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Assessing *Teucrium polium* L. from chemical profiling to antioxidant, anticancer, α-amylase, and lipase activities

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Teucrium polium **L, known as felty germander or Palestinian Ja'adeh, primarily flourishes in Mediterranean countries such as Palestine, Jordan, and Turkey. There has been a significant increase in the investigation and analysis of essential oils derived from medicinal plants due to their potential biological and therapeutic applications. This study will investigate the chemical composition of essential oil derived from the dried leaves of *T. polium* L. and evaluate its in vitro antioxidant, anticancer, *α*-amylase, and lipase inhibitory properties. The essential oil extracted from the dried leaves of *T. polium* L. *via* hydrodistillation was analyzed using gas chromatography-mass spectrometry (GC-MS) to determine their chemical composition. Sixteen compounds were identified in the essential oil of *T. polium* L., with E-nerolidol (27.11%), geranyl acetone (23.26%), germacrene D (19.08%), *β*-caryophyllene (17.78%), *α*-caryophyllene (3.35%), and bicyclogermacrene (3.08%) constitute the principal components. This essential oil, abundant in oxygenated sesquiterpenes, demonstrated notable antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) at a concentration of 20.08 ± 1.00 µg/mL. Furthermore, it exhibited significant anticancer effectiveness against the MCF-7 and B16F10 cell lines, with IC_{50} values recorded at 30.56 µg/mL and 32.75 µg/mL, respectively. Nevertheless, it demonstrated restricted lipase activity and insufficient *α*-amylase activity. The findings suggest that *T. polium* L. essential oil is a potential source of natural antioxidants and has anticancer properties, making it a viable candidate for pharmaceutical and cosmetic use.**

Keywords *Teucrium polium* L., Essential oil, Chemical composition, MCF-7, B16F10, Medicinal plants

Essential oils and other phytochemicals extracted from plants, spices, and herbs have a long history of use in aromatherapy, cooking, and medicine[1–3]. Despite recent advances in pharmacology and medicine, natural products, particularly essential oils (EOs), continue to be vital in modern healthcare[3,4]. Worries about disease transmission *via* food, the rise of drug resistance, the negative effects of synthetic compounds, and their high costs have sparked an interest in using plant-derived chemicals, particularly EOs, for medicinal and therapeutic purposes[1–4]. EOs are a mixture of volatile, lipophilic, and odoriferous molecules made mostly of monoterpenes and sesquiterpenes, with smaller amounts of aromatic and aliphatic compounds, that are produced by the secondary metabolism of aromatic plants[4]. These intricate mixtures can consist of terpenoids, alcohols, ethers, esters, ketones, aldehydes, epoxides, phenols, and heterocycles, all of which can come from more than 60 plant families[4–6].

The *Lamiaceae* family is crucial in traditional medicine due to its secondary metabolites, especially essential oils, which exhibit diverse qualities and are utilized across numerous industries, including food, cosmetics, fragrances, and pharmaceuticals[7,8]. *Teucrium L.*, commonly known as 'germander,' is one of the largest genera in the *Lamiaceae* family, encompassing over 300 species[9,10]. Approximately nine species are present in Palestine, including *T. polium* L[11]., which is widely distributed in the region and referred to as Jaa'deh. This little, adolescent, fragrant shrub (20–50 cm in height) is located in arid and rocky hills and deserts in most Mediterranean countries, southwestern Asia, north Africa, and Europe[9,10]. It is distinguished by thick clusters of white flowers and oval leaves with undulating margins and has been utilized for millennia in traditional medicine to address gastrointestinal issues such as colic, migraines, and nephrolithiasis[10,12]. It is utilized to aid digestion, eliminate waste, and calm muscles[10,13]. In traditional Palestinian medicine, hot water leaf extracts are

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used to treat heart and intestinal problems, and brewed leaves are eaten after a meal to help with diarrhea and muscle spasms[14]. Numerous extracts of *T. polium* L. have exhibited notable medicinal properties, encompassing antimicrobial activity, anti-inflammatory effects, antioxidant capabilities, malaria prophylaxis, fever alleviation, ulcer healing, analgesic effects, and potential reductions in blood glucose, blood pressure, and cholesterol levels[10–15]. Furthermore, research on several cancer cell lines has yielded encouraging outcomes for cancer therapy[16]. A recent study on the biological and pharmacological properties of essential oils derived from plants used in traditional medicine, such as *T. polium* L., has revealed that the geographical origin of these plants has a significant impact on their chemical composition and biological performance[10]. The literature review indicated that *T. polium* L EO possesses various chemotypes, including 8-cedren-13-ol, β-caryophyllene reference 24/valerianol, β-pinene reference 25/3-carene, γ-murolene reference 26/β-caryophyllene, t-cadinol, (E)-nerolidol reference 15/α-pinene, β-pinene reference 27/t-cadinol, and germacrene D[17,18].

Although there is considerable global knowledge about *T. polium* L., the chemical composition and biological consequences of its essential oil in Palestine remain mostly unexplored. This study seeks to assess the chemical composition of the essential oil derived from *T. polium* L. leaves cultivated in Jericho hills, the Earth's lowest elevation, along with its anticancer, antioxidant, lipase, and *α*-amylase activities. Considering the traditional use of *T. polium* L. in folk medicine, the identification of the major components and investigating their biological activity is essential to ensure effective and safe use of the plant.

Materials and methods

Species collection and identification

The leaves of *T. polium* L. were collected from Jericho, the lowest point on Earth at 392 m below sea level (latitude 3˚53'07.5" N and longitude 59˚40'10.9" W) in early April. The formal identification of the plant was done by Dr. Nidal Jaradat, and voucher specimens were deposited in An-Najah National University's Herbal Products Laboratory with the code Pharm-PCT-2813. Plant specimens kept in the herbarium of An-Najah National University to be available as scientific reference material for publicity.

Extraction of *T. polium* L. essential oil

The collected leaves were dried in a shaded location at room temperature (25 ± 3 °C) and humidity (55 ± 4 RH). The dried leaves were mechanically crushed into tiny pieces to assist in the extraction of the EO. 200 g of powdered leaves were subjected to hydrodistillation for 2 h using an electric heating mantle at 110 °C. The disperse oil was extracted with diethyl ether (Et_2O, 50 mL x 2), the combined organic layers were dried over $MgSO_4$, and ether was carefully removed under reduced pressure at 35 °C, yielding 0.9 g of pale-yellow oil. The oil was stored in a sealed container in the dark at room temperature.

Qualitative and quantitative analysis of the extracted EO

Gas chromatography-mass spectrometry (GC-MS) analysis was employed to assess the chemical composition of the extracted essential oil (EO) both qualitatively and quantitatively, following the methodology previously outlined by Al-Maharik et al. and the protocol of Maurya et al.[19,20]. This analysis utilized a low-polarity Perkin Elmer-5-MS capillary column with specific dimensions: an inner diameter of 0.25 mm, a length of 30 m, and a width of 0.25 µm. 1 µL of EO samples, prepared at a concentration of 1 mg/mL in acetonitrile, were injected. Helium was employed as the carrier gas with a flow rate of 1 mL/min, maintained at a pressure of 20.41 psi in split mode, utilizing a split ratio of 1:50. The injector was maintained at a temperature of 250 °C, while the transfer line was also held at a temperature of 250 °C. The oven was initially set to a temperature of 50 °C for 5 min, after which it was gradually increased to 280 °C at a rate of 4 °C per minute. It was then kept at a constant temperature of 280 °C for a period of 10 min. The whole duration of the run was 67.5 min, including a 10-minute period after the run for conditioning. The detection was performed utilizing a Perkin Elmer Clarus 560 mass spectrometer (Shelton, CT 06484 USA). The acquisition was performed *via* electron ionization (EI) mode, employing an ionization voltage of 70 eV in a standard scanning mode covering a mass range of 40 to 500 m/z. The identification of individual metabolites was achieved by comparing their relative retention indices (RRI) and mass spectral data with the MS library, NIST webbook, and relevant literature sources, as well as analyzing their fragmentation patterns. The compounds that were detected were represented as the percentages of the peak area of each individual component in relation to the total peak area of the EO. The RRI for each phytoconstituent was determined by comparing their retention times to those of a reference solution of n-alkanes (C_7–C_{30}) at a concentration of 0.1 mL/mL in acetonitrile, using the same experimental conditions as the EO samples. The relative proportions of distinct substances were calculated from the peak regions obtained using gas chromatography using a normalization method, without the application of correction factors.

DPPH free radical scavenging assay

The antioxidant effectiveness of extracted essential oil was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, using established protocols described by Sharifi-Rad et al. and Victor et al.[13,21]. A freshly prepared methanolic solution of 0.1 mM DPPH free radicals was combined and thoroughly mixed with a methanolic solution of essential oil at varying concentrations in a 3:1 ratio, accordingly. The mixes were incubated for 30 min in darkness at room temperature. The absorbance was recorded at 517 nm using a UV-visible spectrophotometer (LABINDIA®, India). Trolox (Sigma-Aldrich, Burlington, MA, USA) was utilized as a positive control, and the blank solution was generated by substituting the EO solution with methanol. The ability to scavenge DPPH radicals was determined using the subsequent formula: Scavenging capacity = $(A_{control} - A_{sample})/A_{control} \times 100$ (1), where $A_{control}$ denotes the absorbance of DPPH radical without any additives, and A_{sample} represents the absorbance of DPPH radical with oil samples and control solutions of

varying concentrations. A graph depicting scavenging activity as percentage inhibition was constructed against the concentrations of essential oil and standards to ascertain their IC_{50} values.

α-Amylase inhibition assay

The inhibitory efficacy of the EO against α-amylase was assessed using the Worthington enzyme method described by Jaradat et al. and Shodehinde et al.[22,23]. The EO was dissolved in 10% DMSO to create dilutions of 10, 50, 70, 100, 500, and 1000 µg/mL. 0.2 mL of EO solution and 0.2 mL of 0.02 M sodium phosphate buffer (pH 6.9, 0.006 M NaCl) containing α-amylase solution (1.0 U/mL) (Sigma-Aldrich, St. Louis, MO, USA) were incubated at 30 °C for 10 min. Subsequent to preincubation, 0.2 mL of a 1% starch solution was introduced to 0.02 M sodium phosphate buffer (pH 6.9 containing 0.006 M NaCl). The reaction mixture was thereafter incubated at 25 °C for 10 min. Subsequently, 0.2 mL of dinitrosalicylic acid (DNS) (Alfa-Aesar, Lancashire, UK) color reagent was introduced to terminate the reaction. Subsequently, each solution was diluted by the addition of 5 mL of distilled water and incubated in a boiling water bath for 5 min, followed by cooling to room temperature. The absorbance was quantified at 540 nm (Shimadzu-UV-1800, Nakagyo-ku, Japan). Acarbose (Sigma-Aldrich, Burlington, MA, USA) served as a positive control and was prepared according to the aforementioned procedure. The α-amylase inhibitory activity was determined using the formula: α-amylase inhibition = $(A_B\text{-}A_E)/A_B\times100$, where A_B represents the absorbance of the blank solution and A_E denotes the absorbance of the essential oil (EO). A graph depicting α-amylase inhibition was constructed against the concentrations of oil and standards to ascertain their IC_{50} value.

Porcine pancreatic lipase inhibition assay

The lipase inhibition activity was assessed using the methodology outlined in the study of Slanc et al.[24]. The EO were initially dissolved in 10% DMSO to produce a stock solution, which was subsequently diluted to prepare concentrations of 50, 100, 200, 300, 500, and 1000 µg/mL. Lipase (Sigma, St. Louis, MO, USA) was diluted in a 75 mM buffer solution at pH 8.5. A *p*-nitrophenyl butyrate (PNPB) (Sigma-Aldrich, Schnelldorf, Germany) solution was prepared by dissolving 20.9 mg in 2 mL of acetonitrile. Subsequently, 100 µL of test solutions were combined with 200 µL of lipase solution (0.8 µg/mL), and a Tri-HCl solution was added to achieve a total volume of 1 mL. The mixture was maintained in darkness at 37 °C for 15 min; subsequently, 20 µL of *p*-nitrophenyl palmitate (4 µg/mL) was added and incubated for 30 min at 37 °C. The analyses were conducted at a wavelength of 450 nm (Shimadzu-UV-1800, Nakagyo-ku, Japan). Orlistat (Sigma-Aldrich, Germany) was utilized as a positive control, adhering to the above outlined procedure. The lipase inhibition activity was calculated using the following equation: lipase inhibition = $(A_B\text{-}A_E)/A_B\times100$, where A_B represents the absorbance of the blank solution and A_E denotes the absorbance of the EO. A graph depicting lipase inhibition was constructed in relation to the concentrations of oil and standards to ascertain their IC_{50} value.

Anticancer activity of *T. polium* L. EO

The *T. polium* L. EO was assessed for its cytotoxic effects on different cancer cell lines: the murine melanoma B16F10 [CRL-6475; American Type Culture Collection (ATCC), Manassas, VA, USA], human breast (MCF-7; ATCC HTB-22), and mouse Embryonic Fibroblast-1 (MEF-1; ATCC CRL-2214) cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM, high-glucose containing L-glutamine, phenol red (Fuji Film Wako, Osaka, Japan), 10% fetal bovine serum (FBS; G.E. Healthcare, Chicago, IL, USA), and 1% penicillin/streptomycin (P/S) (Nacalai Tesque, Kyoto, Japan)[25]. B16F10 or MCF-7 cells (2×10^5 cells/well) were seeded in 6-well plates (TPP, Switzerland) and kept overnight before the addition of DMSO (EO control) or *T. polium* EO at concentrations ranging from 5 to 100 µg/mL EO. Viable cells were counted at 24 h using trypan blue (cat. 207-17081; Fuji Film Wako). All compounds were initially dissolved in DMSO, and the final concentration of DMSO was less than 1% in all of the considered concentrations of the applied compounds. Taxol was used as a positive control of 10ng/ml with both cell lines; MCF-7 and B16F10. The 50% inhibition of cell growth (IC_{50}) was used as the analysis parameter calculated by Graph Pad Prism (GraphPad Software). The IC_{50} values from at least two independent experiments were compared with the control and expressed as the mean ± SD. The analysis of two groups was performed using the student t-test, and P-values < 0.05 were statistically significant.

Statistical analysis

Each experiment was conducted in triplicate. In statistics, the mean and standard deviation are presented. Following the analysis of variance or student's t-test, Tukey HSD post-hoc tests were conducted. *P*-values less than 0.05 were deemed to indicate significance.

Results and discussion

Essential oil composition

GC-MS was used to assess the chemical makeup of the EO that was extracted by hydro-distillation from dried *T. polium* L. leaves that were gathered in Jericho, a unique place, in early April 2023, providing a 0.45% yield (Fig. 1; Table 1). Jericho, in the Jordan Rift Valley, is one of the lowest and driest populated areas on Earth, at an elevation of approximately 258 m below sea level. The sediment and water at this site exhibit chemical characteristics that are significantly influenced by the arid desert climate. The high rates of evaporation result from low annual precipitation and elevated temperatures, leading to the accumulation of minerals and ions in the soil and groundwater. These factors affect the growth of vegetation, soil fertility, and microbial activity. Despite these conditions, Jericho is considered one of the most fertile locations in the region. Table 1 displays the names, retention times (RT), retention indices (RI), and percentages of the detected components of the EO. The GC chromatogram and mass spectrum of the peak facilitated the identification of sixteen components in *T. polium* L. essential oil, comprising 99.51% of the total oil composition. The primary components included E-nerolidol

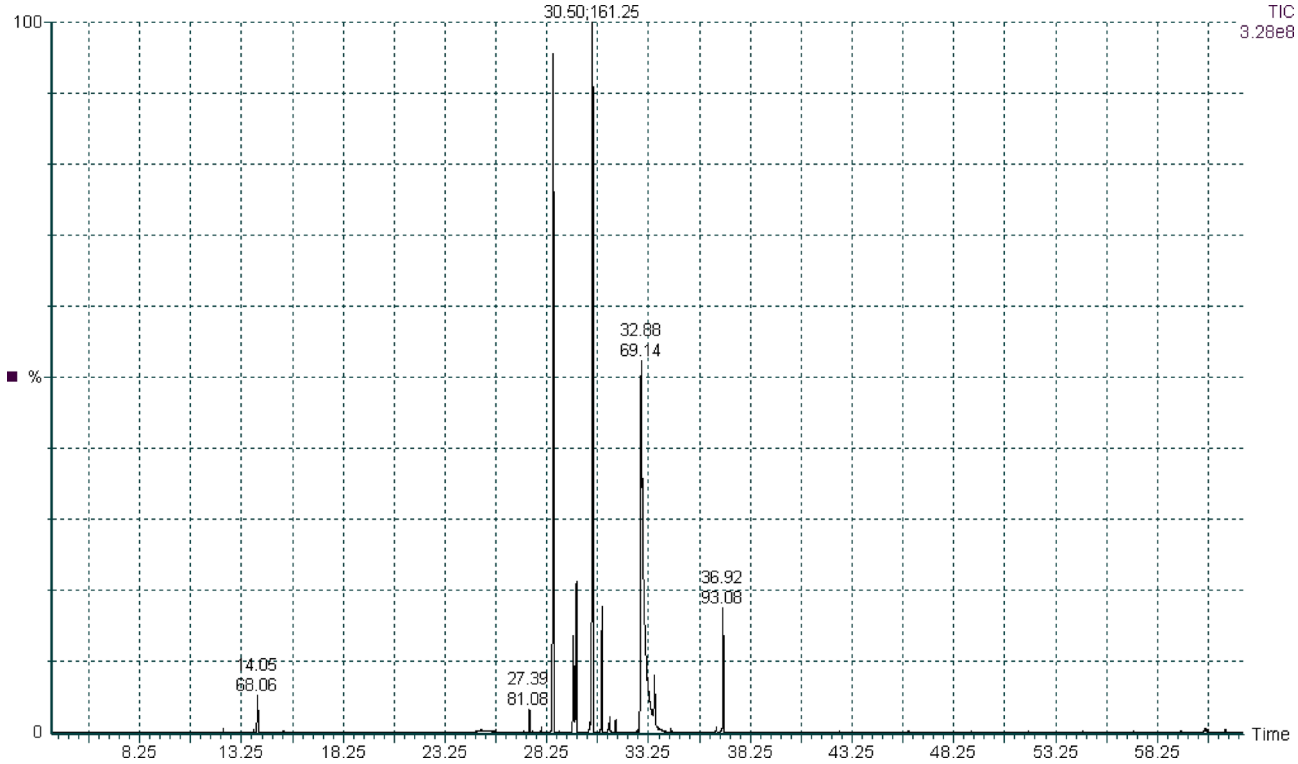

Fig. 1. GC-chromatogram of the *T. polium* L. essential oil.

(27.11%), geranyl acetone (23.26%), germacrene D (19.08%), *β*-caryophyllene (17.78%), *α*-caryophyllene (3.35%), and bicyclogermacrene (3.08%). The constituents of the EO can be classified into four categories, with sesquiterpene hydrocarbons representing the largest group at 43.95%, followed by oxygenated sesquiterpenes at 30.79%, oxygenated monoterpenes at 23.26%, and Monoterpene hydrocarbons at 1.06%.

A review of the literature reveals that EO derived from the aerial parts of *T. polium* L., grown in various climates and regions, demonstrate considerable variation, particularly in their primary components. While most essential oil demonstrated elevated sesquiterpene levels[15,26], an essential oil derived from *T. polium* L. in Corsica was notable for its substantial monoterpene concentration of 74.70%[27]. The EO of *T. polium* L. from different places have varied chemical compositions, although they all belong to the same overarching category. An analysis of the EO extracted from the aerial parts of *T. polium* L. in Jordan revealed 37 unique constituents, with 8-cedren-13-ol comprising 24.80%, *β*-caryophyllene 8.70%, germacrene D 6.83%, sabinene 5.24%, *α*-humulene 4.34%, allo-aromadendrene 4.54%, and δ-cadinene 3.51% as the principal components[28]. Sesquiterpene hydrocarbons constitute 44.71% of the total oil, making them the predominant class, followed by oxygenated sesquiterpenes (41.46%)[28]. Farahbakhsh documented the identification of 64 compounds in the EO of *T. polium*'s aerial parts, sourced from Iran, with valerianol (21.44%), *β*-pinene (12.97%), epi-*α*-bisabolol (9.86%), *α*-pinene (6.70%), caryophyllene (4.71%), limonene (3.45%), and carvone (3.85%) constituting the principal components[29]. El Atki et al.[30] reported the identification of twenty-two constituents accounting for 94.49% of the total EO extracted *via* hydrodistillation from *T. polium* L. aerial parts collected in Morocco, with 3-carene (16.49%), *γ*-murolene (14.03%), *α*-pinene (9.94%), *α*-phellandrene (6.93%), and caryophyllene (7.51%) being the principal constituents. Sesquiterpene hydrocarbons make up the major group, accounting for 54.30% of total oil, followed by monoterpene hydrocarbons at 38.23%[30]. The EO obtained from *T. polium* L. in the eastern part of Turkey had a noteworthy composition, with oxygenated sesquiterpenes accounting for 30.70%, followed closely by sesquiterpene hydrocarbons at 30.40% and monoterpene hydrocarbons at 11.20%[15]. The principal compounds found in EO were *β*-caryophyllene (8.80%), *τ*-cadinol (6.20%), (E)-nerolidol (5.00%), *α*-cadinol (5.40%), and *α*-pinene (4.70%), all with concentrations below 10%[15]. GC/MS analysis of the EO from the aerial parts of *T. polium* L., taken during the flowering stage in June from Tunisia[31], resulted in the identification of 71 compounds, accounting for 89.66% of the total oil. The primary constituents of EO included *α*-pinene (17.04%), *β*-pinene (12.68%), and limonene (6.65%), succeeded by *β*-myrcene (6.07%) and germacrene D (5.89%)[31]. In contrast to major literature reports, EO from Tunisia comprised a substantial amount of monoterpene hydrocarbons (48.73%), followed by sesquiterpene hydrocarbons (20.14%), oxygenated monoterpenes (14.00%), and a mere 5.54% of oxygenated sesquiterpenes[31]. The EO of *T. polium* subsp. capitatum aerial parts collected in Algeria was primarily composed of sesquiterpene hydrocarbons (31.20%) and oxygenated sesquiterpenes (25.50%). The predominant constituents included *τ*-cadinol (18.30%), germacrene D (15.30%), and *β*-pinene (10.50%), with additional components being carvacrol (5.50%), bicyclogermacrene (5.50%), and *α*-pinene (4.10%)[17]. Although there are some similarities between our findings and those of previous research on the composition of EO, the percentages of compounds demonstrated substantial variations.

No.	Compounds	RT	$RI^{calc.}$	$RI^{lit.}$	Area%
1	Myrcene	12.35	987	988	0.05
2	*p*-Cymene	13.84	1022	1023	0.03
3	Sylvestrene	14.05	1027	1027	0.96
4	*γ*-Terpinene	15.30	1056	1058	0.02
5	2-Methoxy-4-vinylphenol	25.07	1312	1309	0.45
6	*α*-Copaene	27.12	1374	1376	0.03
7	*β*-Bourbonene	27.39	1382	1384	0.54
8	Sesquithujene	27.98	1399	1405	0.09
9	*β*-Caryophyllene	28.57	1418	1419	17.78
10	Geranyl acetone	29.56	1450	1450	23.26
11	*α*-caryophyllene	29.70	1454	1454	3.35
12	Germacrene D	30.50	1480	1482	19.08
13	Bicyclogermacrene	30.95	1495	1495	3.08
14	E-Nerolidol	32.88	1560	1561	27.11
15	Caryophyllene oxide	33.55	1582	1581	1.12
16	β-Sinensal	36.92	1702	1706	2.56
	Total identified%				99.51
	Class of compounds				
	Monoterpene hydrocarbons				1.06
	Oxygenated monoterpenes				23.26
	Sesquiterpene hydrocarbons				43.95
	Oxygenated sesquiterpenes				30.79
	Others				0.48

Table 1. Phytochemical composition of *T. polium* L. essential oil. RT = Retention time; $RI^{calc.}$ = calculated Retention index; $RI^{lit.}$ = Retention index according to NIST database.

The majority of studies have identified *α*- and *β*-pinene[15,17,27–31], and a few have demonstrated the presence of carvacrol[21,28,31] in certain *T. Polium* L. essential oil. Nevertheless, they were absent from the oil examined in our investigation. The literature suggests that the essential oil of *T. polium* L. manifest a significant degree of chemical variation. Various ecological parameters, such as plant age, developmental stage, geographical origin, and climatic and genotypic factors, were likely associated with the variations in the chemical compositions of oil[26,31,32].

Antioxidant activity of the *T. polium* L. EO

Using the well-established DPPH assay, which has been previously described[21,37], the free radical scavenging activity of *T. polium* L. EO was evaluated. DPPH• is a stable free radical capable of accepting an electron or hydrogen from an antioxidant, resulting in the formation of a stable molecule. Figure 2 demonstrates that the essential oil of *T. polium* L. displays significant antioxidant capacity, as evidenced by an IC_{50} value of 20.08 ± 1.00 µg/mL, which is less effective than the positive control Trolox (IC_{50} = 1.94 ± 0.10 µg/mL). The EO's enhanced antioxidant capacity is attributed to its chemical constituents, which are distinguished by a substantial concentration of oxygenated sesquiterpenes, particularly the high concentration of E-nerolidol and the presence of 2-methoxy-4-vinylphenol. cis-Nerolidol exhibited substantial scavenging capacity against the DPPH radical, with an IC_{50} value of 1.48 mM[38]. Our results correspond with those of Bakari et al. concerning the DPPH radical scavenging ability of *T. polium* L. essential oil from Tunisia, which demonstrated an IC_{50} value of 20 ± 1.01 µg/mL[31]. The significant antioxidant activity was ascribed to the presence of *α*-pinene, *β*-pinene, *p*-cymene, and borneol, each exhibiting antioxidant capabilities. In a Moroccan investigation, El Atki et al.[30] found that the essential oil of *T. polium* L., primarily composed of t-cadinol (18.3%), germacrene D (15.30%), and *β*-pinene (10.50%), has DPPH radical scavenging action with an IC_{50} value of 7.2 ± 0.55 mg/mL.

Anticancer property of the *T. polium* L. EO

The cytotoxic effects of the examined EO were evaluated against breast cancer (MCF-7) and melanoma (B16F10) cell lines, as illustrated in Figs. 3. This study is the first to evaluate the anticancer activity of *T. polium* L. EO on MCF-7 and B16F10 cell lines. *T. polium* L. EO demonstrated significant cytotoxic effects on MCF-7 and B16F10 cell lines, showing a dose-dependent inhibition of cell viability. The IC_{50} values were 30.56 µg/mL for MCF-7 cells and 32.75 µg/mL for B16F10 cells, indicating that it is less potent than Taxol (the positive control), which has IC_{50} values of 0.068 µg/mL for MCF-7 cells and 0.058 µg/mL for B16F10 cells. The notable anticancer activity could be attributed to the high abundance of E-nerolidol in the tested EO. The literature review indicated

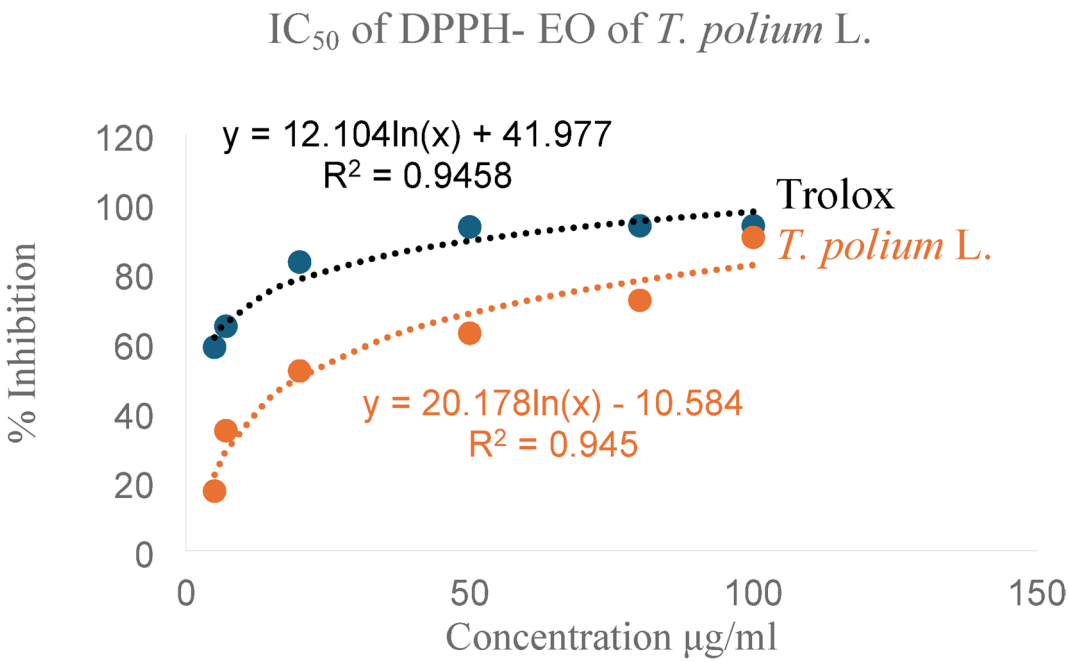

Fig. 2. A plot of the percent inhibition vs. the concentrations of Trolox and *T. polium* L. EO.

that nerolidol demonstrated significant cytotoxicity, with IC_{50} values of 2.96 and 3.02 µg/mL against HT-20 breast carcinoma cells and HeLa cells, respectively, suggesting its potential as a potent anti-cancer agent against these two tumor cell lines[33]. Moreover, trans-nerolidol, extracted from the leaf EO *Zornia brasiliensis* Vogel, exhibited notable cytotoxic effects against several cancer cell lines, specifically B16-F10 (mouse melanoma), HepG2 (human hepatocellular carcinoma), HL-60 (human promyelocytic leukemia), and K562 (human chronic myelocytic leukemia), with IC_{50} values of > 25, >25, 21.99, and 17.58 µg/mL, respectively[34].

A prior investigation in Iran revealed that *T. polium* L. EO, characterized by its principal constituents—10-epi-*γ*-eudesmol (41.70%), spathulenol (8.20%), elemol (7.10%), *α*-pinene (4.50%), and *β*-caryophyllene (3.10%)—exhibited an antiproliferative effect on the HT29 cell line, with an IC_{50} value of 66.87 ± 1.37 µg/mL after 48 h[35]. Menichini et al. reported that the EO of *T. polium* ssp. capitatum, sourced from Greece, demonstrated a cytotoxic effect on colon adenocarcinoma (CACO-2), amelanotic melanoma (C32), and large lung carcinoma (COR-L32) cell lines, with the primary constituents being carvacrol (10.10%), caryophyllene (9.80%), torreyol (7.60%), and caryophyllene oxide (5.00%), and IC_{50} values of 52.70 ± 2.10, 91.20 ± 2.10, and 104 ± 2.10 µg/mL, respectively[36].

Evaluation of the anti-lipase, and anti-α-amylase activities

Obesity results from a disparity between the organism's caloric requirements and food intake, causing disturbances in lipid metabolism[38]. This disorder can be prevented and treated by reducing fat hydrolysis in the gastrointestinal tract through the suppression of pancreatic lipase[38]. Orlistat is a pharmaceutical agent that inhibits pancreatic lipase activity; yet it may result in significant adverse effects, including the development of renal disorders[39]. The inquiry into plant-derived substitutes for orlistat has revealed that polyphenols and essential oil display possible inhibitory effects on pancreatic lipase. In our study, we assessed the lipase inhibitory effect of *T. polium* L. EO for the first time by measuring the hydrolysis of *p*-nitrophenyl butyrate (PNPB). With an IC_{50} of 368.15 ± 18.40 µg/mL, the EO of *T. polium* L. showed significant lipase inhibitory activity, however it was less effective than the positive control orlistat (IC_{50} = 93.87 ± 0.40 µg/mL), as shown in Fig. 4. To our knowledge, no studies have assessed the antilipase effect of *T. polium* L. EO, making ours the inaugural examination.

α-Amylase catalyzes the hydrolysis of complex polysaccharides into oligosaccharides and disaccharides, which are then further degraded by *α*-glycosidase into monosaccharides. Inhibiting the *α*-amylase enzyme, crucial for glucose metabolism, represents a potential therapeutic strategy for type 2 diabetes and obesity[40]. EO extracted from medicinal plants have shown the capacity to inhibit *α*-amylase and *α*-glucosidase in both in vitro and in vivo investigations, indicating their antidiabetic effectiveness. Our analysis of *T. polium* L. EO demonstrated a limited α-amylase inhibitory activity compared to the positive control acarbose, with an IC_{50} of 715 ± 35.80 and 56.42 ± 1.28 µg/mL, respectively, as depicted in Fig. 5.

Conclusion

The hydrodistillation of desiccated *T. polium* L. leaves yielded essential oil, consisting of 17 recognized components that represented 99.54% of the total oil. The principal constituents were E-nerolidol, geranyl acetone, germacrene D, β-caryophyllene, α-caryophyllene, and bicyclogermacrene. Despite its restricted lipase and inferior α-amylase activities, the essential oil demonstrated significant antioxidant effectiveness against 1,1-diphenyl-2-picrylhydrazyl (DPPH). Furthermore, it exhibited notable cytotoxicity against breast cancer

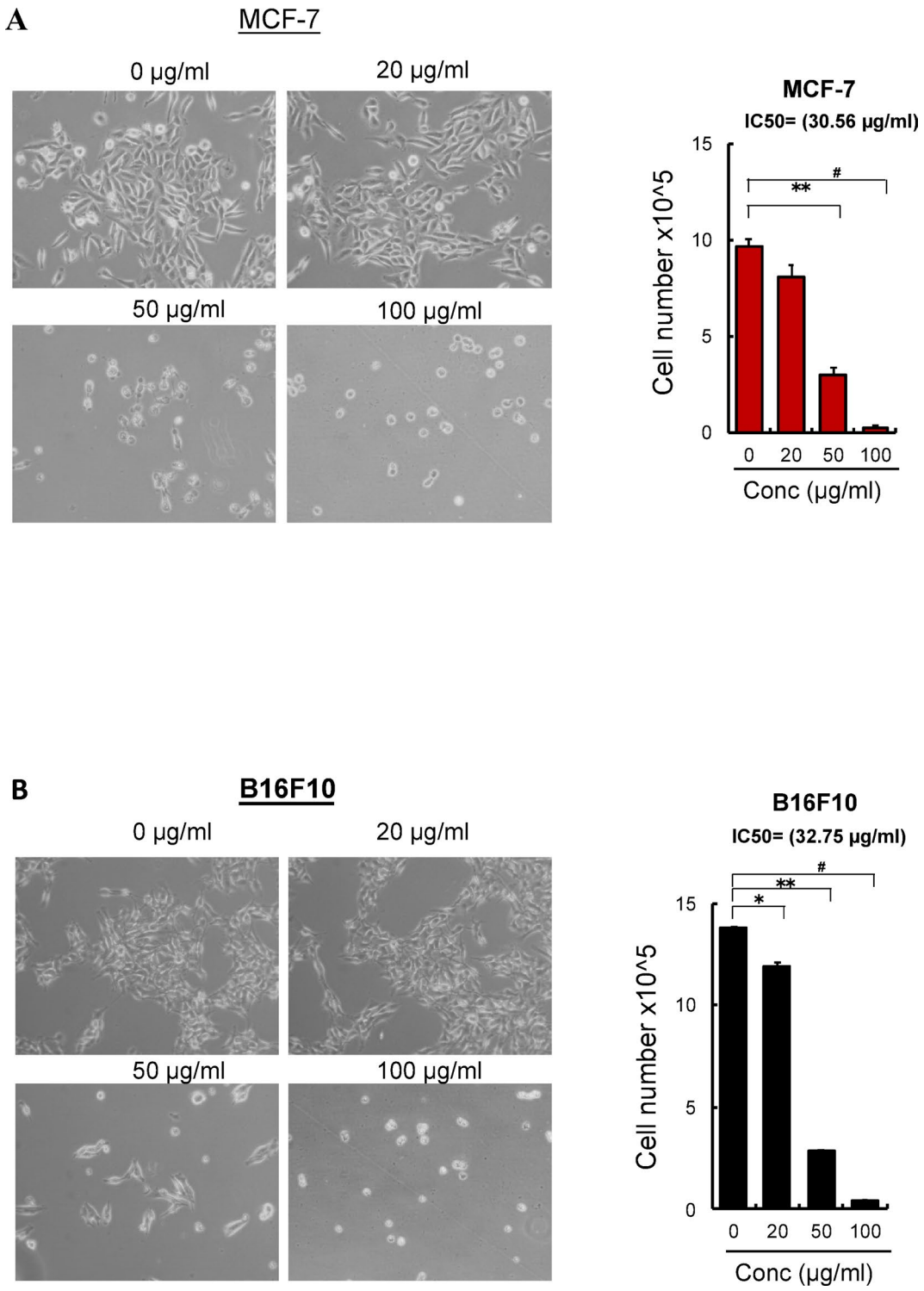

Fig. 3. *T. polium* EO was added in varying concentrations to MCF-7 cells **(A)** and B16F10 **(B)** cell lines. Macroscopic images of MCF-7 cells **(A)** and B16F10 **(B)** cells under an inverted microscope after 24 h treatment with *T. polium* EO or DMSO as a control ($n = 3$ groups). (Magnification 20x). After 24 h, viable cells ($n = 3$/group) were counted using Trypan blue. Data are expressed as mean ± SEM (unpaired Student's t-test or One-way ANOVA $^*p < .05$). The linear regression curve was used to obtain the IC_{50} value, which is as follows: Y = a*X + b, IC50 = (0.5-b)/a.

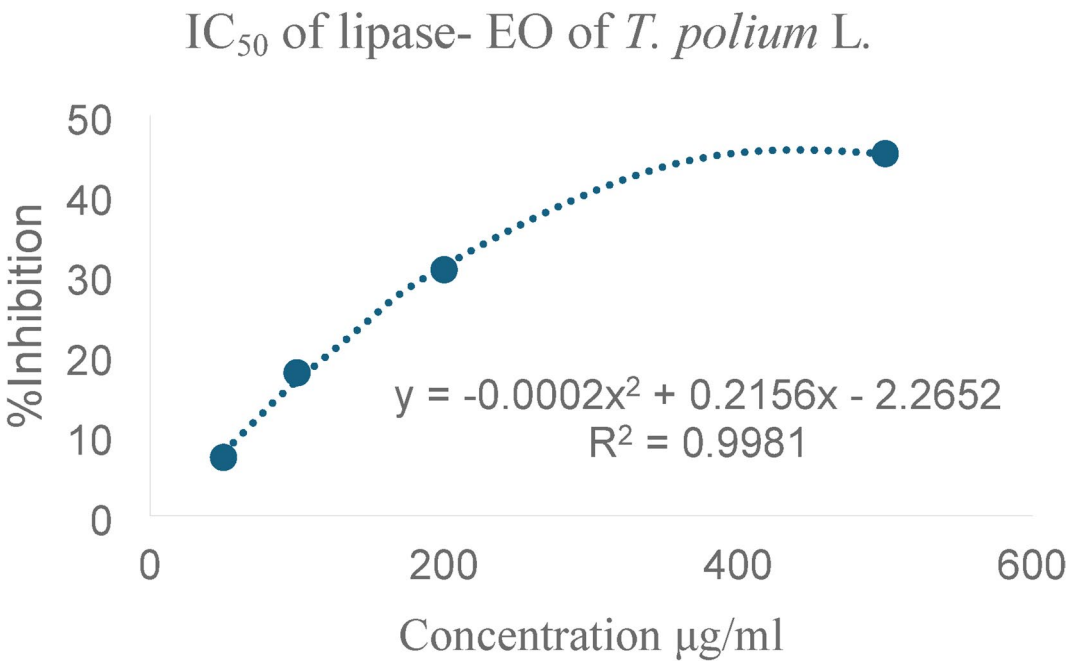

Fig. 4. A plot of Lipase percentage inhibition vs. the concentration of *T. polium* L. EO.

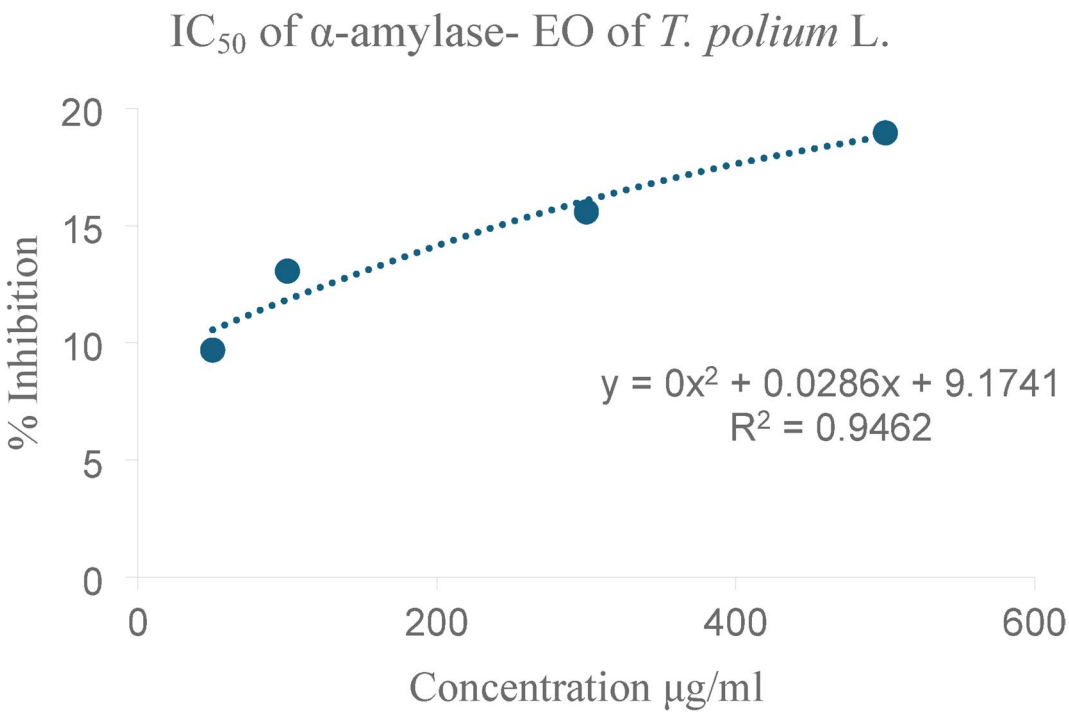

Fig. 5. A plot of the percentage inhibition of α-amylase vs. the concentration of *T. polium* L. EO.

(MCF-7) and melanoma (B16F10) cell lines. The essential oil of *T. polium* L. are attractive candidates for the production of useful bioactive molecules, as well as anticancer and antioxidant agents for medicinal and pharmacological uses. Future research should focus on in vivo studies to assess the efficacy of this oil, utilizing appropriate animal models to further investigate their therapeutic potential.

Data availability

All data generated or analysed during this study are included in this published article.

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References

1. Bolouri, P. et al. Applications of essential oils and plant extracts in different industries. *Molecules* **24**, 8999. https://doi.org/10.3390/molecules27248999 (2022).
2. Giannenas, I., Florou-Paneri, P., Christaki-Sarikaki, E., Sidiropoulou, E., Bonos, E. The history of herbs, medicinal and aromatic plants, and their extracts: past, current situation and future perspectives.*Feed additives*.1-18 (Academic Press, 2020).Doi:10.1016/B978-0-12-814700-9.00001-7.
3. Diniz do Nascimento, L. et al. Bioactive natural compounds and antioxidant activity of essential oils from spice plants: new findings and potential applications. *Biomolecules* **10**, 988. https://doi.org/10.3390/biom10070988 (2020).
4. Cimino, C. et al. Essential oils: pharmaceutical applications and encapsulation strategies into lipid-based delivery systems. *Pharmaceutics* **13**, 327. https://doi.org/10.3390/pharmaceutics13030327 (2021).
5. Raut, J. S. & Karuppayil, S. M. A status review on the medicinal properties of essential oils. *Ind. Crop Prod.* **62**, 250–264. https://doi.org/10.1016/j.indcrop.2014.05.055 (2014).
6. De Sousa, D. P. *et al.* Essential oils: chemistry and Pharmacological activities. *Biomolecules* **13**, 1144. https://doi.org/10.3390/biom13071144 (2023).
7. Uritu, CM. *et al.* Medicinal Plants of the Family Lamiaceae in Pain Therapy: A Review. *Pain Res Manag*. **2018**, 7801543 (2018). doi:10.1155/2018/7801543
8. Ramos da Silva, LR. et al. Lamiaceae essential oils, phytochemical profile, antioxidant, and biological activities. Evid based complement alternat Med et al. 6748052 (2021). https://doi.org/10.1155/2021/6748052
9. Ciocarlan, A. et al. Chemical profile, elemental composition, and antimicrobial activity of plants of the Teucrium (Lamiaceae) genus growing in Moldova. *Agronomy* **12**, 772. https://doi.org/10.3390/agronomy12040772 (2022).
10. Bahramikia, S., Hassan Gavyar, H. & Yazdanparast, P. *Teucrium polium* L: an updated review of phytochemicals and biological activities. *Avicenna J. Phytomed.* **12**, 224–240. https://doi.org/10.22038/AJP.2021.19155 (2022).
11. Ali-Shtayeh, M. S., Jamous, R. M. & Abuzaitoun, S. Y. Analysis of floristic composition and species diversity of vascular plants native to the state of Palestine (West bank and Gaza Strip). *Biodivers. Data J.* **10**, 80427. https://doi.org/10.3897/BDJ.10.e80427 (2022).
12. Abdollahi, M., Karimpour, H. & Monsef-Esfehani, H. R. Antinociceptive effects of *Teucrium polium* L. total extract and essential oil in mouse writhing test. *Pharmacol. Res.* **48**, 31–35. https://doi.org/10.1016/S1043-6618(03)00059-8 (2003).
13. Sharifi-Rad, M. et al. *Teucrium polium* (L.): phytochemical screening and biological activities at different phenological stages. *Molecules* **27**, 1561. https://doi.org/10.3390/molecules27051561 (2022).
14. Ali-Shtayeh, M. S., Jamous, R. M. & Jamous, R. M. Traditional Arabic Palestinian ethnoveterinary practices in animal health care: A field survey in the West bank (Palestine). *J. Ethnopharmacol.* **182**, 35–49. https://doi.org/10.1016/j.jep.2016.02.005 (2016).
15. Gülsoy Toplan, G. et al. Phytochemical composition and Pharmacological activities of *Teucrium polium* L. collected from Eastern Turkey. *Turk. J. Chem.* **46**, 269–282. https://doi.org/10.3906/kim-2107-13 (2021).
16. Sheikhbahaei, F., Khazaei, M. & Nematollahi-Mahani, S. N. *Teucrium polium* extract enhances the anti-angiogenesis effect of Tranilast on human umbilical vein endothelial cells. *Adv. Pharm. Bull.* **8**, 131–139. https://doi.org/10.15171/apb.2018.016 (2018).
17. Kerbouche, L., Hazzit, M., Ferhat, M. A., Baaliouamer, A. & Miguel, M. G. Biological activities of essential oils And ethanol extracts of *Teucrium polium* subsp. Capitatum (L.) briq. And Origanum floribundum Munby. *J. Essent. Oil Bearing Plants*. **18**, 1197–1208. https://doi.org/10.1080/0972060X.2014.935065 (2015).
18. Maurya, A. K., Vashisath, S., Aggarwal, G., Yadav, V. & Agnihotri, V. K. Chemical diversity and α-glucosidase inhibitory activity in needles essential oils of four Pinus species from Northwestern himalaya, India. *Chem. Biodivers.* **19**, e202200428. https://doi.org/10.1002/cbdv.202200428 (2022).
19. Al-Maharik, N., Jaradat, N., Al-Hajj, N. & Jaber, S. Myrtus communis L.: essential oil chemical composition, total phenols and flavonoids contents, antimicrobial, antioxidant, anticancer, and α-amylase inhibitory activity. *Chem. Biol. Technol. Agric.* **10**, 41. https://doi.org/10.1186/s40538-023-00417-4 (2023).
20. Maurya, A. K., Aggarwal, G., Vashisath, S., Kumar, V. & Agnihotri, V. K. Chemodiversity and α-glucosidase activity of Eucalyptus species from Northwestern himalaya, India. *Chem. Biodivers.* **20**, e202300223. https://doi.org/10.1002/cbdv.202300223 (2023).
21. Bsharat, O. et al. Chemical profiling and biological assessment of essential oil from Artemisia herba-alba. *Sci. Rep.* **15**, 31538. https://doi.org/10.1038/s41598-025-17221-9 (2025).
22. Jaradat, N. et al. Eucalyptus camaldulensis Dehnh leaf essential oil from palestine exhibits antimicrobial and antioxidant activity but no effect on porcine pancreatic lipase and α-Amylase. *Plants* **12**(3805), https://doi.org/10.3390/plants12223805 (2023).
23. Shodehinde, S. A., Ademiluyi, A. O., Oboh, G. & Akindahunsi, A. A. Contribution of Musa paradisiaca in the Inhibition of α-amylase, α-glucosidase and Angiotensin-I converting enzyme in streptozotocin induced rats. *Life Sci.* **133**, 8–14. https://doi.org/10.1016/j.lfs.2015.03.026 (2015).
24. Slanc P.*et al.* Screening of selected food and medicinal plant extracts for pancreatic lipase inhibition. *Phytother Res.* **23**, 874-7 (2009). doi: 10.1002/ptr.2718. PMID: 19107742
25. Salama, Y. & Al-Maharik, N. Micromeria fruticosa and foeniculum vulgare essential oils inhibit melanoma cell growth and migration by targeting MMP9 and NFkB signaling. *Chem. Biol. Technol. Agric.* **11**, 6. https://doi.org/10.1186/s40538-023-00522-4 (2024).
26. Sadeghi, H., Jamalpoor, S. & Shirzadi, M. H. Variability in essential *oil of Teucrium polium L. of different latitudinal popul*ations. *Ind. Crops Prod.* **1**, 130–134. https://doi.org/10.1016/j.indcrop.2014.01.015 (2014).
27. Cozzani, S. et al. Chemical composition of essential oil of *Teucrium polium* subsp. Capitatum (L.) from Corsica. *Flavour Fragr. J.* **20**, 436–441. https://doi.org/10.1002/ffj.1463 (2005).
28. Aburjai, T., Hudaib, M. & Cavrini, V. Composition of the essential oil from Jordanian Germander (*Teucrium polium* L). *J. Essent. Oil Res.* **18**, 97–99. https://doi.org/10.1080/10412905.2006.9699398 (2006).
29. Farahbakhsh, J., Najafian, S., Hosseinifarahi, M. & Gholipour, S. Essential oil composition and phytochemical properties from leaves of Felty Germander (*Teucrium polium* L.) and spearmint (Mentha spicata L). *J. Essent. Oil Bearing Plants*. **24**, 147–159. https://doi.org/10.1080/0972060X.2021.1896976 (2021).
30. El Atki, Y. et al. Phytochemistry, antioxidant and antibacterial activities of two Moroccan *Teucrium polium* L. subspecies: preventive approach against nosocomial infections. *Arab. J. Chem.* **13**, 3866–3874. https://doi.org/10.1016/j.arabjc.2019.04.001 (2020).
31. Bakari, S. et al. Chemical composition and in vitro evaluation of total phenolic, flavonoid, and antioxidant properties of essential oil and solvent extract from the aerial parts of *Teucrium polium* grown in Tunisia. *Food Sci. Biotechnol.* **24**, 1943–1949. https://doi.org/10.1007/s10068-015-0256-z (2015).
32. Maurya, A. K. et al. Comparative studies of essential oils composition and cytotoxic activity of Valeriana Jatamansi Jones. *J. Essent. Oil Res.* **33**, 584–591. https://doi.org/10.1080/10412905.2021.1966846 (2021).
33. Chan, W. K. et al. A sesquiterpene alcohol with multi-faceted Pharmacological and biological activities. *Molecules* **21**, 529. https://doi.org/10.3390/molecules21050529 (2016).
34. Costa, E. V. et al. Antitumor properties of the leaf essential oil of Zornia Brasiliensis. *Planta Med.* **81**, 563. https://doi.org/10.1055/s-0035-1545842 (2015).

35. Hashem-Dabaghian, F., Shojaii, A., Asgarpanah, J. & Entezari, M. Anti-mutagenicity and apoptotic effects of *Teucrium polium* L. essential oil in HT29 cell line. *Jundishapur J. Nat. Pharm. Prod.* **15**, https://doi.org/10.5812/jjnpp.79559 (2020).
36. Menichini, F. et al. Phytochemical composition, anti-inflammatory and antitumour activities of four Teucrium essential oils from Greece. *Food Chem.* **115**, 679–686. https://doi.org/10.1016/j.foodchem.2008.12.067 (2009).
37. Bsharat, O., Salama, Y., Al-Hajj, N. & Al-Maharik, N. Chiliadenus iphionoides: from chemical profiling to anticancer, antioxidant, α-amylase, and α-glycosidase activities. *PLoS One*. **20**, e0327632. https://doi.org/10.1371/journal.pone.0327632 (2025).
38. Friedman, M. I., Sørensen, T. I. A., Taubes, G., Lund, J. & Ludwig, D. S. Trapped fat: obesitypathogenesis as an intrinsic disorder in metabolic fuelpartitioning. *Obes. Rev.* **25**, 13795. https://doi.org/10.1111/obr.13795 (2024).
39. Heck, A. M., Yanovski, J. A. & Calis, K. A. Orlistat, a new lipase inhibitor for the management of obesity. *Pharmacotherapy* **20**, 270–279. https://doi.org/10.1592/phco.20.4.270.34882 (2000).
40. Kashtoh, H. & Baek, K. H. Recent updates on phytoconstituent alpha-glucosidase inhibitors: an approach towards the treatment of type two diabetes. *Plants (Basel)*. **11**, 2722. https://doi.org/10.3390/plants11202722 (2022).

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Author contributions
NM design of the work, (ES, YS and NM) data collection, NA and NM data analysis, NM and NA and OB writing and reviewing.

Declarations

Competing interests
The authors declare no competing interests.

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