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# Phytochemical Profile and Insecticidal Potential of Two Halophytes, *Atriplex halimus* and *Suaeda fruticosa*, Against *Tribolium confusum*: In Vitro and In Silico Approaches

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## ABSTRACT

This study aimed to investigate the phytochemical profile and evaluate the in vitro and in silico insecticidal activities of methanolic extracts from two halophytes, *Atriplex halimus* (*A. halimus*) and *Suaeda fruticosa* (*S. fruticosa*). The investigations included phytochemical screening, determination of total phenolic, flavonoid, and tannin contents, as well as compound identification by high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (HPLC-ESI-QTOF-MS). The insecticidal activity was assessed through direct-contact bioassays against adults of *Tribolium confusum*. In addition, a molecular docking study was performed targeting the acetylcholinesterase enzyme (AChE). Phytochemical screening revealed the presence of several bioactive compounds in both plant extracts. *A. halimus* showed the highest flavonoid content, whereas *S. fruticosa* exhibited the highest levels of total phenolics and tannins, corresponding to superior insecticidal effect, as evidenced by its lower lethal concentration 50% (LC<sub>50</sub>) value after 96 h of treatment. HPLC-ESI-QTOF-MS analysis in negative ion mode led to the identification of 24 bioactive constituents in *A. halimus* and 29 in *S. fruticosa*. The in silico study confirmed that most of the identified polyphenols exhibited binding affinity toward insect acetylcholinesterase, including kaempferol 3-O-rutinoside-7-O-glucoside, naringin, feruloyltyramine, and rutin.

## 1 | Introduction

In Algeria, cereal production in 2024 was estimated at approximately 4.1 million tonnes, close to the national average, reflecting the impact of uneven climatic conditions throughout

the growing season [1]. Despite their economic importance, cereal crops remain highly vulnerable to numerous insect pests, which can cause substantial damage and yield losses [2]. Among the most destructive storage pests is *Tribolium confusum* (Duval) (Coleoptera: Tenebrionidae), commonly known as the confused

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flour beetle. This species infests stored cereals worldwide, proliferates rapidly on grains, and renders them unsuitable for consumption [3].

To control storage pests, chemical insecticides are widely used due to their rapid and effective action. However, intensive application raises concerns regarding human health, environmental safety, and the development of insect resistance [4]. Consequently, attention has shifted toward safer and more eco-friendly alternatives, particularly plant-derived insecticides, which are recognized as promising tools for sustainable pest management [3].

The extensive saline ecosystems along Algeria's Mediterranean coast and inland regions, including sebkhas and chotts, are extreme environments where high summer evaporation leads to salt accumulation at the soil surface, severely limiting plant growth [5]. Nevertheless, halophytes have evolved remarkable physiological and biochemical adaptations, allowing them to thrive under such harsh conditions [6]. These plants are valued for diverse applications, including human nutrition, phytotherapy, animal feed, oil extraction, and fiber production [7]. Importantly, halophytes synthesize a wide range of secondary metabolites, particularly polyphenols, which are known for their insecticidal properties [8, 9].

*Atriplex halimus* L. and *Suaeda fruticosa* Forssk. Ex J.F. Gmel are two halophytic medicinal species of the *Chenopodiaceae* (currently *Amaranthaceae*) family, widely distributed across arid and semi-arid Mediterranean regions [10, 11]. Previous studies have reported diverse biological activities for these species, including antioxidant, antimicrobial, antifungal, anti-inflammatory, anticancer, and antidiabetic effects [12, 13].

To date, the insecticidal potential of these halophyte species in the studied region remains largely unexplored, and the available data are still limited. Therefore, the present study was designed to investigate the phytochemical profile and insecticidal potential of aqueous-methanolic extracts of *S. fruticosa* and *A. halimus*. The phytochemical characterization included preliminary phytochemical screening and the quantification of total phenolic, flavonoid, and tannin contents. Furthermore, the identification of bioactive compounds was performed using high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (HPLC-ESI-QTOF-MS). The insecticidal activity of the extracts was evaluated against *T. confusum*. In addition, to elucidate the interaction patterns and possible mechanisms underlying the observed biological activity, the major phytochemicals tentatively identified in the extracts were further investigated through *in silico* molecular docking studies. This approach provides valuable insights into the potential development of natural botanical insecticides derived from halophytes.

## 2 | Results and Discussion

### 2.1 | Phytochemical Screening

The qualitative phytochemical analysis (Table 1) revealed that the methanolic leaf extracts of *A. halimus* and *S. fruticosa* contained

a diverse range of bioactive secondary metabolites, with marked differences in their distribution and intensity. Both species tested positive for alkaloids, phenolic compounds, flavonoids, tannins, and saponins. *S. fruticosa* generally exhibited more intense reactions than *A. halimus*, particularly for phenolic compounds and tannins. Coumarins were exclusively detected in *S. fruticosa*, while glycosides were present only in *A. halimus*. Steroids were notably absent in both species. These findings are consistent with previous phytochemical characterizations as reported by [14, 15].

### 2.2 | Total Phenolic, Flavonoid, and Tannin Contents

Quantitative phytochemical analysis performed on methanolic extracts showed noticeable variation among the studied species (Table 2). *S. fruticosa* was distinguished by a marked richness in total phenolic content ( $119.33 \pm 7.24$  mg GAE/g DW) and tannin content ( $4.51 \pm 0.09$  mg CE/g DW), clearly exceeding those found in *A. halimus* extract ( $22.23 \pm 0.89$  mg GAE/g and  $3.53 \pm 0.15$  mg CE/g, respectively). In contrast, *A. halimus* exhibited a higher flavonoid concentration, reaching  $73.07 \pm 0.19$  mg QE/g, compared with  $63.92 \pm 0.011$  mg QE/g in *S. fruticosa*.

In the present study, *A. halimus* extract collected from Relizane presented a higher total phenolic content than that previously reported for the same species from Bechar [15, 16], but lower than the values obtained from ethanolic leaf harvested in Sig, Mazagran, and Biskra [17]. The total flavonoid content was relatively comparable to values reported by [13, 18], whereas the total tannin content remained lower than those described in the same studies [13, 18]. For *S. fruticosa*, both total phenolic and flavonoid contents were higher than those reported in samples collected from Oran and El-Oued (Algeria) [19, 20]. Furthermore, the tannin content reported by [19] was also lower than that obtained in the present study. The variations observed between our results and previous findings may be attributed to several factors, including genetic variability, environmental conditions such as salinity, light intensity, water availability, and soil characteristics, as well as differences in ecological and climatic conditions [16, 17]. Furthermore, harvest season, extraction methods, solvent polarity, and laboratory procedures may significantly influence both the yield and composition of extracted bioactive compounds [15].

### 2.3 | Analysis of Polyphenols by HPLC-QTOF-ESI-MS

Figure 1A,B and Tables 3 and 4 summarize the base peak chromatograms and the characteristics of the compounds identified in *A. halimus* and *S. fruticosa* extracts, including molecular formulae, retention times, molecular ions ( $m/z$ ), and molecular masses. Metabolic profiling revealed a high level of chemical diversity, highlighting both shared and species-specific metabolites. Based on QTOF-MS analysis combined with authentic standards, specialized databases (PhenolExplorer and CAS SciFinder), and previously reported literature data [21, 22], a total of 24 compounds were identified in *A. halimus* and 29 compounds in *S. fruticosa*. In addition to secondary metabolites, several primary metabolites were detected, such as sugars

**TABLE 1** | Phytochemical screening of methanolic leaf extracts of *A. halimus* and *S. fruticosa*.

Phytochemical compositions	<i>A. halimus</i>	<i>S. fruticosa</i>
Alkaloids	+ <sup>b</sup>	++ <sup>c</sup>
Reducing sugars	- <sup>a</sup>	++
Glycosides	+	-
Proteins and Amino acids	+++ <sup>d</sup>	+++
Phenolic compounds	++	+++
Flavonoids	+++	++
Tannins	++	+++
Saponins	++	+++
Steroids	-	-
Coumarins	-	+

<sup>a</sup>Absence.

<sup>b</sup>Low presence.

<sup>c</sup>Moderate presence.

<sup>d</sup>Strong presence

**TABLE 2** | Total phenolic, flavonoid, and tannin contents in methanolic extracts of the studied plant species.

	Phenolic content (mg GAE/g DW) <sup>a</sup>	Flavonoid content (mg QE/g DW) <sup>b</sup>	Tannin content (mg CE/g DW) <sup>c</sup>
<i>A. halimus</i>	22.23 ± 0.89 <sup>a</sup>	73.07 ± 0.19 <sup>b</sup>	3.53 ± 0.15 <sup>a</sup>
<i>S. fruticosa</i>	119.33 ± 7.24 <sup>b</sup>	63.92 ± 0.01 <sup>a</sup>	4.51 ± 0.09 <sup>b</sup>

Data are presented as the mean of three replicates ± standard deviation. Means followed by different letters (a and b) are significantly different with ( $p \leq 0.05$ ).

<sup>a</sup>Milligrams of Gallic acid equivalents per gram of dry weight.

<sup>b</sup>Milligrams of quercetin equivalents per gram of dry weight.

<sup>c</sup>Milligrams of catechin equivalents per gram of dry weight.

(glucose and D-sedoheptulose), amino acid derivatives (N-acetyl-L-phenylalanine), and lipids (palmitoleic, azelaic, malyngic, coronaric acids, and cratenacin), reflecting essential physiological and metabolic functions. Nine metabolites were common to both species, including gluconic, malic, citric, and succinic acids, pinellic, salicylic, and 17-hydroxylinolenic acids, oxo-tridecanoic acid sulphate, and monomethyl embelin, suggesting the presence of a shared metabolic core potentially associated with adaptation to similar environmental conditions. Despite these similarities, pronounced differences were observed in secondary metabolite composition, particularly among phenolic compounds. *S. fruticosa* exhibited a pronounced enrichment in phenolics, representing more than 50% of the identified metabolites. This abundance included several phenolic acids, such as sinapic, *p*-coumaric, and protocatechuic acids, as well as multiple caffeic acid derivatives, notably diacetyl caffeic acid and fertaric acid.

In addition, kaempferol and quercetin were mainly detected in glycosylated forms (rutin, quercetin 3-O-sambubioside, kaempferol 3-O-rutinoside-7-O-glucoside, and isorhamnetin-3-O-glucoside), along with the flavanone naringenin, further highlighting the flavonoid richness of the extract. These findings differ from those reported by [20], who identified gallic acid, vanillic acid, caffeic acid, *p*-coumaric acid, vanillin, rutin, and quercetin in extracts of the same species. Such differences may be attributed to variations in environmental conditions, genetic diversity among plant populations, and metabolic differences

associated with species adaptation within the *Chenopodiaceae* family [20].

In contrast, *A. halimus* exhibited a comparatively less diverse phenolic profile, mainly characterized by caffeic acid 3-sulfate, dihydroxybenzoic acid derivatives, and protocatechuic acid 4-glucoside. Hydrated quercetin was identified as the predominant flavonoid in this species. These observations are consistent with previous reports [23], supporting the hypothesis that *A. halimus* possesses a distinct secondary metabolite pattern compared with *S. fruticosa*.

The presence of kaempferol, rutin, and quercetin in our samples is of particular interest, given their documented insecticidal properties. These compounds have been reported to interfere with insect development and feeding behavior, especially in the control of insect larvae, which represent the most destructive stage for crops [24].

## 2.4 | Insecticidal Activity

Figure 2A,B (A,B) illustrates the temporal evolution of mortality in adult populations of *T. confusum* exposed to different concentrations of hydromethanolic plant extracts. The results showed that both plant extracts induced significantly higher mortality rates compared with the control treatment. The highest mortality

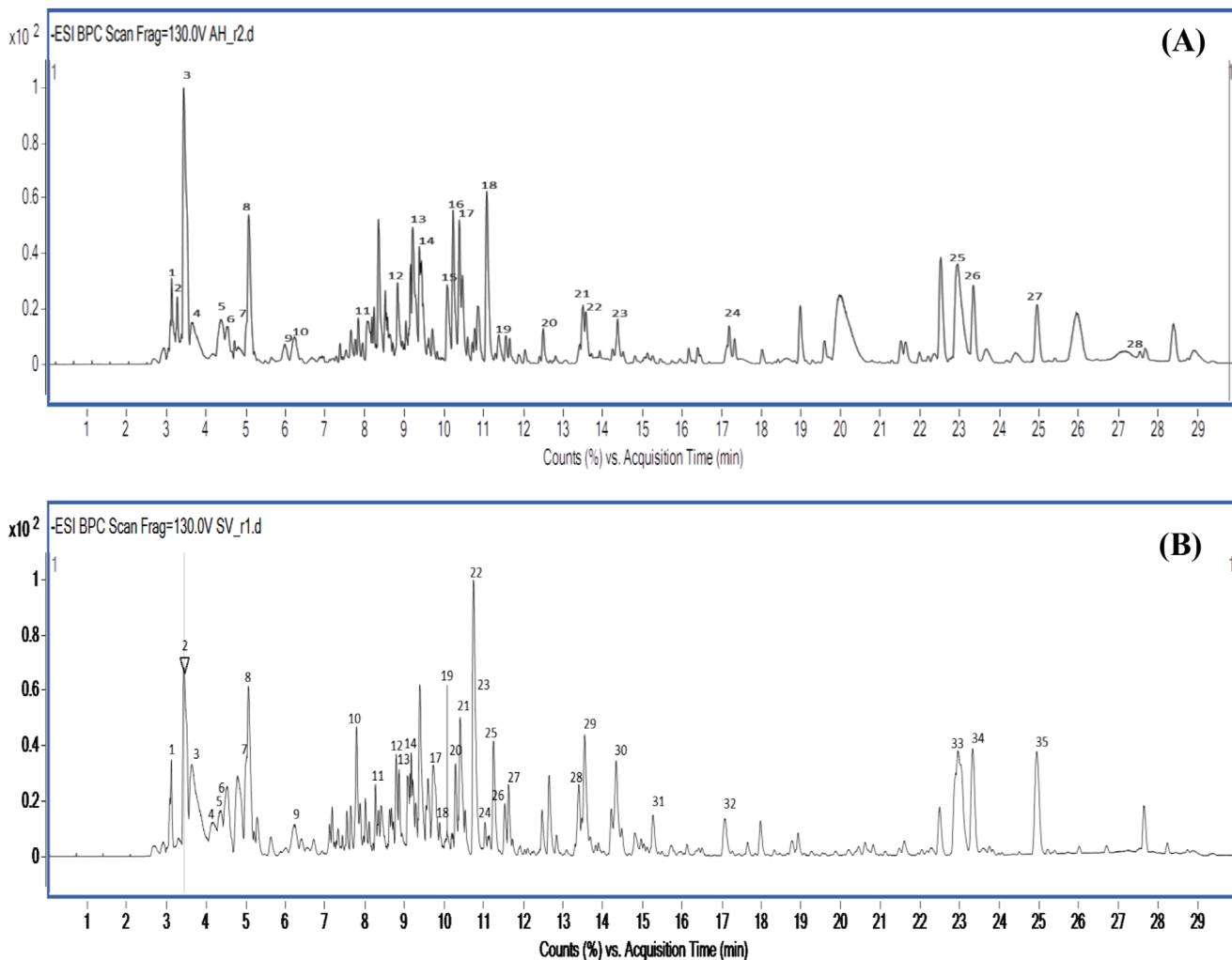


FIGURE 1 | Base peak chromatograms of (A) *A. halimus* and (B) *S. fruticosa*.

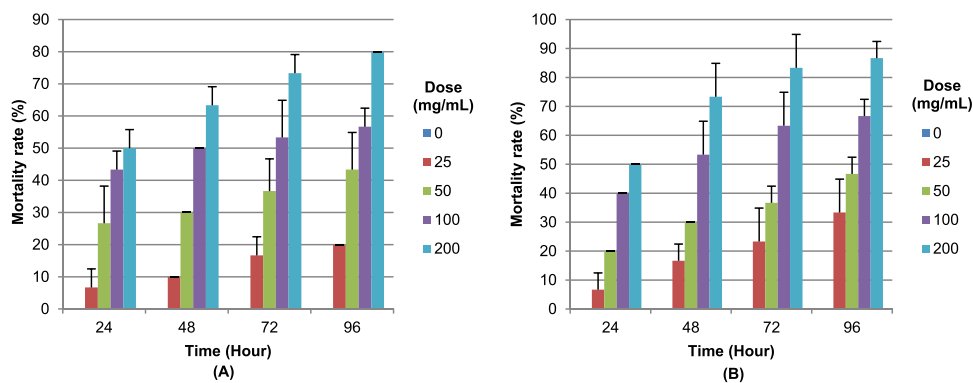


FIGURE 2 | Evaluation of adult *T. confusum* mortality rate exposed to different concentrations of methanolic extracts (A) *A. halimus* and (B) *S. fruticosa*.

levels were recorded after 96 h of exposure at 200 mg/mL, reaching  $86.66 \pm 5.77\%$  and  $80.00 \pm 00\%$  for *S. fruticosa* and *A. halimus* extracts, respectively. In contrast, minimal mortality ( $6.66 \pm 5.77\%$ ) was observed after 24 h at the lowest tested concentration (25 mg/mL), indicating limited acute toxicity during short exposure periods.

Each column represents the mean of three replicates, and error bars indicate the standard deviation. No mortality was observed in the control group (0%).

On the other hand,  $LC_{50}$  values (Table 5) decreased progressively with increasing exposure time. After 24 h, relatively high

**TABLE 3** | Identified compounds in the methanolic extract of *A. halimus* by HPLC-ESI-QTOF-MS analysis.

<b>N</b>	<b>Formula</b>	<b>R<sub>t</sub> (min)</b>	<b>[M-H]<sup>-</sup> (m/z)</b>	<b>Molecular mass</b>	<b>Compound</b>
1	C <sub>6</sub> H <sub>12</sub> O <sub>7</sub>	3.122	195.0512	196.0585	Gluconic acid
2	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	3.265	179.0565	180.0637	Glucose
3	C <sub>7</sub> H <sub>14</sub> O <sub>7</sub>	3.444	209.0671	210.0744	D-Sedoheptulose
4	C <sub>4</sub> H <sub>6</sub> O <sub>5</sub>	3.663	133.0144	134.0217	Malic acid
5	C <sub>5</sub> H <sub>7</sub> NO <sub>3</sub>	4.368	128.0355	129.0428	Pyroglutamic acid
6	C <sub>4</sub> H <sub>6</sub> O <sub>5</sub>	4.519	133.0146	134.0219	Malic acid
7	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	5.035	191.019	192.0269	Citric acid
8	C <sub>5</sub> H <sub>7</sub> NO <sub>3</sub>	5.077	128.0356	129.0428	Pyrrolidinemonocarboxylic acid
9	C <sub>5</sub> H <sub>8</sub> O <sub>5</sub>	5.994	147.0303	148.0375	Citramalic acid
10	C <sub>4</sub> H <sub>6</sub> O <sub>4</sub>	6.229	117.0195	118.0268	Succinic acid
11	C <sub>13</sub> H <sub>16</sub> O <sub>9</sub>	7.821	315.0726	316.0799	Protocatechuic acid 4-glucoside
12	C <sub>13</sub> H <sub>24</sub> O <sub>7</sub> S	8.812	323.1173	324.1246	2-Octyl-2-sulfopentanedioic acid
13	C <sub>9</sub> H <sub>8</sub> O <sub>7</sub> S	9.197	258.9921	259.9995	Caffeic acid 3-sulfate <sup>a</sup>
14	C <sub>9</sub> H <sub>8</sub> O <sub>7</sub> S	9.387	258.9923	259.9996	Caffeic acid 3-sulfate <sup>a</sup>
15	C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>	10.063	153.0198	154.0271	Dihydroxybenzoic acids
16	C <sub>6</sub> H <sub>6</sub> O <sub>6</sub>	10.2	173.0094	174.0166	Trans-aconitic acid
17	C <sub>15</sub> H <sub>14</sub> O <sub>9</sub>	10.342	337.0569	338.0641	Quercetin dihydrate <sup>a</sup>
18	C <sub>13</sub> H <sub>24</sub> O <sub>6</sub> S	11.048	307.1227	308.13	Oxo-tridecanoic acid sulphate
19	C <sub>8</sub> H <sub>8</sub> O <sub>5</sub> S	11.354	215.0022	216.0095	Alpha-sulfophenylacetic acid
20	C <sub>18</sub> H <sub>19</sub> NO <sub>4</sub>	12.469	312.1246	313.1318	Feruloyltyramine
21	C <sub>7</sub> H <sub>6</sub> O <sub>3</sub>	13.474	137.0246	138.0318	Salicylic acid
22	C <sub>6</sub> H <sub>6</sub> O	13.476	93.0348	94.0421	Phenol
23	C <sub>18</sub> H <sub>34</sub> O <sub>5</sub>	14.35	329.2336	330.2409	Pinellic acid
24	C <sub>18</sub> H <sub>28</sub> O <sub>4</sub>	17.116	307.1921	308.1993	Monomethyl ambelin
25	C <sub>18</sub> H <sub>30</sub> O <sub>3</sub>	22.979	293.2127	294.22	17-Hydroxylinolenic acid
26	C <sub>18</sub> H <sub>30</sub> O <sub>3</sub>		293.2127	294.22	17-Hydroxylinolenic acid
27	C <sub>18</sub> H <sub>32</sub> O <sub>3</sub>	24.94	295.228	296.2353	Coronaric acid
28	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	27.556	253.2173	254.2246	Palmitoleic acid

All identified compounds showed mass errors lower than 5 ppm (<5 ppm).

<sup>a</sup>Confirmed using authentic reference standards.

LC<sub>50</sub> values were recorded for both extracts, reaching 169.66 ± 8.85 mg/mL for *Suaeda fruticosa* and 164.75 ± 26.22 mg/mL for *A. halimus*. After 96 h of exposure, these values markedly decreased to 84.73 ± 0.00 mg/mL and 50.03 ± 12.65 mg/mL, respectively. At intermediate exposure times, *S. fruticosa* consistently exhibited lower LC<sub>50</sub> values (108.12 ± 16.004 mg/mL at 48 h; 82.72 ± 2.36 mg/mL at 72 h), compared with *A. halimus* (116.49 ± 18.77 mg/mL at 48 h; 89.63 ± 21.91 mg/mL at 72 h). The consistently lower LC<sub>50</sub> values observed for *S. fruticosa* indicate a higher overall insecticidal potency relative to *A. halimus*. This enhanced activity is likely due to its higher content of phenolic compounds and flavonoids, as revealed by phytochemical profiling.

The insecticidal activity observed in the present study is consistent with previous reports, highlighting the potential of these

species as natural bioinsecticides. For instance, [25] demonstrated the efficacy of a 5% hydromethanolic extract of *A. halimus* against *Dactylopius opuntiae* adults, achieving a mortality rate of 90.00 ± 10% after 8 days of exposure, with an estimated LC<sub>50</sub> of 2.10 mg/mL. Similarly, [26] reported significant larvicidal and adulticidal activities of the methanolic extract of *S. fruticosa* against the Mediterranean fruit fly (*Ceratitis capitata*) after 24 h of treatment, with LC<sub>50</sub> values of 7.1 mg/g of food for larvae and 0.034 g/mL for adults. A comparative study by [8] further demonstrated that the application of *A. halimus* and *S. fruticosa* extracts prepared using different solvents allows an excellent control of the Cotton Aphid (*Aphis gossypii*), emphasizing that solvent selection strongly influences the insecticidal activity, as reflected in LC<sub>50</sub> values variations under similar experimental conditions. Although LC<sub>50</sub> values reported in previous studies

**TABLE 4** | Identified compounds in the methanolic extract of *S. fruticosa* by HPLC-ESI-QTOF-MS analysis.

<i>N</i>	Molecular formula	<i>R<sub>t</sub></i> (min)	[ <i>M-H</i> ] <sup>-</sup> ( <i>m/z</i> )	Molecular mass	Compound
1	C <sub>6</sub> H <sub>12</sub> O <sub>7</sub>	3.113	195.0516	196.0589	Gluconic acid
2	C <sub>11</sub> H <sub>12</sub> O <sub>5</sub>	3.458	223.046	224.0534	Sinapic acid
3	C <sub>4</sub> H <sub>6</sub> O <sub>5</sub>	3.657	133.0148	134.0221	Malic acid
4	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	4.177	191.02	192.0273	Citric acid
5	C <sub>5</sub> H <sub>7</sub> NO <sub>3</sub>	4.355	128.0357	129.043	L-Pyroglutamic acid
6	C <sub>4</sub> H <sub>4</sub> O <sub>4</sub>	4.523	115.0042	116.0115	Fumaric acid
7	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	5.025	191.0201	192.0273	Citric acid
8	C <sub>5</sub> H <sub>7</sub> NO <sub>3</sub>	5.072	128.0356	129.0424	L-Pyroglutamic Acid
9	C <sub>4</sub> H <sub>6</sub> O <sub>4</sub>	6.224	117.0194	118.0266	Succinic acid
10	C <sub>14</sub> H <sub>20</sub> O <sub>8</sub>	7.802	315.1086	316.1159	Vanilloside
11	C <sub>14</sub> H <sub>14</sub> O <sub>9</sub>	8.426	325.0566	326.064	Fertaric acid
12	C <sub>33</sub> H <sub>40</sub> O <sub>20</sub>	9.091	755.206	756.2133	Kaempferol 3-O-rutinoside 7-O-glucoside
13	C <sub>16</sub> H <sub>18</sub> O <sub>10</sub>	9.288	369.0834	370.0907	Fraxin
14	C <sub>26</sub> H <sub>28</sub> O <sub>16</sub>	9.346	595.1308	596.1382	Quercetin 3-O sambubioside <sup>a</sup>
15	C <sub>12</sub> H <sub>12</sub> O <sub>4</sub>	9.392	219.0668	220.074	Eugenitin
16	C <sub>13</sub> H <sub>12</sub> O <sub>6</sub>	9.395	263.0563	264.0636	Diacetyl caffeic acid
17	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	9.741	609.1472	610.1545	Rutin
18	C <sub>13</sub> H <sub>12</sub> O <sub>6</sub>	9.89	263.0566	264.0637	Diacetyl caffeic acid
19	C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>	10.103	153.0198	154.027	Protocatechuic Acid
20	C <sub>22</sub> H <sub>22</sub> O <sub>12</sub>	10.361	477.1045	478.1118	isorhamnetin-3-O-glucoside <sup>a</sup>
21	C <sub>11</sub> H <sub>13</sub> NO <sub>3</sub>	10.414	206.0825	207.0899	N-Acetyl-L-phenylalanine
22	C <sub>27</sub> H <sub>32</sub> O <sub>14</sub>	10.748	579.1742	580.1816	Naringin
23	C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>	10.867	163.0401	164.0474	Coumaric acid
24	C <sub>13</sub> H <sub>12</sub> O <sub>6</sub>	11.141	263.0566	264.0639	Diacetyl caffeic acid
25	C <sub>13</sub> H <sub>24</sub> O <sub>6</sub> S	11.251	307.122	308.13	Oxo-tridecanoic acid sulphate
26	C <sub>9</sub> H <sub>16</sub> O <sub>4</sub>	11.534	187.0977	188.105	Azelaic acid
27	C <sub>29</sub> H <sub>32</sub> O <sub>15</sub>	11.628	619.1677	620.175	Cratenacin
28	C <sub>7</sub> H <sub>6</sub> O <sub>3</sub>	13.476	137.0245	138.0318	Salicylic acid
29	C <sub>18</sub> H <sub>32</sub> O <sub>5</sub>	13.548	327.218	328.2253	Malyngic acid
30	C <sub>18</sub> H <sub>34</sub> O <sub>5</sub>	14.34	329.2336	330.241	Pinellic acid
31	C <sub>16</sub> H <sub>12</sub> O <sub>7</sub>	15.27	315.0513	316.0586	Isorhamnetin <sup>a</sup>
32	C <sub>18</sub> H <sub>28</sub> O <sub>4</sub>	17.081	307.1919	308.1993	Monomethyl embelin
33	C <sub>18</sub> H <sub>30</sub> O <sub>3</sub>	22.986	293.2124	294.2197	17-Hydroxylinolenic acid
34	C <sub>18</sub> H <sub>30</sub> O <sub>3</sub>	23.332	293.2124	294.2197	17-Hydroxylinolenic acid
35	C <sub>18</sub> H <sub>32</sub> O <sub>3</sub>	24.948	295.2279	296.2352	13-Hydroxyoctadecadienoic Acid

All identified compounds showed mass errors lower than 5 ppm (<5 ppm).

<sup>a</sup>Confirmed using authentic reference standards.

differ from those obtained in the present work, such discrepancies may be explained by differences in insect species, exposure duration, experimental conditions, bioassay methods, and variations in extract chemical composition.

## 2.5 | Docking Results

To correlate the experimental results of the phytochemical study and the inhibitory activity observed against *T. confusum* of

**TABLE 5** | LC<sub>50</sub> values of plant extracts against *T. confusum* adults at four times intervals.

Time	LC <sub>50</sub> <sup>1</sup> (mg/mL)	
	<i>A. halimus</i>	<i>S. fruticosa</i>
24 h	164.75 ± 26.22	169.66 ± 8.85
48 h	116.49 ± 18.77	108.12 ± 16.004
72 h	89.63 ± 21.91	82.72 ± 2.36
96 h	84.73 ± 00	50.03 ± 12.65

Data are presented as the mean ± standard deviation of three replicates with ( $p \leq 0.05$ ).

<sup>1</sup>Median lethal concentration.

**TABLE 6** | Binding free energy values Amino acids and types of interactions involved in effectors and ligands binding calculated through the molecular docking of the major polyphenols identified in *A. halimus*.

Compounds	Binding energies (kcal/mol)	Amino acids and interaction types	Distance (Å)
Feruloyltyramine	-9.5	TYR A: 71: Conventional hydrogen bond	2.39
Quercetin dihydrate	-8.3	ASP A: 375: Pi-donor hydrogen Bond TYR A: 324: Pi-donor hydrogen Bond	2.50 2.90
Caffeic acid 3-sulfate	-7.2	TYR A: 324: Pi-donor Hydrogen Bond	2.92
Protocatechuic acid 4-glucoside	-6.6	ARG A: 259: Pi-Alkyl	5.08
Dihydroxybenzoic acid	-6.3	PHE A: 371: Pi-Pi T-shaped	5.08
Salicylic acid	-6.0	TRP: 83 A: Pi-Pi T-shaped TRP: 83 A: Pi-Pi T-shaped GLY: 150 A: Pi-sigma	5.36 5.49 3.75
Phenol	-5.0	TYR: 370 A: Pi-Pi T-shaped TYR: 374 A: Pi-Pi stacked	5.31 4.91

RMSD values < 2.0 Å.

molecules already identified by HPLC-ESI-QTOF in the extracts, docking constitutes a very reliable tool to predict the binding free energy and evaluate the affinity of this molecule with its biological target.

As depicted in Tables 6 and 7, molecular docking studies performed on extracts of *A. halimus* and *S. fruticosa* revealed a variable negative charge affinity of selected phenolic and flavonoid compounds for the target protein, ranging from -10.2 to -5.0 kcal/mol, reflected by their binding energies. All RMSD values were < 2.0 Å, indicating good agreement between docked and crystallographic poses.

For *A. halimus*, feruloyltyramine (-9.5 kcal/mol) and quercetin dihydrate (-8.3 kcal/mol) showed the highest affinities with AChE, forming classical and Pi-donor hydrogen bonds with key amino acids such as TYR71, ASP375, and TYR324, with optimal distances of 2.39-2.92 Å, suggesting stable and specific interactions (Figure 3). In the case of *S. fruticosa* (Figure 4), Kaempferol 3-O-rutinoside 7-O-glucoside exhibited the highest binding energy (-10.2 kcal/mol), primarily via stacked Pi-Pi interactions with TRP321, while other flavonoids such as Naringin (-9.5 kcal/mol) and Rutin (-8.7 kcal/mol) showed

Pi-alkyl and Pi-sigma interactions with VAL318, TYR73, and ASP375. Phenolic acids such as coumaric acid and Isorhamnetin also contributed via Pi-donor and conventional hydrogen bonds at short distances (~2.8-2.9 Å), while smaller compounds like salicylic acid and sinapic acid interacted mainly via Pi-Pi or Pi-sigma interactions at longer distances (3.75-5.49 Å), indicating moderate affinity. Overall, these results suggest that glycosylated flavonoids and certain phenolic acids from both species have significant potential to modulate the Acetylcholinesterase (AChE) via stable interactions, with *S. fruticosa* compounds showing superiority in terms of maximum binding energy, while *A. halimus* shows particularly strong hydrogen interactions. These findings also support the results observed in in vitro studies.

### 3 | Conclusions

This study demonstrates that the methanol extracts of *S. fruticosa* and *A. halimus* contain considerable amounts of total phenolics, flavonoids, and tannins, and exhibit notable insecticidal activity. HPLC-ESI-QTOF-MS profiling enabled the identification of several important secondary metabolites. Both extracts

**TABLE 7** | Binding free energy values Amino acids and types of interactions involved in effectors and ligands binding calculated through the molecular docking of the major polyphenols identified in *S.fruticosa*

<b>Compounds</b>	<b>Binding energies (kcat/mol)</b>	<b>Amino acids and interaction types</b>	<b>Distance (Å)</b>
Kaempferol 3-O-rutinoside 7-O-glucoside	-10.2	TRP A:321: PiPi stacked	4.67
		TRP A:321: PiPi stacked	4.72
		TRP A:321: PiPi stacked	5.1
		TRP A:321: PiPi stacked	5.28
Naringin	-9.5	VAL A: 318: Pi-alkyl	4.08
Rutin	-8.7	ASP A: 375: Carbon-hydrogen bond	3.32
		TYR A: 73: Pi-sigma	3.61
		TYR A: 73: Pi-alkyl	5.49
Coumaric acid	-8.4	TYR A: 324: Pi-donor Hydrogen Bond	2.88
Fertaric acid	-6.4	THR A: 469: Conventional hydrogen bond	2.71
		GLU A: 485: Carbon-hydrogen bond	3.54
		TYR A: 324: Pi-Donor Hydrogen Bond	2.87
Isorhamnetin	-8.5	TYR A: 324: Pi-Donor Hydrogen Bond	2.87
Isorhamnetin-3-O-glucoside	-8.3	TRP A: 321: PiPi Stacked	4.19
		TRP A: 321: PiPi Stacked	4.52
		TRP A: 321: PiPi Stacked	4.33
		TRP A: 321: PiPi Stacked	4.89
		TYR A: 73: PiPi Stacked	5.99
Quercetin 3-O-sambubioside	-8.2	TYR A: 71: PiPi Stacked	5.67
		TYR A: 324: PiPi Stacked	4.92
		TRP A: 321: PiPi-shaped	4.31
		TRP A: 321: PiPi-shaped	4.43
		TRP A: 321: PiPi-shaped	4.69
Cratenacin	-8.1	TRP A: 321: PiPi-shaped	4.94
		XTYR A: 71: Pi-Donor hydrogen bond	2.60
		TRP A: 321: PiPi-shaped	5.11
		TRP A: 321: PiPi stacked	3.86
		TRP A: 321: PiPi stacked	4.33
Fraxin	-6.9	TRP A: 321: PiPi-shaped	4.63
		TYR A: 324: PiPi-shaped	4.67
		ASP A: 356: Conventional hydrogen bond	1.97

(Continues)

TABLE 7 | (Continued)

Compounds	Binding energies (kcal/mol)	Amino acids and interaction types	Distance (Å)
ARG A: 259: Conventional hydrogen bond	3.08		
ASP A: 358: carbon-hydrogen Bond	3.46		
Eugenitin	-6.3	TRP A: 83: Pi-sigma	3.92
		TRP A: 83: PiPi-shaped	5.92
		GLY A: 150: Pi-sigma	3.51
		SER A: 238: Conventional hydrogen bond	1.94
		TRP A: 271: Pi-alkyl	4.01
		TRP A: 271: Pi-alkyl	4.39
		PHE A: 330: Pi-alkyl	4.79
		PHE A: 371: PiPi-shaped	4.74
		PHE A: 440: Pi-alkyl	5.29
		TYR A: 37: PiPi-shaped	5.54
		TYR A: 370: Pi-alkyl	5.23
Sinapic acid	-6.1	TRP A: 321: PiPi stacked	4.02
		TRP A: 321: PiPi stacked	4.32
Diacetyl caffeic acid	-6.0	ILE A: 82: Pi-sigma	3.50
		LEU A: 471: Pi-alkyl	5.23
Salicylic acid	-6.0	TRP A: 83: Pi-Pi T-shaped	5.36
		TRP A: 83: Pi-Pi T-shaped	5.49
		GLY A: 150: Pi-sigma	3.75
		TYR A: 370: Pi-Pi T-shaped	5.31

RMSD values < 2.0 Å.

showed moderate cytotoxicity against *T. confusum*. Furthermore, molecular docking studies confirmed that selected compounds can effectively inhibit AChE, a key enzyme in insect neurotransmission, and detailed their interactions with the enzyme active site. These findings highlight the potential of these halophytic plants as eco-friendly alternatives to synthetic pesticides and support their possible application in sustainable pest management strategies.

## 4 | Experimental Section

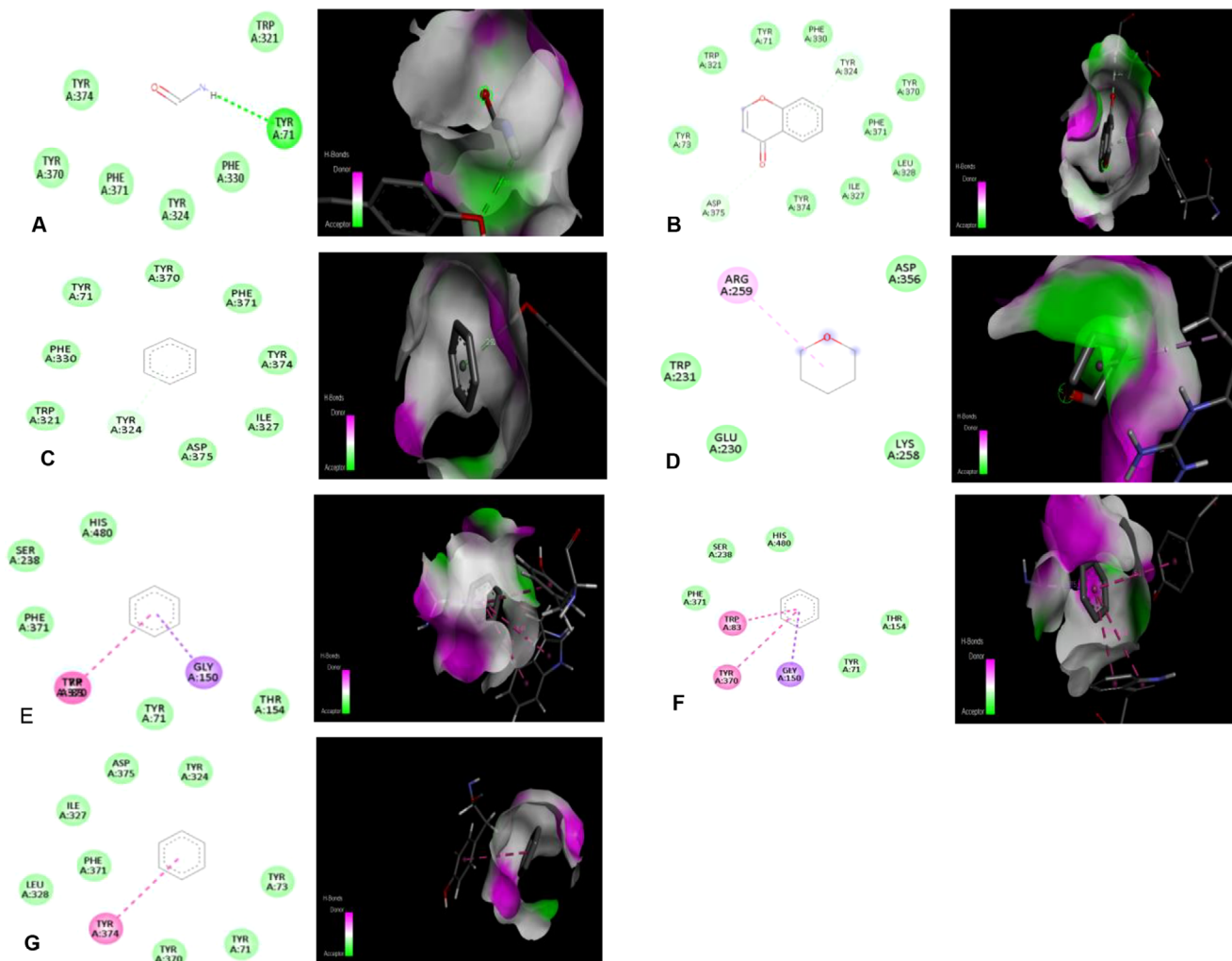
### 4.1 | Plant Material Collection

The aerial parts of *A. halimus* and *S. fruticosa* were collected in January 2024 from natural saline habitats located in northwestern Algeria. *A. halimus* was harvested from Belassel (Relizane, Algeria; 35°44'12.7"N, 0°32'23.9"E), characterized by a soil pH of 8.1 and salinity of 12.48 dS/m. *S. fruticosa* was collected from

Sebkha (Oran, Algeria; 35°36'11.5"N, 0°41'16.2"W), with a soil pH of 7.4 and salinity of 21.15 dS/m. The plant material was identified in the Department of Biology, Oran 1 University, Algeria, by Dr. Yamina HALFAOUI, using standard taxonomic keys [27]. Voucher specimens were deposited under the numbers RE-2024-01 (*A. halimus*) and RE-2024-02 (*S. fruticosa*) at the herbarium of the Faculty of Natural and Life Sciences, University of Relizane, Algeria. The collected leaves were washed and air-dried at room temperature away from direct sunlight. The dried plant material was then finely ground into a uniform powder using an electric grinder. The resulting powder was stored in glass jars until further use.

### 4.2 | Insect Collection and Rearing

Adult of *T. confusum* were obtained from a stock of infested soft wheat grains collected in the Sidi M'hamed Benali area (Relizane, Algeria) and reared in glass jars containing wheat flour as a food



**FIGURE 3** | Intermolecular interaction of the 2D (left) and the 3D (right) structures of acetylcholinesterase (AChE) protein with identified compounds of *A. halmus* extract(A) feruloyltyramine, (B) quercetin dihydrate, (C) caffeic acid 3-sulfate, (D) protocatechuic acid 4-glucoside, (E) dihydroxybenzoic acid, (F) salicylic acid, and (G) phenol.

substrate. Each jar was covered with a fine mesh screen to ensure adequate ventilation and prevent insect escape. The cultures were maintained at  $28 \pm 2^\circ\text{C}$  and 70% relative humidity, following the protocol described by [28].

### 4.3 | Extracts Preparation

A total of 2.5 g of plant powder was mixed with 100 mL of methanol–water solution (75:25, v/v) under continuous stirring at room temperature for 24 h. After maceration, the mixtures were filtered through Whatman filter paper N°:1, and the filtrates were concentrated under reduced pressure using a rotary evaporator (LabTech- EV400) at  $40^\circ\text{C}$ .

### 4.4 | Qualitative Phytochemical Screening

Preliminary phytochemical screening of the methanolic plant extracts was conducted to detect major classes of phytoconstituents, following standard procedures described by [29, 30].

### 4.5 | Detection of Alkaloids

Alkaloids were tested by adding a few drops of 2% aqueous picric acid solution to 1 mL of plant extract. The formation of a yellow to yellow–orange precipitate indicated the presence of alkaloids.

### 4.6 | Detection of Reducing Sugars

Reducing sugars were detected by mixing 1 mL of each extract with equal volumes of Fehling’s A and B solutions, followed by heating in a water bath for 10 min. A red precipitate indicated their presence.

### 4.7 | Detection of Glycosides

Glycosides were identified by adding 400  $\mu\text{L}$  of glacial acetic acid, one drop of 5%  $\text{FeCl}_3$ , and concentrated  $\text{H}_2\text{SO}_4$  along the sides of the test tube to 1 mL of extract. The formation of a brown ring indicated a positive result.



#### 4.8 | Detection of Proteins and Amino Acids

Proteins and amino acids were revealed by adding a drop of saturated ninhydrin solution in acetone to the extract, followed by heating. The appearance of a purple color indicated their presence.

#### 4.9 | Detection of Phenolic Compounds

Phenolic compounds were detected by adding 1–2 drops of 5% aqueous ferric chloride solution ( $\text{FeCl}_3$ ) to 1 mL of extract. A blue–black or green–black coloration indicated their presence.

#### 4.10 | Detection of Flavonoids

Flavonoids were identified using the Shinoda test, in which a few fragments of magnesium ribbon were added to 1 mL of extract, followed by the dropwise addition of concentrated HCl. The formation of a red-to-pink color indicated the presence of flavonoids.

#### 4.11 | Detection of Tannins

Tannins were determined using the Braymer test, where distilled water and three drops of 10% ferric chloride solution were added to 1 mL of extract. A blue–green coloration indicated the presence of tannins.

#### 4.12 | Detection of Saponins

Saponins were identified using the foam test, in which 2 mL of extract was vigorously shaken with 5 mL of water. The formation of a stable foam persisting for more than 10 min indicated the presence of saponins.

#### 4.13 | Detection of Steroids

Steroids were detected using the Salkowski test. One milliliter of extract was treated with 2 mL of chloroform, followed by careful addition of 1 mL of concentrated  $\text{H}_2\text{SO}_4$  along the sides of the test tube. A red or reddish-brown color in the lower layer indicated a positive result.

#### 4.14 | Detection of Coumarins

Coumarins were confirmed using the NaOH test, in which a few drops of 10% sodium hydroxide solution were added to 1 mL of extract. A yellow color indicated the presence of coumarins.

#### 4.15 | Total Phenolic Content

The total phenolic content of plant extracts was determined using the Folin-Ciocalteu method according to [31], with some

modifications. Briefly, 20  $\mu\text{L}$  of each sample was mixed with 1.58 mL of distilled water and 100  $\mu\text{L}$  of Folin–Ciocalteu reagent. The mixture was incubated in the dark for 1–8 min at room temperature, followed by the addition of 300  $\mu\text{L}$  of 20% sodium carbonate solution. After incubation for 2 h, absorbance was measured at 760 nm using a UV–vis spectrophotometer (Dynamica HALO XB-10). The total phenolic content was calculated using a Gallic acid calibration curve (0.025–1 mg/mL) and expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

#### 4.16 | Total Flavonoid Content

The total flavonoid content was estimated according to the method described by [31], using the aluminum chloride colorimetric assay. 1 mL of plant extract was mixed with 1 mL of 2% methanolic  $\text{AlCl}_3$  solution and incubated for 30 min at room temperature. The absorbance was measured at 415 nm against using methanol as a blank. Flavonoid concentrations were calculated from a quercetin calibration curve and expressed as milligrams of quercetin equivalent per gram of dry extract (mg QE/g).

#### 4.17 | Total Tannin Content

The total tannin content was determined by the vanillin reagent method as described by [32]. Briefly, 250  $\mu\text{L}$  of each extract was mixed with 1.5 mL of 4% vanillin solution (prepared in methanol) and 750  $\mu\text{L}$  of concentrated hydrochloric acid (HCl). The mixture was incubated at room temperature for 15 min, and absorbance was measured at 500 nm against a blank. Tannin concentrations were calculated from a catechin calibration curve and expressed as milligrams of catechin equivalents per gram of dry weight (mg CE/g DW).

#### 4.18 | HPLC-ESI-QTOF-MS Analysis

Chromatographic analyses were performed using an Agilent 1260 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a Zorbax Eclipse Plus RP-C18 column (150 mm  $\times$  4.6 mm i.d., 1.8  $\mu\text{m}$  particle size). The HPLC system was coupled to a Q-TOF mass spectrometer with an ESI Jet Stream interface (model 6540, Agilent Technologies), operating in negative-ion mode over a mass range of 50–1700  $m/z$ . Data acquisition and processing were carried out using MassHunter software (version B.06.00, Agilent Technologies). The mobile phase consisted of water containing 0.1% formic acid (solvent A) and acetonitrile (solvent B), using a gradient program as follows: 5% B at 0 min, 35% B at 5 min, 85% B at 22 min, and 95% B at 24 min, followed by re-equilibration to 5% B at 25 min and maintained for 5 min, resulting in a total run time of 30 min. The flow rate was set at 0.5 mL/min, the injection volume was 10  $\mu\text{L}$ , and the column temperature was maintained at 25°C. Samples were kept at 4°C during analysis. The mass spectrometer parameters were set as follows: capillary voltage 4000 V, nebulizer pressure 320 psig, fragmentor voltage 130 V, nozzle voltage 500 V, skimmer voltage 45 V, and octopole voltage 750 V. The instrument was calibrated and tuned according to the manufacturer's instructions, with

continuous internal mass calibration using reference ions at  $m/z$  112.985587 (trifluoroacetate) and 1033.988109 (hexakis(1H,1H,3H-tetrafluoropropoxy) phosphazine adduct). Major compounds were detected using molecular feature extraction, filtered against solvent blanks with a relative abundance threshold of 0.2%, and tentatively identified by comparison with phenolic compound databases (Phenol-Explorer, CAS SciFinder) and relevant literature [21, 22]. All detected compounds showed mass errors lower than 5 ppm. In addition, selected compounds, including caffeic acid, quercetin, and isorhamnetin, were confirmed using authentic reference standards analyzed under identical chromatographic conditions by comparing their retention times and accurate mass spectra.

## 4.19 | Evaluation of Insecticidal Activity

The direct contact insecticidal activity of methanolic extracts from halophytic plants against *T. confusum* was performed according to the method described by [33] with some modifications. Four extract concentrations (25, 50, 100, and 200 mg/mL) were prepared using a 2% Tween solution as a dispersing agent. A volume of 0.5 mL of each concentration was applied using a hand-held sprayer on ten adult insects. The treated insects were subsequently transferred to Petri dishes containing filter paper matching the diameter of the dishes. The negative control consisted of 2% Tween solution alone. Aeration was ensured through small holes in the dish lids. Insect mortality was recorded after 24, 48, 72, and 96 h of exposure.

## 4.20 | Estimation of Mortality Rate and Median Lethal Concentration (LC<sub>50</sub>)

Observed mortality rates were corrected using the formula of [34]

$$\text{Mortality rate (\%)} = \frac{(\% \text{ test mortality} - \% \text{ Control mortality})}{100 - \% \text{ Control mortality}} \times 100.$$

If control mortality exceeded 20%, the test was repeated.

The median lethal concentration (LC<sub>50</sub>) for each plant extract was determined using log-Probit regression analysis according to [35].

## 4.21 | In Silico Studies

### 4.21.1 | Protein Preparation

The enzyme of acetylcholinesterase (PDB ID: 6XYS) [36] was obtained from the RCSB Protein Data Bank and saved in PDB file format. The protein structure was prepared by removing water molecules and bound ligands from the three-dimensional (3D) structure using Discovery Studio 2025 Client. Protons and charges were added, then using UCSF Chimera software 1.19 to optimize the protein for further analysis.

### 4.21.2 | Ligands Preparation

Seventeen identified compounds in the HPLC-ESI-QTOF-MS analysis of *A. halimus* and *S. fruticosa* extracts were selected for ligand preparation to target acetylcholinesterase (AChE) receptors. Their corresponding three-dimensional (3D) chemical structures were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in SDF format and converted to PDB format into Open Babel GUI [37].

### 4.21.3 | Molecular Docking

Molecular docking was carried out using PyRx software. Ligand structures were imported into the platform via the integrated Open Babel module and subsequently converted into an AutoDock ligand. The acetylcholinesterase (AChE) protein structure was also converted into the AutoDock macromolecule. Docking simulations were performed using the Vina Wizard tool in PyRx. The grid box was centered at ( $x = 25.88, y = 62.5, z = 12.34$ ) with dimensions of  $22 \times 22 \times 22 \text{ \AA}$  to ensure appropriate coverage of the binding pocket.

To validate the docking protocol, a redocking procedure was conducted by extracting the native co-crystallized ligand and redocking it into the active site under identical docking parameters. The resulting docked conformation was superimposed onto the crystallographic pose using UCSF Chimera, and the root mean square deviation (RMSD) was calculated. An RMSD value below  $2.0 \text{ \AA}$  confirmed the reliability and reproducibility of the docking protocol. The docking results were exported in CSV format, and the ligand–protein interactions were further analyzed and visualized in both 2D and 3D using Discovery Studio Visualizer 2025 Client [37].

### 4.21.4 | Statistical Analysis

All experiments were performed in triplicate ( $n = 3$ ), and results were expressed as mean  $\pm$  standard deviation ( $M \pm SD$ ) using the Statistical Package for the Social Sciences (SPSS) software, version 26.0 (IBM, 2019). Data normality was assessed using the Shapiro–Wilk test, and all datasets satisfied the assumptions of normality ( $p > 0.05$ ); therefore. Total phenolic, flavonoid, and tannin contents were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Insecticidal activity was evaluated using log-probit regression to estimate LC<sub>50</sub> values along with their 95% confidence intervals. Statistical significance was set at  $p \leq 0.05$ .

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### Author Contributions

**Mounira Tabouri:** data curation, formal analysis, investigation, resources; writing – original draft. **Mustapha Djellouli:** conceptualization, methodology, software, supervision, validation, writing – review and editing. **Khadija Belkheir:** validation, writing – review and editing. **Kamel Benotmane:** funding acquisition, investigation, visualization. **Meriem Dahoun:** funding acquisition, investigation. **Abdesselem Si Mohammed:** resources. **Saïd**

**Nemmiche:** formal analysis, software. **Hamdi Bendif:** funding acquisition, writing – review and editing, supervision. **Elfalah Walid:** software, project administration, funding acquisition. **Boufahja Fehmi:** software, project administration, funding acquisition. **Alsalamah Sulaiman A.:** data curation, project administration. **Stefania Garzoli:** writing – review and editing, supervision. **Inmaculada Moscoso-Ruiz:** formal analysis; validation. **Inés Cea-Pavez:** supervision; validation. **Vito Verardo:** supervision; validation. All authors have read and agreed to the published version of the manuscript.

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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

Data will be made available on request.

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