

Article

Chemical Composition, Antioxidant, and Antibacterial Activities of Essential Oil of *Atriplex semibaccata* R.Br. Aerial Parts: First Assessment against Multidrug-Resistant Bacteria

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Abstract: *Atriplex semibaccata* R.Br. is a perennial halophyte that has received much attention for studies of revegetation of marginal lands in arid and semi-arid environments. It was, recently, demonstrated that there are no risks in terms of contamination of essential oil (EO) from growing plant on such land. Interest in exploring the antibacterial and antioxidant potential of *A. semibaccata* EO has consequently been renewed. The objective of this study was to investigate the chemical composition, as well as the antioxidant and antibacterial activities of *A. semibaccata* EO. The antibacterial activity was evaluated against native (drug-sensitive) and multidrug-resistant (MDR) bacteria by testing the EO alone and in combination with conventional antibiotics. The chemical composition of EO was analyzed by gas chromatography/mass spectrometry, 52 chemical compounds were identified, and 2-Methoxy-4-vinyl phenol (48.9%), benzaldehyde (6.7%), and benzyl alcohol (6.3%) were found to be the main constituents of EO. Furthermore, the antioxidant activity was evaluated using a 2,2-diphenyl-1-picrylhydrazyl reducing–scavenging test. The EO from this species possessed high antioxidant activity (938.65 µg TE/g EO). The antibacterial test demonstrated an inhibitory effect on six native and MDR bacterial strains. We found that *Staphylococcus aureus* (Gram+), *Klebsiella pneumoniae* (Gram–), and *Escherichia coli* (Gram–) were more sensitive than MDR strains, with an inhibition zone ranging from 11.16 mm to 12 mm. Moreover, the minimum inhibitory concentration ranged from 3.12 mg/mL to 6.25 mg/mL. The combination of gentamicin and EO revealed a high synergistic effect. The effect on *S. aureus* and *K. pneumoniae* showed lower fractional inhibitory concentration indices of 0.39 and 0.27, respectively. The results also revealed that *A. semibaccata* EO contained compounds with antibacterial potential against MDR bacteria, with antioxidant properties, and with a moderate synergistic effect in combination with gentamicin. The EO from *A. semibaccata* could be considered a new and potential source of natural antioxidant and antibacterial agents. These findings make *A. semibaccata* an excellent choice for the revegetation of marginal lands with the subsequent use of biomass for the production of EO with significant potential in the control of microbial infection.

Keywords: *Atriplex semibaccata*; essential oil; antibacterial; antioxidant; multidrug-resistant bacteria; marginal lands; phosphate mine waste

1. Introduction

The exploitation of marginal and saline land to produce useful biomass has attracted interest worldwide [1–4]. Plant species with valuable biomass in terms of bioenergy, biomaterials, and essential oil (EO) production may play a primary role in the revegetation of these lands, providing environmental and socioeconomic benefits [5–7]. Species *Atriplex* L. genus have been chiefly recommended for the restoration of saline and marginal lands [8].

Over thousands of years, the use of natural products has been part of traditional medicine [9–12]. Several salt-marsh species (*Atriplex* L.) have traditionally been used for medical aims, such as *A. vestita* (Thunb.) Aellen, *A. hortensis* L., *A. halimus* L., *A. confertifolia* (Torr. & Frém.) S. Watson, *A. portulacoides* L., and others [13,14]. Interest in these species is currently increasing due to their eminent content of bioactive compounds [14], such as *A. semibaccata* R.Br.

Given its abundance in arid and semi-arid areas, its abiotic stress tolerance, and its suitability for use in reclamation, the *Atriplex* L. genus is interesting to explore in terms of the antibacterial potential of EO, especially in light of the worldwide spread of multidrug-resistant (MDR) bacteria [15]. MDR bacteria pose an increasing hazard to public health worldwide [16], and bacteria continue to develop resistance to many of the currently available antibacterial drugs [17–19]. However, many plant species have not been screened for antibacterial activity of their EO against such bacteria. Moreover, an avenue that has not been widely explored involves utilizing new pharmaceutical products, which have original and multiple mechanisms of action, synergistically with current agents, which may be more effective against MDR bacteria [20,21]. Importantly, Lal et al. [22] and Zheljzkov et al. [23] demonstrated that EO extracted from vegetal crops grown in contaminated environments were free from the risk of heavy-metal contamination.

A. semibaccata is a perennial Amaranthaceae species [24], originally from Australia and introduced into several regions of the world as a drought- and salt-tolerant forage crop [25]. It became a naturalized plant in Morocco, distributed in the Saharan and middle Atlantic regions, including the Haouz area [25]. *A. semibaccata* is a xero-halophyte species, that tolerates moderate and high salinity (up to 15 dS/m) and considered a pioneer plant in clay and silty loam soils [26].

The current work was undertaken to identify the chemical composition of *A. semibaccata* EO, to evaluate its antioxidant and antibacterial activities against MDR bacteria, and finally, to explore the antibacterial synergistic effect of *A. semibaccata* EO and conventional antibiotics on MDR bacteria. As far as we know, the present novel research investigated the antibacterial activity of *A. semibaccata* OE against MDR bacteria. Moreover, no other prior studies have investigated the synergistic interaction between *A. semibaccata* EO and conventional antibiotics.

2. Materials and Methods

2.1. Plant Material and Essential Oil (EO) Extraction

In March 2019, the aerial biomass (2500 g) of several *A. semibaccata* plants was harvested from an experimental field located at phosphate mine overburdens in the Kettara region, Morocco (470 m above sea level; 31°51'36" N and 8°9'36" W). A specimen of *A. semibaccata* was deposited and conserved under the voucher specimen code MARK-13 000 at the Regional Herbarium "MARK" of the Faculty of Sciences Semlalia, University of Cadi Ayyad, Marrakech, Morocco.

Extraction of *A. semibaccata* EO was carried out four times (4 × 150 g) using the following procedure: The collected aerial biomass (2500 g) was initially air-dried at ≈25 °C for 5 days; thereafter, the dried biomass (1650 g) was subjected to hydrodistillation using

a Clevenger-type apparatus for 4 h. The obtained EO was dried over anhydrous sodium sulphate and stored in darkness at 4 °C until use.

2.2. Gas Chromatography–Mass Spectrometric (GC–MS) Analysis

The EO was analyzed using a Trace GC-MS system from Thermo Scientific™ (Trace 1300 GC, USA), fitted with a TG-5MS column (30 m × 0.25 mm × 0.25 µm) and used in the electron-impact ionization mode. The temperatures of the injector and the detector were set at 230 and 250 °C, respectively, and the electron-impact ionization energy was 70 eV. For analysis, 1 µL of EO was injected in splitless mode into the GC–MS instrument, and helium gas was used as a carrier gas at a flow rate of 1 mL/min. The sample was pre-diluted in acetone at a 1:100 ratio, and the oven temperature was programmed to increase at a rate of 3 °C/min from 60 °C to 230 °C, which was maintained for 10 min. Finally, the chemical components were quantified by external standard method using calibration curves generated by running GC analysis of representative compounds.

2.3. Antioxidant Activity

2.3.1. Free Radical-Scavenging Activity Using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH)

The antioxidant activity of the EO extracted from the aerial parts of *A. semibaccata* was assessed by a 2,2-diphenyl-1-picrylhydrazyl (DPPH) test [27], where 50 µL of the EO diluted at different concentrations in methanol was mixed with 2 mL of methanolic DPPH solution (60 µM). After 20 min of incubation at room temperature in darkness, the absorbance of the samples was measured at 517 nm. A blank containing the same amount of methanol and DPPH solution was used as a negative control, while butylated hydroxytoluene (BHT) and quercetin were used as positive controls. The radical-scavenging activity was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = [(A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100]$$

where A_{blank} is the absorbance of the blank sample (control) and A_{sample} is the absorbance of the EO test sample. The sample concentration providing 50% inhibition (IC₅₀) was calculated by plotting the percentage of inhibition against the concentration of the EO sample ($y = 116.73x - 0.1372$; $R^2 = 0.99$). The analyses were performed in triplicate, and the results were expressed as the mean ± standard deviation (SD). In addition, the radical-scavenging activity was reported as microgram Trolox equivalents per gram of EO (µg TE/g EO).

2.3.2. Reducing-Power Assay

The EO reductive potential was evaluated by following the procedure of Oyaizu [28]. Briefly, 1 mL of different concentrations of samples (EO and control substance) was mixed with phosphate buffer (2.5 mL, 0.2 mM, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was then incubated at 50 °C for 20 min. Then, after incubation, 2.5 mL of trichloroacetic acid 10% was added to stop the reaction. The mixture was centrifuged at 650 × *g* for 10 min. Finally, the supernatant (2.5 mL) was removed and mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride (FeCl₃), and the absorbance was measured at 700 nm. BHT and quercetin were used as positive controls.

The concentration of the sample providing an absorbance of 0.5 (i.e., IC₅₀) was calculated from the graph of the absorbance at 700 nm against sample concentration, and the results were expressed as an average of triplicate measurements.

2.4. Antibacterial Activity

2.4.1. Microorganism Strains

The antibacterial activity of the EO was tested against a panel of pathogenic bacteria namely methicillin-resistant *Staphylococcus aureus* (NCTC 12493), *Escherichia coli* (ATCC 35218), and *Klebsiella pneumoniae* (ATCC 700603), as well as methicillin-sensitive strains of *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *K. pneumoniae* (ATCC 35657),

all of which were provided by the Laboratory of Microbiology and Virology, the Faculty of Medicine and Pharmacy, Cadi Ayyad University.

2.4.2. Antibacterial Screening

The examination of the antibacterial activity of the EO was evaluated using the agar disc-diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) guideline M07-A10 [29]. For this purpose, sterile and saline suspensions at 0.5 McFarland standards were prepared from overnight cultures of the respective bacteria. The bacterial suspension was then streaked on Mueller-Hinton agar plates using a sterile swab. Then, 10 µL of EO at a concentration of 896 mg/mL were applied to sterile filter paper discs (6 mm in diameter) and placed on the surface of the inoculated medium. The plates were maintained at 4 °C for 4 h to allow diffusion of the EO and then incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the growth-inhibition zones after 24 h. Ceftriaxone (30 µg/disc), cefoxitin (30 µg/disc), and gentamicin (15 µg/disc) were used as potent antibiotics for testing MDR bacteria, according to CLSI guideline M02-A12 [30].

2.4.3. Determination of the Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) was determined using the microdilution broth method [31]. A two-fold serial dilution of EO was prepared in 4% dimethyl sulfoxide, and 100 µL of each dilution was added to micro-wells that were previously inoculated with 100 µL of bacterial suspension. The microplates were then incubated for 18–24 h at 37 °C. The MIC was defined as the lowest concentration without visible growth of the tested bacteria, and p-Iodonitrotetrazolium chloride $\geq 97\%$ (Sigma-Aldrich) was used as a microbial growth indicator, while gentamicin was used as a positive control.

2.4.4. Determination of Minimal Bactericidal Concentration (MBC)

The minimal bactericidal concentration (MBC) was determined according to CLSI guideline M07-A10 [29]. In brief, 0.1 mL of the suspension from wells without apparent microbial growth after incubation during MIC tests was spread on Mueller-Hinton agar in Petri dishes. The Petri dishes were then incubated at 37 °C for 24 h. The lowest concentration of EO at which incubated bacteria were completely killed was taken as the MBC.

2.4.5. Synergistic Interaction between EO from *A. Semibaccata* and Conventional Antibacterials

The synergistic effect of *A. semibaccata* EO and the antibacterial agent gentamicin was assessed using a MIC microdilution [21]. This test was achieved using strains that are sensitive to the conventional antibiotic. MICs of antibacterial agents were determined in the presence of EO at a final concentration of MIC/4 for gentamicin. Briefly, 50 µL serial dilutions of gentamicin were added to microwells previously seeded with 100 µL of cell suspension at 10⁸ colony-forming units/mL and containing 50 µL of EO at MIC/4. The microplates were incubated at 37 °C for 18–24 h.

The analysis of the effect of the combination of gentamicin and EO was calculated and expressed in terms of the fractional inhibitory concentration index (FIC_I) using the following formula [32]:

$$\text{FIC}_I = \text{FIC}(\text{EO}) + \text{FIC}(\text{GT})$$

where GT is gentamicin,

$$\text{FIC}(\text{EO}) = \text{MIC of EO in combination with GT} / \text{MIC of EO alone}$$

and

$$\text{FIC}(\text{GT}) = \text{MIC of GT in combination with EO} / \text{MIC of GT alone}$$

To interpret FIC_I , the system proposed by Didry et al. [32] was adopted; that is, total synergism was found when $FIC_I \leq 0.5$, partial synergism when $0.5 < FIC_I \leq 0.75$, no effect when $0.75 < FIC_I \leq 2$, and antagonism when $FIC_I > 2$. The gain in antibacterial activity was also calculated and determined as the ratio of the MIC for gentamicin alone to the MIC for gentamicin in combination with EO.

3. Results and Discussion

3.1. EO Composition

Hydrodistillation of the aerial parts of *A. semibaccata* by the Clevenger-type apparatus yielded a dark green and strong-smelling EO, with a density of 0.9 g/mL, and a freezing point above -21 °C. In addition, the average yield was $0.09 \pm 0.001\%$ (*w/w*) based on dried weight.

The GC–MS of the EO resulted in the identification of 52 compounds, representing approximately 83.3% of the total oil (Table 1). The main compound was 2-methoxy-4-vinylphenol at 48.9%, followed by benzaldehyde (6.8%), benzyl alcohol (6.3%), and o-xylene (2.1%).

Table 1. Chemical composition of essential oil obtained from aerial parts of *Atriplex semibaccata* R.Br. as determined by gas-chromatography–mass-spectrometric analysis.

No.	Compound	Content %	RT	RI Exp.	RI Lit.
1	Benzyl alcohol	6.3	8.3	1040	1037
2	Cyclohexanone	0.4	12.3	945	891
3	3,10-Dioxatricyclo [4.3.1.0(2,4)]dec-7-ene	0.6	7.4	964	964
4	1-Methylcycloheptanol	1.5	10.9	1010	1009
5	Cycloocta-2.5-dien-1-ol	0.2	4.0	1103	1112
6	3,4-Dimethylcyclohexanol	0.7	10.7	1126	1126
7	(2-Bromoethyl)cyclohexane	0.2	27.5	1176	1176
8	Cyclohexanone, 2-(2-butynyl)-	0.1	20.8	1264	1267
9	1-Cyclohexene-1-carboxaldehyde, 5,5-dimethyl-3-oxo	0.1	12.2	1285	1285
10	Bicyclo[3.1.0]hexane-6-methanol,2-hydroxy-1,4,4-trimethyl-	0.2	14.2	1322	1330
11	2-Butanone, 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-	0.5	23.4	1424	1425
12	1,3-Heptadiene, 2,3-dimethyl	0.4	10.2	868	866
13	2,4-Heptadienal, (<i>E,E</i>)-	0.4	7.6	1005	1012
14	Damascenone	0.5	22.1	1338	1820
15	Linalool oxide	1.2	9.6	1067	1061
16	Linalool	0.2	10.6	1098	1099
17	Endo-borneol	0.1	13.1	1165	1162.6
18	<i>m</i> -Cymen-8-ol	0.6	13.9	1176	1182
19	Safranal	0.4	14.5	1212	1207
20	Ascaridole epoxide	0.2	18.7	1220	1234
21	<i>Cis-p</i> -mentha-1(7),8-dien-2-ol	0.1	4.8	1227	1185
22	<i>Cis-p</i> -Mentha-1(7),8-dien-2-ol	0.2	13.3	1231	1175
23	<i>p</i> -Cymen-7-ol	0.3	20.0	1289	1287
24	<i>Trans</i> - α -ionone	0.8	26.2	1422	1430
25	Geranyl acetone	0.2	24.9	1453	1455
26	Ionone	0.3	17.7	1493	1425.6
27	Linalool oxide	0.2	9.7	1513	1446
28	3-Hydroxy- β -damascone	0.2	31.2	1618	1640
29	L-Menthone	0.2	12.7	1148	1136

Table 1. Cont.

No.	Compound	Content %	RT	RI Exp.	RI Lit.
30	Isospathulenol	1.1	22.2	1624	1625.6
31	Hexahydrofarnesyl acetone	0.4	39.9	1697	1833
32	<i>p</i> -Xylene	0.5	4.4	865	863.5
33	<i>o</i> -Xylene	2.1	4.2	890	882.4
34	Benzaldehyde	6.8	6.1	977	976
35	2-Nitroheptenol	0.1	5.6	1127	1147.1
36	2-Methoxy-4-vinylphenol	48.9	9.2	1320	1316.9
37	Vanillin	0.5	22.6	1393	1394
38	Benzene acetaldehyde	0.7	8.7	1036	1043
39	1,2-Benzenedimethanol	1.9	15.2	1392	1385
40	2(4 <i>H</i>)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	0.4	27.8	2466	2316
41	2,3-Pinane diol	0.2	6.9	1244	1244
42	2-Decenal, (E)-	0.3	5.3	1260	1259
43	2-Decen-1-ol, (E)-	0.1	5.0	1268	1273.3
44	Edu lan II	0.2	16.8	1328	1326
45	Megastigmatrienone	0.3	29.8	1435	1455
46	Diethyl phthalate	0.3	30.5	1590	1563
47	2 <i>H</i> -Pyran, tetrahydro-2-(12-pentadecynyl oxy)-	0.3	9.0	2245	-
48	Maleimide	0.3	15.7	2602	2244
49	Dihydroedulan II	0.2	18.3	1496	1526
50	Eicosatetraenoic acid, phenylmethyl ester	0.1	7.8	2270	3003
	Other compounds	16.7	-	-	
	Alcohol [No. 1]				
	Alicyclic hydrocarbons [No. 2–12]				
	Aliphatic hydrocarbons [No. 13]				
	Oxygenated monoterpene hydrocarbons [No. 14–29]				
	Oxygenated sesquiterpenes [No. 30–31]				
	Phenolic compound [No. 32–39]				
	Others [No. 40–50]				

RT: Retention times on DB-5 column; RI exp.: Retention index relative to C9-C22 *n*-alkanes on DB-5 column; RI lit.: Retention indices reported in the literature taken from NIST 08.

The chemical analysis of *A. semibaccata* EO revealed the major presence of 2-methoxy-4-vinylphenol, which, as far as we know, has never been found in EO from other plants of this genus. This compound is a phenolic derivative, exerting a potent anti-inflammatory effect, and it can block the growth of mammalian cells by arresting the cell cycle [33,34]. In another study, EOs from *A. semibaccata* and *A. undulata* (Moq.) D. Dietr. were found to have three compounds in common: 3-Hydroxy-beta-damascone, beta-ionone, and vanillin [35]. Boutaoui et al. [35] demonstrated that extracts from aerial parts of *A. mollis* Desf. contained vanillin, and Chouitah et al. [35] showed that the EO from *A. lentiformis* (Torr.) S. Wats. contained linalool and 2,3-pinane diol. We also found some of these compounds as minor components of the EO from *A. semibaccata*.

Furthermore, from *A. hortensis* leaves, Bylka et al. [36] isolated rare sulphated flavonoids, and Bylka [37] succeeded in isolating new acetylated flavonol glycosides from *A. littoralis* L. In addition, a previous study indicated the presence of naringin, naringenin 7-O-glucoside, isorhamnetin 3-O-rhamnosyl (1-6) glucopyranoside, and isorhamnetin 7-O-glucopyranoside in *A. farinose* Forssk. [38]. More recently, Awaad et al. [39] isolated two new flavonoids from *A. lentiformis* (Torr.) S. Wats., namely, quercetin 6,4'-dimethoxy-3-

fructo-rhamnoside and quercetin 4'-methoxy-3-fructo-rhamnoside. According to the same authors, all six compounds exhibited antioxidant activity.

3.2. Antioxidant Activity

Table 2 presents the results regarding the assay of antioxidant activity of *A. semibaccata* EO. The EO from *A. semibaccata* displayed high radical-scavenging activity (450 ± 3.39 $\mu\text{g}/\text{mL}$ EO) compared to ethanol and chloroform extracts of *A. lindleyi* Moq., (345.70–332.46 mg/mL) [40]. However, our results are consistent with those provided by Kamal et al. [41] for *A. laciniata* L. This activity was found to be less impressive than that of quercetin and BHT (IC₅₀ values of 1.07 ± 0.01 $\mu\text{g}/\text{mL}$ and 4.21 ± 0.08 $\mu\text{g}/\text{mL}$, respectively).

Table 2. Antioxidant activity of essential oil (EO) from aerial parts of *Atriplex semibaccata* R.Br by three different assays.

	DPPH		
	IC ₅₀ ($\mu\text{g}/\text{mL}$)	$\mu\text{g TE}/\text{g EO}$	Reducing Power (IC ₅₀ , $\mu\text{g}/\text{mL}$)
EO	450 ± 3.39	938.65 ± 9.68	84 ± 2.5
BHT	4.21 ± 0.08	—	7.09 ± 0.1
Quercetin	1.07 ± 0.01	—	2.29 ± 0.1

BHT: Butylated hydroxytoluene; DPPH: 2,2-diphenyl-1-picrylhydrazyl; IC₅₀: concentration providing 50% inhibition; TE: Trolox equivalent. Values represent the average \pm standard deviations for triplicate analyses.

Aissi et al. [42] reported that EO from *Pistacia lentiscus* L. (Anacardiaceae) had high activity (993.4 $\mu\text{g TE}/\text{g EO}$), which is close to the value obtained for EO from *A. semibaccata* (938.65 $\mu\text{g TE}/\text{g EO}$). Furthermore, Awaad et al. [39], Benhammou et al. [43], Gamal et al. [44], and Souda et al. [40] reported that extracts from several species belonging to *Atriplex* L. For example *A. farinosa* Forssk., *A. nummularia* Lindl., *A. lindleyi*, *A. lentiformis* (Torr.) S. Watson, and *A. halimus* exhibited antioxidant activity.

3.3. Antibacterial Activity

The antibacterial properties of *A. semibaccata* EO and conventional antibiotics were investigated against six pathogenic bacterial strains, including MDR strains (Figure 1 and Table 3). The findings disclosed that the EO of *A. semibaccata* had an antibacterial effect to different degrees on all the tested strains, including MDR strains, albeit to different degrees. The diameters of the inhibition zones lay between 11.16 ± 0.76 mm and 20.66 ± 0.57 mm, whereas the conventional antibiotics did not display any activity against the MDR strains.

On the basis of the results reported in Table 4 and Figure 2, the MIC and MBC values for *A. semibaccata* EO were in the range of 3.12 to 6.25 mg/mL. Native bacteria were found to be more sensitive than MDR bacteria, with an appropriate MIC of 3 mg/mL. Concerning the MDR bacteria, methicillin-resistant *S. aureus* and *K. pneumoniae* were inhibited at a MIC and an MBC of 6.25 mg/mL, while the EO repressed the growth of *E. coli* at an MIC value of 3.12 mg/mL. The inactivity of gentamicin against the MDR strains is explained by the resistance of these strains to this agent [45], taking into account that for sensitive strains to gentamicin, MICs start from 2 $\mu\text{g}/\text{mL}$. The chemical architecture of the bacterial cell membrane is the main factor involved in its responding negatively or positively to antibacterial agents [45].

The present study demonstrates promising results since the MIC values were found to be equal to the MBC values, indicating a bactericidal effect on both native and methicillin-resistant *S. aureus* and *K. pneumoniae*, and native *E. coli* (ATCC 25922).

According to Chambers and Deleo [45], and Garcia-Alvarez et al. [45], the resistance of methicillin-resistant *S. aureus* is essentially related to the production of an auxiliary penicillin-binding protein, PBP2a, which renders it resistant to all β -lactams, except for the novel class of cephalosporins. Previous findings suggested that an outbreak of infection with *K. pneumoniae* occurred as a result of the generation of the production of extended-spectrum β -lactamase (ESBL) [46]. ESBL plays the main role in increasing the antibacterial

resistance of *K. pneumoniae* [47]. Despite the multitude of antibiotic types that have been developed, the molecular mechanisms of *K. pneumoniae*'s resistance to antibacterial drugs remain unclear and need to be elucidated [48,49].

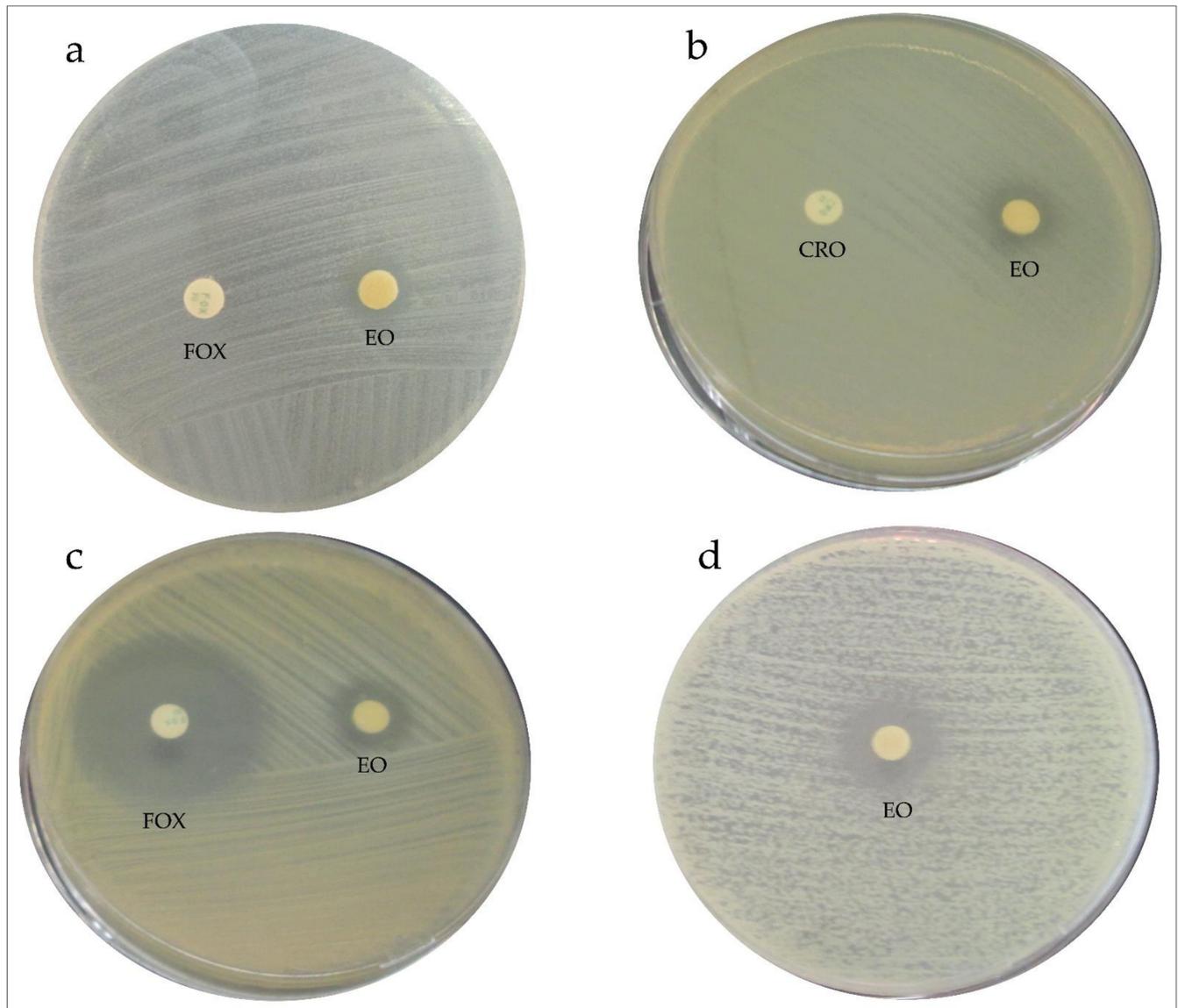


Figure 1. Antibacterial activity of essential oil (EO) from *Atriplex semibaccata* R.Br. compared with ceftriaxone (CRO) and cefoxitin (FOX) against (a) methicillin-resistant *Staphylococcus aureus* (NCTC 12493), (b) *Escherichia coli* (ATCC 35218), (c) native *S. aureus* (ATCC 25923), and (d) *Klebsiella pneumoniae* (ATCC 700603) on Mueller-Hinton agar.

To the best of our knowledge, this is pioneering research that examined the antibacterial potential of *A. semibaccata* EO against MDR bacteria. Therefore, our results can only be compared and discussed regarding closely related species. Benzarti et al. [50] reported that *A. semibaccata* was previously tested as an antifungal agent. Moreover, according to Siddiqui et al. [13] and Ksouri et al. [14], numerous species of *Atriplex* L., such as *A. hortensis*, *A. canescens* (Pursh) Nutt (\equiv *A. fruticosa* Nutt.ex Moq), *A. lindleyi* subsp. *inflata* (F.Muell.) Paul G. Wilson (\equiv *A. inflata* F.Muell.), *A. muricata* Humb. & Bonpl. Ex Willd. (\equiv *A. parvifolia* Kunth.), *A. undulata*, *A. vestita*, and *A. portulacoides*, have been reported as sources of antifungal, antiviral, and antibacterial compounds through their extracts (e.g., in EO) or their chemical constituents.

Table 3. Antibacterial activity of essential oil from *Atriplex semibaccata* R.Br. and antibiotics using the disc-diffusion method.

Microorganism	Designated Strain Code	Essential Oil (10 µL/disc)	Diameter of Inhibition Zone (mm)			
			Cefoxitin (30 µg/disc)	Ceftriaxone (30 µg/disc)	Gentamicin (15 µg/disc)	
Native	<i>Staphylococcus aureus</i>	ATCC 25923	20.66 ± 0.57	32 ± 0	NT	28 ± 1
	<i>Klebsiella pneumoniae</i>	ATCC 35657	15 ± 1	NT	26 ± 1	NI
	<i>Escherichia coli</i>	ATCC 25922	15.33 ± 0.57	NT	27 ± 0	24.3 ± 0.4
Methicillin-resistant	<i>Staphylococcus aureus</i>	NCTC 12493	11.16 ± 0.76	NI	NT	NI
MDR	<i>Klebsiella pneumoniae</i>	ATCC 700603	11.73 ± 0.64	NT	NI	NI
	<i>Escherichia coli</i>	ATCC 35218	12 ± 0.45	NT	NI	NI

ATCC: American Type Culture Collection; NCTC: National Collection of Type Cultures (Public Health England); NI: No inhibition, NT: not tested; MDR: multidrug-resistant. Diameter of inhibition zone includes the disc diameter (6 mm). Values are expressed as the mean ± standard deviation.

Table 4. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) for the antibiotic gentamicin and *Atriplex semibaccata* R.Br. essential oil against different strains of pathogenic bacteria.

Microorganism	Designated Strain Code	Essential Oil		Gentamicin		
		MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	
Native	<i>Staphylococcus aureus</i>	ATCC 25923	3.12	3.12	0.01	0.01
	<i>Klebsiella pneumoniae</i>	ATCC 35657	3.12	3.12	0.04	0.04
	<i>Escherichia coli</i>	ATCC 25922	3.12	3.12	0.02	0.02
Methicillin-resistant	<i>Staphylococcus aureus</i>	NCTC 12493	6.25	6.25	-	-
MDR	<i>Klebsiella pneumoniae</i>	ATCC 700603	6.25	6.25	-	-
	<i>Escherichia coli</i>	ATCC 35218	3.12	6.25	-	-

ATCC: American Type Culture Collection; NCTC: National Collection of Type Cultures (Public Health England); MDR: multidrug-resistant; (-): inactive.

The antibacterial potency of *A. semibaccata* EO might be explained by the fact that its main compound, 2-methoxy-4-vinylphenol, has antibacterial potency [51,52]. Furthermore, the significant presence of other chemical components such as benzaldehyde and benzyl alcohol, also contributes to its antibacterial properties [53–55]. Benzaldehyde has been reported to have a bactericidal effect on human pathogens [56,57]. Moreover, benzyl alcohol is one of most frequently employed antibacterial preservatives in commercial peptide and protein products [55,58].

3.4. Synergistic Interactions between *A. semibaccata* EO and Conventional Antibiotics

Drug synergism between conventional antibacterial agents and plant EOs is a new approach to defeating the defense systems of microorganisms [21,59]. For this reason, our research attempted to explore potential interactions between EO of *A. semibaccata* and gentamicin as a conventional antibiotic.

The antibacterial effects of the EO with combined conventional antibiotics on selected pathogenic bacteria were explored by the checkboard method, and the results are presented in Table 5. The FIC and the FIC_I were calculated to evaluate the synergistic activity of the EO in combination with gentamicin. The gain reported in the MIC of gentamicin in

combination with *A. semibaccata* EO is also summarized in Table 5. Gentamicin exhibited a strong synergistic interaction with *A. semibaccata* EO, achieving a gain of four-fold for native strains of both *S. aureus* ($FIC_I = 0.39$) and *K. pneumoniae* ($FIC_I = 0.27$; Table 5).

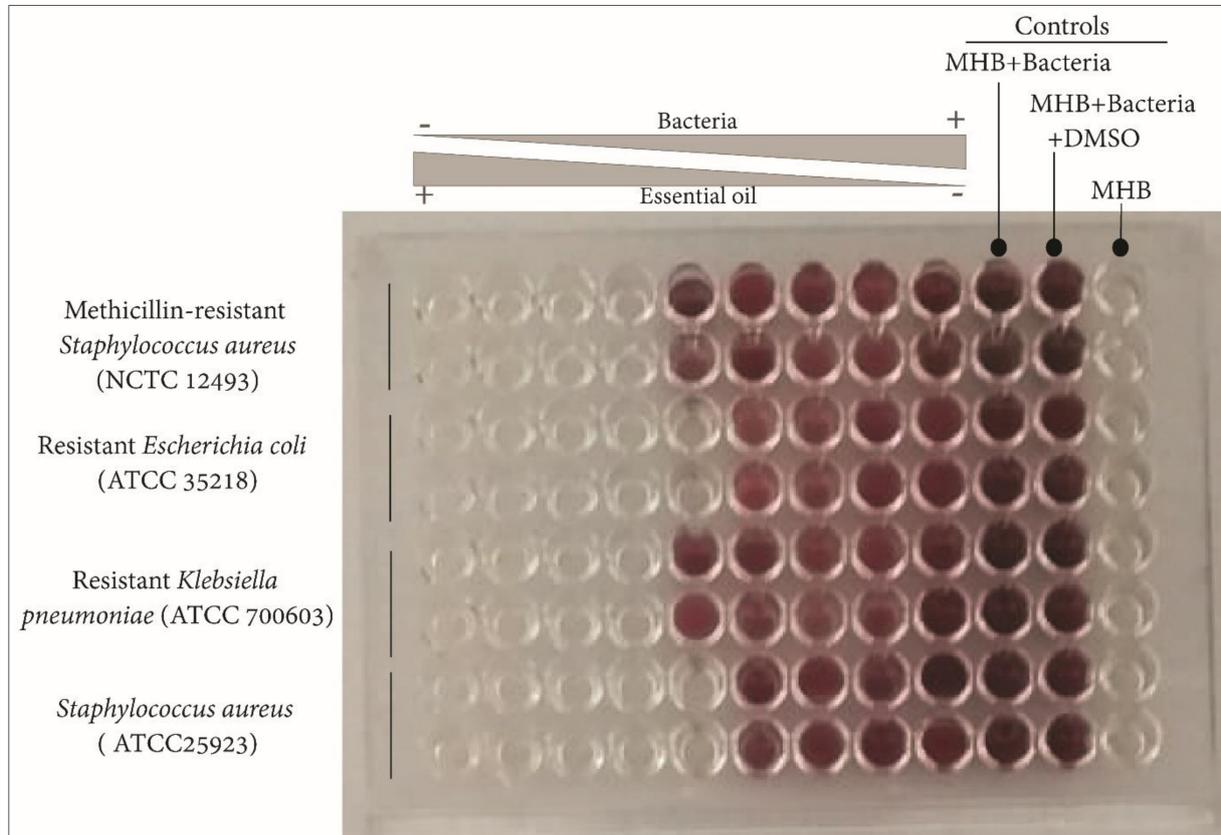


Figure 2. Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using the microdilution method. DMSO: Dimethyl sulfoxide; MHB: Mueller-Hinton broth.

Table 5. Synergistic interaction between *Atriplex semibaccata* R.Br. essential oil and the antibiotic gentamicin against selected pathogenic bacteria.

	Microorganism	Designated Strain Code	FIC_I	Gain
Native	<i>Staphylococcus aureus</i>	ATCC 25923	0.39 ^a	4
	<i>Klebsiella pneumoniae</i>	ATCC 35657	0.27 ^a	4
	<i>Escherichia coli</i>	ATCC 25922	1.33 ^b	3.33

ATCC: American Type Culture Collection; FIC_I : fractional inhibitory concentration index. ^a Complete synergism; ^b no effect. Gain: The ratio of the minimal inhibitory concentration for gentamicin alone to that for gentamicin in combination with essential oil.

Results obtained here cannot be compared to other authors' findings because, as far as we are aware, no previous study has investigated the synergistic interaction between the EO from *A. semibaccata* and an antibiotic. The present study demonstrated that the interaction between the EO of *A. semibaccata* and a standard antibiotic (gentamicin) was notably effective using lower doses (MIC/4). EO of *A. semibaccata* therefore offers high potential for the development of further antibacterial agents for use in the treatment of certain diseases [60].

EOs have been found to act in different ways at multiple levels, and microorganisms have been found to be incapable of overcoming the antibacterial activity of EOs, unlike when they are treated with many conventional antibacterial, which have only one restricted site or mechanism of action [61,62]. Furthermore, numerous authors have demonstrated

that the antibacterial activity of EOs in combination with other compounds is more effective than that of the individual constituents alone [21,63–65]. These combinations reduced the minimum efficient dose of an antibiotic [66].

4. Conclusions

This study found that the EO obtained from the aerial parts of *A. semibaccata* had antioxidant and antibacterial activities against MDR bacteria. The results also confirm that the combination of EO and gentamicin, as a classic antibiotic, has a synergistic interaction against bacterial strains, despite not having clinically relevant effects. Furthermore, this EO was found to be rich in bioactive compounds, mainly, 2-methoxy-4-vinylphenol, and a naturally occurring phenolic compound with potent properties. However, future research on the chemical composition of EO of *A. semibaccata* should consider the potential effects of a multitude of parameters, given that it depends on geographical location, genetic factors, plant material, climate, soil, harvesting period, and method of storage and extraction. Although *A. semibaccata* R.Br. is well adapted to arid and semi-arid climatic conditions, and the moderate antibacterial activities of its EO were demonstrated in vitro, future in vivo investigations are necessary to validate these findings, by testing the EO and the cytotoxicity of its major components at different concentrations on several cell lines to confirm its effectiveness and safety.

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