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Experimental Research Article

Chemical composition, phenolic content, antioxidant, anticancer, and antimicrobial activities of *Teucrium polium* l. essential oil

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ABSTRACT

Introduction: Medicinal plants perform a prominent function in improving sustainable healthcare systems and benefits because of enriching bioactive components in the pharmacology industry. The present research aims to explore potential medicinal attributes of *Teucrium polium* L. essential oil (TEO), focusing on antioxidant, anticancer and antimicrobial attributes.

Methods: TEO was extracted by hydrodistillation and constituents were identified using gas chromatography-mass spectrometry and Fourier transform infrared spectroscopy. Total phenolic (TPC) and flavonoid (TFC) contents were quantified using Folin-Ciocalteu and aluminium chloride methods. Antioxidant potential was assessed through 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and ferric ions reducing antioxidant power (FRAP) assays. Anticancer activity was evaluated on Caco-2 cells using the dimethylthiazol-diphenyltetrazolium bromide colorimetric technique. Antimicrobial properties were determined by disk diffusion and well diffusion methods, minimum inhibitory and bactericidal concentration, and also scanning electron microscopy to examine bacterial cell morphology.

Results: In addition, TPC and TFC were reported as 198.1 ± 4.19 mg gallic acid equivalent /g dry weight (DW) and also 56.23 ± 9 mg rutin (RE)/g DW, respectively. The promising antioxidant function of TEO was proved using achieved IC_{50} from DPPH (94.35 ± 3.2 µg/mL), ABST (83.3 ± 5.1 µg/mL) and FRAP (2.8 ± 0.02 µg ascorbic acid equivalent /g). Additionally, an acceptable cytotoxicity was detected by TEO according to the obtained results. The lowest (1.8 mm) and highest (13.8 mm) inhibition zones for TEO were observed against *Escherichia coli* (*E. coli*) at a concentration of 20 mg/mL and *Staphylococcus aureus* at 80 mg/L level in disk diffusion agar, respectively. The structure of *E. coli* was depicted using a scanning electron microscopy evaluation after treating by TEO, which indicated several effects on the membrane and denaturalization for bacterial cell.

Conclusion: Overall, the significant antimicrobial, antioxidant, and anticancer functions of TEO not only confirm multifunctional bioactivity but also highlight a critical therapeutic opportunity. Unlike prior studies that evaluated isolated activities, this research demonstrates the integrated pharmacological potential for TEO, positioning it as a promising candidate for the development of novel herbal medicines and complementary therapeutic agents.

Keywords:
Antimicrobial
Antioxidant
Anticancer
Essential oil
Teucrium polium L.

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Introduction

Medicinal plants have long played a critical role in healthcare systems due to their wide range of bioactive compounds and relatively low toxicity compared with synthetic pharmaceuticals (Hoseini et al., 2023; Nouri, 2022). They serve as valuable natural resources for developing new therapeutic agents to address key pharmacological challenges such as increasing microbial resistance, oxidative stress–related disorders, and cancer progression (Soltanbeigi et al., 2024; Pandey et al., 2020). In this context, plant-derived essential oils (EOs) and extracts (EXs) have attracted growing attention for their potent antimicrobial, antioxidant, and anticancer properties (Davoudi and Ramazani, 2024; Sati and Qubbaj, 2021). These activities are largely attributed to their rich composition of secondary metabolites—terpenoids, phenolics, and flavonoids—that act through multiple biochemical pathways, including radical scavenging, enzyme inhibition, and modulation of cellular signaling (Farnejad et al., 2022).

Teucrium polium L., a member of the Lamiaceae family, is widely distributed in Mediterranean regions, North Africa, Europe, and Southwest Asia (Sabzeghabaie and Asgarpanah, 2016). Traditionally, this plant has been used as an antispasmodic, analgesic, anti-inflammatory, antihypertensive, and wound-healing agent (Othman et al., 2017). Phytochemical analyses have revealed the presence of various compounds such as tannins, terpenoids, saponins, flavonoids, sterols, β-caryophyllene, diterpenoids, caryophyllene oxide, and germacrene derivatives, which collectively contribute to its wide-ranging pharmacological effects (Chabane et al., 2021; Darabi et al., 2025; Noumi et al., 2020).

Despite extensive research on plant essential oils with antioxidant and antimicrobial potential (Chabane et al., 2021; Darabi et al., 2025), investigations into *T. polium* essential oil (TEO) remain limited and fragmented. Most studies have focused on isolated biological activities rather than providing an integrated pharmacological assessment. The biological functions of TEO are closely related to its dominant constituents: spathulenol, a sesquiterpene alcohol capable of donating hydrogen atoms to neutralize free radicals; β-pinene, which stabilizes unpaired electrons; and β-caryophyllene, known to disrupt bacterial membranes and enhance cell permeability (Farahbakhsh et al., 2021). Phenolic components further reinforce these effects by binding sulfhydryl groups in microbial enzymes, which contributes to TEO's strong antibacterial activity (Othman et al., 2017; Mooliani and Nouri, 2021). The synergistic interactions among terpenoid and phenolic fractions are thus central to the multifunctional therapeutic potential of TEO.

Given the growing demand for natural agents capable of combating oxidative stress, microbial resistance, and cancer progression, a comprehensive evaluation of TEO is warranted. The present study aims to systematically explore the multifunctional bioactivity of *T. polium* essential oil by (i) extracting and characterizing its chemical constituents through gas chromatography (GC) and Fourier transform infrared spectroscopy (FTIR), (ii) quantifying total phenolic (TPC) and flavonoid (TFC) contents, (iii) determining antioxidant capacity via DPPH, ABTS, and FRAP assays, (iv) evaluating anticancer potential against Caco-2 human colon carcinoma cells using the MTT assay, and (v) assessing antimicrobial efficacy against several pathogenic bacteria using disk and well diffusion, minimum inhibitory (MIC) and bactericidal (MBC) concentration assays, and scanning electron microscopy (SEM). Integrating these approaches will allow the establishment of a comprehensive pharmacological profile of TEO, thereby supporting its potential application as a natural antioxidant, anticancer, and antimicrobial agent for use in pharmaceutical and food industries.

Materials and methods

Chemicals, culture media, and a comprehensive assessment of microbial activity

The DPPH, ABTS, Folin-Cicalteu reagent, dimethyl sulfoxide (DMSO), tryptic soy broth, Mueller Hinton agar (MHA), Mueller Hinton broth (MHB), nutrient agar, and Sabouraud dextrose agar and broth were purchased from Sigma-Aldrich.

In these tests, *S. typhimurium* ATCC 14028, *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *E. coli* ATCC 25922, *L. monocytogenes* ATCC 19111, and *Sh. dysentery* ATCC 13313 as pathogen bacteria were purchased from the National Center of Biological and Genetic Resources in Iran.

TEO extraction

TEO plant collected from local gardens in Shiraz city, which recognized by traditional taxonomist and extraction was performed by hydrodistillation using

a Clevenger-type apparatus. Briefly, 20 g dried and powdered aerial parts were immersed in 200 mL water (solvent-to-sample ratio 10:1, v/w) and subjected to distillation at atmospheric pressure. The distillation was maintained at 100 ± 2 °C for 3 h to ensure complete recovery of volatile constituents and obtained TEO was collected, dried over anhydrous sodium sulfate to remove residual water, and stored in a dark glass vial at 4 °C until further analysis (Chabane et al., 2021). Because *T. polium* essential oil (TEO) is hydrophobic, it was first dissolved in a small amount of DMSO (final concentration ≤1 %, v/v) and then diluted with sterile distilled water or the corresponding assay medium. This approach ensured uniform dispersion and eliminated solubility-related variability.

Identification of TEO constituents

GC determination:

TEO sample with a 0.1 µL volume was diluted in hexane (ratio of 10:100), which was injected into a Thermo Fischer capillary gas chromatograph that was directly coupled to a mass spectrometer system (model GC ULTRA S/N 20062969; Polaris QS/N 210729). An HP-5MS non-polar fused silica capillary column with dimensions of 60 m × 0.32 mm × 0.25 µm film thickness was used. Heating rate was set at 2 °C/min from an initial temperature to final 260 °C for achieving complete separation of TEO constituents. The ionization energy applied was 70 eV and helium gas applied as the carrier gas with 1 mL/min flow rate. The ion source temperature was set to 200 °C and a scan mass range of m/z 40 to 650 and also an interface line at 300 °C employed. The components were characterized by determining their retention indices relative to a homologous series of n-alkanes (C_8-C_{20}) from Fluka, Buchs/sg and Switzerland. Additionally, recorded mass spectra for constituents were matched with those stored in spectrometer database (NIST MS Library v. 2.0) and bibliography for identification purposes (Fard and Nouri 2023).

FTIR spectroscopy:

The dried TEO sample was injected into FTIR (Bruker Tensor 27) and spectrum was obtained in 400 to 4000 cm^{-1} range (Sabzeghabaie and Asgarpanah 2016).

Measurement of TPC and TFC

These are the main constituents of medicinal plants and are responsible for their antimicrobial and antioxidant effects. Folin-Ciocalteu reagent and gallic acid (at 0 to 200 mg/mL concentration) standards were used to measure TPC. In the present method, 0.1 mL Folin-Ciocalteu reagent, water and 1 % w/v TEO sample at similar levels 2 mL blended, which is then placed at ambient temperature for 3 min to allow the reaction to occur. After 2 h, 0.3 mL sodium carbonate was added to the mixture and the solution absorbance was measured at 765 nm using a spectrophotometer (Bausch & Lomb, Germany). The recorded absorbance values were used to calculate TPC of TEO sample, and for TFC determination, aluminum chloride procedure was employed, and also a standard curve was drawn using quercetin at concentrations ranging from 0 to 200 mg/mL (Pacifico et al., 2012).

A comparative antioxidant potential by DPPH, ABTS and ferric reducing antioxidant power (FRAP) assays

DPPH radical scavenging activity:

TEO sample (1 mL) was mixed with DPPH ethanol solution (90 mL), and after 30 min of incubation (25 °C), absorbance (wavelength 517 nm) was read by a spectrophotometer and employed to measure antiradical activity by Eq 1 (Stankovic et al., 2012):

$$\mathrm{DPPH}_{\mathrm{RS\ activity}} = [1 - \mathrm{A_1}/\mathrm{A_0}] \times 100$$

Where A_0 and A_1 are related to absorption for EO and control (distilled water instead of sample), respectively. The inhibition concentration 50 % (IC_{50}) of TEO was calculated as the inhibition percentage against DPPH radicals and antioxidant activity for tert-butylhydroquinone (TBHQ) was evaluated as a positive control.

ABTS radical scavenging method:

Initially, reaction solution containing 7 mM ABTS and 2.4 mM potassium persulfate (1:1 ratio) was prepared, kept at 24 °C and in darkness for 14 h, then diluted with ethanol until absorbance reached to 0.70 at 734 nm. The 200 mL TEO (at 10, 100, 200, 300 and 500 ppm concentrations) were combined with 2 mL free radical solution and after 30 min of reaction, absorbance was recorded at 734 nm wavelength (Noumi et al., 2020).

FRAP method:

First, a mixture was prepared by combining 2.5 mL TEO sample at concentrations ranging from 50 to 10 ppm, 2.5 mL ethanol containing phosphate buffer (2.5 mL, 0.2 M and pH 6.6) and 2.5 mL potassium ferricyanide (1 %). The mixture was incubated at 50 °C for approximately 20 min and to stop the reaction, 2.5 mL trichloroacetic acid (10 %) was added to solution and then centrifuged. From supernatant, 2.5 mL was taken and mixed with 2.5 mL distilled water and 0.5 mL ferric chloride. The reduction activity of sample was measured at 700 nm wavelength using a spectrophotometer, and sample percentage compared to control was calculated through following Eq 2 (Zhu et al., 2011).

$$\% \text{ reducing activity} = [(1 - \text{sample absorbance}) / \text{control absorbance}] \times 100$$

The MTT assay

The effect of TEO on cytotoxicity was studied through MTT method, which Caco-2 cells were added to a solution including Dulbecco modified eagle medium (high glucose, fetal bovine serum and 10 % volume) and penicillin/streptomycin) and incubation was performed at 37 °C (95 % constant humidity) for 21 days. Caco-2 cells (1×10^4 cells per well) were seeded into 96-well plates and allowed to attach for 24 h. Cells were then treated with TEO at concentrations up to 200 µg/mL for 24 h. After treatment, 30 µL MTT solution (0.5 mg/mL) was added per well and incubated for 3 h at 37 °C. The reaction was stopped with 200 µL DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to untreated controls and cytotoxicity was reported in terms of IC_{50} and cell survival curves were drawn through control cells (Noumi et al., 2020).

DDA and WDA procedure

Six bacterial strains were used: *E. coli*, *S. typhimurium*, *S. aureus*, *S. epidermidis*, *L. monocytogenes* and *S. dysenteriae*. DDA is considered a conventional and appropriate method for measuring antimicrobial function of EOs. The TEO concentration was prepared at 20, 40, 60 and 80 mg/mL and also sterilized using a filter. Discs were immersed in TEO solution for 20 min and a microbial suspension (equivalent to 1.5×10^8 CFU/mL or 0.5 McFarland standard) was spread evenly on culture medium. The disks impregnated with TEO were placed on medium surface and culture then incubated at 37 °C for 24 h. The antimicrobial effect was expressed as inhibition zone (mm) and chloramphenicol antibiotic was used as a positive control (Chabane et al., 2021).

In WDA method, following steps are performed: A petri dish containing culture medium was prepared and microbial suspension was spread evenly on medium surface. Wells with 6 mm diameter were created on agar surface and 20 µL TEO with different concentrations (20, 40, 60 and 80 mg/mL) were added. The petri dish was then incubated for 24 h at 37 °C, diameter of growth inhibiting zones (mm) around wells was measured as response and a larger zone diameter indicated a greater inhibitory TEO effect (Unlu et al., 2010).

MIC and MBC determination of EO

The overnight cultures of 5 bacterial strains were prepared in MHB medium at 37 °C to produce stock cultures. The microdilution assay was used to evaluate MIC of TEO and diluted TEO solutions obtained using MHB medium at 3.9, 7.81, 15.62, 31.25, 62.5, 125, 250 and 500 mg/mL concentrations. Then, 70 µL of each TEO solution was added to 96-well microtitre plate followed by 70 µL MHB culture addition. Next, equivalent to a 0.5 McFarland standard containing 10^8 CFU/mL and 70 µL bacterial suspension added to each well, which included 210 µL totally. A similar process was carried out for control and all microtitre plates then incubated at 37 °C for approximately 22 to 24 h in an incubator.

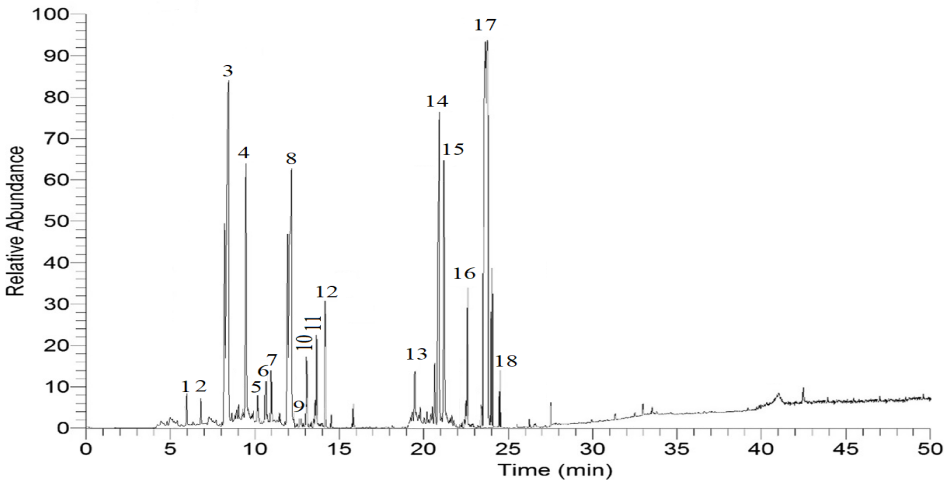
The first well without turbidity indicating no visible bacterial growth reported as MIC of TEO. The 5 µL from wells without bacterial growth was transferred to plates containing MHA culture medium and incubated for 22 to 24 h at 37 °C to determine MBC. Several concentrations were reported as MBC because no bacterial population was observed (Hematian et al., 2020).

Evaluation of microorganism structure

SEM images were prepared to evaluate TEO impact on structural characteristics of *E. coli* strain. The stock culture of mentioned bacteria under assessment inoculated into a main medium containing TEO at MIC concentration. Incubation carried out at 37 °C and resulting culture medium then centrifuged at 5000 g for approximately 5 min. Next, microorganism sample washed with 0.1 M phosphate buffer saline at pH 7.0 and purified sample fixed in a 2.5 % glutaraldehyde solution, kept

TABLE 1: Identification for compositions of *Teucrium polium* L. essential oil through gas chromatography

Number	Compositions	Retention time (min)	Area (%)
1	α-Thujene	6.07	1.87
2	α-Pinene	6.92	1.05
3	β-Pinene	8.27	10.07
4	β-Myrcene	9.65	8.76
5	Limonene	10.09	1.75
6	Benzene, (1-methyl)	10.83	1.12
7	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	11.02	2.13
8	β-Caryophyllene	12.14	8.41
9	Benzene	12.85	0.72
10	α-Humulene	13.05	3.64
11	Alloaromadendrene	13.63	3.89
12	γ-Cadinene	14.15	4.08
13	δ-Cadinene	19.45	2.11
14	Germacrene D	21.56	9.21
15	Germacrene B	21.72	8.81
16	Sesquisabinene hydrate	22.68	5.06
17	Spathulenol	23.91	12.98
18	Linalool	24.71	2.26



at 4°C for 2 h; and then dried with water and ethanol. Finally, sample structure was evaluated using a SEM (Zeiss (LEO) 1450 VP model, Germany) after being coated with a gold layer (Falah et al., 2022).

Statistical analysis

Data were analyzed by Minitab software (version 20) via one-way ANOVA and Tukey test at confidence level of 95 % ($p < 0.05$) was applied to determine differences between data means, so it was necessary to note that experiments were conducted at three replications.

Results

Identification of TEO constituents

The chromatographic profile of TEO detects in Table 1 and reveals 18 identified constituents, which account-

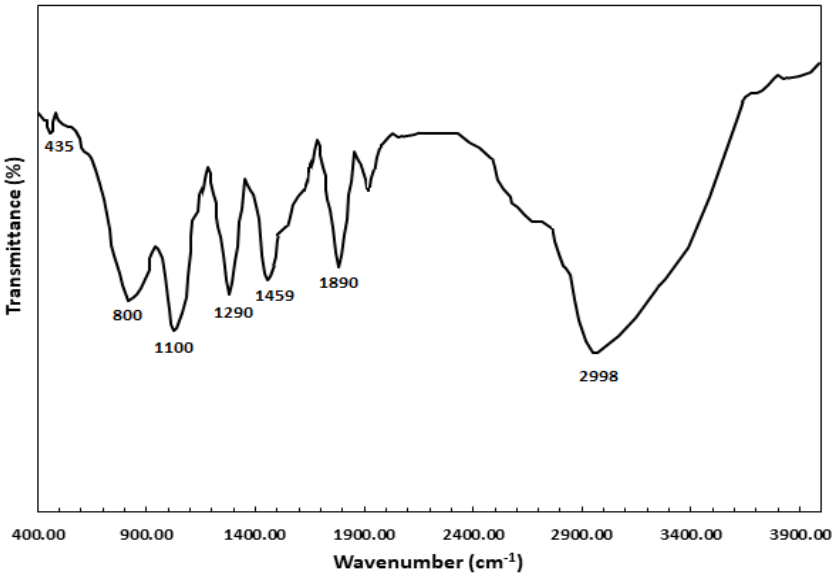


FIGURE 1. Monitoring of Fourier-transform infrared spectroscopy for Teucrium polium L. essential oil

ed for 87.92 % total GC area. Among these components, spathulenol (12.98 %), β-pinene (10.07 %), germacrene D (9.21 %), germacrene B (8.81 %), β-myrcene (8.76 %) and β-caryophyllene (8.41 %) were recognized as the major. Consequently, sesquiterpene and sesquiterpenoid fractions were found to be the most abundant surpassing levels of monoterpenes.

Detection of functional groups in TEO was guided by FTIR and representative peaks from 400 to 4000 cm^{-1} (Figure 1) corresponding to hydroxyl (O-H) relating alcohol groups or carboxylic acids (~3200 to 3600 cm^{-1}) stretching modes and alkyl (nCH, ~2850 to 2950 cm^{-1}). The 1890 cm^{-1} peak was ascribed to C=O vibrations in aldehyde groups and signal 1459 cm^{-1} described C=C stretching vibrations of aromatic ring.

TPC, TFC and antioxidant potential of TEO

The obtained results illustrated that 198.1 ± 4.19 mg GAE/g DW as TPC and 56.23 ± 9 mg RE/g DW for TFC were calculated.

In these methods, TBHQ used as positive control and graph related to DPPH radical inhibitory activity by TEO (artificial antioxidant) depicts in Figure 2a. IC_{50} levels for TEO and TBHQ samples were 94.35 ± 3.2 and 59.62 ± 0.4 µg/mL, respectively. The antiradical activity of TBHQ was about 1.6 times higher than TEO sample, which indicated promising antioxidant function. Based on obtained graph from ABTS assay, IC_{50} for TEO and TBHQ samples was 83.3 ± 5.1 and 59.43 ± 2.18 µg/mL, respectively (Figure 2b). For FRAP method, amount of antioxidant activity (2.8 ± 0.02 AAE/g EO) and TBHQ (3.46 ± 0.032µg AAE/g EO) were evaluated. The FRAP was only about 23 % lower than positive control (vitamin C and TBHQ), which demonstrated high antioxidant activity.

Anticancer potential

The graph related to effect of TEO different concentrations on survival of Caco2-cells outlines in Figure 3. There were 95.34, 71.31, 45.6, 29.21, 13.3 and 5.41 % in concentrations of 0, 10, 25, 50, 100 and 200 mg/mL, which IC_{50} was equal to 15.5 ± 0.9 µg/mL. The increase in TEO (200 µg/mL) reduced survival percentage and proliferation, which influenced on elevated cancer cells.

Antibacterial assay

In the current study, antimicrobial activity was determined through DDA and WDA methods and measuring MIC and MBC of pathogenic bacteria. In the DDA method, the lowest and highest inhibition zones were observed against *E. coli* and *S. aureus* at 20 mg/L and 80 mg/L, respectively (Table 2). The control antibiotic, such as chloramphenicol, was included in the current study, and the halo created by TEO suggested promising antimicrobial activity. In the WDA method, the minimum (1.8 mm, 20 mg/mL TEO) and maximum (13.8

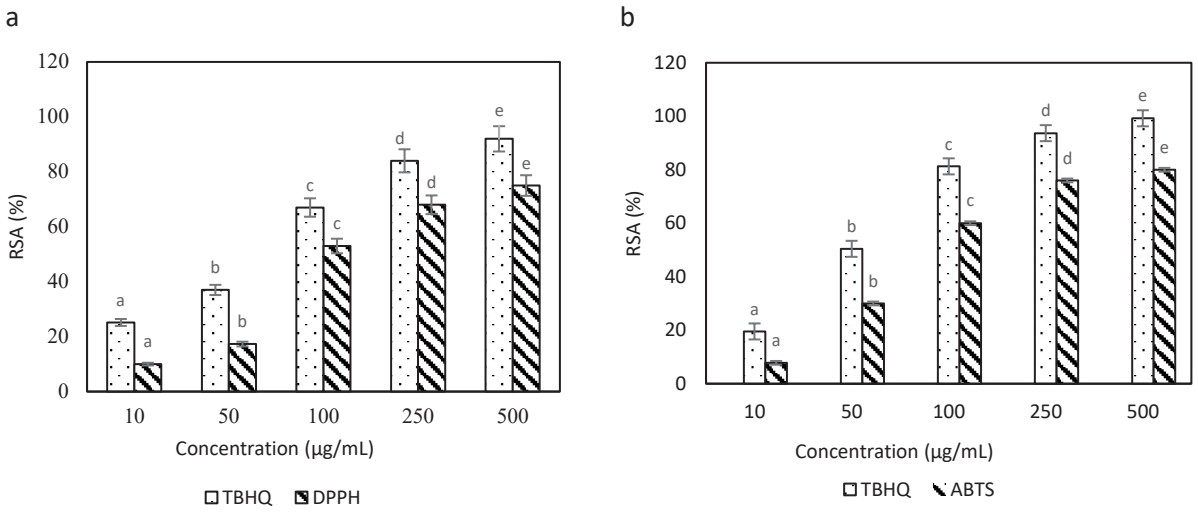


FIGURE 2. Determination of radical scavenging activity (RSA) percentage of Teucrium polium L. essential oil on (a) DPPH and (b) ABTS radicals
[a-e] indicate the difference between different concentrations in an antioxidant and data represent mean ± standard deviation (n = 3).

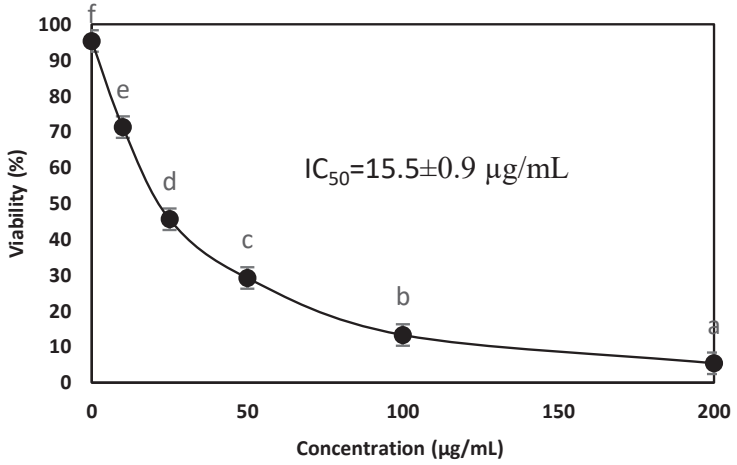


FIGURE 3. Cytotoxic effect of various *Teucrium polium L.* essential oil concentrations on survival of Caco-2 cell line
[a-f] indicate the difference between different concentrations and data represent mean ± standard deviation (n = 3).

mm, 80 mg/mL TEO) inhibitory zones against *E. coli* and *S. aureus* had been seen, respectively (Table 2).

The results indicated that inhibition significantly enhanced in both methods by increasing TEO concentration based on DDA and WDA methods, and also *E. coli* exhibited the highest sensitivity among tested strains. Following *E. coli*, *S. typhimurium*, *S. dysenteriae*, *L. monocytogenes*, *S. epidermidis* and *S. aureus* strains exhibited varying levels of sensitivity to TEO.

The MIC and MBC results for TEO against target microorganisms depicts in Figure 4 According to results, the minimum and maximum values of MIC and MBC had been obtained for *E. coli* (0.450 and 3.450 µL/mL). These results demonstrated varying degrees of susceptibility among different target microorganisms and *E. coli* (gram-negative) bacteria indicated the highest resistant to compared to other strains. The MIC and MBC assays were performed only for the 5 pathogenic bacterial strains.

Comparative study of TEO effect on E. coli bacteria

The SEM images of *E. coli*, the most resistant patho-

TABLE 2: The average inhibition zone (mm) of *Teucrium polium L.* EO against pathogenic bacteria, based on DDA and WDA

Bacteria	Concentration (mg/mL of extract)				
DDA	20	40	60	80	Chloramphenicol concentration (80 mg/mL)
Sh. dysentery	6.5±0.3[a]	7.3±0.5[b]	8.5±0.2[c]	9.3±0.2[d]	24.2±0.2[e]
E. coli	1.1±0.1[a]	1.3±0.1[b]	5.2±0.3[c]	7.8±0.1[d]	21.5±0.2[e]
S. aureus	8.5±0.2[a]	10.1±0.5[b]	12.6±0.2[c]	13.7±0.3[d]	25.4±0.1[e]
S. typhimurium	5.5±0.1[a]	6.5±0.5[b]	8.2±0.1[c]	8.8±0.2[d]	23.6±0.4[e]
L. monocytogenes	6.8±0.2[a]	8.1±0.5[b]	9.7±0.5[c]	11.4±0.1[d]	24.5±0.2[e]
S. epidermidis	8.1±0.2[a]	9±0.5[b]	11.5±0.2[c]	13.2±0.1[d]	26.2±0.1[e]
WDA	20	40	60	80	Chloramphenicol concentration (80 mg/mL)
Sh. dysentery	6.2±0.1[a]	7.7±0.3[b]	8.9±0.2[c]	9.7±0.1[d]	24.2±0.2[e]
E. coli	1.8±0.1[a]	6.4±0.1[b]	6.5±0.1[c]	7.5±0.2[d]	21.5±0.3[e]
S. aureus	7.9±0.2[a]	9.8±0.2[b]	12.6±0.3[c]	13.8±0.1[d]	25.4±0.2[e]
S. typhimurium	5.9±0.1[a]	6.8±0.2[b]	8.1±0.1[c]	9.2±0.2[d]	23.6±0.3[e]
L. monocytogenes	6.7±0.1[a]	7.7±0.1[b]	9.4±0.2[c]	11.7±0.2[d]	24.5±0.3[e]
S. epidermidis	7.3±0.2[a]	8.5±0.2[b]	10.9±0.2[c]	11.2±0.2[d]	26.2±0.2[e]

[a-e] indicate the difference between different concentrations at $p < 0.05$ and data represent mean ± standard deviation (n = 3).

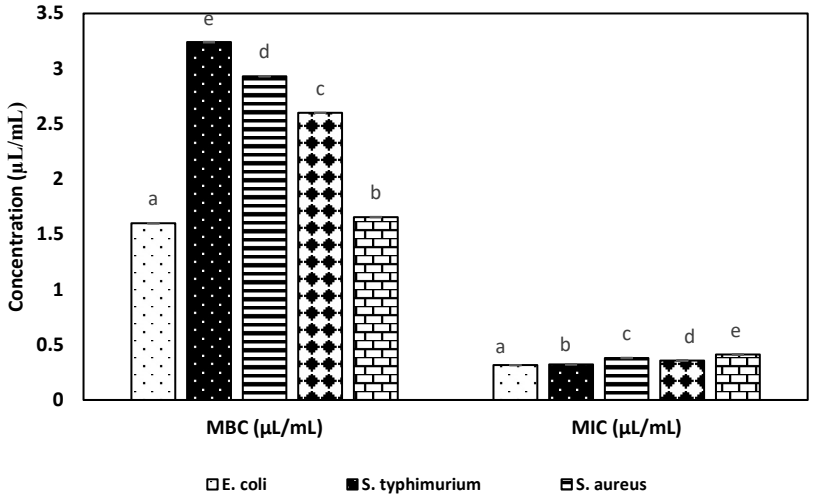


FIGURE 4. Minimum inhibitory concentration and minimum bactericidal concentration of Teucrium polium L. essential oil for some pathogenic bacteria
[a- d] indicate the difference between various strains, and data represent mean ± standard deviation (n = 3).

gen before and after treatment with TEO, reveal significant structural changes (as depicted in Figure 5). In untreated bacteria, structure appeared intact and rod-shaped, which was characteristic of *E. coli*; however, upon exposure to TEO, several alterations in *E. coli* framework were evident.

Discussion

The EOs primarily consist of secondary metabolites, which can be challenging to access due to their interactions with the matrix, and various methods such as solvent extraction are employed to separate these constituents (Davoudi and Ramazani 2024; Sati and Qubbaj 2021). The identified constituents in the structure of Teucrium polium subsp. aurum included 19.13% caryophyllene (Fard and Nouri 2023); similarly, spathulenol is the main compound identified in TEO (Nouri 2022). Carvacrol (10.1%), germacrene (3.1%), and α-cadinene (2.5%) have been isolated from TEO indigenous to Karpathos Island (Al-Otaibi and AlMotwaa,

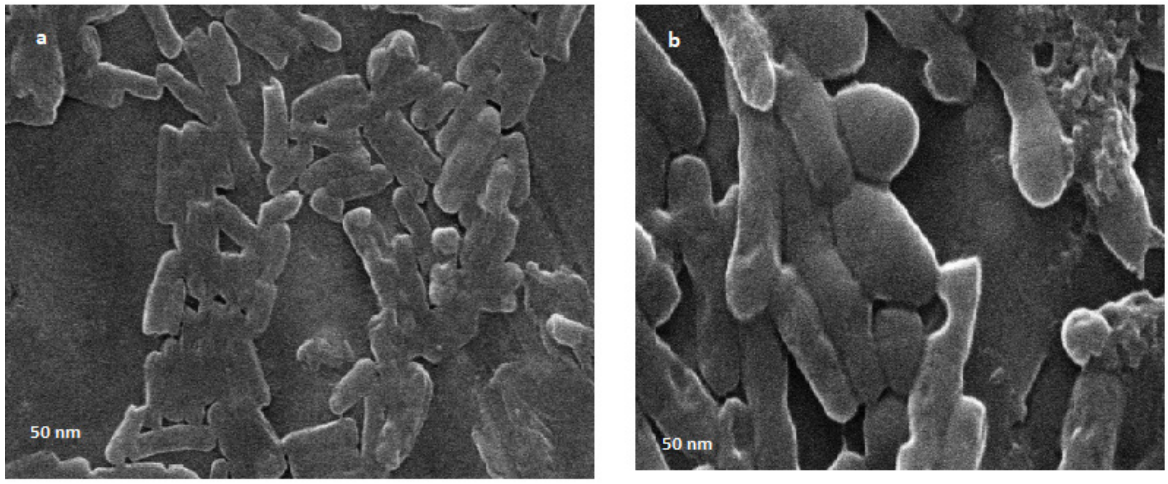


FIGURE 5. Scanning electron microscopy of (a) untreated Escherichia coli and (b) treated with Teucrium polium L. essential oil at minimum inhibitory concentration value for 24 h

2022). Additionally, TEO from Saudi Arabia identified α-cadinol (5.93%), β-pinene (4.58%), β-gurjurene (4.43%), and α-pinene (3.79%) as constituents (Guetat and Al-Ghamdi 2014). In another study conducted on TEO, the main constituents reported were 24.80% 8-cedren-13-ol, 8.70% β-caryophyllene, 6.83% germacrene D, 5.24% sabinene, 4.34% α-humulene, 4.54% alloaromadendrene, 3.51% γ-cadinene, and 6.90% unidentified sesquiterpene hydrocarbon (Chabane et al., 2021). For Teucrium polium subsp. gabesianum from Tunisia, evaluation illustrated that β-pinene (35.97%) and α-pinene (13.32%) were the dominant volatile constituents (Othman et al., 2017). In the case of TEO grown in Iran, identified substances were 6.97% α-pinene, 12.97% β-pinene, 2.19% myrcene, and 3.45% limonene (Farahbakhsh et al., 2021). In another study, the results depicted that major compounds in TEO were 19.82% β-pinene, 18.33% germacrene D, and 6.83% α-cadinol (Guetat and Al-Ghamdi 2014). These constituents contribute to the overall chemical composition of the respective TEOs from different regions.

The FTIR peak presence at 1100 cm^{-1} wavenumber indicated C-O bonds related to alcohols, carboxylic acids, esters, and ethers, and also CH_2 groups' stretching vibrations of alkanes were determined at 541 cm^{-1} (Noumi et al., 2020; Othman et al., 2017), which were similar to the present study's. TEO contained hydroxyl, carboxyl, carbonyl, and functional groups for the phenol ring, and also spectrum peaks in the current study reflect the presence of these components, which was in line with previous research (Chabane et al., 2021; Sabzeghabaie and Asgarpanah, 2016).

The present study confirmed that TEO possesses strong antioxidant potential, supported by high TPC and TFC and also consistent radical-scavenging effects across multiple assays. These findings align with previous reports on TEO, although differences in antioxidant strength have been noted between plant populations from Iran, North Africa, and the Mediterranean (Al-Otaibi and AlMotwaa, 2022; Pacifico et al., 2012). Such variability is likely influenced by environmental factors, extraction techniques, and plant chemotypes, all of which affect phenolic composition (Chabane et al., 2021). The observed activity can be attributed primarily to sesquiterpenes such as spathulenol and β-pinene, both of which are known to neutralize free radicals by donating hydrogen atoms (Sabzeghabaie and Asgarpanah 2016). The presence of flavonoids further enhances this effect through metal-chelating and chain-breaking antioxidant mechanisms (Chabane et al., 2021). Importantly, the multifunctional antioxidant profile of TEO suggests potential applications in mitigating oxidative stress, which is implicated in aging, neurodegenerative disorders, and cardiovascular diseases; thus, TEO may serve as a natural source for both food preservation and therapeutic applications (Al-Otaibi and AlMotwaa, 2022; Pacifico et al., 2012). These constituents varied depending on extraction method, generally containing higher levels of

phenolics compared to other plant parts (Chabane et al., 2021; Pacifico et al., 2012). In different research studies, a wide range of phenolic constituents had been reported for plant EOs (Chabane et al., 2021; Sabzeghabaie and Asgarpanah, 2016). Since antioxidant substances work by donating hydrogen atoms and breaking free radical chains as well as in the FRAP method, the antioxidant impact of TEO was measured through its ability to convert ferric (Fe^{3+}) into ferrous (Fe^{2+}) form (Chabane et al., 2021). Phenolic constituents, especially flavonoids, are secondary metabolites of plants that have significant antioxidant and chelating attributes (Mooliani and Nouri 2021). The level of antioxidant activity was directly influenced by obtained constituents (Othman et al., 2017). The ability for flavonoids to inhibit events caused by free radicals is determined by their chemical structure and pattern of hydroxyl group substitutions (Fard and Nouri 2023). Flavonoids with ortho-dihydroxy substitution in ring B, a hydroxyl group at position 3, a double bond between carbons 2 and 3 (C2-C3), and also a carbonyl group at carbon 4 (C4) in ring C are particularly effective for scavenging free radicals and donating hydrogen atoms (Guetat and Al-ghamdi 2014). The antioxidant potential of TEO can be attributed to high sesquiterpenes and TPC, where spathulenol, a sesquiterpene alcohol, acts as a hydrogen donor, neutralizing free radicals and inhibiting lipid peroxidation (Chabane et al., 2021). β-Caryophyllene has been reported to activate the Nrf2 signaling pathway, thereby stimulating the expression of antioxidant enzymes such as superoxide dismutase and catalase, which enhance endogenous defense systems (Guetat and Al-Ghamdi, 2014). Germacrene D exhibits free radical–scavenging activity, primarily through stabilization of unpaired electrons in reactive oxygen species, and combined mechanisms may explain the strong radical-scavenging activity for TEO across multiple assays (Farahbakhsh et al., 2021).

The lowest cytotoxic concentration of 200 µg/mL was noticed for mammary gland carcinoma (Walker 256/B cells) and prostate cancer (MatLyLu) by *Teucrium polium L*. EX (Noumi et al., 2020). Various research illustrated the effectiveness of *Teucrium polium L*. and *Teucrium* persicum EXs in preventing the proliferation of PC-3 breast and prostate cancer cells (Chabane et al., 2021; Tafrihi et al., 2014). In another study, *Teucrium capitatum* and *Teucrium creticum* were effective against MCF-7 breast cancer cells (Husein et al., 2014), and *Teucrium ramosissimum* had anticancer potential by inhibiting proliferation of K562 (Sghaier et al., 2016). Plant phytochemical substances have been found to enhance the influences of therapeutic drugs (Hoseini et al., 2023). *Teucrium polium* could inhibit the apoptotic effects of certain anticancer drugs like doxorubicin and vinblastine on various cancer cell lines, including Saos-2, A431, SW480, and Skmel-3 (Noumi et al., 2020). Numerous studies demonstrated that the combined impact of phytochemicals was more potent than each individual component alone, which exhibited synergistic behavior and intensified therapeutic potential (Hoseini et al., 2023; Pandey et al., 2020). Phytochemicals in *Teucrium polium* could enhance the efficacy of anticancer drugs and potentially improve their outcomes in treating cancer (Chabane et al., 2021). The nanoparticles for TEO were demonstrated to have anticancer potential effects against the intestinal membrane cells (Al-Otaibi and Al-Motwaa 2022). The anticancer effects observed in this study may be linked to the ability of TEO constituents to regulate cell survival and death pathways. β-Caryophyllene has been shown to induce apoptosis in cancer cells through mitochondrial dysfunction and activation of caspase-3, while also causing cycle arrest at the G1 phase (Al-Otaibi and AlMotwaa 2022). Spathulenol has been reported to trigger apoptosis via reactive oxygen species generation and activation of intrinsic apoptotic pathways involving Bax/Bcl-2 modulation (Noumi et al., 2020). Germacrene D demonstrates antiproliferative effects by interfering with cell cycle progression and enhancing oxidative stress in tumor cells (Al-Otaibi and AlMotwaa 2022). Together, these mechanisms support the cytotoxic activity of TEO against colon cancer cells observed in this study.

TEO exhibited broad-spectrum antimicrobial effects, with the highest activity against *S. aureus* and notable structural disruption of *E. coli* confirmed by SEM (Asemani et al., 2024). These results are consistent with earlier findings showing that *Teucrium* species exert antimicrobial effects through terpenoid-mediated disruption of microbial membranes; however, gram-negative bacteria such as *E. coli* generally displayed higher resistance, reflecting the protective role of their outer membrane and lipopolysaccharide layer (Alarjani and Skalicky, 2021; Asemani et al., 2024). The antimicrobial potential of TEO is likely the synergistic interactions among terpenoids and phenolics, which together

destabilize bacterial membranes, increase permeability, and inhibit key enzymatic pathways (Sati and Qubbaj, 2021). Importantly, the strong activity against clinically relevant pathogens such as *S. aureus* underscores the therapeutic potential of TEO as a natural antimicrobial agent (Davoudi and Ramazani 2024). Beyond pharmaceutical applications, these properties suggest that TEO could serve as a safe preservative in food systems, contributing to both microbial safety and shelf-life extension; hence, the antimicrobial potential of these plant materials is considered of great significance (Hoseini et al., 2023).

In line with antimicrobial results of the present study, TEO nanoparticles exhibited similar antibacterial activities against 10.46 ± 0.366 mm *S. aureus* and 12.53 ± 0.338 mm *Salmonella enterica* (Asemani et al., 2024). In another study, TEO was highly active against *S. aureus* C15 with an inhibition zone of 21 ± 2 mm and MIC values ranging between 15 ± 1.25 and 75 ± 12.5 µg/mL (Alarjani and Skalicky 2021). Various factors impact the antimicrobial behavior of EOs, which include type and antimicrobial substances (phenolics, quinones, beta-carotene, flavonoids, and tannins) and actions and mechanisms against microorganisms (Sati and Qubbaj 2021). Generally, phenolic constituents are extremely important antimicrobials in plant EOs; usually, their mechanism is based on enzymatic inhibition of oxidized constituents or blocking sulfhydryl groups in proteins (Othman et al., 2017). Indeed, antimicrobial functions of TEO were not solely attributed to individual constituents but related to synergistic effects of various components, which worked together to enhance overall activities (Chabane et al., 2021). One of the mechanisms related to inactivated bacteria is the release of substances from the cell wall, and higher phenolic substances have been shown to improve this process (Sati and Qubbaj 2021). Phenolics have the ability to disrupt walls and membranes, leading to increased cell permeability (Mooliani and Nouri 2021). In particular, gram-negative bacteria possess a complex cell membrane structure, including lipopolysaccharides, which includes two layers of phospholipids (Falah et al., 2022). The outer layer in gram-negative bacteria acts as a barrier, making it more difficult for hydrophobic constituents from EO to penetrate the cell membrane and exert their antimicrobial effects (Bendif et al., 2018). The characteristic that contributes to higher resistance was observed in gram-negative compared to gram-positive bacteria (Falah et al., 2022). Furthermore, constituents present in EO can induce cell death through various mechanisms such as enzymatic inhibition of oxidized substances, reaction with sulfhydryl groups, and non-specific protein modification (Davoudi and Ramazani 2024).

These SEM changes included cell wall perforation, leakage of cellular contents, microbe cell folding, and rod structure disruption (Falah et al., 2022). The observed perforations in the cell wall indicated the impact of TEO and disruption of integrity may lead to the release of substances, contributing to bacteria inactivation (Bendif et al., 2018). The phenolic substances play a crucial role, and higher concentrations can enhance the leakage of intracellular components by altering cell membranes, thereby increasing bacteria permeability (Falah et al., 2022). As a result, critical membrane functions that regulate cellular exchange and metabolite synthesis are affected by these changes (Bendif et al., 2018). The antimicrobial activity of TEO is largely attributable to terpenoid constituents and β- Caryophyllene disrupts microbial membranes by inserting into lipid bilayers, causing increased permeability and leakage of essential intracellular metabolites (Othman et al., 2017). Spathulenol has been reported to interfere with bacterial enzyme systems through interaction by sulfhydryl groups, leading to metabolic inhibition (Bendif et al., 2018). Germacrene D contributes to antimicrobial effects by destabilizing membrane integrity and impairing energy metabolism in pathogens (Senani et al., 2024). The synergistic interactions likely underlie the observed broad-spectrum antibacterial activity of TEO, particularly strong effects against *S. aureus* (Othman et al., 2017).

Conclusion

The evaluation for TEO using GC and FTIR revealed spathulenol (12.98%), β-pinene (10.07%), and germacrene B (9.21%) as major constituents, and the chemical composition suggests potential bioactive attributes. Furthermore, EO exhibited significant antioxidant activity, as indicated by high TPC and TFC. The TEO demonstrated effective inhibition of free radicals such as DPPH and ABTS and also exhibited a high FRAP factor. These results highlighted the antioxidant potential of TEO, which could help protect against oxidative stress-related damage. In addition to antioxidant activity, TEO

demonstrated promising anticancer attributes, which assessed cell viability and cytotoxicity. The MTT results indicated a suitable level of cytotoxicity, suggesting TEO potential as an anticancer agent. The antimicrobial activity suggested that TEO had the potential to inhibit the growth of microorganisms, making it a promising candidate for use as an antimicrobial additive. Overall, comprehensive evaluation of TEO demonstrated adequacy and potential for various applications in the pharmacology industry, and these findings suggested that TEO be utilized as a safe antioxidant and anticancer and antimicrobial additive in industrial food applications.

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Conflict of interest

No conflict of interest declared by authors.

Ethical approval

Ethical clearance was obtained from the Internal Ethical Clearance in compliance with institutional research ethics policies or similar, based on their institutional norms.

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