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Exploring the *Iris haynei* essential oil: analysis of phytochemical composition, evaluation of cytotoxicity, antimicrobial properties, and AMPA receptor modulation

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Abstract

Background The complex composition of essential oils (EOs) derived from plants offers potential therapeutic approaches for treating various medical conditions, including neurological disorders and infectious diseases. This research aimed to comprehensively analyze the phytochemical composition of *Iris haynei* EO and evaluate its pharmacological properties, specifically its cytotoxicity, antimicrobial activity, and modulation of AMPA receptors.

Results The analysis of *I. haynei* EO, conducted using gas chromatography and mass spectroscopy, identified diethyl phthalate, α-terpineol, and benzyl acetate as the main components. Cytotoxicity experiments on several cancer cell lines revealed a dose-dependent impact. Electrophysiological recordings on HEK293T cells expressing AMPARs showed varying levels of inhibition across different subunits, with receptors encoding GluA2 exhibiting the most significant effects. The EO also demonstrated significant antibacterial activity against both Gram-positive and Gram-negative bacteria, as well as fungi.

Conclusions The findings of this study underscore the potential therapeutic benefits of *I. haynei* EO, particularly in treating neurological conditions associated with AMPA receptor dysfunction and exhibiting antimicrobial activity against infectious agents. The promising results warrant further investigation into the pharmacological effects of the EO, suggesting a novel approach for addressing cerebral ischemia and related neurological diseases.

Keywords *Iris haynei*, Phytochemistry, Cytotoxicity, Antimicrobial, AMPA receptors, GluA2

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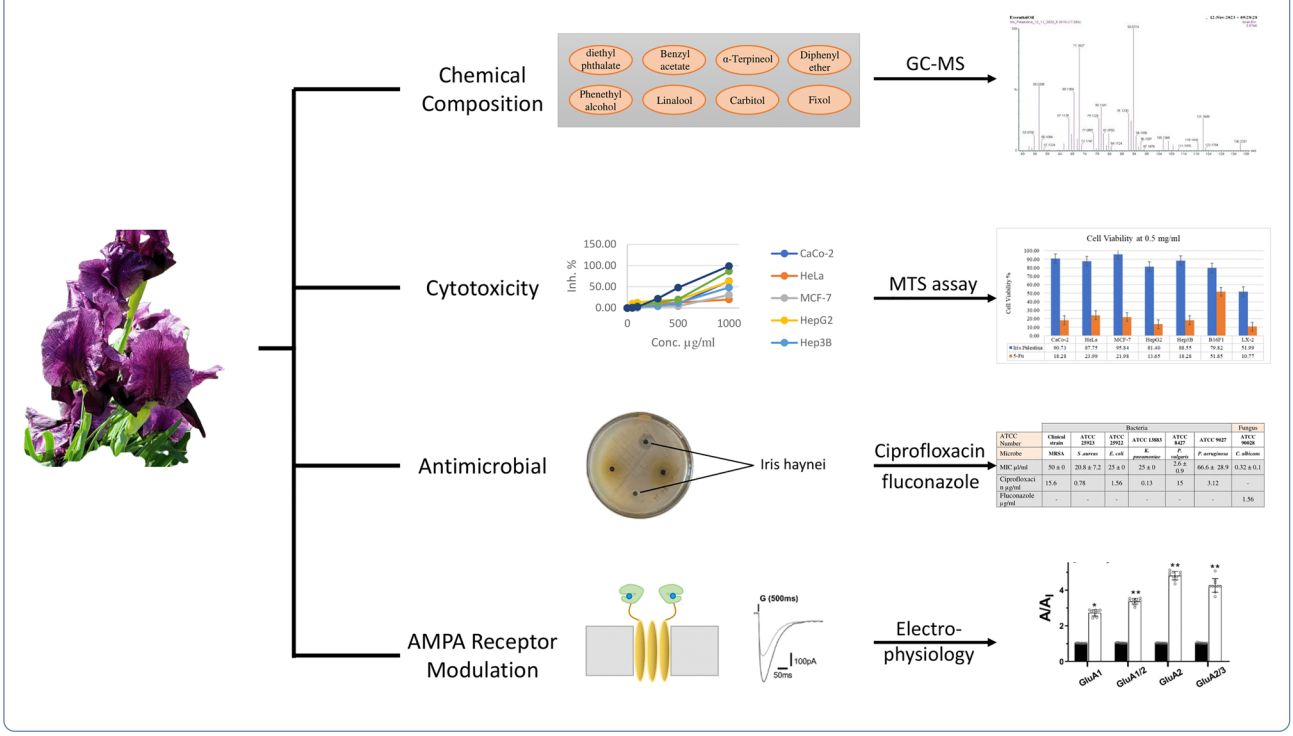
Graphical Abstract



Background

Iris haynei, or Gilboa iris, is a species in the Iris family known for its vibrant purple flowers with a dense network of veins and dots. Native to the Gilboa mountain and the West Bank region in Palestine, this rhizomatous perennial reflects the rich biodiversity of the eastern Mediterranean. Its greyish-green, linear to lanceolate leaves and captivating fragrance contribute to its unique esthetic appeal. Thriving in Mediterranean shrublands, woodlands, and rocky hillsides at 150–550 m, *I. haynei* demonstrates remarkable adaptability [1, 2]. Despite being classified as vulnerable, its ability to flourish in various environmental settings, from shaded areas to sun-drenched rocky terrains, underscores its ecological resilience and versatility [3].

Traditionally, *I. haynei* has been deeply integrated into the cultural and medicinal practices of its native regions. Various parts of the plant, especially the rhizomes, have been utilized for their health-promoting properties, finding their place in preparing traditional remedies for various ailments [4]. This longstanding use in ethnomedicine highlights the plant's significant role in the heritage and healing practices of local communities, where it has been cherished not just for its ornamental beauty but also for its contribution to traditional healing modalities, bridging the gap between natural beauty and therapeutic utility.

The EOs are a concentrated hydrophobic liquid containing volatile aroma compounds from the plant, representing a significant aspect of its chemical composition. This oil is typically extracted through distillation, which involves passing steam through the plant material to vaporize the volatile compounds, which are then condensed and collected. This method captures the plant's essence, encapsulating its aromatic and bioactive compounds [5]. The chemical composition of EO is characterized by an intricate combination of ingredients, such as terpenes, esters, and alcohols, each playing a role in the oil's unique scent and possible healing powers. This intricate chemical composition invites a deeper exploration of its applications and benefits, particularly in natural products and alternative therapies [6]. The diverse array of chemical constituents not only defines the unique aroma and flavor profile of plants but also suggests a range of pharmacological activities, such as antimicrobial [7], anti-inflammatory [8], and antioxidant effects [9], which could have significant implications for its use in natural medicine and aromatherapy.

The exploration of EOs, including that of *I. haynei*, within therapeutic contexts highlights a deep-seated

reverence for the healing powers of nature that transcends cultural and temporal boundaries, blending ancient wisdom with modern scientific inquiry. This ongoing research into Eos' complex chemical compositions unveils many bioactive molecules, each harboring the potential for diverse therapeutic applications. Among these, the potential modulation of AMPA receptors by constituents found in *I. haynei*'s EO presents a fascinating avenue for neuropharmacological research, particularly in cerebral ischemia.

AMPA receptors, essential ionotropic glutamate receptor family components, are fundamental to fast synaptic transmission within the central nervous system, orchestrating rapid excitatory signals crucial for learning, memory, and cognitive functionality. These receptors exhibit distinct kinetic properties, including rapid activation and deactivation upon glutamate binding, which enables them to mediate quick synaptic responses integral to high-frequency neuronal signaling [10, 11]. The modulation of AMPA receptor kinetics, such as changes in channel opening times and the rate of glutamate unbinding, can significantly influence neural plasticity and the efficacy of synaptic transmission. This dynamic regulation of AMPA receptors is central to the adaptive changes in the brain's neural circuitry, underscoring their pivotal role in studying neurological disorders, including cerebral ischemia [12, 13]. In the context of cerebral ischemia, characterized by reduced cerebral blood flow, there is a disruption in normal glutamatergic signaling, leading to a surge in excitotoxicity, primarily due to the overactivation of AMPA receptors. This pathological overstimulation alters the usual kinetics of AMPA receptors, exacerbating calcium and sodium influx into neurons, which can culminate in neuronal injury and death. The strategic modulation of AMPA receptor kinetics, therefore, emerges as a vital therapeutic approach to mitigate the excitotoxic damage associated with ischemic brain tissue, potentially offering neuroprotection and improving outcomes following ischemic stroke events like many other emerging antagonists of AMPA receptor [14, 15].

The bioactive compounds in *I. haynei* EO may interact with AMPA receptors, allowing neuroprotection by modulating receptor activity to reduce excitotoxicity during cerebral ischemia. This approach aligns with exploring natural products for neuroprotective strategies against cerebral ischemia and other neurological conditions. The relationship between *I. haynei* EO, AMPA receptor modulation, and cerebral ischemia treatment highlights the importance of integrating natural product research, neuropharmacology, and clinical neuroscience. It emphasizes a multidisciplinary strategy in discovering new nature-derived therapeutic agents, potentially advancing the management of cerebral ischemia and contributing to the broader understanding of neuroprotection in neurodegenerative disease contexts [16].

The primary objectives of this comprehensive study are multifaceted and aimed at unraveling the intricate interactions between *I. haynei* EO and key biological targets. First, the study seeks to elucidate the inhibitory effects of *I. haynei* EO on AMPA receptors, pivotal elements in synaptic transmission and neural plasticity, which play a significant role in the pathophysiology of various neurological disorders. By investigating how the bioactive compounds within *I. haynei* EO modulate AMPA receptor activity, the research aims to contribute valuable insights into these natural compounds' potential neuroprotective properties, especially in conditions such as cerebral ischemia. Simultaneously, the study is designed to explore the cytotoxic effects of *I. haynei* EO on cancer cells, delving into the potential anticancer properties of its complex chemical composition. This line of inquiry seeks to expand the understanding of the EO's bioactivity against cancer cell lines and identify specific compounds responsible for these effects, thereby opening avenues for developing novel anticancer therapies derived from natural products. The growing fear of antibiotic resistance has motivated an investigation into new antimicrobial drugs. *Iris* species are widely known for their therapeutic capabilities as medicinal herbs [17]. *I. haynei*, found in the Palestinian environment, shows promise for its possible antibacterial activity, which could be valuable in the fight against infectious diseases. An integral part of the study also involves a detailed analysis of the chemical composition of *I. haynei*, aiming to identify and characterize the array of compounds present in the EO. This comprehensive chemical profiling will be the foundation for understanding the pharmacological activities observed, linking specific constituents with their biological effects on AMPA receptors, microbial infections, and cancer cells.

Materials and methods

Plant materials

During the flowering season, healthy, dust-free, fresh *I. haynei* (9.3 kg) flowers were collected from the Jenin region of Palestine in June 2023, authenticated by Prof. Jaradat Nidal, Pharmacognosist, Department of Pharmacy, An-Najah University, where a voucher specimen (Pharm-PCT-1269) was deposited in the Natural Product Laboratory. After removing the sepals, the harvested flowers were dried in a shaded place for 14 days. After drying, the weight of the flowers was reduced to 2.3 kg.

Essential oil extraction procedure

The EO of *I. haynei* dried flowers was extracted using the microwave-ultrasonic method, following the procedure

described by Jaradat et al. [18]. The extraction involved exposing the powder suspension to ultrasound and microwaves to enhance the process. In a 1 L round-bottom flask, 80 g of dried flower powder was mixed with 800 ml of distilled water and placed in a microwave-ultrasonic apparatus. The extraction was performed at 1000 W for 40 min at 100 °C and repeated thrice for the same plant sample. The obtained *I. haynei* EO, with an average yield of 1.55% (w/w), was chemically dried using magnesium sulfate and stored at 2–8 °C.

Essential oil characterization

To characterize the *I. haynei* EO quantitatively and qualitatively, we utilized the approach outlined in Ref. [19]. Gas chromatographic (GC) studies were conducted using an HP 5890 series II gas chromatograph equipped with a Perkin Elmer Elite-5-MS fused silica capillary column (30 m × 0.25 mm, film thickness 0.25 m). The helium flow rate was maintained at 1.1 mL/min, while the injector and oven temperatures were set to 250 °C and 50 °C for 5 min, respectively. The mass spectroscopy (MS) scan time ranged from 8 to 44 min, covering a mass range of 50.00 to 300.00 m/z. The mass spectra were collected at 70 eV using electronic ionization. Retention indices (RIs) were estimated using a standard combination of normal alkanes injected as a reference (C_6–C_{27}) under the specified conditions, utilizing the equation recommended by the International Union of Pure and Applied Chemistry (IUPAC) (https://goldbook.iupac.org/te+rms/view/R05360):

$$\mathrm{RI} = 100 \times (((\mathrm{tR}(i) - \mathrm{tR}(z))/(\mathrm{tR}(z+1) - \mathrm{tR}(z))) + z)$$

where the carbon atoms numbers in the smaller *n*-alkane and tR(*i*), tR(*z*), and tR(*z*+1) are the retention times of the desired molecule, the smaller *n*-alkane, and the larger n-alkane, respectively, briefly, the EO compositions were identified by comparison of their retention indices (RT) to the RT of a series of *n*-alkanes (C_6–C_{27}; Sigma, Milan, Italy) with those reported by Ref. [20] and listed in Ref. [21].

Cytotoxicity assay

The breast (MCF-7), liver (Hep3B, HepG2), cervical (HeLa), and colon (Caco-2) cancer cells were grown in the RPMI 1640 medium, and LX-2 and B16F1 cells were grown in the DMEM medium. All cells were incubated at 37 °C in a humidified environment with 5% CO2, and 2.6 × 104 cells/ml were then seeded into a 96-well plate. After 24 h, cells were exposed to various concentrations of the tested EO (1000, 500, 300, 100, and 50 µg/ml) for 72 h. The Cell-Tilter 96® aqueous one solution cell proliferation (MTS) bioassay was used to evaluate the viability of the cells following culture. The MTS assay was based on adding 90 µl of medium, and 10 µL of MTS solution was added to each well. The plates were incubated for 2–4 h at 37 °C before the absorbance was determined using a UV–Vis spectrophotometer at 490 nm [22]. The *I. haynei* EO cytotoxicity assays were conducted in triplicate, and the results were presented as means (±) standard deviation (SD). A p value less than 0.05 was considered statistically significant.

Electrophysiological recordings

HEK293T cells expressing AMPARs (flip isoform) from various subunits, provided by S.F. Heinemann at the Salk Institute, were used. Transient transfections were performed with jetPRIME (Polyplus: New York, NY) [13]. Electrophysiological measurements were made using the whole-cell patch-clamp technique [23, 24]. Data were acquired using Integrated Patch Clamp Amplifiers with a Data Acquisition System (I.P.A., Sutter Instruments, Novato, CA). A piezoelectric translator (Automate Scientific, Berkeley, CA) operated a two-barrel theta glass pipette for rapid solution exchange. One barrel contained the wash solution, and the other contained *I. haynei* EO (800 µg/ml) mixed with glutamate. EO concentration was determined through incremental testing, starting at 100 µM and increasing to a maximum of 800 µg/ml. Electrode resistance was 2–4 µΩ.

Data analysis entailed fitting the current decline from 90 to 95% of the peak to the baseline current using two exponentials, allowing for calculating time constants for deactivation (τ_w deact) and desensitization (τ_w des). The weighted tau (τ_w) was computed as

$$\tau_w = (\tau f \text{ x af}) + (\tau s \text{ x as})$$

where af and as represent the amplitudes of the fast (τf) and slow (τs) exponential components, respectively. Currents of deactivation and desensitization were measured using 10 mM of glutamate for 1 ms and 500 ms, respectively, at a potential of − 60 mV, pH 7.4, and room temperature (20–23 °C). Each experiment involved ten cells to calculate the mean of inhibition, desensitization, and deactivation. Control recordings were conducted with glutamate alone to ascertain the effect of *I. haynei* EO on the cells and confirm cellular health. Data analysis was performed using Igor Pro7 (Wave Metrics, Inc). Statistical differences between experimental groups and the wild type were assessed using one-way analysis of variance (ANOVA). Significance levels were set as follows: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.05$; ns, insignificant, with $^{***}p < 0.05$ denoting statistical significance.

Antimicrobial evaluation

The antimicrobial activity of the *I. haynei* EO on microbial strains (clinically confirmed Methicillin-Resistant *Staphylococcus aureus* (MRSA) strain, *Staphylococcus aureus* (ATCC 25,923), *Klebsiella pneumonia* (ATCC 13,883), *Proteus vulgaris* (ATCC 8427), *Escherichia coli* (ATCC 25,922), *Pseudomonas aeruginosa* (ATCC 9027) and *Candida albicans* (ATCC 90,028)) cultivated for 18–24 h in a culture plate was evaluated using the broth microdilution method as mentioned before [25, 26]. The choice of bacterial strains for the antimicrobial assay was based on their clinical relevance and common occurrence in infections. MRSA and *S. aureus* were selected due to their significance in skin and soft tissue infections. *K. pneumoniae* and *E. coli* were included for their roles in urinary tract infections and other hospital-acquired infections [27, 28]. *P. vulgaris* and *P. aeruginosa* are known for their resistance to multiple antibiotics and presence in various infections. *C. albicans* was chosen to assess the antifungal properties of the EO, given its relevance in fungal infections [29, 30].

I. haynei EO was diluted in a 5% dimethyl sulfoxide solution at 200 µL/mL concentration. Using a 96-well plate and under an aseptic environment, EO solution was serially diluted ten times in sterile Muller Hinton broth (MHB) or RPMI media for *C. albicans*.

Micro-wells numbered 1 to 11 were inoculated with the test microorganisms using a sterile fresh microbial inoculum in MHB. Upon inoculation, the final microbial cell concentrations were around 5×10^5 colony-forming units (CFU)/mL for the studied bacterial isolates. The antibacterial effectiveness of *I. haynei* EO against each tested organism was assessed three times. The inoculated plates were placed in an incubator set at 35 °C. The incubation time was 24 h, except for *C. albicans,* which was 48 h. The minimum inhibitory concentration (MIC) of the *I. haynei* EO was the lowest concentration, where no microbial growth was seen in the micro-well. Multiple controls were employed in the MBD test.

Positive controls: Ciprofloxacin was employed as a standard antibacterial agent to confirm the inhibitory activity against microbial growth in the testing technique, while fluconazole was used as a standard antifungal agent to confirm the inhibitory activity against fungal microorganisms. Each tested microbe is placed in micro-well number 11 of a 96-well plate containing *I. haynei* EO-free MHB and the test microbe. This is done to confirm that the test microbe can grow in our experimental conditions.

Negative controls: The initial negative control contained only the growth medium (Muller Hinton Broth or RPMI media) without the essential oil or microbial inoculum

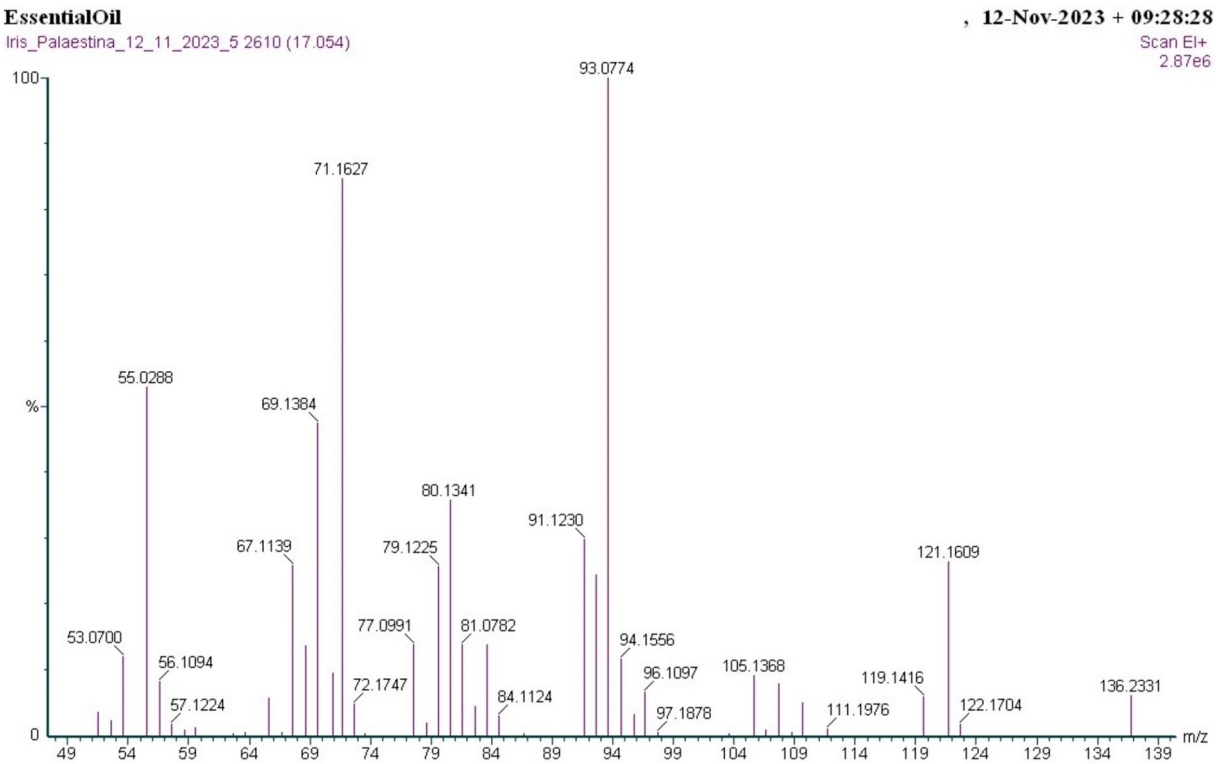


Fig. 1 Gas chromatography–mass spectrometry chromatogram of *I. haynei* EO

(Micro-well number 12). In the negative control context, an additional control was included, which consisted of the EO (without microbe) being serially diluted in the MHB. This control verified that any observed antimicrobial activity was solely due to the EO's antimicrobial properties and not caused by contamination or other external factors [31–34].

Results

Phytochemistry

In this investigation, the chemical composition of *I. haynei* EO was examined using chromatography (GC) coupled with mass spectrometry (MS) techniques (Fig. 1). Table 1 displays the elution sequence of the identified compounds on the Elite-5-MS fused silica capillary column, along with their percentages, retention time, calculated kovats retention index, and literature kovats retention index for *I. haynei* EO. We identified 29 molecules from the *I. haynei* EO, which accounted for 100% of the total oil. The analysis revealed that diethyl phthalate (65.87 ± 0.11), *α*-terpineol (14.16 ± 0.07%), and benzyl acetate (5.46 ± 0.1%) were the most abundant compounds.

Cytotoxicity

Table 2 provides IC_{50} values for different cell lines (CaCo-2, HeLa, MCF-7, HepG2, Hep3B, B16F1, and LX-2) and

Table 1 Chemical composition of *I. haynei* EO from Palestine

Number	Compound	Retention time	$KI_{(cal.)}$[a]	$KI_{(lit.)}$[b]	Concentration (% w/w), ± SE
1	Carbitol	12.87	999	998	1.18 ± 0.04
2	2-Ethylhexan-1-ol	14.14	1029	1029	0.24 ± 0.003
3	Benzyl alcohol	14.33	1033	1033	1.17 ± 0.02
4	Linalool	17.06	1097	1098	0.60 ± 0.01
5	Phenethyl alcohol	17.54	1110	1110	2.31 ± ± 0.1
6	Fenchol	17.81	1116	1117	0.06 ± 0.002
7	1-Terpinenol	18.5	1134	1134	0.41 ± 0.01
8	*cis-β*-Terpineol	19.02	1147	1148	0.28 ± 0.01
9	Benzyl acetate	19.5	1160	1161	5.46 ± 0.1
10	*β*-Terpineol	19.75	1166	1165	0.11 ± 0.01
11	Borneol	19.9	1170	1171	0.18 ± 0.01
12	Methyl phenylacetate	19.98	1172	1174	0.34 ± 0.01
13	(-)-Terpinen-4-ol	20.21	1178	1179	0.21 ± 0.01
14	*α*-Terpineol	20.81	1193	1192	14.16 ± 0.07
15	*γ*-Terpineol	20.98	1198	1198	1.97 ± 0.04
16	Citronellol	21.97	1223	1222	0.41 ± 0.01
17	Bergamol	22.75	1243	1245	0.04 ± 0.001
18	Geraniol	22.83	1245	1246	0.21 ± 0.01
19	Anisaldehyde	22.98	1249	1249	0.48 ± 0.01
20	Benzyl propionate	23	1250	1250	0.31 ± 0.01
21	Fixol	24.08	1277	1278	0.71 ± 0.01
22	Methyl anthranilate	25.92	1338	1336	0.11 ± 0.03
23	*α*-Terpinyl acetate	26.13	1344	1342	0.18 ± 0.01
24	Diphenyl ether	27.91	1397	1397	1.94 ± 0.09
25	*α*-Ionone	28.62	1420	1420	0.19 ± 0.01
26	Rosacetol	32.46	1551	1551	0.07 ± 0.001
28	Phthalic acid diethyl ester (Diethyl phthalate)	33.72	1572	1570	65.87 ± 0.11
27	Benzophenone	34.84	1624	1624	0.49 ± 0.01
29	Jasminal	35.3	1641	1642	0.31 ± 0.01
	SUM				100%

Quantitative and qualitative GC–MS analyses of EO were performed in triplicate using HP 5890 series II gas chromatograph equipped with a Perkin Elmer Elite-5-MS fused silica capillary column. The run was performed under conditions similar to those of the GC–MS. [a] Calculated Kovats retention index determined relative to the retention time of a series of *n*-alkanes (C_6–C_{27}).[b] Literature Kovats retention index based on NIST-14 mass spectral reference library

Table 2 Cytotoxicity IC_{50} values (µg/ml) for *I. haynei* EO and 5-Fluorouracil against CaCo-2, HeLa, MCF-7, HepG2, Hep3B, B16F1, and LX-2 cells lines

	CaCo-2	HeLa	MCF-7	HepG2	Hep3B	B16F1
I. haynei EO	915.47 ± 2.87	> 1000	> 1000	874.95 ± 2.64	> 1000	757.88 ± 3.18
5-Fu	3.72 ± 0.84	2.16 ± 1.01	1.29 ± 0.45	4.23 ± 1.78	13.28 ± 2.21	12.91 ± 1.36

various treatments, including *I. haynei* EO and anticancer drug 5-Fluorouracil (5-FU). The results indicate that *I. haynei* EO has the highest cytotoxic effect against melanoma tumor cells (B16F1) with an IC_{50} dose of 757.88 ± 3.18 µg/ml, followed by hepatocellular HepG2 cells and colon CaCo-2 carcinoma cells with IC_{50} doses of 874.95 ± 2.64 and 915.47 ± 2.87 µg/ml, respectively. However, the cytotoxic effect of *I. haynei* EO against hepatoma (Hep3B), breast cancer (MCF-7), and colon cancer (CaCo-2) cells was > 1000 µg/ml.

To verify the reliability of the MTS assay, this research examined the effectiveness of 5-Fluorouracil as a potent chemotherapy drug that showed strong efficacy against all the cancer cells tested. Furthermore, the cytotoxic effect of the substance on the LX-2 human hepatic stellate cell line was very damaging. It suppressed the proliferation of these cells with a dosage of 0.74 ± 0.33 µg/ml, whereas the IC50 value for *I. haynei* EO was 515.08 ± 3.96.

Cell viability percentage was calculated for CaCo-2, HeLa, MCF-7, HepG2, Hep3B, B16F1, and LX-2 cells at 0.5 mg/ml concentrations, as shown in Fig. 2. Cancer cells with the highest cell viability were B16F1 (79.82%), HepG2 (81.40%), HeLa (87.75%), and Hep3B (88.55%). At the same concentration, the EO cell viability on LX-2 normal cells was 51.99%.

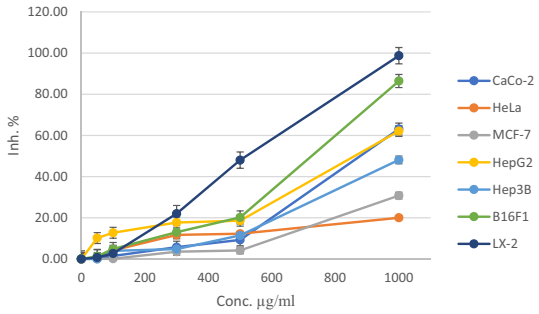


Fig. 3 Percentage inhibition of *I. haynei* EO (0–1000 µg/mL) on seven cancer cell lines

Figure 3 shows that *I. haynei* flower EO at a concentration of 1000 µg/ml inhibited the growth of CaCo-2, HeLa, MCF-7, HepG2, Hep3B, B16F1, and LX-2 cells. However, the highest inhibitory percent was observed against B16F1 (86.43 ± 3.18%), CaCo-2 (63.14 ± 2.87%), and HepG2 (62.15 ± 2.64%).

Table 3 displays the findings of the antibacterial activity of *I. haynei* EO and the antibiotics (Ciprofloxacin and Fluconazole) used as controls in the micro broth dilution technique. According to minimum inhibitory concentration (MIC) values of 20.8 ± 7.2 µl/ml for *S. aureus*

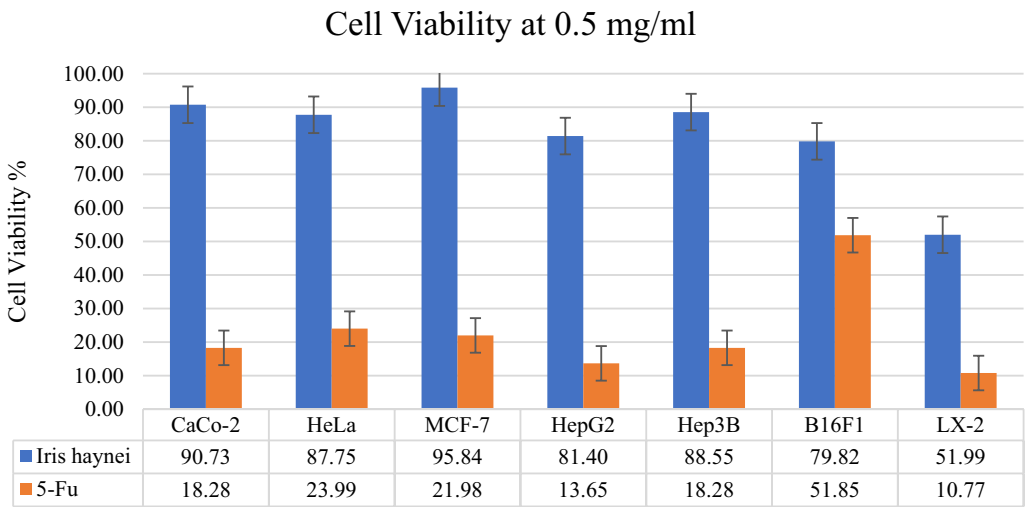


Fig. 2 Cell viability percentages of *I. haynei* EO and 5-Fluorouracil against CaCo-2, HeLa, MCF-7, HepG2, Hep3B, B16F1 cancer cells and LX-2 normal cells at 0.5 mg/ml concentrations

Table 3 Antimicrobial activity (MIC values) of *I. haynei* EO, Ciprofloxacin, and Fluconazole against the tested microbial strains

	Bacteria						Fungus
ATCC number	**Clinical strain**	**ATCC 25923**	**ATCC 25922**	**ATCC 13883**	**ATCC 8427**	**ATCC 9027**	**ATCC 90028**
Microbe	**MRSA**	***S. aureus***	***E. coli***	***K. pneumoniae***	***P. vulgaris***	***P. aeruginosa***	***C. albicans***
MIC (µl/ml)	50 ± 0	20.8 ± 7.2	25 ± 0	25 ± 0	2.6 ± 0.9	66.6 ± 28.9	0.32 ± 0.1
Ciprofloxacin (µg/ml)	15.6	0.78	1.56	0.13	15	3.12	–
Fluconazole (µg/ml)	–	–	–	–	–	–	1.56

and 50 ± 0 µl/ml for clinically relevant MRSA strains, *I. haynei* EO has significant activity against gram-positive bacteria. The MIC values for the activity against gram-negative organisms varied from 2.6 ± 0.9 to 66.6 ± 28.9 µl/ml. Among these species, *P. vulgaris* exhibited the greatest potency. The antifungal activity against the *C. albicans* strain was assessed, and the MIC was found to be 0.32 ± 0.1 µl/ml.

AMPA receptor modulation by *I. haynei* essential oil

Our investigation initiated with a thorough assessment of the impact of *I. haynei* EO on the activity of various AMPA receptor subunits, encompassing both homomeric and heteromeric configurations, namely GluA1, GluA2, GluA1/2, and GluA2/3. Remarkably, our findings unveiled that the application of *I. haynei* EO at a concentration of 800 µg/ml elicited a modest reduction in whole-cell current across the tested subunits, indicative of inhibition (Fig. 4a). To delve deeper into the mechanistic insights, we introduced the concept of the A/A_I ratio, enabling a comparative analysis of currents induced by glutamate (A) versus those following *I. haynei* EO administration (A_I) (Fig. 4b). Notably, GluA2-containing AMPA receptor subunits (GluA1/2, GluA2, and GluA2/3) exhibited a more pronounced inhibition than homomeric GluA1. Specifically, while *I. haynei* EO inhibited GluA1 by nearly 2.5-fold, it exerted inhibitory effects of approximately 3.5-fold, 4.8-fold, and 4.3-fold on GluA1/2, GluA2, and GluA2/3, respectively. These observations underscore the differential susceptibility of AMPA receptor subunits to modulation by *I. haynei* EO, hinting at intricate mechanisms underlying its pharmacological actions.

As our inquiry deepened into the ramifications of *I. haynei* EO on AMPA receptor subunit activity, we employed the whole-cell patch-clamp technique to scrutinize the influence of *I. haynei* EO on the kinetics of AMPA receptor subunits. Specifically, we focused on recording deactivation rates (τ_w deact) and desensitization rates (τ_w des). Notably, our results revealed a discernible modulation of kinetics by *I. haynei* EO, with the

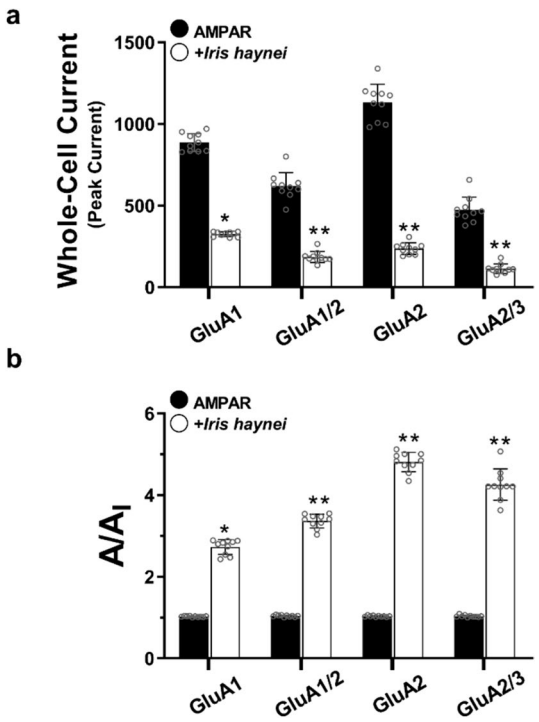


Fig. 4 Dynamic showcase revealing the Impact of *I. haynei* essential oil on AMPA receptor subunits. Panel **a** presents the whole-cell current responses (in pA) of AMPA receptor subunits expressed in HEK293T cells. The responses are illustrated in the presence of glutamate alone (represented in black) and after the application of 800 µg/ml of *I. haynei* extract (represented in white). Panel **b** depicts the A/A_I ratio terrain, providing a full picture. Here, 'A' indicates the current reaction to glutamate alone, as opposed to 'A_I,' which depicts the response after administering *I. haynei* EO. The experimental conditions underscore the controlled environment, notably a holding potential of − 60 mV, a pH level of 7.4, and a consistent temperature of 22 °C. This perspective aims to encompass the entirety of the A/A_I ratio dynamics, shedding light on the impact of *I. haynei* EO on the initial glutamate-induced responses. Statistical scrutiny, facilitated by one-way analysis of variance (ANOVA), unfolds with 'ns' signaling non-significance, * $p < 0.05$; ** $p < 0.01$. Each dataset draws from scrutinizing 10 cells, and the data unfolds as means ± SEM. The dots gracefully poised above each column encapsulate the unique current generated by each of the 10 cells following exposure to the extract, adding a nuanced layer to our exploration

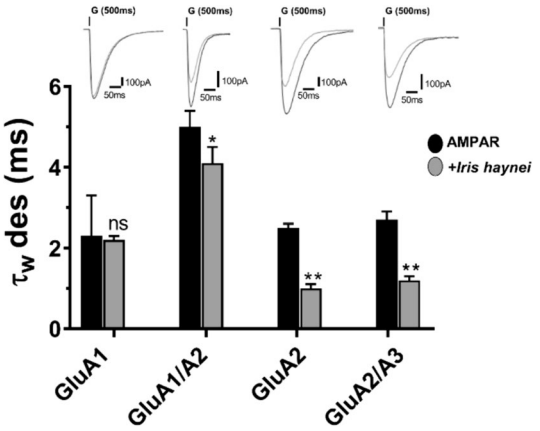


Fig. 5 Effect of *I. haynei on* AMPA receptor desensitization rates in the presence of glutamate. The desensitization rates (τ_w des) for AMPA receptor subunits were measured under two conditions: in the presence of glutamate alone (shown by black) and in the presence of *I. haynei* EO (represented by gray). A 500 ms protocol was used to identify the desensitization rate. The traces for the four examined subunits are shown for each situation. The measurements were recorded at a potential of − 60 mV, a pH of 7.4, and a temperature of 22 °C. Statistical analysis was performed using one-way analysis of variance (ANOVA) for comparison. Significant differences are denoted as * $p < 0.05$; ** $p < 0.01$, while "ns" indicates non-significance. Each experiment included the evaluation of 10 cells, and the presented data are expressed as means ± SEM, providing a comprehensive overview of the observed effects of *I. haynei* EO on AMPA receptor kinetics

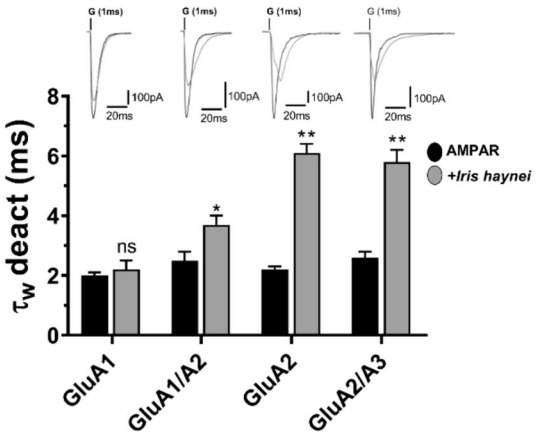


Fig. 6 The impact of *I. haynei* EO on AMPA receptor deactivation in the presence of glutamate. The deactivation rates (τ_w deact) are depicted under two conditions: in the presence of glutamate alone (depicted in black) and in the presence of *I. haynei* EO (depicted in gray), using a 1 ms protocol to capture the deactivation rate. All recordings were conducted at − 60 mV, pH 7.4, and 22 °C. Statistical analysis was performed using one-way analysis of variance (ANOVA), indicating significance levels with * $p < 0.05$; ** $p < 0.01$, and "ns" denotes results that were not significant. Each experiment involved the evaluation of 10 cells, and the presented data represent means ± standard error of the mean (SEM)

most pronounced effects observed in GluA2-containing subunits (Figs. 5 and 6). GluA2 demonstrated a notable 2.7-fold increase in deactivation rate (as depicted in Fig. 6), concomitant with a substantial 2.5-fold decrease in desensitization rate (Fig. 5). Similarly, within the heteromeric subunit category, GluA2/3 exhibited greater susceptibility than GluA1/2, manifesting a 2.2-fold reduction in desensitization rate and a 2.2-fold increase in deactivation rate. The influence of *I. haynei* EO on GluA1/2 kinetics resulted in a 1.5-fold increase in deactivation rate and a 1.2-fold decrease in desensitization rate. However, the impact on GluA1 kinetics was not statistically significant. These intriguing findings unveil the nuanced modulation of AMPA receptor subunit kinetics by *I. haynei* EO, shedding light on the intricate mechanisms underlying its pharmacological effects.

Discussion

I. haynei, a rhizomatous perennial in the Iridaceae family, contains various bioactive compounds such as phenols, ketones, and terpenes. Our analysis identified 29 molecules, making up 100% of the oil extracted from *I. haynei*. Diethyl phthalate was the predominant compound at (65.87 ± 0.11), followed by α-terpineol at 14.16 ± 0.07% and benzyl acetate at 5.46 ± 0.1%. Phthalates, a class of chemicals found in various organisms, including plants, bacteria, and fungi, have garnered significant attention since 1967 due to their multifaceted biological potential. Over 52 phthalate ester derivatives have been identified across taxonomic groups, demonstrating many activities ranging from anti-melanogenic and anti-inflammatory to antiviral and larvicidal. Despite their wide-ranging applications, phthalates possess the unique property of being easily metabolized and excreted from the human body, with elimination half-lives measured in hours. This swift metabolism distinguishes them from other contaminants, preventing their accumulation and rendering them virtually undetectable in humans. Meanwhile, benzyl acetate, although commonly synthesized chemically for industrial purposes, also occurs naturally in EOs of various plant species, including jasmine, *Hibiscus rosa*, and *Cananga odorata* [35, 36]. This dual origin underscores its significance as a naturally occurring compound with potential therapeutic benefits.

While the major components of the EO—diethyl phthalate, α-terpineol, and benzyl acetate—were identified, discussing their known biological activities in more detail could help hypothesize their possible mechanisms of action in the observed pharmacological effects. Diethyl phthalate has been reported to possess anti-inflammatory and antiviral activities, which might contribute to the neuroprotective and antimicrobial effects observed in this study [37]. In addition, studies have shown that

diethyl phthalate can affect neurotoxicity-related gene expression and acetylcholinesterase activity, indicating potential neurological impact [38]. This insight enhances our understanding of the neuropharmacological relevance of diethyl phthalate. α-Terpineol is known for its antioxidant, anti-inflammatory, and antimicrobial properties, suggesting its role in modulating AMPA receptor activity and providing antibacterial effects [39]. Benzyl acetate, with its anti-inflammatory and antimicrobial activities [40], could further enhance the EO's efficacy against microbial infections and inflammation-related neuronal damage. The chemical properties of these compounds, such as their ability to penetrate cell membranes and interact with specific cellular targets, likely play a significant role in their biological outcomes.

The *Iridaceae* family encompasses a diverse array of plants with significant therapeutic potential. Among them is *Gladiolus dalenii* Van Geel, which stands out for its traditional use in treating various ailments, including headaches, epilepsy, convulsions, and intestinal spasms [41]. Particularly noteworthy is its historical use in Cameroon for treating epilepsy and schizophrenia, primarily utilizing plant bulbs [41]. Recent studies have shed light on its antidepressant-like properties, comparable to fluoxetine, particularly in depressive states associated with epilepsy. Another notable member of the Iridaceae family is *Iris tectorum* Maxim, which is utilized in traditional Chinese medicine for its heat-relieving and detoxifying properties [42]. This plant has long been employed in treating sore throats, with its rhizomes yielding six iridals, featuring an unusual 3,6-dihydro-2H-pyran moiety. These compounds have exhibited neuroprotective activities against serum-deprivation-induced PC12 cell damage, showcasing the therapeutic potential of *Iris* species [42].

Furthermore, using EOs derived from *Iris* species like *I. haynei* has gained attention as adjunctive therapy for various neurological conditions. *I. haynei* EO, containing compounds such as diethyl phthalate, α-terpineol, and benzyl acetate, has demonstrated effects on AMPA receptors, particularly potent on GluA2-containing subunits. Considering the crucial role of AMPA receptors in neurological function, modulation of their activity and kinetics by EOs presents a promising avenue for exploring novel therapeutic interventions [43]. Notably, dysfunction or misregulation of AMPA receptors has been implicated in various neurological disorders. RNA editing at the Q/R site of GluA2 is crucial for maintaining normal receptor function, and alterations in this process have been associated with conditions like Alzheimer's disease [44]. Impaired GluA2 Q/R site editing in Alzheimer's disease may contribute to synapse loss and neurodegeneration, highlighting the potential significance of AMPA receptor modulation by compounds like those found in *I. haynei* EO as a potential therapeutic strategy for neurological diseases.

The differential effects observed in our study, where GluA2-containing subunits were more affected by *I. haynei* EO than homomeric GluA1, may be attributed to the specific interactions between the bioactive compounds in the EO and the unique structural features of these subunits. GluA2 subunits have distinct properties, such as RNA editing at the Q/R site, affecting their permeability to calcium ions and overall kinetics. The presence of compounds such as diethyl phthalate and *α*-terpineol in *I. haynei* EO might interact more effectively with these subunits, leading to more significant modulation. Further research is needed to elucidate the precise mechanisms of these interactions and how they contribute to the observed effects.

I. haynei EO's bioactivity goes beyond neuroprotective potentials to antibacterial potency, providing a comprehensive approach to natural therapy. The analysis revealed the antibacterial properties of *I. haynei* EO. Remarkably, this action was shown against various microorganisms, including Gram-positive and Gram-negative bacterial strains and the *C. albicans* strain. The findings demonstrate significant efficacy, particularly against *S. aureus*, *P. vulgaris*, and *C. albicans*, with MIC values of 20.8 ± 7.2, 2.6 ± 0.9, and 0.32 ± 0.1 µl/ml, respectively. As far as we know, there is a scarcity of information on *I. haynei*. The results of our study on the effectiveness of *Iris* species EOs in killing bacteria were in line with previous studies on the antimicrobial properties of these oils [45]. Unlike the evidence on other extracts from the *Iris* species, such as ethanol extract, little to no detectable antibacterial activity was reported [17, 42, 46]. The investigation of *I. haynei* EO aligns with results from comparable research on other plant oils, highlighting the many possibilities of botanical extracts for medicinal uses [47, 48].

Exploring *I. haynei* EO and its diverse pharmacological properties underscores the intricate relationship between natural compounds and human health. This study sheds light on the potential therapeutic applications of *I. haynei* EO in addressing neurological disorders and combating microbial infections and invites a broader conversation about integrating traditional herbal wisdom with contemporary scientific methodologies. The compelling evidence presented herein advocates for a more inclusive approach toward understanding botanical extracts' full spectrum of benefits. As we continue to unravel the complexities of plant-based therapeutics, the findings from this research pave the way for future investigations that may further delineate the mechanisms underlying the pharmacological actions of EOs and their constituents. In doing so, we contribute

to the rich tapestry of natural product research and underscore the potential of holistic health strategies that harness the power of nature in promoting well-being and combating disease.

Conclusion

This research reveals the therapeutic capabilities of *I. haynei* EO indigenous to the Jenin area of Palestine. The examination of *I. haynei* EO revealed diethyl phthalate, *α*-terpineol, and benzyl acetate as their primary constituents, each contributing to the oil's distinct characteristics and medicinal efficacy. The cytotoxicity studies showed that the EO can impact various cancer cell lines in a way that is dependent on the dosage, indicating its potential for cancer treatment. *I. haynei* EO demonstrated broad-spectrum antibacterial and antifungal effects. Electrophysiological studies have shown that the EO specifically suppresses AMPA receptor subunits, with a particular impact on those that contain GluA2. This suggests a novel approach for treating neurological disorders associated with AMPA receptor dysfunction, such as cerebral ischemia. The research presents data supporting the traditional medicinal use of *I. haynei*. The proposal suggests doing more inquiry into the pharmacological processes of the substance by examining its interactions with important biological targets, such as AMPA receptors. This study presents noteworthy discoveries about the potential neuroprotective properties of organic compounds. The positive results indicate the need for further exploration into the pharmacological effects of *I. haynei* EO. This work can potentially provide new and innovative methods for treating cerebral ischemia and other related neurological illnesses. This has the potential to combine ancient herbal knowledge with modern scientific investigation to develop new health remedies.

Abbreviations

EO	Essential oil
$KI_{(cal.)}$	Calculated Retention Index
$KI_{(lit.)}$	Literature Kovats Retention Index
RT	Retention time
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
HEK293T	Human embryonic kidney 293 cells
AMPARs	AMPA receptors
GC	Gas chromatography
MS	Mass spectroscopy
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
ATCC	American type culture collection
CFU	Colony-forming units
MHB	Muller Hinton Broth
IC_{50}	Half-maximal inhibitory concentration
5-FU	5-Fluorouracil
RIs	Retention Indices
τw deact	Time constant for deactivation
τw des	Time constant for desensitization
τw	Weighted tau
ANOVA	One-way analysis of variance
SEM	Standard error of the mean

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00636-3.

Supplementary Material 1.

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Author contributions

N. J.: conceptualization, validation, investigation, writing—review and editing, visualization, supervision, project administration. M. Q.: conceptualization, validation, investigation, writing—original draft, writing—review and editing, visualization, supervision, project administration. M. H.: validation, investigation, writing—original draft, writing—review and editing, visualization. M. Q.: validation, investigation, writing—original draft, writing—review and editing, visualization. N. A.: validation, investigation, writing—original draft, writing—review and editing, visualization. S. B.: validation, writing—review and editing. M. B.: validation, writing—review and editing. J. B.: validation, writing—review and editing. N. S.: validation, writing—review and editing. M. I.: validation, writing—review and editing.

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Availability of data and materials

No datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate

All the procedures utilized in this investigation, including the *Iris haynei* plant, followed the relevant institutional, national, and international guidelines and legislation. The plant was identified and authenticated at An-Najah National University.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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