial strains. All of the bacterial strains were cultured in nutrient broth (Sigma Aldrich, Cat. no. 7014), and the disc diffusion assay was conducted in accordance with the procedure described in Rad et al., 2021. The samples were grown at 37°C at 200 rpm until their turbidity reached 0.3 at 600 nm. Then, 100 µL of overnight bacterial culture was used to inoculate 10 mL of nutrient broth. The homogeneous bacterial culture containing 5 mL of each bacterial strain was then added to individual nutrient agar plates, and each plate was gently stirred to ensure that the culture was distributed equally on the agar. Four agar plates were inoculated with each bacterial strain. Before the test solution discs were applied to the agar, all inoculated plates were left unsealed in the bio-safety cabinet to allow the remaining liquid to absorb into the agar. A set of four discs of a 6 mm diameter, containing various test solutions, were placed onto the agar surface of each inoculated plate. Three of those discs contained the *S. baryosma* test solutions from the extracts of the aerial parts, while the positive control disc contained 10 µg of Meropenem and was purchased from Condalab, Madrid, Spain. The diameters of the incubation zones were then measured after each plate had been incubated at 37°C overnight.

Determination of the minimum inhibitory concentration (MIC)

The MIC of the AEESB against the potential pathogenic bacteria strain (*Staphylococcus aureus*) was estimated using the agar microdilution method (Tsukatani et al., 2012). Briefly, *Staphylococcus aureus* was cultivated for 24 h at 30°C in Mueller–Hinton broth (MHB, Oxoid, Basingstoke, UK). The MDK33 suspension was adjusted with sterile saline solution until it achieved a concentration of 1x 107 CFU/mL. A 96-well microtiter plate was then filled with 50 µL of MHB and 50 µL of inoculum. A 5 mg/mL stock solution of plant extract in DMSO was prepared, and twofold dilutions (5-500 µg/mL) of the stock plant extract in MHB were well mixed with test bacterial strains in the wells. By adding MHB, the volume in each well was adjusted to 150 µL. The microplates were incubated for 24 h at 30°C in a rotary agitator (160 rpm). For maximum growth, MHB with plant extract was utilized as a negative control, and MHB with inoculum was employed as a positive control. The p-iodonitrotetrazolium (MP Biomedicals, Salon) was used to determine the inhibitory concentration. Bacterial growth was examined after incubation by adding 10 µL of (0.1 mg/mL) p-

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iodonitrotetrazolium violet to each microtiter well and reincubating at 30°C for 1 h. The change in color from violet to orange-red highlighted that the cells were still alive. The inhibitory concentration was similar to the oxidation of metabolically active cells. A Bio-Rad ELISA reader was used to measure the prepared 96-well microtiter plates at 630 nm. The analysis was carried out in triplicate. The MIC value was determined by taking the concentration of the first well that showed no turbidity (Mousavi et al., 2011).

Activity against biofilms

In order to estimate the antibiofilm activity of the AEESB, trypticase soy broth (TSB) amended with 0.1% glucose (w/v) was inoculated with the bacterial strains. After incubation at 30°C for 18 h, cells were harvested by centrifugation at 10,000 rpm for 10 min. Cell pellets were washed with PBS. Exactly 100 µL of the bacterial suspensions (~ OD_{600} = 0.1) in TSB was added to 96-well plates, which were incubated at 37°C for 24 h under static conditions. After the biofilms were incubated with plant extract at the defined concentrations for 1 h, 0.1% crystal violet was added to each well to stain the biofilms for 10 min. Next, 30% (v/v) acetic acid was added to each well to dissolve the crystal violet before measuring the optical density of each sample at 540 nm using a microplate reader (Rayto, Germany) (Stepanovic et al., 2007 and Amin et al., 2022). The experiments were conducted in triplicate.

DNA fragmentation assay

S. aureus cells were inoculated in 12-well plates. After incubation overnight, the medium was replaced with a fresh one, and IC50 doses of test sample extracts were added. After 24 h of incubations, cells were precipitated by centrifugation, washed with PBS, and counted. Then, cells (5×10^7) were treated with lysis buffer (10 mM Tris (pH 8), 10 mM EDTA, and 0.5% Triton X-100) for 2 h at 37°C. Genomic DNA was treated with RNase A (20 µg/mL) for 1 h at 37°C and then mixed with proteinase K (50 µg/mL) for 2 h at 50°C. After phenol, chloroform, and isoamyl alcohol (25:24:1) were used to remove the DNA, the cells were washed with 70% ethanol, left to dry in the air, soaked in distilled water, and separated in a 1.5% agarose gel with 10 mg/mL of EtBr. Under a UV transilluminator, the DNA fragmentation pattern was revealed, and a gel documentation device was used for imaging.

HPLC analysis of polyphenolic compounds in AEESB

HPLC analysis was achieved using an Agilent 1260 series. The separation was carried out using Eclipse C18 column (4.6 mm × 250 mm i.d., 5 µm). Water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) were the components of the mobile phase, which had a flow rate of 0.9 ml/min. The linear gradient was sequentially written into the mobile phase as follows: 12–15 min (82% A), 15–16 min (82% A), and 16–20 min (82% A) are all within the acceptable range. Absorbance was measured at 280 nm with a multi-wavelength detector. For every one of the sample solutions, a 5 µl injection capacity was used. The temperature in the shaft was kept constant at 40°C. All reference standard compounds were of HPLC grade. Gallic acid had a purity of 99%, chlorogenic acid 98%, catechin 98%, methyl gallate 99%, caffeic acid 99%, syringic acid 98%, pyro catechol 98%, rutin 99%, ellagic acid 99%, coumaric acid 98%, vanillin 99%, ferulic acid 99%, naringenin 98%, daidzein 98%, querectin 99%, cinnamic acid 99%, apigenin 98%, kaempferol 99%, and hesperetin 95%.

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The concentration of sample = Area of sample / Area of standard × concentration of standard.

GC/MS analysis of volatile compounds in AEESB

The volatile compounds in AEESB was analyzed using a GCMS-QP2010 SE instrument (SHIMADZU, Japan) with a direct capillary column Rtx–5MS (30 m × 0.25 mm × 0.25 μm film thickness). The temperature of the column oven was initially held at 60°C for 2 min, then increased by 10°C/min to 300°C and held for 4 min. A 300°C injector temperature was maintained. Helium was employed as a carrier gas at a constant flow rate of 2 ml/min. using an AOC-20S autosampler connected to a GC in split mode, 1 μl samples were automatically injected. The m/z 20–500 EI mass spectra were gathered in full scan mode at a 70 eV ionization voltage. Temperatures of 200 and 300°C were chosen for the ion source and transmission lines, respectively. By comparing the components' retention times and mass spectral database of authentic compounds to those of the National Institute of Standards and Technology (NIST 05) library, the components were identified (Olivia et al., 2021). The relative percent amount of each component was calculated by comparing its average peak area to total area. MS solution software, provided by the supplier, was used to control the system and to acquire the data (Olivia et al., 2021).

Statistical analysis

The standard deviation (SD) of three replicates is used to illustrate the data. One-way analysis of variance (ANOVA) was used to determine statistical significance, and the LSD post hoc test was used to compare means in Microsoft Excel. At $p < 0.05$, differences in averages were considered statistically significant.

Results

The inhibitory effects of the AEESB against S. aureus

The effects of various AEESB concentrations on the growth of *S. aureus* are presented (*Table 1; Fig. 1*). The bacterial strain's survival percentage reduced as the plant aqueous ethanolic extract concentration increased. In comparison to the untreated control, good growth was detected at 5 μg/mL of the plant extract, whereas a substantial suppression (78%) was found at 500 μg/mL. Plant extract exhibited substantial antibacterial activity against *S. aureus,* with the minimum inhibitory concentration (MIC) being 43 μg/mL (*Table 1; Fig. 1*).

Table 1. *Characterization of AZC66*

Bacteria	Treatment	Inhibition zone
S. aureus	S1	9.0 [a]
S. aureus	S2	8.0 [a]
S. aureus	S3	7.0 [a]
S. aureus	MEM 10	16.0 [b]

According to the L.S.D. test, means followed by the same letter within a column are not statistically different at the 0.05 level of probability. S1: 100% of aqueous ethanolic extract of *Salsola baryosma* (AEESB), S2: 50% of AEESB concentration and S3: 25% of AEESB concentration

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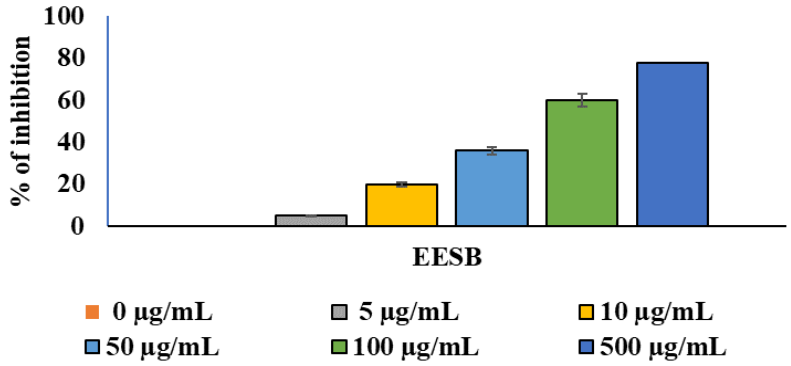

Figure 1. *Effects of different AEESB concentrations (µg/mL) on S. aureus growth. The AEESB concentrations used were 5 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL, and µg/mL. Data are represented by the mean of three replicates ± standard deviation (SD). *: Significant difference at $p < 0.05$*

Crystal violet testing is applied as a method for measuring the production of biofilms (Suleiman et al., 2021). The effects of various concentrations of AEESB (5 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL, and µg/mL) were evaluated, and the results are presented in *Figure 2*. It can be seen that the treated cells exhibited a significantly reduced amount of biofilm development in comparison to the control, as depicted in *Figure 2*. While there was a higher concentration of AEESB, there was a correspondingly lower percentage of biofilm development. When compared to the control, the amount of biofilm formation that occurred following treatment with AEESB at 10 µg/mL, 50 µg/mL, 100 µg/mL, and 500 µg/mL was significantly reduced by 50%, 80%, 92%, and 94%, respectively. Similar antibacterial action was shown by EEBS against existing biofilms (24 h old) generated by *S. aureus*. Biofilm formation of 100% in the control corresponds to an OD530 of 0.48 ± 0.05, where no AEESB was added (0 µg/mL), and the bacterial strain was able to form a biofilm without inhibition. Significant inactivation of biofilms was observed at doses of 50 µg/mL, which was in general accordance with the MIC value (*Fig. 3*).

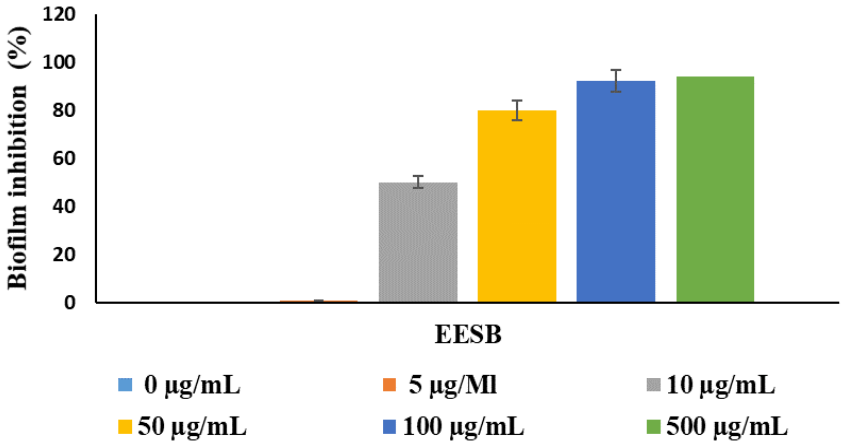

Figure 2. *Effects of different AEESB concentrations (µg/mL) on biofilm formation (%) of S. aureus. Various concentrations of AEESB were used: 5 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL, and µg/m mL. Data are represented by the mean of three replicates ± standard deviation (SD). *: Significant difference at $p < 0.05$*

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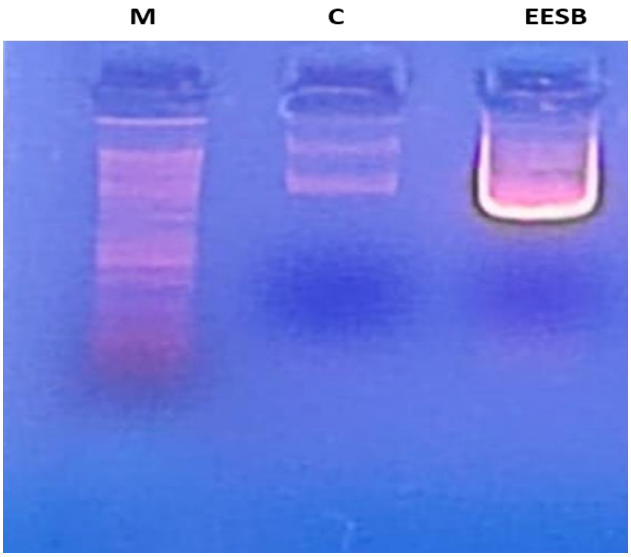

Figure 3. *Effect of the AEESB at the MIC (43 µg/mL) on the DNA integrity of S. aureus. C stands for untreated S. aureus DNA*

DNA fragmentation can be studied using the standard "DNA ladder" technique, in which DNA is taken from apoptotic cells and separated in an agarose gel. As demonstrated in *Figure 3*, treatment of the genomic DNA of *S. aureus* with AEESB at the MIC (43 µg/mL) resulted in chromosomal DNA breakdown into tiny internucleosomal fragments, while the untreated DNA did not breakdown.

Identification of polyphenolic compounds in AEESB using HPLC

According to the findings in *Figure 4* and *Table 2*, the polyphenolic components in the AEESB had significant concentrations of phenolic acids, with ferulic acid (3055.87 µg/g), gallic acid (1749.35 µg/g), caffeic acid (421.89 µg/g), and syringic acid (374.83 µg/g) being the most abundant. Rutin (911.41 µg/g), naringenin (539.40 µg/g), and daidzein (265.42 µg/g) were the major flavonoids.

Identification of volatile compounds in AEESB via GC–MS analysis

The volatile compounds in AEESB, identified using GC–MS analysis, are presented in *Table 3* and *Figure 5*. The data revealed high percentages of oleic acid (34.86%), l-(+)-ascorbic acid 2,6-dihexadecanoate (13.25%), and butylated hydroxytoluene (8.24%), followed by (2-phenyl-1,3-dioxolan-4-yl) methyl -9-octadecenoate (3.7%) and cyclononasiloxane, octadecamethyl (3.55%).

Discussion

Staphylococci are widespread members of the human skin microbiome, but they are also among the leading bacteria responsible for severe infections around the world (Kranjec et al., 2021). Overuse of antibiotics may result in spreading of antibiotic resistance. Alternatively, natural products from wild halophytic plants could combat the pathogenic microbes without adverse impacts on health and environment. In this study, the activity of AEESB against *Staphylococcus aureus* was evaluated using disc

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diffusion, determination of the MIC, biofilm formation, and DNA fragmentation assays. Additionally, the key phenolic and volatile compounds in the AEESB were identified using HPLC and GC–MS, respectively.

Table 2. *Polyphenolic compounds in AEESB using HPLC*

Retention time (min)	Compounds	Conc. (µg/g)	Compounds nature
3.331	Gallic acid	1749.35	Phenolic acid
4.309	Chlorogenic acid	29.26	
4.674	Catechin	139.75	Flavonol
5.508	Methyl gallate	0.00	Phenolic acid
5.768	Caffeic acid	421.89	
6.456	Syringic acid	374.83	
6.691	Pyro catechol	0.00	Phenolic compound
7.968	Rutin	911.41	Flavonoid glycoside
8.636	Ellagic acid	0.00	Phenolic acid
9.054	Coumaric acid	33.22	Phenolic acid
9.665	Vanillin	0.00	Phenolic aldehyde
10.189	Ferulic acid	3055.87	Phenolic acid
10.513	Naringenin	539.40	Flavanone
12.185	Daidzein	265.42	Hydroxyisoflavone
12.446	Querectin	45.29	Flavonol
13.987	Cinnamic acid	27.23	Phenolic acid
14.454	Apigenin	0.00	Flavone
15.055	Kaempferol	11.11	Flavonol
15.559	Hesperetin	20.92	Flavanone

The minimum inhibitory concentration (MIC) and the zone diameter of Inhibition have an inversely proportional relationship. When a bacterium is more susceptible to an antimicrobial agent, the minimum inhibitory concentration (MIC) of that agent is lower, and the zone of inhibition is greater. In contrast, the minimum inhibitory concentration (MIC) will be larger and the zone of inhibition will be smaller the more resistant the microorganism is.

The minimum inhibitory concentration (MIC) of 43 µg/mL for AEESB's antibacterial activity against *Staphylococcus aureus* provided evidence of the above-explained relationship. This MIC value was lower than the value obtained with *S. villosa* and *S. persia* which had MICs of 6.25 mg/mL and 1.56 mg/mL, respectively (Gupta and Guliani, 2022). However, comparable results were obtained by Oueslati (Oueslati et al., 2017), who reported that ethyl acetate extract from the roots of *S. imbricata* displayed a potential antibacterial activity against *Staphylococcus aureus*, as evidenced by the relatively low MIC value (16 ug/mL) (Oueslati et al., 2017). The variabilities in the MIC values could be attributed to a number of factors, including the plant species used, the plant part employed for extraction, the extraction conditions, the type of solvent used, and the bacterial strain tested. Consequently, the chemical composition, including the active ingredients, varied, and the potential modes of action against microbial cells diversified. Furthermore, flavonoids have been proposed to have their antibacterial activities mediated via the inhibition of nucleic acid synthesis,

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cytoplasmic membrane function, energy metabolism, and attachment and biofilm formation, as well as the inhibition of porin on the cell membrane and membrane alteration (Thebti et al., 2023).

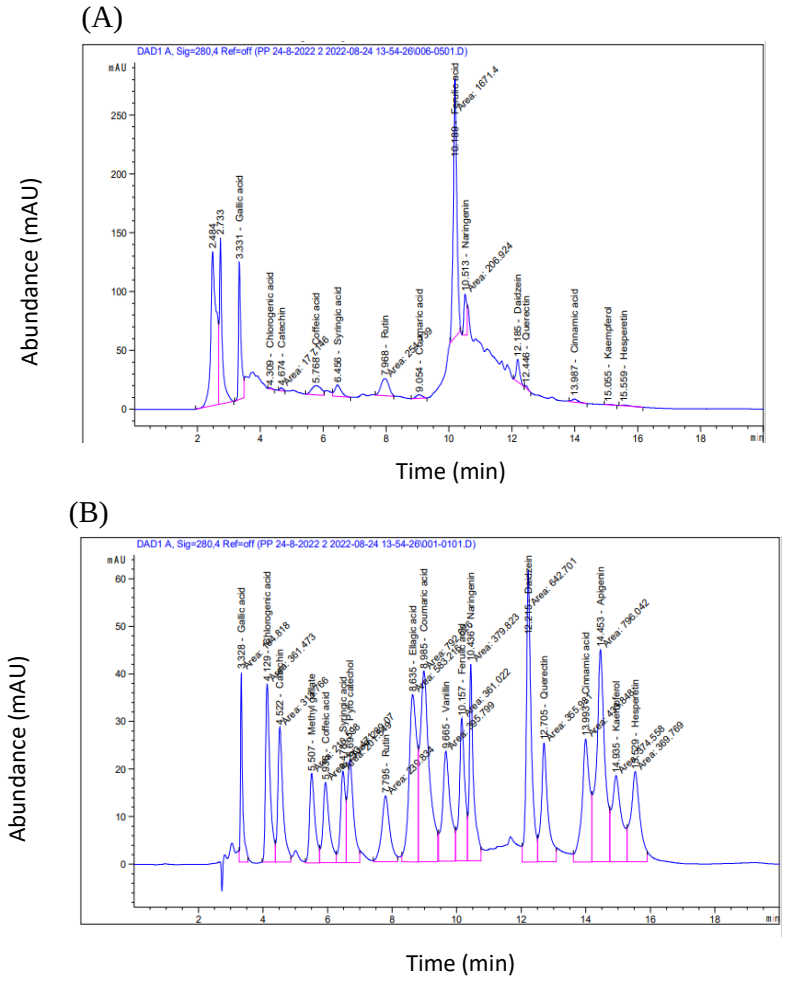

Figure 4. *(A) HPLC profile of the polyphenolic compounds in AEESB. (B) HPLC profile of the reference standard of polyphenolic compounds*

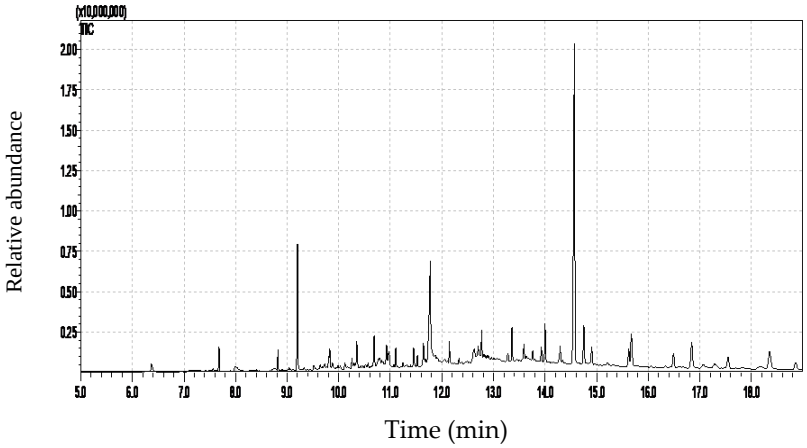

Figure 5. *GC–MS spectral analysis of AEESB*

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Table 3. *GC–MS spectral analysis of AEESB*

Peak No.	R. T. (min.)	Area %	Compounds
1	7.677	1.57	Cyclohexasiloxane, dodecamethyl-
2	8.82	1.12	1,2-Benzenediol
3	9.199	8.24	Butylated Hydroxytoluene
4	9.804	0.83	5.beta.,6.beta.-Epoxy-7.alpha.-bromocholestan-3.beta.-ol
5	9.826	0.97	18-Methyl-nonadecane-1,2-dio, trimethylsilyl ether
6	10.255	0.76	1-Heptadec-1-ynyl-cyclopentanol
7	10.348	2.82	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl-
8	10.686	2.14	Cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5.alpha.)-
9	10.926	1.55	6-Methyl-cyclodec-5-enol
10	10.983	1.54	2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-diol
11	11.105	1.16	2-Pentadecanone, 6,10,14-trimethyl-
12	11.451	1.28	Methoprene
13	11.524	0.8	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.alpha.,7a.beta.,7b.alpha.)]-
14	11.64	1.56	8-Hexadecenal, 14-methyl
15	11.774	13.25	l-(+)-Ascorbic acid 2,6-dihexadecanoate
16	12.144	1.27	18-Methyl-nonadecane-1,2-dio, trimethylsilyl ether
17	12.703	0.5	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione
18	12.769	1.57	Dihydromorphine, di(trimehylsilyl) ether
19	13.357	2.18	Cyclononasiloxane, octadecamethyl-
20	13.589	0.99	2H-Pyran-2-one, tetrahydro-6-tridecyl-
21	13.761	0.61	Tetrapentacontane, 1,54-dibromo-
22	13.927	1.08	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-
23	13.997	2.8	2-Methyl, 3,13-octadecadienl-ol
24	14.291	0.98	2-methyltetracosane
25	14.569	34.86	Oleic Acid
26	14.751	3.55	Cyclononasiloxane, octadecamethyl
27	14.902	1.4	2-methylhexacosane
28	15.622	1.65	Diisooctyl phthalate
29	15.673	3.7	(2-phenyl-1,3-dioxolan-4-yl) methyl -9-octadecenoate
30	16.842	3.27	Glycidyl oleate

Due to the lipophilic nature of certain compounds present in Salsola extract, including ferulic acid one possible explanation is that interference with the cell membrane destroys the membrane's integrity. The selective permeability, efflux pumping systems, ATPase machinery activities, and RNA synthesis of the bacterial cells are adversely affected, leading to bacterial death (Yin et al., 2019).

It has been reported that *Staphylococcus aureus* NorA, tetK and MsrA efflux pumps are blocked, and antibacterial action is conferred by ferulic acid and its esterified derivatives (Pinheiro et al., 2021 and Pinheiro et al., 2022). It is generally accepted that an extrusion mechanism might cause an antibiotic's intracellular concentration to decrease because the drug is ejected from the cell by an energy-dependent process called active efflux, which is mediated by efflux transmembrane protein pumps, conferring drug resistance. However, impairing this efflux system might have implications for the shape and fluidity of cell membranes. All of these mechanisms contribute to the potent antibacterial activities exhibited by these compounds (Song et al., 2023).

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Qualitative phytochemical examination of five halophytes, including *Salsola villosa* in Qassim, revealed the presence of saponins, tannins, sterols, carbohydrates, and flavonoids in all species. *S. villosa* had the greatest total phenolics and total flavonoids (135.2 mg GAE/g and 18.2 mg QE/g) among the five species. *H. bottae* had the most antioxidants, followed by *S. villosa* (IC50 263.7 and 290.7 μg/mL). *S. villosa* also showed high action against *Staphylococcus aureus* (Gupta and Guliani, 2022).

Cell aggregates that attach to an interface or a surface and become entrenched in a self-produced matrix of extracellular components, such as polysaccharides and proteins, are what make up bacterial biofilms (Yadav et al., 2017). The formation of a biofilm is essential to the continued existence of bacteria, particularly in difficult settings such as those that contain antimicrobial chemicals (Yadav et al., 2017). According to the results of our research, AEESB exhibited antibiofilm-forming activity that was equivalent to its antibacterial activity. The development of antibiotic resistance in bacteria has been linked to their ability to form biofilms, rather than their existence as free bacterium. Antimicrobial agents cannot reach bacterial cells when a biofilm is present because the biofilm has the ability to render antimicrobials ineffective (Yadav et al., 2017). It is well-documented that biofilm formation is a multistep process that involves adhesion, maturation/proliferation, and separation. Biofilm-associated *S. aureus* infections also involve nonspecific antibiotic resistance through biofilm development (Kranjec et al., 2021). The use of AEESB to prevent the formation of biofilms would therefore limit the emergence of antibiotic resistance.

One of the most fundamental characteristics of microbial death is DNA fragmentation. The leakage of an intercellular component, DNA, indicates membrane disruption. In comparison to untreated cells, our results demonstrated a definite formation of smear in 72 h of combination treated with bacterial cells. Previous reports indicated that herbal components have demonstrated antibacterial action via DNA fragmentation. For example, the treatment of certain pathogenic bacteria such as *Staphylococcus aureus*, *E. coli*, and *Klebsiella pneumonia* with Caesalpinia coriaria glycosides and flavonoids showed obvious production of damaged DNA. *Syzygium cumini* ethanolic leaf extract has also been demonstrated to have antibacterial activity via an underlying mode of action involving DNA fragmentation. The antibacterial activity of *S. cumini* methanolic seed extract was also demonstrated by DNA fragmentation activity (Yadav et al., 2018). Some carbohydrates, such as chitosan, have also been shown to be effective against *S. aureus* by DNA fragmentation (Song et al., 2023). It is worth mentioning that genomic DNA fragmentation is an irreversible process that kills cells. Endonucleases preferentially cleave linker DNA into mono- and oligonucleosomal DNA fragments.

Our results indicate that *S. baryosma* extract contains the major compounds of the phenolic acids (ferulic acid, gallic acid, caffeic acid, and syringic acid) and flavonoids (rutin, naringenin, and daidzein), as reported in a previous study which found *Salsola imbricata* to have the highest concentrations of phenolics and flavonoids (360 mg/g of the extract as gallic-acid-equivalents/GAE and 70.5 mg/g of the extract as rutin-equivalents/RE) (Al-Omar et al., 2020). In addition, Kaempferol and quercetin were isolated from the root, shoot, and fruit of *S. baryosma* (Yadav et al., 2017). GC–MS analysis also proved that oleic Acid, l-(+)-ascorbic acid 2,6-dihexadecanoate, butylated hydroxytoluene, (2-phenyl-1,3-dioxolan-4-yl) methyl -9-octadecenoate and Cyclononasiloxane, octadecamethyl are the main components in *S. baryosma* extract. These compounds were isolated from *Salsola imbricate* and *S. arabica* in the previous study (Elwekeel et al., 2023).

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Conclusion

This study showed the potential antibacterial activity of the aqueous ethanolic extract of *Salsola baryosma* (AEESB) against *Staphylococcus aureus*, as evidenced by the disc diffusion, biofilm formation, and DNA fragmentation assays. The MIC was 43 μg/mL. The observed antibacterial activity is attributed to the identified polyphenolic and volatile compounds enriched in AEESB. The use of AEESB to prevent biofilm development would thus minimize the emergence of antibiotic resistance and open the door for the future deployment of AEESB constituents for potentially valuable applications.

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