Future Journal of Pharmaceutical Sciences

RESEARCH

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Chemical composition and antifungal activity of *Teucrium Leucocladum* Boiss. essential oils growing in Egypt using two different techniques

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Abstract

Background *Teucrium Leucocladum* Boiss. (TL) (family Lamiaceae), indigenous to Sinai, Egypt, and Mediterranean region, is considered a rich source of essential oils (EOs). This study aimed to extract the aerial parts essential oils utilizing hydro-distillation (HD) and microwave-assisted extraction (MAE), and analyze the volatile constituents by Gas Chromatography–Mass Spectrometry (GC/MS). The antifungal and cytotoxic potentials against *Candida albicans* (*C. albicans*) and non-small cell lung adenocarcinoma A549, triple-negative breast cancer MDA-MB-231 cell lines, respectively, were likewise estimated. Subsequently, the three main compounds were docked into the crystal structure of *Candida albicans N*-myristoyltransferase (NMT) with myristoyl-COA and peptidic inhibitor (PDB 1IYK), and predictions of human absorption, distribution, metabolism, and excretion (ADME) were performed to assess the drug-likeness of the compounds.

Results The chemical profile consisted of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes. The MAE oil sample (TLM) yield was found to be double that of the HD oil sample (TLH). TLM afforded an inhibitory diameter (13 mm) comparable to the ketoconazole (20 mm), TLM 100 mg/mL showed the strongest antifungal potential against *C. albicans*. The cytotoxic assay revealed moderate activity against A549 and MDA-MB-231. In silico studies using molecular docking were processed on the major components in which nerolidol had the best-fitting energy to inhibit *C. albicans* (− 7.21 kcal/mol), while ADME results established a promising first step for the potential drug bioavailability.

Conclusion In this research, essential oil acquired from the aerial parts proved to contain monoterpenes and sesquiterpenes, which are classes of compounds known for their versatile usage in medicine. In vivo studies on *Teucrium Leucocladum* Boiss. active metabolites against clinical strains of fungi need to be further studied, as do the effects of combining the active compounds with antifungal agents to combat antimicrobial resistance.

Keywords Essential oil, GC/MS, *Candida albicans*, Cytotoxic activity, Molecular docking

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Background

Since the dawn of humanity, essential oils have been utilized for plenty of reasons. They have numerous positive attributes as they are traditionally used to enhance the flavor and fragrance of prepared foods, as well as perfumes and cosmetics. Beyond their sensory contributions, essential oils exhibit significant biological potentials, including larvicidal action, analgesic and anti-inflammatory effects, antioxidant, antifungal, and anticancer activities. Moreover, essential oils have been integrated into medicinal practices, showcasing their therapeutic potential [1]. The diverse range of activities exhibited by essential oils (EOs) can be attributed to their complex composition, comprised of various constituents such as terpenes, terpenoids, and phenylpropanoids. Notably, within these intricate chemical profiles, only two or three major components typically constitute a significant proportion, ranging from 20 to 70% of the total substance [2].

The chemical composition of EOs is significantly influenced by various factors, including the geographical location of the plant containing the EOs, the seasonal timing of harvest, storage conditions, and the extraction methodology employed. These variables collectively contribute to the unique and dynamic molecular profiles of EOs. The numerous biological and therapeutic activities exhibited by essential oils, coupled with their relative safety and capacity to synergistically interact with other compounds, qualify the utilization of essential oils as naturally derived medicinal compounds [3].

Traditional medicines and modern pharmaceutical developments alike are predominantly derived from plant sources, utilizing active metabolites known for their low to negligible toxicity. These bioactive compounds have demonstrated efficacy in treating a wide spectrum of disorders. In recent years, there has been a conspicuous surge in research initiatives concentrating on medicinal plants, with a specific emphasis on investigating their potential as agents exhibiting anticancer and antimicrobial properties. This heightened scientific interest reflects the continual exploration of plant-based compounds as significant reservoirs for the advancement of novel drugs, particularly in the fields of oncology and infectious diseases [4–6].

Family Lamiaceae (mint family) comprises 236 genera and 6900–7000 species of aromatic plants. Herbs, whether perennial or annual, shrubs, and trees are all members of this family [7]. Many species of this family are considered sources of essential oils (EOs) [8]. Significantly, members of the Lamiaceae family are extensively utilized as medicinal plants in various folk traditions. Lamiaceae largest genus in the Mediterranean area is *Teucrium* [8].

Teucrium (Lamiaceae) is an aromatic [9], polymorphic [7] genus, comprising more than 300 species [8] represented mostly by perennial, bushy, or herbaceous plants growing in temperate zones, particularly in Central Asia and the Mediterranean basin [10]. Chemical investigations of genus *Teucrium* members have shown that those plants are very rich sources of active principles, especially essential oils [11].

Teucrium leucocladum (TL) Boiss. is an aromatic low shrub ranging from 20 to 50 cm long, indigenous to the Sinai Peninsula [12] and is considered one of the most used traditional medicinal plants in Palestine, Egypt, and the Mediterranean region for the treatment of hyperglycemia (aqueous extract of aerial parts) and colon spasms among other ailments [13].

The major objective of the current research was to comprehensively characterize the complete chemical profile of the EOs extracted from (stem and leaf parts) of *Teucrium leucocladum* Boiss. for the first time. This characterization employed two distinct techniques: microwave-assisted extraction (MAE) and hydro-distillation (HD), resulting in the production of TLM and TLH, respectively. GC–MS was used to analyze the prepared essential oils. Additionally, the study involved the assessment of the potential activity of the oil samples against *Candida albicans* and three different cancer cell lines.

Results

Chemical composition

Both techniques produced yellow-colored oils from *Teucrium leucocladum* Boiss. with a distinct characteristic odor. The percentage yield of the essential oils, however, varied significantly depending on the procedure, affording 0.5 and 1.2% (v/w) for HD and MAE, respectively. Our results are consistent with published data revealing that hydro-distillation provides yields less than microwave-assisted extraction [14]. The results of GC chromatograms are represented in Fig. 1.

While MAE exhibited a higher oil yield, it presented a lower number of metabolites compared to hydro-distillation, as indicated in Table 1. Moreover, the yields emphasized that the selection of the extraction technique markedly impacted the chemical composition of the extracted essential oils. Seventy-three components were identified during hydro-distilled oil preparation, whereas 32 components were detected in the oil prepared by microwave-assisted extraction (MAE), constituting 95.53% and 94.33%, respectively.

The identified constituents are categorized mainly into four groups: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes (Fig. 2). Oxygenated sesquiterpenes were the major group in oils prepared by both methods

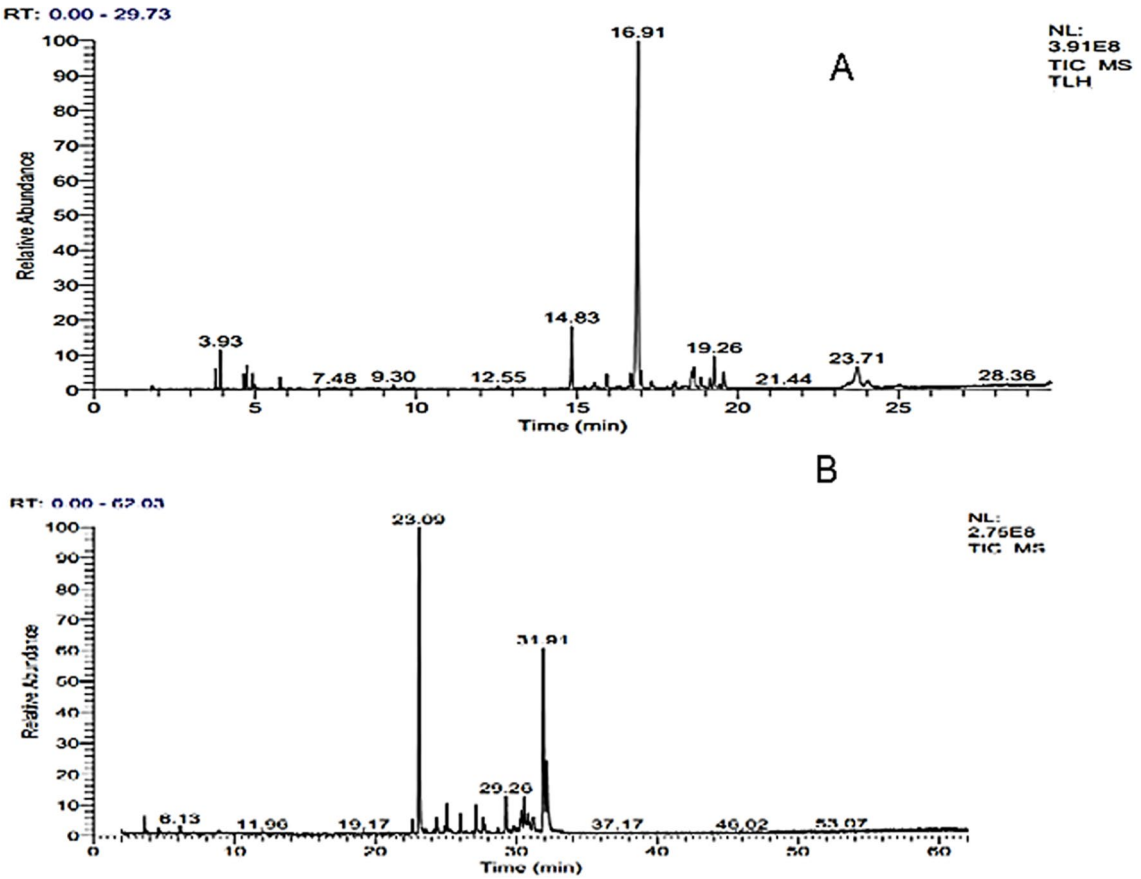

Fig. 1 GC chromatograms of the essential oils of *Teucrium leucocladum* Boiss. extracted via hydro-distillation (**A**), and microwave-assisted extraction (**B**)

at about 61.26% and 42.85% in TLH and TLM, respectively. The sesquiterpene alcohol nerolidol was the most prominent compound amounting to 50.02% in the HD oil sample, while the sesquiterpene alcohol levomenol and the monoterpene alcohol *trans*-2-Caren-4-ol dominated TLM at 21.40% and 33.92%, respectively.

α-Pinene and β-Pinene were the major monoterpene hydrocarbons observed in HD sample 2.59 and 1.77%, respectively, while α-thujene was major in MAE at 1.18%. For sesquiterpene hydrocarbons, valencene was the major observed in the HD sample at 2.00%, while cadinene was the major one in the MAE sample with a percentage of 3.25.

Despite that the hydro-distilled oil of *T. leucocladum* Boiss. was formerly assessed [12], this is the first time to evaluate the oil prepared via microwave-assisted extraction. The structures of certain identified oil components are illustrated in Fig. 3.

Screening of the antifungal activity

In vitro antifungal activity of TLH and TLM oil samples was tested against a type of yeast-forming fungi *C. albicans* using the agar well diffusion method. For each oil, two concentrations 50 and 100 mg/mL were used in the procedure. The concentration and method of oil extraction exerted a notable influence on the extent of inhibition of *C. albicans* growth, as illustrated in Fig. 4. TLM, at concentrations of 100 mg/mL and 50 mg/mL, demonstrated the highest antifungal potential, yielding inhibition diameters of 13 mm and 10 mm, respectively, surpassing the inhibition diameter observed for the standard drug ketoconazole (20 mm). TLH, at a concentration of 100 mg/mL, exhibited a weaker but still noticeable inhibition diameter of 10 mm, while no inhibition was observed for TLH at a concentration of 50 mg/mL.

Our results imply that *Teucrium Leucocladum* Boiss. essential oil might have the ability to function as an antifungal medication.

Cytotoxic activity

Evaluation of the cytotoxic activity via resazurin reduction assay [15] was accomplished against non-small cell lung adenocarcinoma A549, triple-negative breast cancer MDA-MB-231, and colon adenocarcinoma Caco-2

Table 1 Chemical composition of the isolated essential oils

No	Compound	[a]R_t HD	[b]R_t MAE	[c]RI_{Exp}	Area%		Molecular Formula
					HD[d]	MAE[e]	
Hydrocarbon components							
(A) Monoterpene hydrocarbons							
1	α-Thujene	3.78	3.59	856	1.35	1.18	$C_{10}H_{16}$
2	α- Pinene	3.93	NA	865	2.59	NA	$C_{10}H_{16}$
3	2,4(10)-Thujadien	4.15	NA	878	0.07	NA	$C_{10}H_{14}$
4	Sabinene	4.64	4.64	904	1.14	0.50	$C_{10}H_{16}$
5	β-Pinene	4.76	NA	908	1.77	NA	$C_{10}H_{16}$
6	α-Myrcene	4.91	NA	914	1.06	NA	$C_{10}H_{16}$
7	α-Terpinene	5.51	NA	935	0.11	NA	$C_{10}H_{16}$
8	o-Cymene	5.77	6.13	945	1.02	0.83	$C_{10}H_{14}$
9	α-Ocimene	6.08	NA	956	0.13	NA	$C_{10}H_{16}$
10	Δ-3-Carene	6.37	NA	966	0.25	NA	$C_{10}H_{16}$
11	α-Terpinolene	6.93	NA	986	0.06	NA	$C_{10}H_{16}$
(B) Sesquiterpene hydrocarbons							
12	α-Copaene	13.01	NA	1144	0.12	NA	$C_{15}H_{24}$
13	Elemene	13.33	NA	1151	0.16	NA	$C_{15}H_{24}$
14	*trans*-Caryophyllene	13.97	NA	1166	0.20	NA	$C_{15}H_{24}$
15	*trans*-Farnesene	14.49	NA	1178	0.15	NA	$C_{15}H_{24}$
16	Gymnomitrene	14.67	NA	1182	0.07	NA	$C_{15}H_{24}$
17	α-Gurjunene	15.02	NA	1190	0.13	NA	$C_{15}H_{24}$
18	Germacrene D	15.24	NA	1195	0.38	NA	$C_{15}H_{24}$
19	α-Selinene	15.44	NA	1198	0.20	NA	$C_{15}H_{24}$
20	α-Bisabolene	15.66	NA	1205	0.25	NA	$C_{15}H_{24}$
21	α-cadinene	15.93	NA	1211	1.72	NA	$C_{15}H_{24}$
22	Valencene	18.57	NA	1269	2.00	NA	$C_{15}H_{24}$
23	*cis*-α-Farnesene	19.13	NA	1281	0.94	NA	$C_{15}H_{24}$
24	Aromadendrene	20.68	22.61	1316	0.12	1.49	$C_{15}H_{24}$
25	Bicyclo-Elemene	NA	24.13	1393	NA	0.41	$C_{15}H_{24}$
26	α-Muurolene	NA	24.35	1398	NA	2.37	$C_{15}H_{24}$
27	α-Amorphene	NA	24.92	1411	NA	0.60	$C_{15}H_{24}$
28	Cadinene	NA	25.09	1415	NA	3.25	$C_{15}H_{24}$
29	α-Humulene	NA	26.02	1436	NA	2.15	$C_{15}H_{24}$
Oxygenated components							
(A) Oxygenated monoterpenes							
30	Thujol	5.90	NA	949	0.08	NA	$C_{10}H_{18}O$
31	*cis*-Sabinene hydrate	6.87	NA	984	0.07	NA	$C_{10}H_{18}O$
32	1-Octen-3-yl-acetate	7.29	NA	999	0.19	NA	$C_{10}H_{18}O_2$
33	Linalool	7.48	NA	1004	0.23	NA	$C_{10}H_{18}O$
34	α-Thujone	7.78	NA	1012	0.17	NA	$C_{10}H_{16}O$
35	*trans*-Verbenol	8.51	NA	1032	0.17	NA	$C_{10}H_{16}O$
36	*cis*-Verbenol	8.63	NA	1035	0.14	NA	$C_{10}H_{16}O$
37	Pinocarvone	8.84	NA	1040	0.07	NA	$C_{10}H_{14}O$
38	*trans*-2-Pinanol	NA	8.85	1041	NA	0.41	$C_{10}H_{18}O$
39	Terpinen-4-ol	9.30	NA	1053	0.52	NA	$C_{10}H_{18}O$
40	Myrtenal	9.58	NA	1060	0.12	NA	$C_{10}H_{14}O$
41	α-Terpineol	9.78	NA	1065	0.17	NA	$C_{10}H_{18}O$
42	*p*-Menth-4(8)-en-3-one (Beta- Pulegone)	9.92	NA	1069	0.12	NA	$C_{10}H_{16}O$
43	Δ-(7)-Methenone-2	10.79	NA	1092	0.13	NA	$C_{10}H_{16}O$

Table 1 (continued)

No	Compound	[a]R_t HD	[b]R_t MAE	[c]RI_{Exp}	Area%		Molecular Formula
					HD[d]	MAE[e]	
44	*trans-p*-Mentha-2,8-dienol	14.83	NA	1186	6.54	NA	$C_{10}H_{16}O$
45	lilac alcohol epoxide	16.34	NA	1220	0.29	NA	$C_{10}H_{18}O_3$
46	*trans*-2-Caren-4-ol	NA	23.09	1370	NA	33.92	$C_{10}H_{16}O$
47	*trans-p*-2,8-Menthadien-1-ol	NA	31.13	1557	NA	1.11	$C_{10}H_{16}O$
(B) Oxygenated sesquiterpenes							
48	Farnesene epoxide	15.55	NA	1202	0.93	NA	$C_{15}H_{24}O$
49	Cedrenol	16.07	NA	1214	0.11	NA	$C_{15}H_{24}O$
50	Isoaromadendrene epoxide	16.14	NA	1215	0.14	NA	$C_{15}H_{24}O$
51	Humulene-1,2-epoxide	16.67	NA	1227	1.54	NA	$C_{15}H_{24}O$
52	Caryophyllene oxide	17.32	NA	1241	1.17	NA	$C_{15}H_{24}O$
53	Globulol	17.80	NA	1252	0.21	NA	$C_{15}H_{26}O$
54	α-Santalol	18.06	NA	1258	0.77	NA	$C_{15}H_{24}O$
55	Bisabolol oxide A	18.29	NA	1263	0.20	NA	$C_{15}H_{26}O_2$
56	α-Bisabolol Oxide-B	18.64	NA	1270	2.10	NA	$C_{15}H_{26}O_2$
57	α-Cadinol	18.85	NA	1275	1.51	NA	$C_{15}H_{26}O$
58	Spathulenol	18.97	NA	1278	0.24	NA	$C_{15}H_{24}O$
59	Santalol, E-*cis*, epi-α-	19.43	NA	1288	0.38	NA	$C_{15}H_{24}O$
60	6-Epi-shyobunol	19.56	NA	1291	1.86	NA	$C_{15}H_{26}O$
61	2-Pentadecanone	21.44	NA	1333	0.08	NA	$C_{15}H_{30}O$
62	4-Epi-cubedol	NA	25.28	1419	NA	0.46	$C_{15}H_{26}O$
63	Alloaromadendrene oxide	NA	26.41	1445	NA	0.35	$C_{15}H_{24}O$
64	Cubenol	NA	27.63	1474	NA	1.86	$C_{15}H_{26}O$
65	Epiglobulol	NA	28.67	1498	NA	0.70	$C_{15}H_{26}O$
66	Lanceol	NA	29.25	1512	NA	4.25	$C_{15}H_{24}O$
67	Limonen-6-ol, pivalate	NA	29.77	1524	NA	0.55	$C_{15}H_{24}O2$
68	Longiborneol	NA	29.86	1526	NA	0.54	$C_{15}H_{26}O$
69	Gossonorol	NA	30.34	1538	NA	2.90	$C_{15}H_{22}O$
70	tau-Muurolol	NA	30.80	1549	NA	1.53	$C_{15}H_{26}O$
71	Ledene oxide-(II)	NA	31.20	1559	NA	1.37	$C_{15}H_{24}O$
72	Ledol	NA	32.16	1582	NA	6.94	$C_{15}H_{26}O$
73	Nerolidol	16.91	NA	1232	50.02	NA	$C_{15}H_{26}O$
74	Levomenol (α-bisabolol)	NA	31.91	1576	NA	21.40	$C_{15}H_{26}O$
Other volatiles							
75	*P*-Methyl anisole	4.07	NA	873	0.05	NA	$C_8H_{10}O$
76	6-Methyl-5-hepten-2-one	5.00	NA	917	0.36	NA	$C_8H_{14}O$
77	1-Octen-3-ol	5.08	NA	920	0.06	NA	$C_8H_{16}O$
78	Nonanal	7.42	NA	1003	0.07	NA	$C_9H_{18}O$
79	4-Acetyl-1-methylcyclohexene	8.18	NA	1023	0.23	NA	$C_9H_{14}O$
80	α-Terpinyl acetate	12.55	NA	1133	0.31	NA	$C_{12}H_{20}O_2$
81	Glutaric acid, di(3-(2-methoxyethyl) heptyl) ester	16.23	NA	1217	0.24	NA	$C_{25}H_{48}O_6$
82	Oxiraneoctanoic acid, 3-octyl-, methyl ester, *trans*-	16.57	NA	1225	0.29	NA	$C_{19}H_{36}O_3$
83	2-Acetoxy-1,8-cineole	17.00	NA	1234	1.09	NA	$C_{12}H_{20}O_3$
84	Geranyl-*p*-cymene	18.38	NA	1265	0.42	NA	$C_{18}H_{26}$
85	7-Cyano-6-methoxy-1,4,5-trimethyl-indole	19.85	NA	1297	0.18	NA	$C_{13}H_{14}N_2O$
86	9,12-Octadecadienoyl chloride, (Z, Z)-	23.42	NA	1377	0.28	NA	$C_{18}H_{31}ClO$
87	Dl-(9-Octadecenoyl)-Glycerol	23.71	NA	1383	4.05	NA	$C_{39}H_{72}O_5$
88	9-Octadecenoic acid	24.04	NA	1391	1.27	NA	$C_{18}H_{34}O_2$
89	Glycidol stearate	25.01	NA	1412	0.37	NA	$C_{21}H_{40}O_3$

Table 1 (continued)

No	Compound	[a]R_t HD	[b]R_t MAE	[c]RI_{Exp}	Area%		Molecular Formula
					HD[d]	MAE[e]	
90	Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-dodeca-2,6-dienyl ester	NA	30.56	1543	NA	3.26	$C_{17}H_{30}O_4$
Total identified volatiles					95.53	94.33	
Monoterpene hydrocarbons					9.55	2.51	
Sesquiterpene hydrocarbons					6.44	10.27	
Oxygenated Monoterpenes					9.01	35.44	
Oxygenated Sesquiterpenes					61.26	42.85	
Other volatiles					9.27	3.26	

[a] R_t HD: retention time for hydro-distillation. [b]R_t MAE: retention time for microwave-assisted extraction. [c]RI_{Exp}: retention index was determined experimentally relative to C8–C28 n-alkanes for all compounds. [d]HD: hydro-distillation, [e]MAE: microwave-assisted extraction, NA: not available

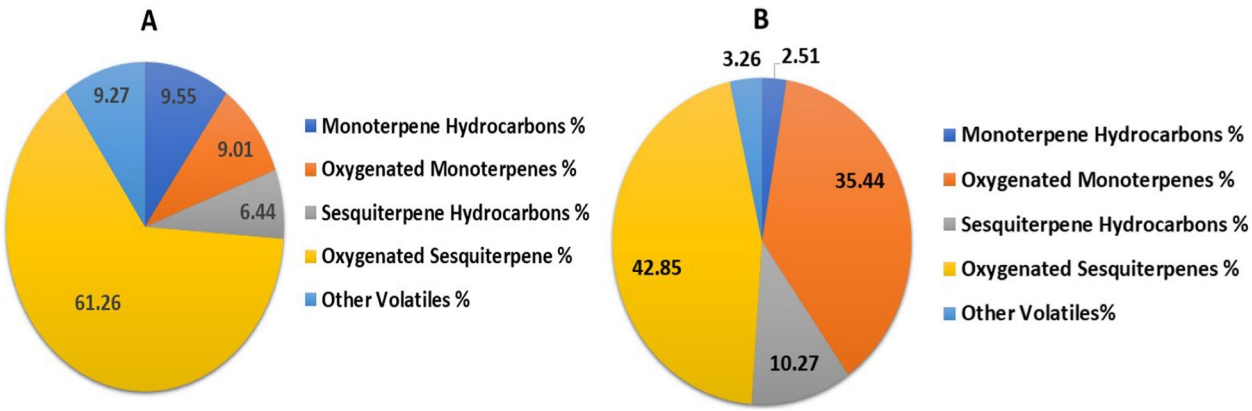

Fig. 2 Percentage of the classes of volatile components in the essential oil of *Teucrium leucocladum* Boiss. extracted via (**A**) hydro-distillation, and (**B**) microwave-assisted extraction

(ATCC) using doxorubicin HCL as a positive control. Preliminary screening of the oil samples TLH and TLM against cell viability of A549 and MDA-MB-231 cell lines at two concentrations (20 and 200 µg/mL) revealed a promising effect at the high concentration (200 µg/mL) for both samples verifying that the inhibition was dose-dependent, whereas lower sensitivity was demonstrated against Caco-2.

Eventually, the fifty percent inhibitory concentration (IC_{50}) determined for cell lines with promising cytotoxic activities, revealed that IC_{50} of TLM was 142.0 and 185.0 µg/mL against A549 and MDA-MB-231, respectively, while TLH exhibited IC_{50} 192.0 and 190.0 µg/mL against A549 and MDA-MB-231, respectively.

Molecular docking studies

The process of discovering and developing new drugs involves looking for the metabolites of herbal medicines that act as disease inhibitors [16]. Given its ability to provide information about the atomic-level interactions between tiny molecules and proteins, molecular docking has become an indispensable tool in the drug discovery process [17].

Many researches have proved that the NMT gene [18] is essential for vegetative growth and survival of *C. albicans.* Research in genetics and biochemistry has established that *N*-myristoyltransferase (NMT) is a promising target for antifungal medications. Numerous studies have indicated that the NMT enzyme in *C. albicans* provides important data for the design of the inhibitor [19].

Results revealed that binding energies of the examined compounds namely levomenol, nerolidol, and *trans*-2-caren-4-ol (Table 2, Fig. 5) were −5.53, −6.62, and −7.21, respectively approaching that of the reference drug (ketoconazole) −9.25.

The ADME, Lipinski's rule of five and BOILED-Egg techniques

The prediction of human absorption, distribution, metabolism, and excretion (ADME) properties, as well as the estimation of therapeutic dose and exposure, has become an essential component of compound optimization

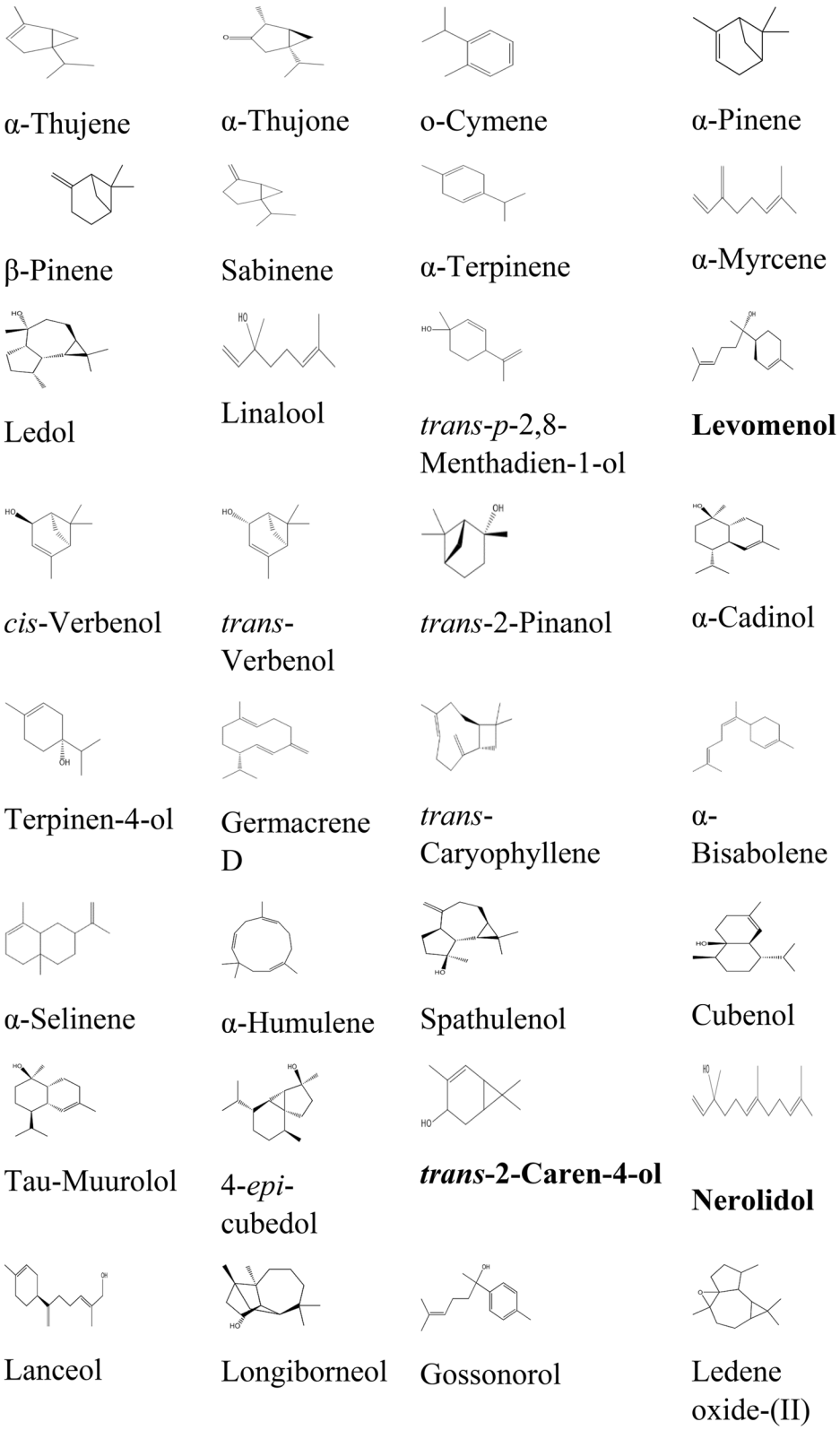

Fig. 3 Two-dimensional structures of the identified compounds of the essential oils (EOs) extracted using hydro-distillation (HD) and microwave-assisted extraction (MAE) methods of *Teucrium leucocladum* Boiss. oils by GC–MS analysis

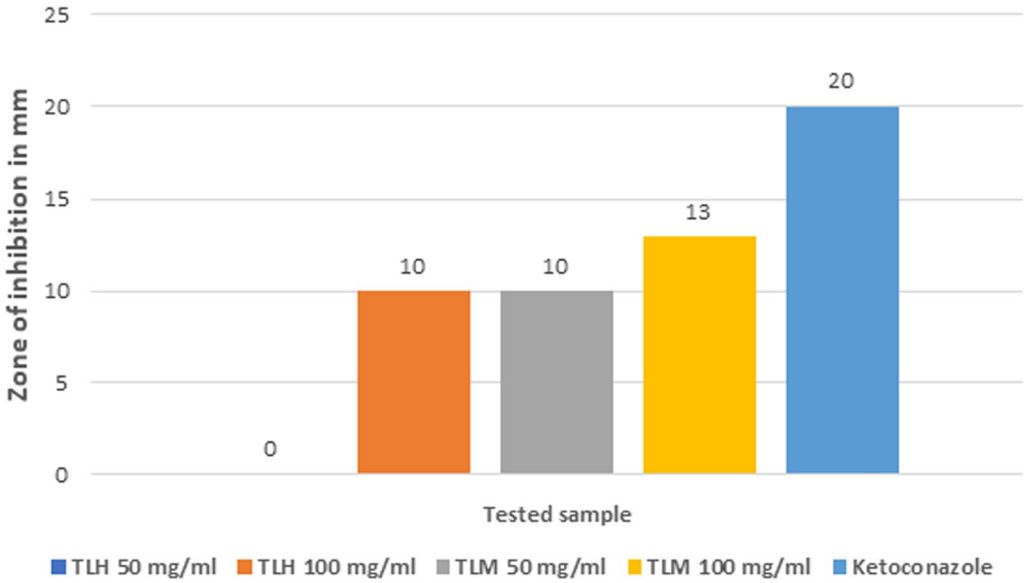

Fig. 4 Antifungal Inhibition diameters represented in mm of TLM and TLH samples against Ketoconazole

Table 2 Results of docking simulations of the main identified components in TLH and TLM samples

No.	Name	Lowest Binding energy	KI[a]
1	Levomenol	−7.21	5.18 µM
2	Nerolidol	−6.62	14.01 µM
3	*trans*-2-Caren-4-ol	−5.53	87.84 µM
4	Ketoconazole	−9.25	164.76 nM

KI[a] Inhibition concentration of the best score in docking, µM micromolar, nM nanomolar

during the drug discovery process. This practice is vital for enhancing the efficiency and success of drug development by providing valuable insights into how a compound is likely to be absorbed, distributed, metabolized, and eliminated within the human body. Accurate predictions in these areas enable researchers to optimize compounds for better bioavailability, efficacy, and safety, ultimately guiding the selection of promising candidates for further development and clinical testing. This integrated approach contributes significantly to the rational design and prioritization of drug candidates, facilitating a more streamlined and effective drug discovery process [20].

Poor ADME characteristics for a certain compound are considered triggers for a cascade of failures of most medicines in clinical experiments. The outcomes indicated variation in the physiochemical parameters of the three compounds with differences in the BBB (blood–brain barrier) and (HIA) human intestine absorption ranges, so we were encouraged to follow Lipinski's rule of five [21], which was published in 1997 by Christopher A. Lipinski [22]. It is a standard practice to assess the drug-likeness of a chemical compound and establish whether it possesses characteristics to be active for humans when administered orally.

As shown in Table 3, these 5 parameters totally and completely complied with the examined compounds (*trans*-2-caren-4-ol, nerolidol, and levomenol) in which the polarity, lipophilicity, solubility, flexibility, and saturation were found within the pink area (Fig. 6) indicating promising bioavailability [23].

BOILED-Egg technique [21] designed for the assessment of the lipophilicity and polarity of small compounds, is supposed to serve as a highly accurate predictive model. This model holds significance in the lead optimization of drugs. Notably, the BOILED-Egg model has the capability to simultaneously predict two crucial ADME parameters: passive gastrointestinal absorption (HIA) and brain access(BBB) [24]. The findings indicate that the three compounds were effectively absorbed, potentially having access to the brain, as depicted by the white region in the results.

Additionally, the compounds exhibited P-glycoprotein permeability (PGP), represented by the red dot. Importantly, all recorded values fell within the acceptable range, meeting the specified criteria [25]. This suggests that the compounds absorption characteristics align with established thresholds, highlighting their favorable pharmacokinetic properties and suitability for further consideration in drug development [24]. Among the three

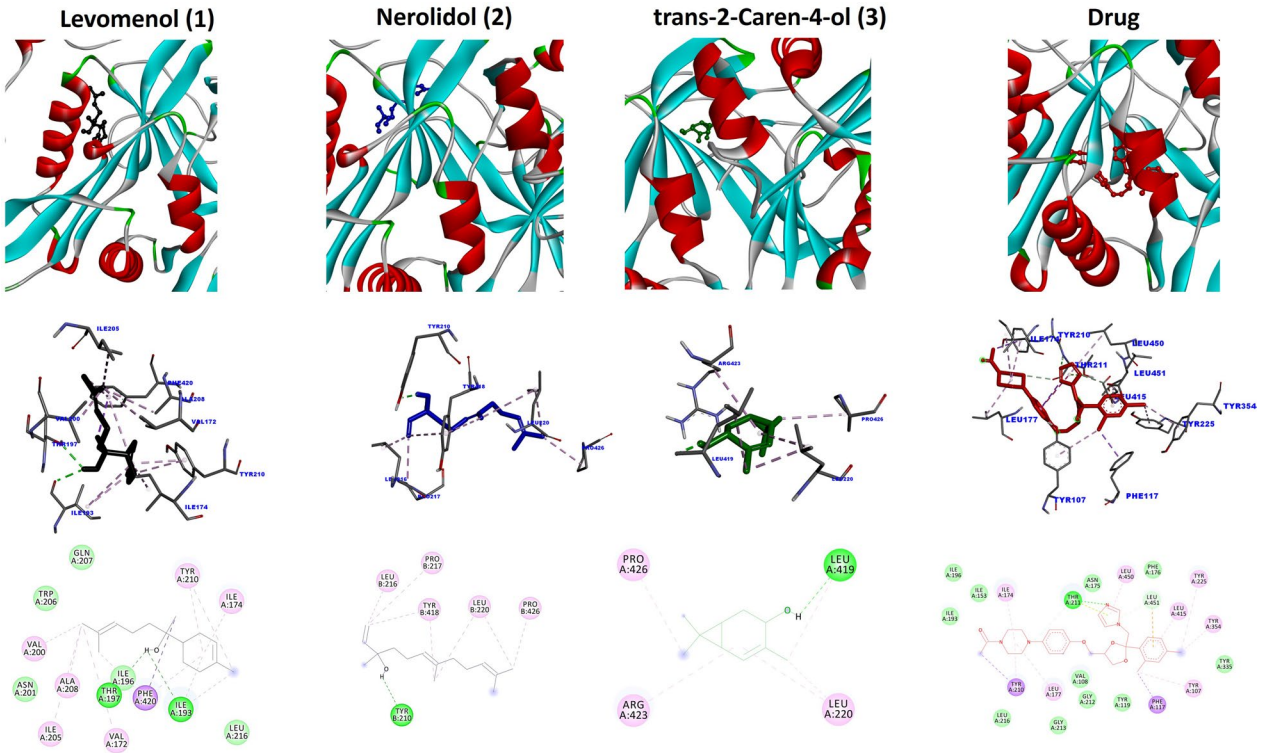

Fig. 5 Docked complexes showing 2D and 3D binding modes of levomenol, nerolidol, *trans*-2-Caren-4-ol, and the reference compound ketoconazole in the active site of *Candida albicans*

compounds, nerolidol revealed the highest water partition coefficient (WLOGP) followed by levomenol and then *trans*-2-caren-4-ol indicating that *trans*-2-caren-4-ol has the least potential to pass the BBB presented in Fig. 7. However, they were nearly similar regarding topological polar surface area (TPSA) with a value of 20.23 Å.

Discussion

Candida albicans is the main fungus associated with infections via medical devices [26]. Contact lenses, joint prostheses, mechanical heart valves, and dentures are all prone to be infected with this fungus. Accordingly, many studies were conducted to search for new and promising agents against this fungus [26]. Worldwide, cancer is the second most common cause of death. Despite continuous improvements in cancer treatment, there are still many undesirable side effects that occur when receiving chemotherapy. Natural remedies, acquired from plants, may limit those unfavorable side effects and still be utilized to treat cancer [27]. The results revealed that the MAE and hydro-distillation produced EOs with dissimilar volatile content, suggesting that altering the extraction procedure may lead to changes in the chemical profile and thus the biological activity. This heightened variation of efficacy is likely attributable to its terpenoid content, specifically the presence of oxygenated sesquiterpenes and monoterpenes.

On one hand, nerolidol was the most prominent compound in the TLH that may contribute to the biological activity. It is a sesquiterpene alcohol well-established with various uses and demonstrates favorable effects on human health; hence, it is regarded as a promising candidate for chemical or drug development across various domains, including agriculture, industry, and medicine [28]. Numerous publications [28, 29] provide proof of nerolidol effectiveness in demonstrating antifungal activity. It was illustrated to have fungicidal effects against *Microsporum gypseum*, *Candida albicans*, and *T. mentagrophytes* [30, 31].

On the other hand, levomenol (α-bisabolol) was observed as one of the major compounds in TLM. Bisabolol was demonstrated to have the ability to prevent the development of hyphae and fungal growth, as well as to change how ergosterol, a crucial structural element of the fungal membrane, is produced. These effects may be key virulence factors in some strains of the yeast *C. albicans* [32].

Trans-2-Caren-4-ol constitutes 33.92% of the overall volatile content in the EOs extracted using the MAE method from TL. Nerolidol is also known as an

Table 3 Lipinski's rule of five for ADME analysis of the investigated compounds

No.	Name	M.wt	Lipophilicity	Hydrogen Bond Donors	Hydrogen Bond Acceptors	No. of Rule Violations	Drug-Likeness
	Lipinski's rule limits	**Less than 500 Dalton**	**Less than 5**	**Less than 5**	**Less than 10**	**Less than 2 Violations**	**Lipinski's rule Follows**
1	Levomenol	222.37	3.56	1	1	0	Yes
2	Nerolidol	222.37	3.86	1	1	0	Yes
3	*trans*-2-Caren-4-ol	152.23	2.30	1	1	0	Yes

antioxidant, chemo-preventive, and antitumor agent. It can modulate the biochemical profiles, work as an antioxidants, detoxification agent, and inhibit tumor development and various types of carcinogenesis [33].

α-Bisabolol was recorded to inhibit the growth of tumors and induce apoptosis in several malignancies, such as acute leukemia, glioblastoma, and pancreatic, prostatic, breast, and liver cancers. Its mechanism of action involves inhibiting the proliferation, invasiveness, and motility of cancer cells [34, 35]. The notable differences in the mechanisms of action of the major components identified in the oil extracts may account for the variations in cytotoxic activity.

Finally, In silico and ADME studies were conducted to validate the potential antifungal activities and to predict the physiochemical properties of the three major compounds, respectively. The NMT enzyme was chosen due to its essential role in the viability of *Candida albicans*, a major contributor to systemic fungal infections in immunocompromised patients.

Consequently, NMT is considered a promising target for antifungal drug development. It was observed that the enzyme adopts an open conformation during substrate binding. The major compounds present in the oils, being nonpeptidic inhibitors, have the capacity to bind to the substrate binding site enveloped by hydrophobic residues. This interaction pattern differs in detail from that observed with conventional peptidic inhibitors [36]. Levomenol demonstrated the highest binding energy approaching that of ketoconazole followed by nerolidol then *trans*-2-Caren-4-ol.

Conclusion

The widespread inefficiency of antifungal therapy to treat various fungal infections and the side effects of conventional chemotherapy has accelerated research into alternative therapeutics. *Teucrium leucocladum* Boiss. is a famous endogenous plant grown in Egypt that exhibits pharmaceutical potential and medicinal value. In this research, the essential oil acquired from the aerial parts proved to contain monoterpenes and sesquiterpenes, which are classes of compounds known for their versatile usage in medicine. In vivo studies on TL active metabolites against clinical strains of fungi need to be further studied, as do the effects of combining the active compounds with antifungal agents to combat antimicrobial resistance. Additionally, deeper investigation is necessary to find a simpler, natural, more advantageous anticancer pharmaceutical product.

Methods

Plant materials

The aerial parts of *Teucrium Leucocladum* Boiss. were collected [18] from St Katherine Protectorate (28∘3203200 N 33∘57030.200 E), South Sinai, Egypt, in April 2022. The specimen was authenticated by Prof. Dr. Ibrahim El-Garf, Department of Botany, Faculty of Science, Cairo University, Egypt, where a voucher specimen (TL-113) has been deposited.

Hydro-distillation of the essential oil

A fresh sample (100 g) of TL dried via air-shading was hydro-distilled via Clevenger-type apparatus for 3 h which was conducted based on the methods presented by the European Pharmacopoeia (1996) [37] (plant: water ratio 1:3, w/v).

Microwave-assisted extraction of the essential oil

The air-shaded dried sample (100 g) of *T. leucocladum* was extracted using a focused microwave apparatus [18]. Then, the oil sample was prepared [18], separated, and dried [18] and the volume of the recovered essential oil was determined [18]. The extracted oil samples were stored in sealed air-tight glass vials at − 20 °C until further analysis. The percentage yield was computed as % v/w using the following equation:

$$\%\text{Yield} = [\text{Oil volume (mL)}/\text{Plant material weight (g)}] \times 100$$

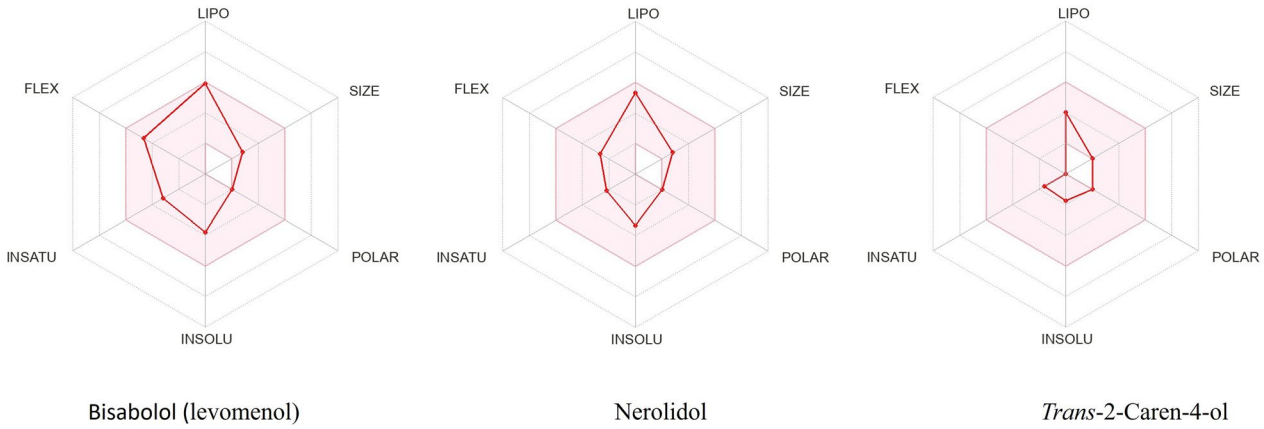

Fig. 6 Radar plot of the examined compounds. POLAR (polarity), LIPO (lipophilicity), INSOLU (solubility), FLEX (flexibility), and IN-SATU (saturation)

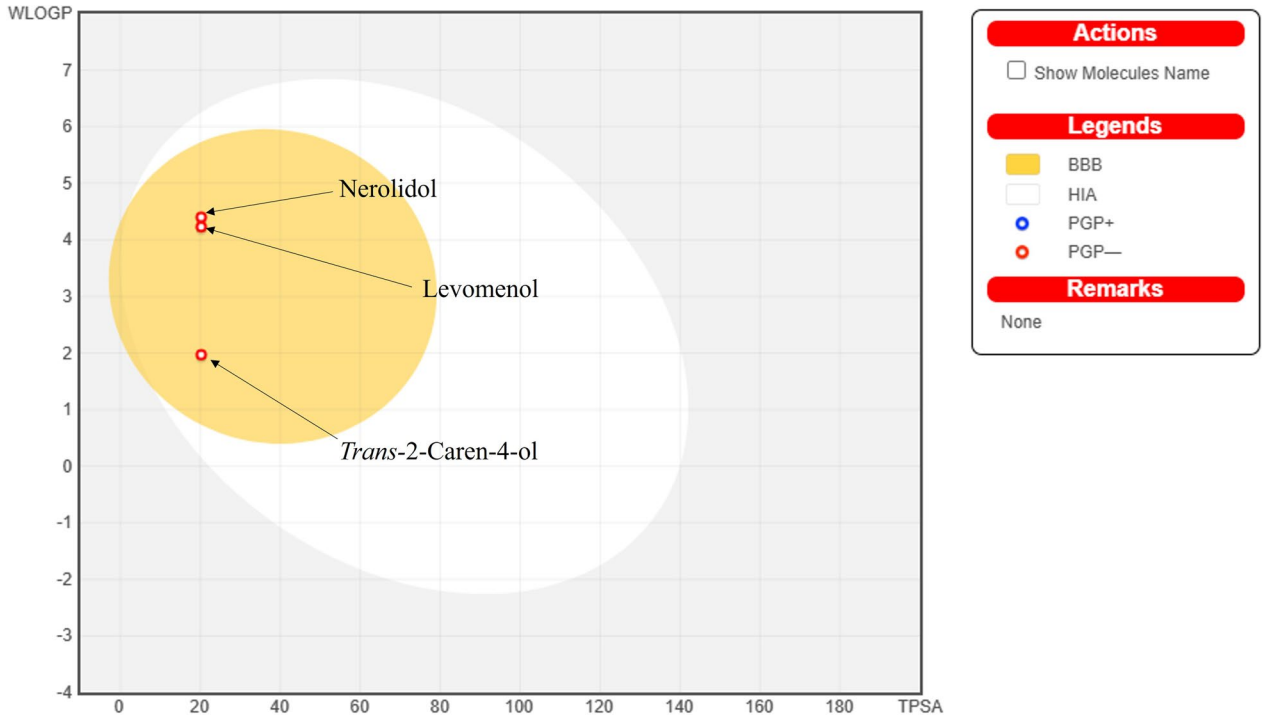

Fig. 7 Evaluation of Levomenol *WLOGP* = 4.23, Nerolidol *WLOGP* = 4.40, and *trans*-2-Caren-4-ol *WLOGP* = 1.97 by the BOILED-Egg method. *Note*: In the 2D graphical representation of a BOILED-Egg, the yolk area corresponds to molecules that are anticipated to passively permeate through the blood–brain barrier (BBB). Conversely, molecules situated in the white region are predicted to undergo passive absorption through the gastrointestinal (GI) tract

GC–MS analysis of the essential oils

Components of the extracted EOs were analyzed and characterized by GC–MS. The GC–MS analysis was performed at the Department of Medicinal and Aromatic Plants Research, National Research Center, Dokki, Giza, Egypt. The gas chromatography–mass spectrometry instrument and the identification of the chemical components of the EOs was achieved according to the previously mentioned method [18].

Antifungal activity

The antifungal activity was performed according to NCCLS recommendations (National Committee for Clinical Laboratory Standards, 1993).

Screening tests regarding the inhibition zone were carried out by the agar well diffusion method [38, 39], and 100 µL of each sample were applied in the wells of diameters 0.6 mm. The experiment was carried out in the Regional Center of Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt. The inoculum suspension was prepared according to the reported method [40].

The EOs were dissolved in dimethyl sulfoxide (DMSO) with two different concentrations (50 and 100 mg/mL) for each oil sample (TLH and TLM). Then, the inhibition zone was measured around each well after 48 h at 28 °C using ketoconazole (100 µg/mL) as positive control for fungi.

Cytotoxic activity

The cytotoxic activities on the oil samples were performed at Polychem Bioassays for Scientific Services (Educational Research Center), Egypt on three cell lines against Doxorubicin HCL (98.0%) as a positive control. All reagents and materials for the cell cultures were purchased from Pan Biotech (Germany). Human cancer cell lines of non-small cell lung adenocarcinoma (A549), Triple-negative breast cancer MDA-MB-231, and colon adenocarcinoma (Caco-2) (ATCC®) were maintained [18]. Doxorubicin (98.0%) was provided and dissolved in PBS at 10 mM [15]. Using an Alamar blue (Resazurin reduction) assay based on a previously published method [15]. Appropriate cell densities of exponentially growing A549, MDA-MB-231, or Caco-2 cells (5000–8000 cells/well) were seeded onto 96-well plates. After 24-h incubation with 5% CO_2 at 37 °C, quadrate wells of cells received screening concentrations of each sample (20 and 200 µg/mL) in the culture medium (final DMSO concentration in medium = 0.1%, by volume). After 48 h of incubation, alamar blue dye in culture medium was added to each well after which the incubation was resumed for further 4 h. At the end of the incubation period, the absorbance at 600 nm was recorded on a microplate reader (Sunrise™ microplate reader, Tecan Austria Gmbh, Grödig, Austria) and was used as a measure of cell viability. In dose-dependent experiments, cells were exposed as above to sample serial dilutions (200, 100, 50, 25, and 12.5 µg/mL) to estimate the dose causing a 50% loss of cell viability compared to the control (IC_{50}) using nonlinear regression curve fit on GraphPad Prism software V8.0. (San Diego, USA) to give significant differences in cytotoxicity, one-way analysis of variance (ANOVA) was used for statistical analysis. Significant differences were indicated as $^{*}p < 0.05$.

Molecular docking studies

The chemical structures of the major metabolites in the essential oils (EOs), namely levomenol, nerolidol, and *trans*-2-caren-4-ol, were obtained by downloading their respective Structure Data Files (SDF files) from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), followed by conversion to PDB format via the free software Avogadro (https://avogadro.cc/) [41, 42].

The protein crystal structure for 1IYK (antifungal) was downloaded from the protein databank (https://www.rcsb.org/) [41] using the previously published molecular docking protocol [42].

The ADME, Lipinski's rule of five and BOILED-Egg techniques

The ADME and pharmacokinetic studies have been determined using SWISS ADME [25] (accessed on 25 July 2023) to evaluate the potential of the three major compounds as promising candidates for pharmaceutical drug development. The expected values for the compounds (levomenol, nerolidol, and *trans*-2-Caren-4-ol) in the radar are represented in Fig. 6.

The three compounds with potential physicochemical characteristics for oral bioavailability were identified by the Swiss ADME molecules bioavailability radar. The pink region represents the ideal spaces for the six physicochemical properties [25]. A chemical is regarded as drug-like if its complete radar plot falls within the pink region. An alternative approach for the assessment [25] involves the use of the BOILED-Egg technique, where levomenol, nerolidol, and *trans*-2-caren-4-ol are anticipated to exhibit brain-penetrating properties and are detected within the yolk [25].

Abbreviations

TL	*Teucrium Leucocladum* Boiss.
EOs	Essential oils
HD	Hydro-distillation
MAE	Microwave-assisted extraction
GC/MS	Gas chromatography–mass spectrometry
A549	Non-small cell lung adenocarcinoma
MDA-MB-231	Triple-negative breast cancer
C. albicans	*Candida albicans*
TLM	The MAE oil sample
TLH	HD oil sample
Caco-2	Colon adenocarcinoma
IC_{50}	The fifty percent inhibitory concentration
NMT	*N*-Myristoyltransferase
BBB	Blood–brain barrier
HIA	Human intestine absorption ranges
PGP	P-glycoprotein permeability
WLOGP	Water partition coefficient
TPSA	Topological polar surface area
Akt	Protein kinase B phosphorylation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-024-00621-5.

Additional file 1. Supplementary material.

Acknowledgements
Not applicable.

Author contributions
E.M.S, M.Y.I, N.F., and T.A.M. contributed to methodology, formal analysis, investigation, and writing–original draft preparation. M-E.F.H and S.H.T. contributed to conceptualization, editing, validation, and supervision. All authors read and approved the final manuscript.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Experiments have been carried out in compliance with the Ethical Committee of Faculty of Pharmacy, Cairo University, Cairo, Egypt, (Committee of Safe Handling and Disposal of Chemicals and Biologicals) of the protocol numbered MP (3124).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 1 February 2024 Accepted: 19 March 2024
Published online: 28 March 2024

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