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Essential oils from *Artemisia judaica*, *Ruta graveolens* and *Salvia palaestina* with antiradical, cytotoxic and AMPA receptor-modulatory activities

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Essential oils (EOs) from the leaves of *Artemisia judaica*, *Ruta graveolens*, *and Salvia palaestina* were analyzed for their phytochemical composition and their antiradical, cytotoxic, and neuromodulatory activities. Using microwave-ultrasonic technology, EOs were extracted from *S. palaestina*, *A. judaica*, and *R. graveolens*, and their antiradical activity (via DPPH assay), cytotoxicity, and neuromodulatory effects (using whole-cell patch-clamp recordings) were evaluated. The main compounds that were explored by GC-MS analysis include piperitone and carvacrol in *A. Judaica*, 2-undecanone in *R. graveolens*, and carvacrol in *S. palaestina*. *S. palaestina* exhibited the greatest antiradical effect with a 50% inhibition concentration of 8.30 µg/mL. Cytotoxicity tests showed that these EOs express dose-dependent activity on HeLa, HepG3, and B16F1 cancer cells. Among them, *S. palaestina* and *A. judaica* were the most potent ones. Patch-clamp recordings showed that *S. palaestina* was the most potent in inhibiting action toward AMPA receptors, thereby reducing currents up to 6-fold and modulating receptor kinetics. *A. Judaica* and *R. graveolens* also inhibited the AMPA receptor activity, but to a lesser extent. These findings point to the important bioactive potential of these EOs, especially *S. palaestina*, which demonstrated high antiradical and AMPA receptor-modulatory activities and, thus, could be of interest for pharmaceutical and neuroprotective uses.

Keywords Phytochemical composition, Antiradical, Cytotoxicity, AMPA receptor, Desensitization

Abbreviations

EO	Essential oil
A. Judaica	*Artemisia judaica*
S. palaestina	*Salvia palaestina*
R. graveolens	*Ruta graveolens*
ROS	Reactive oxygen species
DPPH	2,2-diphenyl-1-picrylhydrazyl
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid
DMSO	Dimethyl sulfoxide
DNSA	3,5-dinitrosalicylic acid
ATCC	American type culture collection
RPMI	Roswell park memorial institute medium
Hep G2	Human hepatocellular carcinoma cell line
B16-F1	Mouse melanoma cell line
HeLa	Human cervical adenocarcinoma cell line
LX-2	Human hepatic stellate cell line

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FBS	Fetal bovine serum
HEK293T	Human embryonic kidney 293T cell line
EGFP	Enhanced green fluorescent protein

Plant essential oils (EOs) possess diverse therapeutic properties, attracting interest in food, cosmetics, and pharmaceutical industries[1]. Oxidative stress is a common pathway exacerbating diseases such as cancer, obesity, and neurological disorders[2–4]. Within the realm of cancer, free radicals has the potential to promote the occurrence of DNA damage and mutations, hence facilitating the genesis and advancement of malignant cells[2–4].

Artemisia judaica L. (Asteraceae) (*A. Judaica*) is a medicinal, aromatic herb traditionally utilized to manage digestive issues, skin problems, and atherosclerosis grown primarily in the Mediterranean region. It is also used as an immunostimulant[5]. A recent study demonstrated that *A. judaica* EO has antibiofilm, anti-inflammatory, and antioxidant properties[5]. *Salvia palaestina* Benth (Lamiaceae) (*S. palaestina*) is a perennial plant that is often found in Palestine, Egypt, Syria, Southern Turkey, Iran, North Iraq, Lebanon, and Jordan[6]. Moderate growth-inhibitory effects of the raw methanol essence of *S. palaestina* against specific types of tumor cells were reported[7]. Additionally, it was found to have a notable affinity for noradrenergic and dopaminergic receptors in the CNS[8]. A therapeutic ointment from *S. palaestina* was created and utilized for wound healing in Turkey[9]. Rutaceae family include *Ruta graveolens (R. graveolens)* which is widely grown and found in various regions across continents, but is particularly prominent in the Mediterranean and parts of Europe. Modern research has confirmed the following therapeutic properties of *R. graveolens*: abortifacient, antifungal, antibacterial, antihistaminic, antihypertensive, anticancer, anthelminthic, antiinflammatory, antifertility, antiarrhythmic, antispasmodic and analgesic activities, besides several activities causing alterations in the central nervous system[10–12].

Phytochemicals, especially medicinal herbs, have been important in discovering new neurologic therapies[13]. Several plant products with excitotoxicity-inhibiting properties implicated in the pathogenesis of neurodegenerative and neuroinflammatory disorders have been described. Because excessive glutamate release is closely associated with neuronal injury, its regulation might constitute a neuroprotective strategy[14,15]. Among various well-known medicinal plants for neuroactive properties, species from *Artemisia*, *Salvia*, and *Ruta* genera have shown prominent modulatory activity on the nervous system[16–18].

Bioactive principles from *Artemisia* species have demonstrated efficacy in decreasing the excitotoxicity of glutamate, hence standing as potential candidates for treating neural impairments associated with the harmful excitotoxic activity of glutamate[19,20]. At the same time, the growing body of evidence points to the effects of oxidative molecules and neuroinflammation in progression of epilepsy-characterized by recurrent seizures contributing to cognitive impairments and neural damage[21]. This has shifted epilepsy research toward the ongoing exploration of alternative therapies targeting neuroinflammation and oxidative injury, including plant-derived antioxidants. Some species of *Artemisia* have long been used in the traditional management of epilepsy, and their anticonvulsant activities have been validated using experimental models of seizure disorders[16]. Besides this, EOs from *Salvia* species showed neuroprotective potential in degenerative diseases through neurotransmitter system modulation, diminution of oxidative stress, and evasion of neuronal death, showing their therapeutic prowess in neurological health[17].

Various species of *Ruta* have been traditionally reported in folk medicine for their therapeutic use against epilepsy. Recent studies indicate that *R. graveolens* has recorded anticonvulsant activities; such an effect may partly be due to the enhancement of GABAergic neurotransmission[18]. Its neuroprotective effects probably emanate from this plant's enormous derived chemicals, including saponins, triterpenes, steroids, alkaloids, and flavonoids[18]. Similarly, neuronal depressant activity was shown by *R. chalepensis*; the latter also supports its folkloric use in seizure treatment[22].

In light of the plant's neuropharmacological profile, the current investigation aimed to extract the EOs from *A. Judaica*, *S. palaestina*, and *R. graveolens* leaves from Palestine and compare EO's constituents and effects 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals to assess EO's the antiradical effects. In addition, we evaluate EO's cytotoxic effects against HepG2, HeLa, and B16F1 cancer cell lines and LX-2 non-cancer cells. The study will also investigate the plants' impact on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor function[23,24]. AMPA receptors are well recognized for their central role in excitatory synaptic transmission and have been associated with the development of several neuropathological conditions, especially those related to excitotoxicity[23,25,26]. We, therefore, set out to examine exactly how these EOs impinge on AMPA receptor activity, focusing in greater detail on their interaction with receptor deactivation and desensitization kinetics.

Experimental methods

Herbals

The leaves of the three wild species, *A. judaica*, *S. palaestina*, and *R. graveolens*, were collected from the Marj Ibn Amer plains of Jenin (32°27′33.95″ N, 35°18′3.10″ E), located between the Nablus and Galilee Mountains in the northern region of Palestine. The leaves gathered in the flowering stage in May 2023. At the time of collection, all species were in full bloom, with *A. judaica* and *S. palaestina* typically flowering from April–June and *R. graveolens* from June–July in the Mediterranean climate of northern Palestine. Voucher specimens (Pharm-PCT-238, Pharm-PCT-2117, and Pharm-PCT-2805, respectively) have been recognized and stored in the Herbal Products Laboratory of An-Najah National University (West Bank/Palestine). The collection was taking place with the permission of local authorities for scientific purposes. The plants leaves were collected by WHO standards for evaluating herbal medicines and legislation. All methods followed applicable institutional, national, and international guidelines and legislation. Prof. Nidal Jaradat verified the herbs. After the required herbs were collected, they were washed well with running water and desiccated in a shaded area at room temperature and typical humidity levels.

Essential oils extraction method

A microwave-ultrasonic technique that included ultrasonication was used to extract the essential oils of *A. Judaica*, *S. palaestina*, and *R. graveolens*. Using an ultrasonic extractor device (Ultrasonic-microwave Cooperative Extractor, CW-2000, China), a powder suspension of the plants under investigation was used during the extraction process. This device comes with a 0.5 L round-bottom flask that holds 500 mL of distilled water and roughly 50 g of herb powder. The flask, which was housed inside the Clevenger apparatus, was placed inside the device. During the extraction process, the microwave-ultrasonic extractor apparatus's output was adjusted by 1000 W.

Furthermore, the instrument's ultrasonic power was adjusted to its maximum settings of 40 kHz and 50 W. At 100˚C, the extraction process was carried out for 20 min. For every herb, this process was done three times. The resulting EOs were collected in a sterile tiny beaker, dehydrated using magnesium sulfate anhydrous, and then stored in firmly sealed dark amber bottles at around 4 °C in a refrigerator (Fig. 1).

Gas chromatography–mass spectrometry (GC–MS) analysis

Essential oil constituents were analyzed using a PerkinElmer Clarus 500 GC coupled to a Clarus 560 mass spectrometer (Shelton, CT, USA). Separation was achieved on an Elite-5 fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was programmed from 50 °C (5 min hold) to 280 °C at 4 °C/min. Helium was used as the carrier gas at a constant flow of 1 mL/min. Samples (0.2 µL) were injected in split mode (1:50) at 250 °C[27–29].

Linear Retention Index (LRIs) were determined using an n-alkane standard mixture (C8–C40) analyzed under identical chromatographic conditions, with compound abundances expressed as % Area based on GC-MS total ion current (TIC), acknowledging the semi-quantitative nature of this approach. Experimental LRIs were calculated according to the van den Dool and Kratz equation for temperature-programmed GC[30]:

$$\mathrm{LRI} = 100[\,\mathrm{n} + \frac{tR\,(x) - tR\,(n)}{tR\,(n+1) - tR\,(n)}$$

where:

$t_R(x)$ is the retention time for the analyte,

$t_R(n)$ and $t_R(n+1)$ are retention times of the n-alkanes eluting immediately before and after the compound,

n is the carbon number of the preceding alkane.

Alkane standards were injected at the beginning and end of each sample batch to verify retention stability. All LRIs are reported as the mean of three injections ($n = 3$).

Identification of compounds was performed using two complementary criteria: comparison of mass spectra with reference spectra from the NIST MS database; and comparison of experimental with literature values, including those reported in Adams, R.P. Identification of Essential Oil Components by GC/MS (4th ed.). Compound identification was accepted only when both mass-spectral match and LRI agreement with literature/Adams library values were satisfied (Tables S4-6).

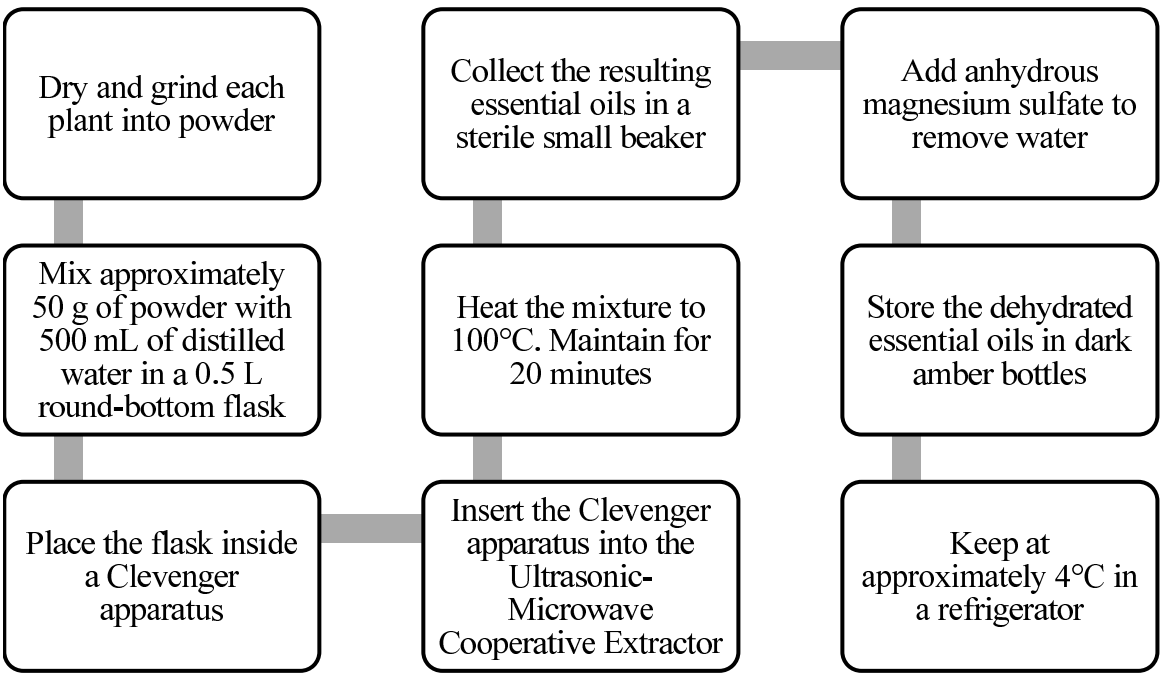

Fig. 1. Microwave-ultrasonic extraction of essential oils from *A. judaica*, *S. palaestina*, and *R. graveolens*.

Antiradical activity

With Trolox as a positive control, the capacity of EOs to scavenge free radicals was assessed by identical serial dilution in methanol (1 mg/mL) to concentrations starting with 3 and finishing with 100 µg/ml. The DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent (Sigma, USA) was dissolved in methanol at a concentration of 0.002% w/v and then combined 1:1 with the working quantities that had been prepared earlier. Using a UV-visible spectrophotometer the absorbance values were obtained after 30 min in dark room at room temperature.

DPPH inhibition (%) = $(abs_{blank} - abs_{sample})/abs_{blank} X100\%$.

Abs: absorbance. 50% Inhibition concentration (IC_{50}) of EOs and Trolox were assessed utilizing the BioDataFit-E1051 program[31].

Cytotoxicity assay

Human hepatic stellate (LX-2) cells, hepatocellular carcinoma (Hep G2), skin tumor (B16-F1), and cervical adenocarcinoma (HeLa) were collected from the ATCC, Rockville, MD, USA, and were all cultured in RPMI 1640 media supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin. After they were grown in a humidified environment at 37 °C and 5% CO_2, the cells were seeded at 5×10^3 cells/well on a 96-well plate and then exposed to 24, 48, and 125 µg/mL concentrations of assessed EO and doxorubicin (positive control). According to the directions on the package, the Cell-Tilter 96® Aqueous One Solution Cell Proliferation (MTS) bioassay (Promega Corporation, Madison, WI) was used to assess the cells' viability. After the treatment, 20 µL of MTS solution was added to each well for every 100 µL of media, and the plates were incubated for two hours at 37 °C. At 490 nm, the absorbance was measured with a UV-Vis spectrophotometer[32,33]. Three duplicates of each experiment were conducted. The means ± standard deviation (SD) are used to display the results. Only when the p-values were less than 0.05 were the results deemed significant.

Electrophysiological recordings

Every AMPA receptor subunit utilized in this investigation was of the flip isoform. The Salk Institute in La Jolla, California's S.F. Heinemann kindly donated the GluA1-3 (Q-form/flip) templates. We utilized Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum (FBS), 0.1 mg/ml streptomycin, and 1 mM sodium pyruvate to cultivate HEK293T cells (purchased from Sigma, Germany). These cells, which were supplied by Biological Industries in Beit-Haemek, Israel and sourced from Sigma, Germany, were cultivated in a humidified incubator with 5% CO_2 at 37 °C.

Wild-type AMPA receptor DNA was added downstream of an internal ribosome entry site in the pRK5 plasmid to aid transfection. In order to help track expression, this DNA additionally encoded an enhanced green fluorescent protein (EGFP) sequence, using resources from Clontech, Palo Alto, California. For optimal results, a ratio of 1:9 was used to cotransfect the pEGFP-C1 vector with GluA subunit DNA, achieving the most efficient expression levels.

HEK293T cells transiently were transfected with jetPRIME (Polyplus: New York, NY) according to the method previously reported. After 36 h, cells were sorted according to the intensity of their fluorescence and plated onto coverslips coated with laminin for whole-cell patch-clamp recordings. An Integrated Patch Clamp Amplifier system (IPA, Sutter Instruments, Novato, CA) connected to a two-barrel theta glass pipette via a rapid solution exchange system powered by a piezoelectric translator was used to conduct electrophysiological recordings. While the second pipette barrel held extracts of *A. judaica*, *S. palaestina*, or *R. graveolens* combined with 10 mM glutamate, the first barrel was filled with an external wash solution.

An extracellular solution with 2.8 mM KCl, 150 mM NaCl, 2 mM CaCl2, 0.5 mM MgCl2, and 10 mM HEPES, adjusted to pH 7.4, was used for the recordings. An intracellular solution comprising 110 mM CsF, 30 mM CsCl, 4 mM NaCl, 0.5 mM CaCl2, 10 mM EGTA, and 10 mM HEPES, adjusted to pH 7.2, was placed into patch electrodes made of borosilicate glass. Junction potentials at the pipette tip, typically between 200 and 300 µs, were monitored to establish the exchange rate of liquids. Electrode resistances ranged from 2 to 4 MΩ. A two-exponential model was used to fit the current's decline from 90 to 95% of peak to baseline in order to determine the deactivation and desensitization time course. $\tau w = (\tau f \times af) + (\tau s \times as)$ is the formula used to determine the weighted τ, where af and as represent the relative amplitudes of the fast, τf, and slow, τs, components, respectively[34,35].

10 mM glutamate was applied for 1 ms and 500 ms, respectively, to evaluate deactivation and desensitization. At room temperature (20–23 °C), with a holding potential of −60 mV and a pH of 7.4, recordings were made at a sampling rate of 10 kHz. To cut down on high-frequency noise, a low-pass filter of 2 kHz was used. Sutter Instruments' SutterPatch Software v.1.1.1 was used to digitize and process the data. Ten cells per condition, obtained from at least seven to nine separate transfections, were used for recording.

Statistical analysis

Igor Pro7 (WaveMetrics, Inc.) was used for data analysis, and one-way ANOVA was used to compare the treated and wild-type groups statistically. 'ns' is used to indicate non-significant results, and p-values ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) are used to report significance. Tukey's Honestly Significant Difference (HSD) test was used as the post hoc method following the one-way ANOVA. This method was selected due to its robustness in comparing all possible pairs of group means while controlling for the family-wise error rate. It is particularly suitable for our design and aligns with standard practices in the field. GraphPad Prism 6.01 (GraphPad Software) was used to fit concentration-response relationships to the Hill equation. At least three separate experiments are represented in all of the data.

Results

Phytochemical components of the EOs

The current investigation utilized microwave-ultrasonic technology with vibrations to extract pure EOs. The technology enhances the extraction process without using organic solvents and shorter time of extraction, which can maintain the chemical components of EOs without changes in chemical structure or damage, enabling the swift release of EOs. In addition, microwave heating and ultrasonication improve the mass transfer process and break up plant cell structures, leading to higher extraction yields and better-quality products. This approach provides a sustainable and efficient substitute for conventional and Soxhlet extraction methods, with the capacity to substantially decrease processing time and resource usage. Utilizing this technique, *A. Judaica*, *R. graveolens*, and *S. palaestina* leaves yielded 1.1 ± 0.11, 0.89 ± 0.09, and 1.41 ± 0.39% (w/w) of EOs, respectively.

The EO from *A. judaica* leaves contains twenty compounds as showed by GC-MS analysis (Table 1, Fig. S1), the two most abundant of which are piperitone (39.04%) and carvacrol (30.68%).

Instrumental GC-MS analysis of *R. graveolens* leaf EO showed the existence of fourteen organic molecules accounting for 100% of the oil (Fig. S2); the major compounds are 2-undecanone (50.82%) and 2-nonanone (27.00%), as shown in Table 2.

GC-MS screening of results of EO form *S. palaestina* leaf showed the existence of 28 molecules constituting the total oil. The major identified compounds are carvacrol (19.63%), eucalyptol (15.59%), and thujone (15.22%), which belonged to the oxygenated monoterpenoid group of EOs (Table 3, Fig. S3).

DPPH free radical scavenging activity

Effective antiradical capabilities are suggested by the compound's high DPPH free radical scavenging activity, which efficiently neutralizes and stabilizes free radicals to reduce oxidative damage. This test provides important information on the possible health-promoting effects of natural items, medications, and dietary supplements. It is an essential part of the assessment process.

Table 4 shows the IC_{50} values and standard deviations for DPPH free radical scavenging activity of *S. palaestina*, *A. judaica*, and *R. graveolens* EOs and Trolox (a synthetic antioxidant employed as a reference). The concentration of the sample needed to scavenge 50% of the DPPH radicals in the experiment is shown by the IC_{50} values. Lower IC_{50} values indicate higher antiradical potency. With an IC_{50} of 8.30 ± 2 µg/ml, *S. palaestina* exhibits most potent free radical scavenging activity. Following *A. judaica* EO, which has an IC_{50} of 33.65 ± 5 µg/ml, and *R. graveolens* EO has an IC_{50} of 49.23 ± 8 µg/ml. At the same time, Trolox has DPPH free radical scavenging activity with an IC_{50} of 2.7 ± 0.7 µg/ml.

Cytotoxic activity

For cancer management, the presently utilized therapeutic protocols are often linked with the development of multi-anticancer medication resistance, high costs, and serious adverse effects, highlighting the urgent need for cheap, less toxic, and more effective therapeutics[36]. There are many instances of chemicals extracted from plants that are utilized in cancer treatment procedures, and medicinal herbs have long been an alluring and limitless

#	Name	*R.T*	LRI	Literature LRI	Area	% area
1	Artemisia triene	9.34	920.8	922	12,744	0.02
2	α-Pinene	9.76	930.1	930	37,686	0.05
3	Propyl 2-methylbutanoate	10.36	943.4	943	231,802	0.32
4	Camphene	10.47	945.8	946	220,255	0.31
5	Benzoic aldehyde	11.04	958.4	985	151,220	0.21
6	Nopinene	11.71	973.2	973	119,311	0.17
7	Psi-cumene	12.44	989.4	989	387,812	0.54
8	Yomogi alcohol	12.65	994.0	996	195,315	0.27
9	Hemimellitene	13.61	1016.2	1017	258,698	0.36
10	p-Cymene	13.8	1020.7	1021	1,552,523	2.17
11	γ-vinyl-γ-valerolactone	14.35	1033.6	1035	110,497	0.15
12	Artemisia ketone	15.27	1055.3	1057	4,822,088	6.74
13	Linalool	17.05	1097.2	1097	515,146	0.72
14	Isophorone	17.85	1116.0	1116	2,206,581	3.08
15	camphor	18.84	1139.3	1139	8,152,271	11.39
16	Bornyl alcohol	19.86	1163.3	1163.0	1,212,439	1.69
17	Piperitone	22.92	1251.5	1252	27,937,342	39.04
18	Bornyl acetate	23.96	1280.5	1281	761,253	1.06
19	Thymol	24.26	1288.9	1289	715,801	1.00
20	Carvacrol	24.57	1297.5	1297	21,956,852	30.68
	SUM				71,557,636	100.00

Table 1. Phytochemical components of *Artemisia judaica* leaf EO.

No	Name	RT	LRI	Literature LRI	Area	% area
1	α-Pinene	9.74	929.6	930	5,974,772	7.63
2	Camphene	10.48	946.0	946	84,060	0.11
3	β-Pinene	11.69	972.8	970	2,140,032	2.73
4	β-Myrcene	12.28	985.8	986	196,652	0.25
5	3-Carene	13.1	1004.2	1004	1,805,877	2.31
6	α-Terpinene	13.48	1013.2	1013	30,442	0.04
7	o-Cymene	13.79	1020.5	1021	185,461	0.24
8	Limonene	14	1025.4	1025	1,877,550	2.40
9	2-Nonanone	16.65	1087.8	1088	21,144,628	27.00
10	Octyl methyl ketone	20.6	1187.9	1190	1,133,820	1.45
11	4,8-Dimethylnonanol	22.15	1230.1	1230	3,478,869	4.44
12	2-Undecanone	24.26	1288.9	1288	39,802,084	50.82
13	Methyl nonyl carbinol	24.62	1298.9	1298	149,994	0.19
14	Decyl methyl ketone	26.66	1359.9	1361	315,795	0.40
	SUM				78,320,036	100.00

Table 2. Phytochemical components of *Ruta graveolens* leaf EO.

No	Name	RT	LRI	Literature LRI	Area	% area
1	α-Tricyclene	9.27	919.2	919	511,619	0.17
2	α-Thujene	9.45	923.2	923	1,610,123	0.54
3	α-Pinene	9.77	930.3	930	16,692,845	5.63
4	Camphene	10.48	946.0	946	19,638,532	6.62
5	Sabinene	11.53	969.2	970	1,012,492	0.34
6	Nopinene	11.73	973.7	974	24,561,518	8.28
7	β-Myrcene	12.32	986.7	987	6,670,383	2.25
8	α-Terpinene	13.48	1013.2	1013	770,267	0.26
9	*o*-Cymene	13.81	1020.9	1021	10,780,588	3.63
10	Limonene	14.03	1026.1	1026	5,863,840	1.98
11	Eucalyptol	14.15	1028.9	1029	46,257,376	15.59
12	γ-Terpinene	15.28	1055.3	1055	3,686,442	1.24
13	Linalool	17.06	1097.4	1097	1,689,453	0.57
14	Thujone	17.28	1102.6	1103	45,157,016	15.22
15	3-Thujanone	17.71	1112.7	1114	9,851,650	3.32
16	Camphor	18.85	1139.5	1140	23,416,412	7.89
17	Borneol	19.86	1163.3	1163	2,284,130	0.77
18	4-Carvomenthenol	20.2	1171.3	1172	828,012	0.28
19	α-Terpinol	20.76	1184.5	1185	876,662	0.30
20	Bornyl acetate	23.98	1281.1	1281	431,566	0.15
21	Thymol	24.26	1288.9	1289	981,599	0.33
22	Carvacrol	24.6	1298.3	1298	58,258,392	19.63
23	α-Terpinyl acetate	26.1	1343.1	1345	716,415	0.24
24	Caryophyllene	28.54	1417.3	1417	8,450,774	2.85
25	Aromandendrene	29.14	1436.5	1436	530,203	0.18
26	Humulene	29.64	1452.6	1452	3,563,672	1.20
27	Caryophyllene oxide	33.52	1576.9	1577	399,947	0.13
28	Viridiflorol	33.89	1588.8	1589	1,232,491	0.42
	SUM				296,724,419	100.00

Table 3. Phytochemical components of *Salvia palaestina* leaf EO.

source of therapies. As a result, plant-derived anticancer medications such as vincristine, vinblastine, colchicine, taxol, paclitaxel, and others are currently employed in clinics as effective agents.

The MTS assay results revealed that all screened EOs showed cytotoxic effects against HeLa, HepG2, and B16F1 cancer cell lines (Table 5). Various concentrations of EOs were applied to cancer cells from 31.25 to 500 µg/mL. The results showed that all three EOs have a dose-dependent cytotoxic effect on all cancer cells

	IC_{50} values (µg/mL)			
Inhibitory effects against	***S. palaestina* EO**	***A. judaica* EO**	***R. graveolens* EO**	**Positive control**
Free radicals of DPPH	8.3 ± 2	33.65 ± 5	49.23 ± 8	2.7 ± 0.7[a]

Table 4. DPPH-free radicals inhibitory activities IC_{50} values (µg/mL) by *Artemisia judaica*, *Ruta graveolens*, *and salvia palaestina* EO and positive controls.

Cells	*A. judaica* EO	*S. palaestina* EO	*R. graveolens* EO	DOX
HeLa	395.17 ± 3.44	104.98 ± 2.6	830.90 ± 2.52	1.29 ± 1.02
HepG2	401.71 ± 1.04	178.7 ± 1.41	554.47 ± 1.85	0.89 ± 0.75
B16F1	226.35 ± 1.81	73.94 ± 1.62	752.90 ± 0.4	>>0.05
LX-2	75.92 ± 1.43	184.10 ± 0.54	515.2 ± 1.88	>>0.05

Table 5. IC_{50} (µg/mL) of *Artemisia judaica*, *Ruta graveolens*, and *Salvia palaestina* EOs against three cancer cell lines and one non-cancer cell line compared with Doxorubicin.

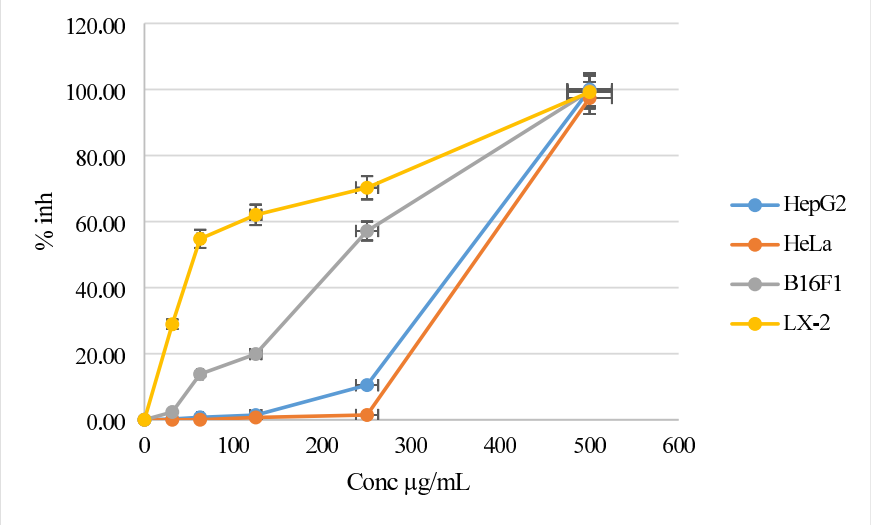

Fig. 2. Cytotoxic effect of EOs extracted from *Artemisia judaica* against HeLa, HepG2, B16F1, and LX-2 cell lines (p values < 0.05). Cell viability (%) versus concentration doses (µg/ml) of EOs.

(Figs. 2, 3, 4). In comparison to the positive control (Doxorubicin) and the negative control (DMSO), the EOs extracted from *S. palaestina* and *A. judaica* have the strongest cytotoxic effects against HeLa, HepG2, B16F1, and LX-2 with IC_{50} values of (104.98 ± 2.6, 395.17 ± 3.44), (178.7 ± 1.41, 401.71 ± 1.04), (73.94 ± 1.62, 226.35 ± 1.81), and (184.10 ± 0.54, 75.92 ± 1.43) µg/mL, respectively.

Inhibition and kinetic modulation of AMPA receptor subunits by *S. Palaestina*, *A. Judaica*, and *R. Graveolens* EOs

In the current study, whole-cell patch clamp recordings of GluA2/3, GluA1, GluA1/2, GluA2, and subunits were tested to assess the effects of *A. Judaica*, *S. palaestina*, and *R. graveolens* EOs. Among all the tested oils, *S. palaestina* was the most inhibitory potent. It reduced the whole-cell AMPA receptor currents 5-fold for GluA1, GluA1/2, and GluA2/3 subunits and about 6-fold for GluA2 (Fig. 5c,d). Furthermore, *S. palaestina* strongly affected the receptor kinetics by increasing almost 3-fold the deactivation rate in all subunits (Fig. 6). The desensitization rate was 4-fold slowed down in GluA2 (Fig. 7e,f), 3-fold in GluA2/3 (Fig. 7g,h), 2.2-fold in GluA1 (Fig. 7a,b), and 1.4-fold in GluA1/2 (Fig. 7c,d; Table S2).

The second EO, *A. judaica*, significantly inhibited AMPA receptor currents about 3.5-fold across all subunits tested (Fig. 5a,b). Regarding the kinetics, *A. judaica* increased the deactivation rate by nearly 2-fold (Fig. 6a,c,e,g). For GluA2 and GluA2/3, the rate of desensitization was lowered by approximately 2-fold (Fig. 7e,g) and that for GluA1 and GluA1/2 by 1.5-fold (Fig. 7a,c; Table S1). *R. graveolens*, although still statistically significant, had the weakest inhibitory effect among the three oils. It decreased AMPA receptor currents 3-fold for all tested subunits (Fig. 5e,f). The deactivation rate increased about 1.5-fold (Fig. 6a,c,e,g), while the desensitization rate decreased about 1-fold for all subunits (Fig. 7a,c; Table S3).

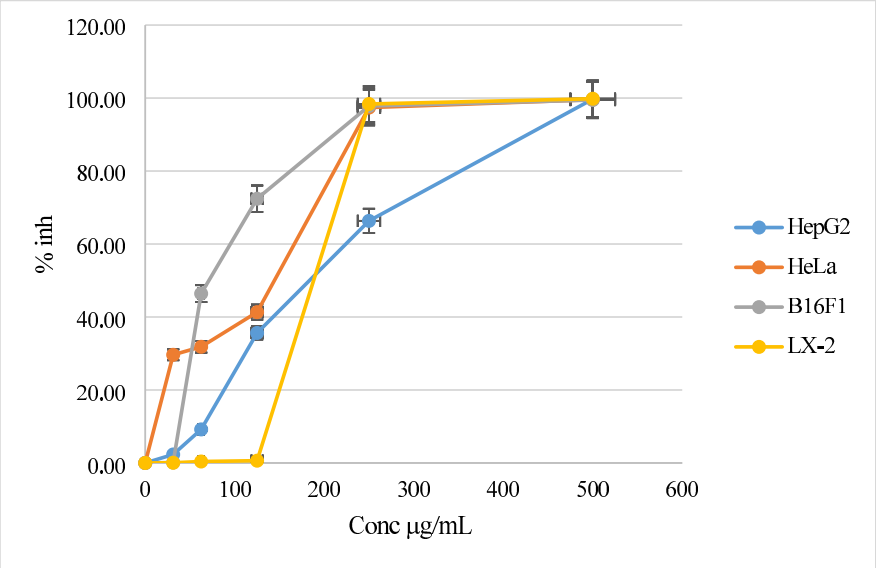

Fig. 3. Cytotoxic effect of EOs extracted from *Salvia palaestina* against HeLa, HepG2, B16F1, and LX-2 cell lines (p values < 0.05). Cell viability (%) versus concentration doses (µg/ml) of EOs.

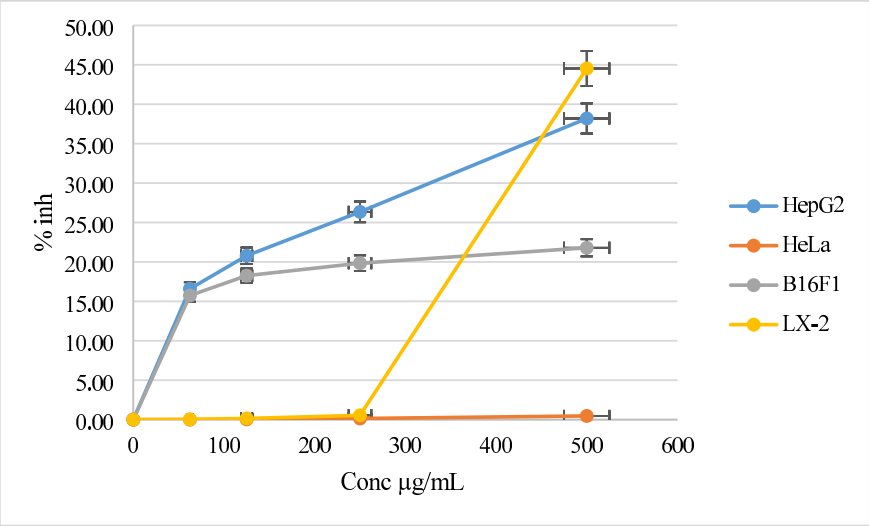

Fig. 4. Cytotoxic effect of EOs extracted from *Ruta graveolens* against HeLa, HepG2, B16F1, and LX-2 cell lines (p values < 0.05). Cell viability (%) versus concentration doses (µg/ml) of EOs.

Discussion

The literature stated that *A. judaica* EO had two different chemical types. Artemisyl skeleton-type compounds characterize one chemical type, while the other lacks these compounds but has relatively high percentages of piperitone and camphor[37,38] (Table 6). The current study's GC-MS analysis found that both types are present, as well as that *Artemisia triene* and *Artemisia ketone* belong to artemisyl skeleton-type compounds besides piperitone EO, which was the major one (39.04%), and instead of camphor, carvacrol (30.68%) was the second major molecule.

Rustaiyan et al. extracted the aerial portions of *S. palaestina* EO from Iran using the hydrodistillation process, yielding 0.26% (w/w). Out of the sixteen compounds they found, the most prevalent ones were β-caryophyllene (38.4%), α-humulene (8.7%), -spathulenol (10.5%), and -germanacrene D (11.2%) (75). Fifty molecules, comprising 99.82% of the total oil, were characterized as a consequence of Al-Jaber et al.'s investigation of the fresh and air-dried *S. palaestina* leaves EO growing wild in Jordan. Among the examined phytochemical groups, sesquiterpene hydrocarbons contributed the most, suppressing 52.66% of the total contents. Germacrene D (21.18%), α-copaene (12.14%), β-cubebene (7.93%), and cadinene (6.43%) were the main contributors. Sabinene

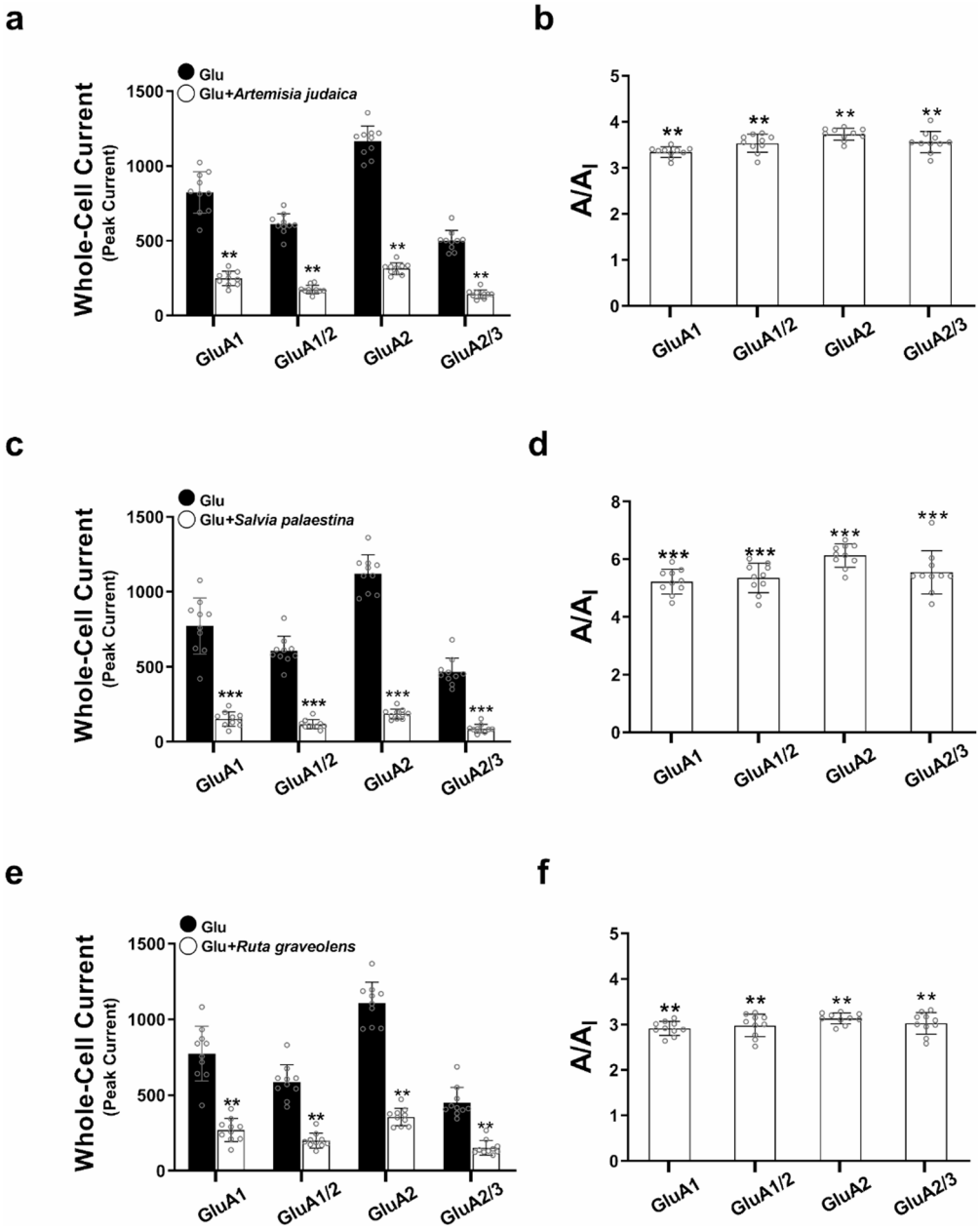

Fig. 5. Inhibition of AMPA Receptor Currents by *A. judaica*, *S. palaestina*, and *R. graveolens* EOs. Representative whole-cell patch-clamp recordings illustrating the actions of *A. judaica*, *S. palaestina* and *R. graveolens* EOs on the peak AMPA receptor-mediated currents in HEK293T cells transiently expressing GluA2/3, GluA1, GluA1/2, GluA2 subunits. Panels (**a**, **c**, and **e**) show currents elicited by glutamate alone (black) compared to currents evoked after treatment with the respective EOs (white). The appropriate A/A_I (normal current/inhibitory current) ratios at each oil are presented in panels (**b**, **d**, and **f**). One-way ANOVA calculated statistical significance levels: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; ns, not significant. Data represent the mean ± SD, $n = 10$ cells per condition analyzed.

(19.08%) was the main constituent of the monoterpene hydrocarbons, which made up 30.97% of the overall oil composition (76).

The chemical components of EO recovered by steam distillation from the aerial portions of *S. palaestina* from Saudi Arabia (yields 1.35%) were described by Tawfek and Al Howiriny. Thirty-four compounds, or 97.1% of the total oil, were identified; the principal constituents were 1,8-cineole (5.2%), linalool (7.8%), guaiol (5.4%), Σ-caryophyllene (16.9%), and sclareol (26.8%) (77). Furthermore, according to a study by Gürsoy et al., 70 distinct compounds were found in *S. palaestina* hydrodistilled EO from Turkey, representing 92.5% of the total oil contents. (E)-caryophyllene (4.5%), pulegone (4.1%), terpineol-4 (4.0%), farnesyl acetone (3.1%), carvacrol, α-copaene (4.0%), and caryophyllene oxide (16.1%) were the main molecules (78).

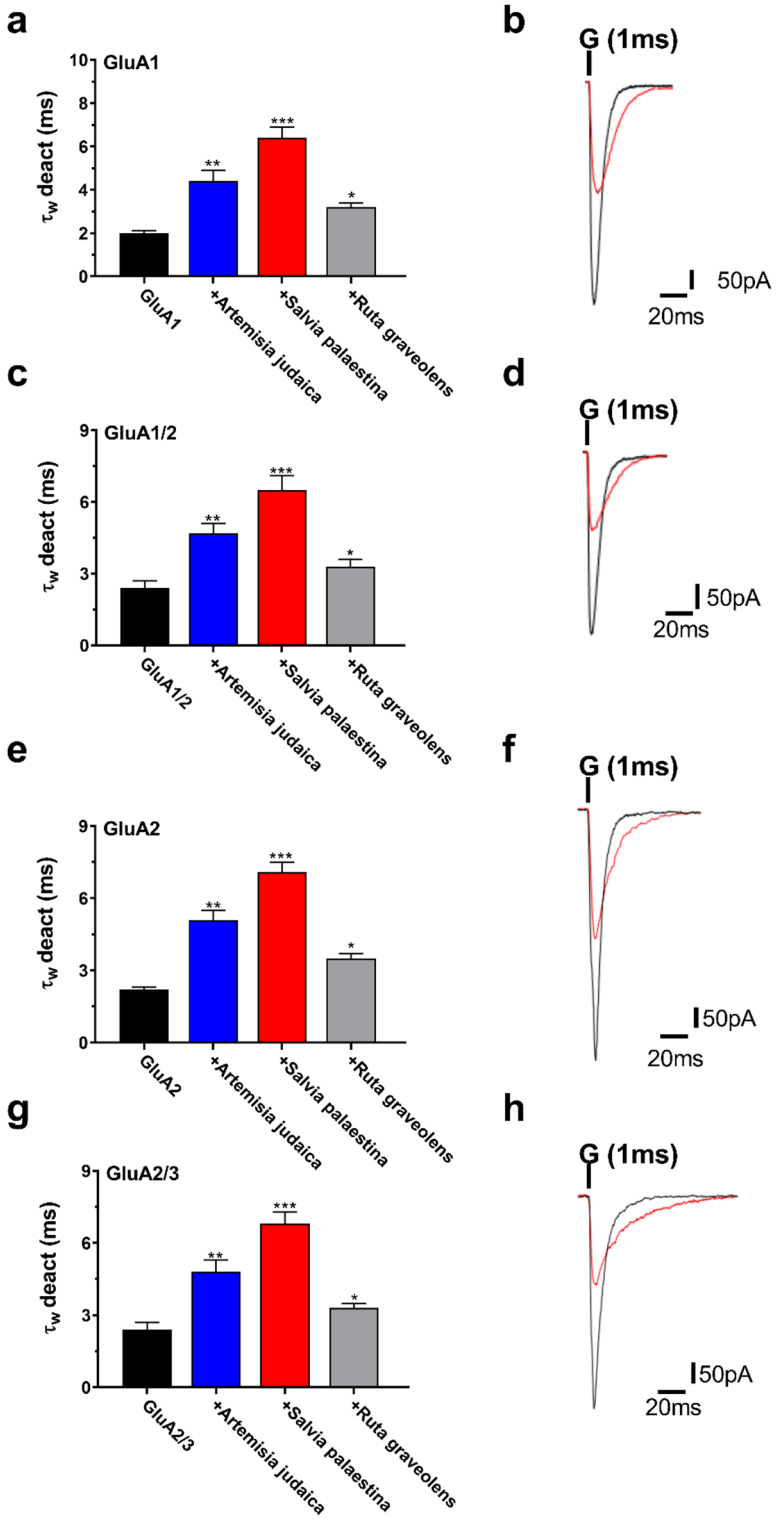

Fig. 6. Effects of *A. judaica*, *S. palaestina*, and *R. graveolens* EOs on AMPA Receptor Deactivation Rates. The deactivation rates of AMPAR subunits GluA2/3, GluA1, GluA1/2, and GluA2 following exposure to *A. judaica* (blue), *S. palaestina* (red), and *R. graveolens* (gray) are shown in Panels (**a**, **c**, **e**, and **g**). Panels (**b**, **d**, **f**, and **h**) display traces of responses both before and after *S. Palaestina* was applied. The recordings were made at −60 mV, 20–23 °C, and a pH of 7.4. One-way ANOVA, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; ns, not significant, was used for statistical comparisons. Mean ± SD is used to display the data ($n = 10$ cells/condition).

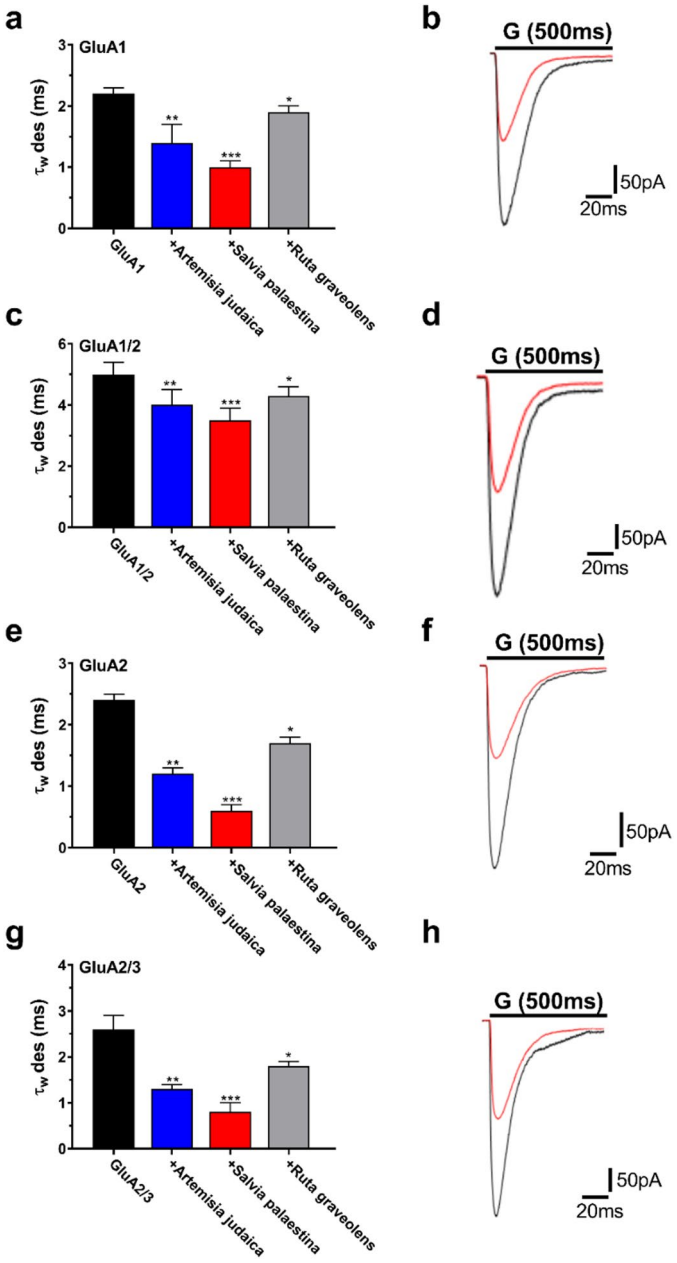

Fig. 7. Effects of *A. judaica*, *S. palaestina*, and *R. graveolens* EOs on AMPA Receptor Desensitization Rates. Panels (**a**, **c**, **e**, and **g**) depict desensitization rates of AMPAR subunits GluA2/3, GluA1, GluA1/2, GluA2 after exposure to *A. judaica* (blue), *S. palaestina* (red), and *R. graveolens* (gray). Traces depicting responses before and after the application of *S. Palaestina* are shown in panels (**b**, **d**, **f**, and **h**). Recordings were done at −60 mV under a pH of 7.4 and temperature of 20–23 °C. Statistical comparisons were performed using one-way ANOVA, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; ns, not significant. Data are presented as mean ± SD ($n = 10$ cells/condition).

The studies above showed that *S. palaestina* EO major compounds differ from region to region. B-caryophyllene and germacrene D were the dominant molecules of *S. palaestina* EO from Iran. In contrast, germacrene D and α-copaene were the abundant molecules of *S. palaestina* EO from Jordan. Moreover, sclareol and Σ-caryophyllene were the plant EO's major components from Saudi Arabia. In addition, caryophyllene oxide and (E)- caryophyllene (4.5%) were the dominant molecules of *S. palaestina* EO from Turkey. Many factors have been stated as influencing parameters affecting EO quality, yields, and components, such as the plant maturity stage, extraction, and drying methods, as well as the plant locations and the environmental conditions from which the plant was collected[39]. A similar pattern of geographic chemotypic diversity has also been reported for *R. graveolens*, with substantial variation in its essential oil composition among different countries, where 2-undecanone and 2-nonanone are most commonly identified as the dominant constituents (Table 7).

Regions	Major constituents	Yields (%)	References
Jordan	Chrysanthenone (4–31%), artemisia ketone (9–24%), camphor (0.3–16%), piperitone (3–15%), and cinnamate (11.0%).	0.4–0.9% (w/w)	[48]
Sinai, Egypt	Camphor (31.4%), endo-borneol (5.72%), and piperitone (29.9%)	0.28% (w/w)	[49]
Saudi Arabia	cis-Thujone (2.5%), thymol (3.5%), trans-sabinyl acetate (3.3%), carvacrol (3.5%), b-eudesmol (13.1%), eudesma-4 (15), 7-dien-1-b-ol (3.5%), and hexadecanoic acid (5.7%)	0.18% (v/w)	[50]
Algeria	Piperitone (66.17%), ethyl cinnamate isomer (6.11%), spathulenol (2.34%), E-longipinane (2.55%)	1.7% (w/w)	[51]
Egypt	Piperitone (49.1%) and camphor (34.5%), borneol (3.90%)		[52]
Libya	cis-Chrysanthenol (9.1%), piperitone (30.2%), and ethyl cinnamate (3.8%).	0.62% (*w*/*w*)	[53]
Northern Qassim region of Saudi Arabia	piperitone (31.99%), terpinene-4-ol, α-thujone, β-thujone, 1,8-cineole, camphor, and linalool were represented at relatively high concentrations of 6.42, 5.94, 3.61, 2.56, 1.92, and 1.21%, respectively,	1.71 ± 0.3% *w*/*w*	[5]

Table 6. The diverse chemical types of *A. judaica* EO from different regions.

Regions	Major constituents	Yield (%)	References
Iran	2-Undecanone (33.9%), 2-heptanol acetate (17.5%), 1-dodecanol (11.0%), geyrene (10.4%), and 2-nonanone (8.8%)	0.4	[54]
Turkey	2-Undecanone (64.8%) and 2-nonanone (13.8%)	1.25%	[55]
Tunisia	2-Undecanone (56.9%), 2-nonanone (23.6%), and 1-nonen (4.4%)	1.67	[56]
Portugal	2-Undecanone (91.0%) and 8-phenyl-2-octanone (7.0%)	0.81%	[57]
Peru	2-Undecanone (40.9%), 2-nonanone (29.0%), and β-caryophyllene (3.4%)	0.27%	[58]
India	2-Undecaone (43.7%), 2-nonanone (16.1%), 2-tridecanone (2.6%), and 2-decanone (2.6%)	1.29%	[59]
Egypt	2-Undecanone (62.0%) and 2-nonanone (18.0%)	0.34%	[60]
Colombia	2-Nonanone (35.4%), 2-undecanone (30.5%), and 2-decanone (3.4%)	1.60%	[61]
China	2-Undecanol acetate (19.2%) and 2-undecanol 2-methylbutyl ester (8.9%)	0.99%	[62]
Algeria	2-Undecanone (55.4%), 2-nonanone (21.6%), 1-nonene (4.4%), and α-limonene (4.3%)	0.18%	[63]

Table 7. The diverse chemical types of *Ruta graveolens* leaf EO from various countries.

In the current experiment, *S. palestina* EO has potent DPPH free radical scavenging activity compared with Trolox (8.30 ± 2 and 2.7 ± 0.7 µg/mL, respectively) over other investigated plants EOs due to the presence of carvacrol, the dominant molecule in *S. palaestina* EO. Carvacrol is the primary component of many Lamiaceae family EOs, which is recognized by its potent antiradical activity similar to Ascorbic acid and vitamin E[40].

At a concentration of 500 µg/mL, a test of HepG2, HeLa, B16F1, and LX-2 cell lines showed that EO from *A. judaica* EO suppressed the screened cell's growth with 99.97, 97.41, 99.51, and 99.20%, respectively (p-value < 0.05). Moreover, at the same *S. palaestina* EO suppressed the screened cell's growth with 99.71, 99.55, 99.46, and 99.81%, respectively (p-value < 0.05). In addition, *R. graveolens* EO inhibited the growth of the tested cells in the same concentration with 38.20, 0.47, 21.79, and 44.53%, respectively.

These extracts have achieved significant AMPA receptor amplitude reduction, along with alteration in the deactivation and desensitization time, which become very important factors in the excitotoxicity-regulating mechanism that is important for the pathologies of diseases such as Alzheimer's disease, Parkinson's disease, and epilepsy. The magnitude of current inhibition observed in this study (3–6-fold reduction) is substantial for crude essential oils. the current work demonstrates that the whole essential oils of *A. judaica*, *S. palaestina*, and *R. graveolens* are sufficient to produce pronounced modulation of AMPA receptor kinetics (see Supplementary Tables S1–S3). Some studies have been conducted on *A. judaica* for its neuromodulatory properties, with much work being done in model systems of neurotoxicity. Several studies reported evidence that EOs and extracts obtained from *A. judaica* exert a protective effect on neuronal cells, which is thought to occur mainly through antioxidant mechanisms and modulation of ion channels[41,42]. The strong AMPA modulation we observed for *A. judaica* EO is consistent with the chemical profile reported for regional Artemisia samples, which commonly include oxygenated monoterpenes (e.g., piperitone, camphor, 1,8-cineole) known to affect ion channel function and synaptic signaling. Piperitone and camphor, both present at high abundance in our samples, have been implicated in previous studies of Artemisia extracts that reported neuroactive and ion-channel modulating effects, providing precedent for potent electrophysiological actions of whole-plant volatile fractions[43].

Like others of the *Salvia* genus, *S. palaestina* has modulatory effects attributed to conditions that involve oxidative stress and synaptic dysfunction. The neuromodulatory properties of *Salvia* compounds were demonstrated in several studies, which showed the modulation of glutamatergic transmission, enhancement of synaptic plasticity, and prevention of neuronal death in models of neurodegeneration[44]. Results from the current study show that *S. palaestina* extracts significantly impact AMPAR desensitization properties and may indicate anti-exitotoxicity by preventing the excessive, sustained activation of AMPARs. Such findings are consistent with previous works proposing that extracts from *Salvia* could reduce glutamate-induced neurotoxicity and, therefore, might be applied in treating disorders like Alzheimer's, where a dysregulation in glutamate takes a major part[44]. The pronounced inhibition is plausibly explained by its major constituents. Carvacrol (19.63%) is a phenolic monoterpene that has been shown to modulate glutamatergic transmission and reduce excitatory

synaptic currents in neuronal preparations, consistent with a direct or indirect suppression of AMPAR-mediated responses[45]. Eucalyptol (1,8-cineole; 15.59%) displays neuroprotective activity in several models and can modulate excitotoxic pathways, while α- and β-thujone (together ≈ 15%) are known to interact with neuronal ligand-gated channels and influence excitability[46].

These effects have also been credited to *R. graveolens*, notably for its modulation activity on ion channels and neurotransmitter systems. Alkaloids and flavonoids, bioactive plant metabolites from *Ruta* species, exert an effect against excitotoxic neuronal injury by modulating the excitatory receptor activity, such as AMPA and NMDA receptors[47]. Such a decrease in AMPA receptor amplitude and an increase in the desensitization process fully agree with previous assumptions that *R. graveolens* may reduce abnormal excitatory transmission and protect neurons against neurodegenerative injuries. This corresponds to the investigations showing that *R. graveolens*, together with some of its closely related species, can act against oxidative stress and excitotoxicity, significant factors of neuronal death in serious pathologies such as epilepsy and Parkinson's disease[47].

Conclusion

This work highlighted the high and promising bioactivity of *A. judaica*, *R. graveolens*, and *S. palaestina* EOs for antiradical, cytotoxic, and neuromodulatory uses. The applied microwave-ultrasonic extraction technique was suitable since it yielded desirable qualitative characteristics in EOs and assessed relevant biological properties. Among all the oils, *S. palaestina* showed the highest antiradical capacity and the strongest inhibitory activity on AMPA receptor activity, placing it as a promising plant for therapeutic use in the treatment of neurological disorders associated with oxidative stress. *A. judaica* also showed a very good profile of AMPA receptor-modulatory activities, whereas *R. graveolens* showed a moderate bioactivity in all the tests performed. These findings provide the groundwork that could be used to extend the research on the medicament activities of these EOs and can be exploited for the design of new drugs targeting oxidative damage, and neurologic dysfunction. Further investigations are needed to evaluate the practical values of the therapeutic applications of *A. judaica*, *R. graveolens*, and *S. palaestina* EOs.

Data availability

The article includes all the utilized data to support the current study's findings.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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