

Original Research

Screening of Chemical Characterization, Antifungal and Cytotoxic Activities of Essential Oil Constituents of *Tagetes erecta* L. from Erbil, Kurdistan Region-Iraq

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Abstract

Ethnopharmacologic relevance: The history of health benefits of *Tagetes* (Asteraceae) dates back at least to the 12th century. *Tagetes erecta*, an important specie from this genus, was widely known for its traditional medicine. Different parts of *T. erecta* are used in folk medicine to cure various types of diseases.

Aim of the study: Considering the lack of scientific studies of *Tagetes*, the present study was aimed to evaluate the chemical composition, antifungal activity of its essential oil against fungi responsible for human infections, as well as its cytotoxicity on HepG2 human liver carcinoma cell lines.

Materials and methods: Clevenger-type was performed to hydrodistillate EOs and chemically analyzed by combination of GC and MS technique, followed by the evaluation of antifungal activity by using the broth microdilution method. The cytotoxicity was evaluated through MTT assay against HepG2 and expressed as IC₅₀.

Results: One hundred and eleven compounds of the total EOs were identified from three parts (shoot, flower, and root). For the first time, more than 60 new compounds such as iso-bergapten, bergapten, (3)-thujanol acetate, sylvestrene, α -vetivone, tridecenol acetate, β -atlantol, and p-cymenene have been isolated from *T. erecta*. Among all yeasts, *C. albicans* was the most sensitive with MICs of 0.08, 0.04, 0.16 $\mu\text{L mL}^{-1}$ for TES, TEF, and TER oil respectively. In addition, maximum apoptosis rate of up to 90% was observed for HepG2 cell line at concentrations ranging between 82 and 122 $\mu\text{g/ml}$, with IC₅₀ value from 11.58 $\mu\text{g mL}^{-1}$ to 19.86 $\mu\text{g mL}^{-1}$.

Conclusion: The findings from this study showed that the chemical composition of *T. erecta* EO varies, depending on the geographical situation, extraction method, environmental factors, and plant

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organ. Our results also support the hypothesis that the antifungal capacity and cytotoxic activity of the EOs can be ascribed to the lipophilic nature and low molecular weights of the constituents of EOs.

Keywords: *Tagetes erecta*, essential oil, GC-MS, new terpenoids, antifungal, cytotoxicity

Introduction

The Aztec marigold *T. erecta* L. (Asteraceae) family and was widely known as an ornamental plant in the Kurdistan region, as well as being used in different fields like cosmetic preparation and medicine. A literature survey revealed that *T. erecta* possesses a wide spectrum of phytochemical constituents that are used as remedies to treat various health problems, including piles, wounds, fevers, stomachic, rheumatism, scabies and liver troubles, and is also utilized for eye treatments [1]. However, one of the most complex mixture components in *T. erecta* is the essential oil with volatile and aromatic properties, which exhibit effective pharmacological activities like anti-inflammatory [2], insecticidal [3], larvicidal [4], antimicrobial [5], antioxidant [6], anticancer [7], as well as allelopathy efficacy [8]. The present study has been undertaken to isolate the EO from TES, TEF, and TER parts of *T. erecta* using the GC-MS technique, and to evaluate their antifungal and cytotoxic activities. However, this is the first publication to provide a list of more than 60 new compounds, which will be discussed in the following sections.

Materials and Methods

Plant Material and Isolation of Essential Oils

The three different parts of *T. erecta* (shoot (TES), flower (TEF), and root (TER)) were sampled fresh every morning in fresh polythene bags, then cut into small pieces and prepared for distillation. A voucher specimen was deposited at the herbarium of Salahaddin University- Hawler under voucher No. 7592. Seven hundred grams of fresh plant parts were submitted to hydrodistillation for 3 hours using a Clevenger-type apparatus to produce oil under laboratory conditions. The oil produced was dried over anhydrous sodium sulfate¹ (Na₂SO₄) and stored in tightly closed dark vials at 4°C until analysis [8].

¹ Anhydrous sodium sulfate (Na₂SO₄) is typically used in organic chemistry as a drying agent. After aqueous extractions, the organic layer always has a certain amount of water left in it. Adding anhydrous sodium sulfate removes this water by forming sodium sulfate hydrate, which conveniently is also a solid, allowing it to be filtered away. Magnesium sulfate (MgSO₄) is a similar drying agent.

Identification of Compounds by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC/MS)

The identity and quantity of particular components of *T. erecta* essential oil were evaluated by the GC-MS analysis method using a Thermoquest-Finnigan gas chromatograph GC equipped with Trace MS detector and fused silica capillary DB-5 column (60 m × 0.25 mm; film thickness 0.25 μm). The measurement records started after 3 min of the run began. The oven temperature was programmed from 60 to 250°C at the rate of 5°C/min. and finally a the temperature of 250°C was kept constant for 10 minutes. Subsequent GC working conditions were as follows: carrier gas was nitrogen with a constant flow rate of 1 mL/min. Ionization voltage was kept at 70 eV. MS working conditions were as follows: temperature of the ion source and the interface were 200 and 250°C, respectively, the mass range was scanned from 43 to 456 m/z. The injector and detector temperatures were 250 and 300°C, respectively [9].

The constituents were identified by calculation of their retention indices under the same chromatographic conditions for n-alkanes (C₆ – C₂₄) and the oil on a DB-5 column. Compounds were identified by comparing their mass spectra library (Wiley and Adams) or with authentic compounds, and for confirmed compounds their GC retention indices were compared with authentic compounds or with those reported [9, 10]. Each sample was then analyzed three times with GC-FID to obtain the percentage concentration of each constituent without performing any correction.

Antifungal Broth Microdilution Susceptibility Testing

The effectiveness of the antifungal activities of the tested essential oils was evaluated using two-fold serial broth microdilution techniques as described by Pfaller and V. Chaturvedi [11], with some modification. Four *Candida* strains (*C. albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis*) were obtained from the Biology Department, College of Science, Salahaddin University, Erbil. 96 well flat-shaped microtitre culture plates were used. Stock solutions of EOs were prepared by dissolving the EOs in 5% dimethyl sulphoxide (DMSO) at a ratio 1:10 to facilitate the dispersion of the oils in the aqueous nutrient medium. Plant EOs of 100 μl from the stock solution (10 μl ml⁻¹) were taken into the first well containing 100 μl of sterile SDB and thoroughly mixed. Serial 11-fold dilutions were performed by transferring 100 μl from well to well (on

row). From the last well, 100 μ l solution was discarded. Thus, the reached concentrations ranged from 5 to 0.005 μ l EO /mL.

At the time of inoculation, the final concentrations of microbial cells were about 0.5-2.5x10³ CFU/ml [12]. Then 20 μ l of test organisms were added into each tube. The final volume of the solution in each tube was made up to 220 μ l. Nystatin (520 ppm) was used for control study. The absorbance for all 96 wells was measured at 630 nm before incubating by ELISA Reader (BioTech, USA). Then the plates were incubated under shaking conditions (100-120 rpm) at 25±2°C for 24 hours. After the incubation time, the absorbance was re-measured at the end of incubation to determine the final absorbance and compare it with the initial absorbance [13]. Each sample assay was carried out in triplicate.

Cytotoxic Activity of the Essential Oils

The cytotoxic activity of *T. erecta* EOs was screened by determination of their IC₅₀s using the (MTT) colorimetric in-vitro assay as described by Oliveira and Alves [7], with slight modifications. The liver (HepG2) cell line was obtained from the Pasteur Institute of Iran. In this study, 1×10⁴ cells/well were seeded in 96 well plates with 90 μ l of DMEM medium supplemented with 10% fetal bovine serum (FBS) containing concentrations of the essential oils that ranged from 5 to 122 μ g/mL and were incubated for 24 hours at 37°C in a CO₂ incubator (5%). Then, 10 μ l of MTT solution (5 mg/ml) was pipetted into each well and the mixture was incubated for 4 hours at 37°C. After incubation, 100 μ l of (DMSO) was added to each well to dissolve the formed formazan from the MTT and incubated overnight at 37°C. Absorbance was then measured at a wavelength of 540 nm and a reference length of 690 nm using the ELISA reader (BioTech, USA). Untreated cells (<1 % DMSO) were used as control. The percentage of the reduction of viability was calculated as follows:

$$\text{Viability \%} = \frac{OD_s}{OD_c} \times 100$$

...where OD_s is the mean value of the measured optical density of the 100% extracts of the sample and OD_c is the mean value of the measured optical density of the control [14].

Five replicate wells were used for each concentration tested, and 50% inhibition of cell growth (IC₅₀) was used as the analysis parameter.

Statistical Analysis

Results are presented as the mean±standard deviation. The one-way ANOVA and Dunnett's multiple comparisons were used to test the significance

of the difference between two mean values. P<0.01 was considered to indicate a statistically significant difference. The half maximal inhibitory concentrations (IC₅₀) were statistically analysed by GraphPad Prism 6 for Windows software package.

Results and Discussion

The Common Volatile Oils of *T. erecta*

The variation in essential oil content and composition of TES, TEF, and TER of *T. erecta* growing in Erbil was analyzed using GC-MS and a GC- flame ionization detector (GC-FID). The identified constituents with their relative content in EOs are summarized in Table 1. Maximal EO content (0.64%) was obtained from TES, followed by TEF (0.48%), and minimal by TER (0.2%). There were 62, 56, and 43 compounds identified from TES-EO, TEF-EO, and TER-EO comprising 93.87%, 90.83%, and 93.22% of the total oil, respectively.

The TES and TEF oil composition was mainly dominated by monoterpenoids 75% and 71% consecutively, representing piperitone (11.58%, 7.5%), piperitenone (8.36%, 11.22%), sylvestrene (5.9%, 6.82%), terpinolene (2.97%, 5.41%), and (Z)- β -ocimene (2.46%, 3.97%) and their major constituents respectively. The second major class of compounds in both parts were sesquiterpenoids, with percentages of 22% and 20% respectively, from which (E)-caryophyllene (5.92%, 7.72%), caryophyllene oxide (4%, 2.87%), (E)-myroxide (3.57%, 5.41%), and spathulenol (1.31%, 1.78%) were the most abundant consecutively. The results have shown similarity with the composition reported by Marques, Morais [4], Oliveira, Alves [7], Laosinwattana, Wichittrakarn [8], Crevelin [15], Resmi, and Nair [16], who emphasized that the monoterpenes are the predominant components of the aerial parts of *T. erecta* ranging between (46.3% to 97.3%), besides some variation in major and minor compounds occurred. Furthermore, major compounds such as methyleugenol (E)-ocimene, undacane, piperitenone oxide, l-limonene, cis-ocimene, (E)-ocimene, limonene, (Z)-myroxide, camphene, α -terpinolene, α -thujene, 1,4-naptoquinone, 2-hexyl-1-decanol, fenchol, eugenol, and 4-terpinyl acetate isolated by Tripathi, Bhatia [5], Oliveira, Alves [7], Crevelin [15], Resmi, Nair [16], Yasheshwar, and Umar [17] were not found in our analysis.

Conversely, the chemical constituents of TER-EO were quite different from the components of the TES and TEF oil, which were characterized by a large number of furanocoumarins, non-volatile compounds, accounting for 59% of the total root oil composition. The second major groups of compounds were sesquiterpenoids, from which cyperene (9.64%), caryophyllene oxide (4.17%), (E)- β -farnesene (2.95), α -vetivone (2.83), and β -bisabolene (2.5) were the most abundant, meanwhile monoterpenes were occupied by only a few amounts of TER-EO representing 3% of the total oil. Worth

Table 1. Essential oil composition of different parts of *T. erecta* L. from Erbil.

#	Compounds	RI ^a	TES		TEF		TER	
			RT ^b	%	RT	%	RT	%
1.	α -Pinene	932	4.09	0.14	4.09	0.34		
2.	Sabinene	969	4.77	0.61	4.76	0.9		
3.	Myrcene	988	5.05	0.29	5.05	0.19		
4.*	(3E)-Hexenyl acetate	1001	5.36	0.07				
5.	α -phellandrene	1002			18.93	0.1		
6.	O-Cymene	1022	5.78	0.32	5.77	0.31		
7.	Limonene	1024					5.88	0.07
8.*	Sylvestrene	1025	5.87	5.9	5.88	6.82		
9.	(Z)- β -Ocimene	1030	6.02	2.46	6.03	3.97		
10.	(E)- β -Ocimene	1044	6.24	0.5	6.24	0.42		
11.	dihydro-Tagetone	1046	6.34	0.38	6.34	0.89		
12.*	cis-Linalool oxide	1067			6.91	0.25		
13.	Terpinolene	1086	7.2	2.97	7.21	5.41		
14.*	p-Cymene	1089	7.26	2.96	7.25	0.89		
15.	Linalool	1095	7.48	0.91	7.47	0.42		
16.*	α -Pinene oxide	1099	7.4	0.21	7.39	0.13		
17.	1,3,8-p-Menthatriene	1108	7.79	0.18				
18.*	(2E,4E)-Octadienol	1113	7.81	0.3	7.8	0.54		
19.*	dehydro-Sabina ketone	1117					8.13	0.05
20.	(Z)-Epoxy-ocimene	1128	8.24	1.5	8.23	1.69		
21.*	trans-Pinocarveol	1135	8.40	0.79	8.38	0.32		
22.	(E)-Tagetone	1139	8.59	1.87	8.57	2.29		
23.	(E)-Myroxide	1140	8.5	3.57	8.47	1.13		
24.	(Z)-Tagetone	1148	8.79	0.8	8.78	1.6		
25.	Borneol	1165	9.18	0.21				
26.	Terpinen-4-ol	1174	9.44	0.65				
27.*	(E)-Isocitral	1177	9.39	0.33	9.39	0.3		
28.	p-cymen-8-ol	1179	9.77	6.15	9.72	5.46		
29.*	p-methyl-Acetophenone	1179	9.71	2.34				
30.*	cis-Pinocarveol	1182			9.42	0.56		
31.	α -Terpineol	1186	9.84	1.16				
32.*	Verbenol	1197	10	0.26				
33.*	cis-4-Caranone	1200	10.1	0.7				
34.	Verbenone	1204			9.77	0.43		
35.	(Z)-Ocimenone	1226			10.81	3.45		
36.*	cis-p-Mentha-1(7),8-dien-2-ol	1227	10.59	0.18				
37.	(E)-Ocimenone	1235	10.82	1.34	11.02	2.13		
38.*	Carvacrol, methyl ether	1241	10.84	0.55				
39.	Car-3-en-2-one	1244	11.04	0.97				
40.	Piperitone	1249	11.49	11.58	11.45	7.5		
41.*	Perilla aldehyde	1269			11.82	0.29		
42.	Isobornyl acetate	1283			12.17	1.32		

Table 1. Continued.

43.*	perilla alcohol	1294			12.33	0.42		
44.*	3-Thujanol acetate	1295	12.35	11.58				
45.	Carvacrol	1298	12.68	0.23				
46.*	(Z)-Patchenol	1316	12.93	0.27				
47.*	iso-Dehydro carveol acetate	1326	13.44	0.15				
48.	Piperitenone	1340	13.74	8.36	13.72	11.22		
49.	α -Longipinene	1350	13.85	1.31	13.83	0.59		
50.	Piperitenone oxide	1366	14.31	0.52	14.3	2.67		
51.*	Longicyclene	1371					14.21	0.58
52.*	α -Copaene	1374					14.46	0.05
53.	Geranyl acetate	1379	14.64	0.14	14.62	0.17		
54.	β -Elemene	1389					14.89	0.35
55.*	(Z)-Jasmone	1392	15.07	0.19	15.06	0.23		
56.	Cyperene	1398					15.13	9.64
57.*	α -Funebrene	1402	15.16	0.23	15.14	0.23		
58.	(Z)-Caryophyllene	1408	15.29	0.71	15.27	0.3	15.25	0.08
59.	(E)-Caryophyllene	1417	15.61	5.92	15.59	7.72	15.57	1.88
60.*	α -trans-Bergamotene	1432					21.56	0.76
61.	Aromadendrene	1439	16.28	0.12			16	0.59
62.	(E)- β -Farnesene	1454	16.44	0.79	16.42	0.99	16.44	2.95
63.*	dehydro Aromadendrane	1460	16.56	0.19	16.53	0.11		
64.*	γ -Gurjunene	1475					16.98	0.28
65.	Germacrene D	1484	17.09	0.16	17.08	0.5	17.15	1.92
66.*	β -Selinene	1489					17.26	0.03
67.*	α -Selinene	1498					17.42	0.22
68.	Bicyclogermacrene	1500			17.45	0.12		
69.*	γ -Patchoulene	1502					17.53	0.43
70.*	β -Bisabolene	1505					17.72	2.5
71.	(E,E)- α -Farnesene	1505			17.66	0.45		
72.*	trans-Cycloisolongifol-5-ol	1513					17.98	0.5
73.	β -Sesquiphellandrene	1521					18.07	0.19
74.*	Italicene epoxid	1547	18.81	0.29	18.79	0.34	18.78	0.38
75.	(E)-Nerolidol	1561	19.04	0.82	19.02	0.6		
76.*	epi-Longipinanol	1562	18.96	0.1	5.36	0.1		
77.	Spathulenol	1577	19.47	1.31	19.43	1.78		
78.	Caryophyllene oxide	1582	19.56	4	19.52	2.87	19.54	4.17
79.*	Fokienol	1596					19.79	0.06
81.*	Caryophylla-4(12),8(13)-dien-5 α -ol	1639	20.8	0.51			20.77	0.89
82.*	Selina-3,11-dien-6 α -ol	1642			20.78	0.41		
83.*	Himachalol	1652	21.18	0.11				
84.*	14-hydroxy-(Z)-Caryophyllene	1666					21.29	0.63
85.*	14-hydroxy-9-epi-(E)-Caryophyllene	1668	7.79	0.18			15.93	0.76
86.*	2Z,6Z-Farnesal	1684					21.71	0.07

Table 1. Continued.

#	Compounds	RI ^a	TES		TEF		TER	
			RT ^b	%	RT	%	RT	%
88.*	Cyperotundone	1695					22.06	0.08
89.*	Tridecenol acetate	1703					22.33	1.16
90.*	cis-Thujopsenal	1708					22.48	0.37
91.*	(2E,6Z)-Farnesol	1714					22.66	0.23
92.*	Isobicyclogermacrenal	1733					22.93	0.05
93.*	(6S,7R)-Bisabolone	1748					23.12	0.43
94.*	β -Acoradienol	1762			24.69	0.35		
95.*	β -Costol	1766	25.45	0.12			23.54	0.21
96.*	β -Bisabolenal	1768					23.73	0.25
97.*	Squanmulosone	1770					23.86	0.77
98.	(E)- α -Atlantol	1777			29.55	0.31		
99.*	α -Vetivone	1842					25.56	2.83
100.*	Flourensadiol	1869	26.83	0.12				
101.	Phytol	1942	27.52	0.08	24.95	7.07		
102.*	Columellarin	1952					28.34	0.25
103.*	2 α -acetoxy-Amorpha-4,7(11)-dien-8-on	1985	34.24	0.37				
104.*	epi-Catalponol	1988					28.82	0.24
105.*	Phyllocladene	2016					29.67	0.05
106.*	iso-Bergapten	2033					26.87	41.84
107.*	Bergapten	2056					32.19	12.64
108.*	n-Heneicosane	2100			29.93	0.18		
109.*	(E)-phytol acetate	2218	30.27	1.84	30.25	0.6		
110.*	Incensole oxide	2279			30.75	0.13		
111.	Dotriacontane	3400			33.42	0.32		
Total			62 (93.87%)		56 (90.86%)		43 (93.22%)	
Monoterpene hydrocarbons			17%		22%		< 0.5%	
Oxygenated Monoterpenes			58%		49%		3%	
Sesquiterpene hydrocarbons			3%		3%		23%	
Oxygenated Sesquiterpenes			19%		17%		14%	
Oxygenated diterpenes			0%		8%		0%	
Furanocoumarins			0%		0%		59%	
Others			3%		<1%		<1%	

^a RI, Retention indices relative to C6 – C24 n-alkanes on the DB-5 column.

^b RT, Retention Time

* New chemical constituents

mentioning is that the thiophenes, which were isolated from the root part in other studies [3, 4, 6, 18], were absent in our analysis. When compared with the literature chart, the results obtained in this investigation showed that the chemical composition of the essential oils from different parts of *T. erecta* differ depending on the parts of the plant used, isolation method, and the environmental conditions.

The New Chemical Components

Analysis of the EOs reveals that they possess very complex GC-MS profiles. The new structures identified were mainly monoterpenoids in TES-EO (26.21%) and TEF-EO (11.6%), represented by monoterpene hydrocarbons (8.86%, 7.71%) and oxygenated monoterpenes (17.35%, 3.89%) respectively (Table 1).

The major constituents distributed in both parts were 3-thujanol acetate, sylvestrene, p-cymene, p-methylacetophenone, and (E)-phytol acetate. Furthermore, the essential oil composition of TER was mainly dominated by non-volatile furanocoumarins represented by iso-bergapten 41.84% and bergapten 12.64%, followed by terpenoids α -vetivone (2.83%), β -bisabolene (2.5%), tridecenol acetate (1.16%), and β -atlantol (1.03%).

Antifungal Activity

The antifungal efficacy of the EOs from different parts of *T. erecta* was tested against *C. albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis* (Fig. 1). The results of the MIC and the minimum fungicidal concentration (MFC) are given in Table 2. Statistically, *T. erecta* EO has restricted significantly all yeast growth compared with the positive control Nystatin (520 ppm). According to the classification of biological activity used by Saha and Kundu [19], and Pessini, Dias, and Filho [20], the

antifungal activities were categorized as weak (above $1.6 \mu\text{l mL}^{-1}$), moderate (0.6 to $1.5 \mu\text{l mL}^{-1}$) and strong (lower than $0.5 \mu\text{l mL}^{-1}$). Hence, a highly significant antifungal activity has been recorded for all the EOs with MIC value ranging from ($0.08 \mu\text{l mL}^{-1}$) to ($0.32 \mu\text{l mL}^{-1}$), with the exception of *C. tropicalis*, which was moderately affected by all EOs (MIC value $1.25 \mu\text{l mL}^{-1}$).

Hence, we found that among all the *Candida* species tested, *C. albicans* was the most affected fungi toward the EO of all *T. erecta*, while *C. tropicalis* was moderately affected. The results are consistent with those obtained by Resmi and Nair [16], and Padalia and Chanda [21]. Previously, the inhibitory activity of the EOs of other *Tagetes* species against *Candida* spp has already been confirmed [22-24]. As a result, this antifungal potential may be associated with the existence of lipophilic compounds, which could interfere with the cytoplasmic membrane depending on the presence of water-soluble terpenoids present in the essential oil [22].

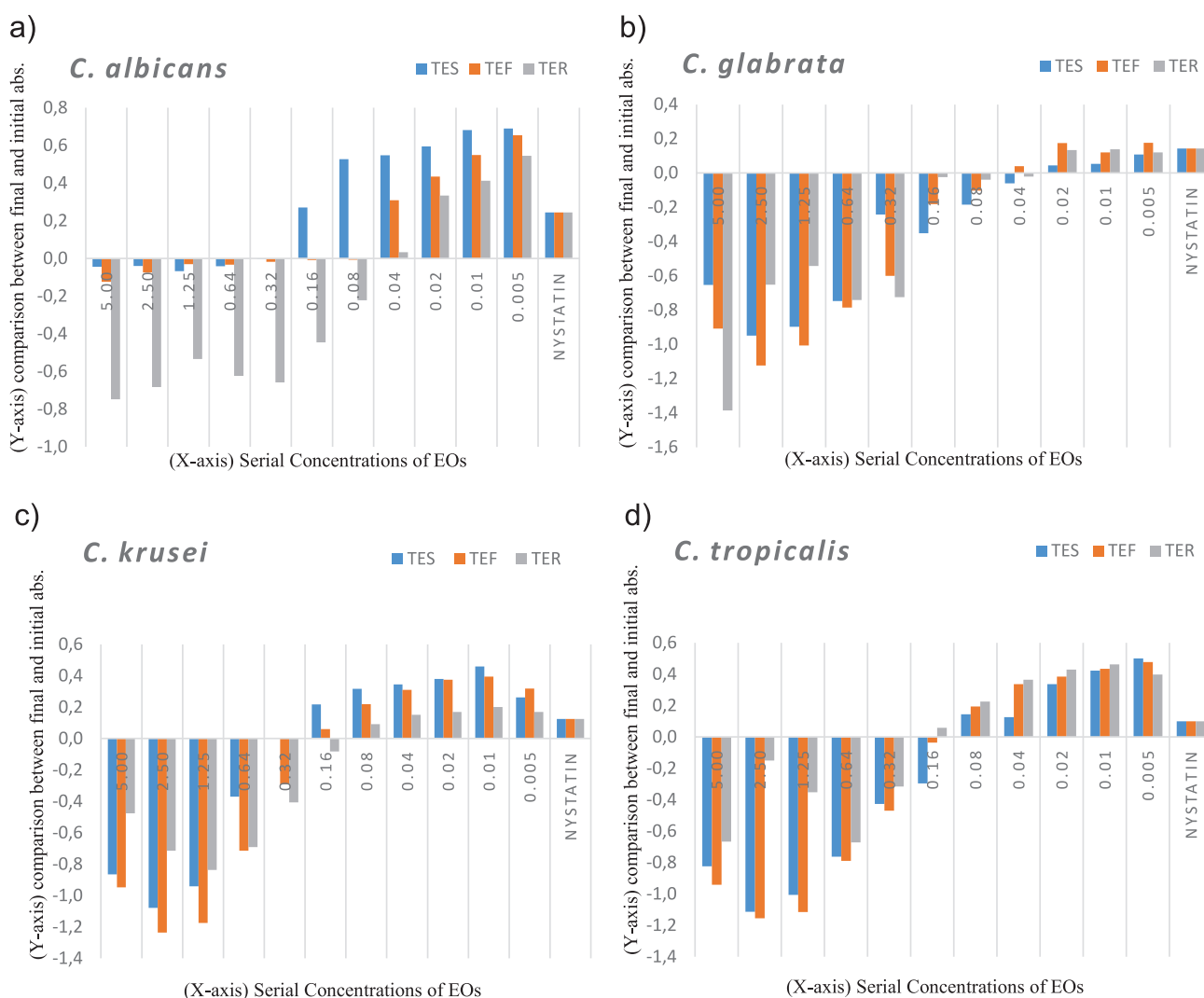


Fig. 1. Antifungal effect of various concentrations ($\mu\text{l mL}^{-1}$) of EOs of three *T. erecta* parts on: a) *C. albicans*, b) *C. glabrata*, c) *C. krusei*, d) *C. tropicalis*.

Table 2. Minimum inhibition concentration (MIC ($\mu\text{l ml}^{-1}$)) and minimum fungal concentration (MFC ($\mu\text{l ml}^{-1}$)) of *T. erecta* EOs against test fungi strains.

Test Fungi	EOs conc. ($\mu\text{l ml}^{-1}$)					
	Shoot		Flowers		Root	
	MIC	MFC	MIC	MFC	MIC	MFC
<i>Candida albicans</i>	0.08	1.25	0.04	0.16	0.16	0.32
<i>Candida glabrata</i>	0.32	0.64	0.32	0.64	0.32	0.64
<i>Candida krusei</i>	0.16	0.32	0.32	0.64	0.64	2.5
<i>Candida tropicalis</i>	1.25	2.5	1.25	2.5	1.25	2.5

Cytotoxic Activity

The cytotoxicity of the *T. erecta* EOs was carried out against HepG2 cell lines at different concentrations to determine growth inhibition rate. A dose-response histogram created between the range of 5 and 122 $\mu\text{g mL}^{-1}$ for the EOs of all three parts (Fig. 2) expresses the decreasing number of viable cells with increasing concentrations of EO. The EOs significantly exhibited high cytotoxicity in comparison with the control (untreated cells). The test samples showing cell viability ranging between 33.64% and 93.1% at 5.12 to 7.6 $\mu\text{g mL}^{-1}$ were considered to be less active at minimum concentration. In addition, the anti-proliferative effect strengthens with an increase in the concentration of the EOs. Maximum apoptosis rate of up to 90% was observed for the HepG2 cell line at concentrations ranging between 82 and 122 $\mu\text{g/ml}$, with IC_{50} value from 11.58 $\mu\text{g mL}^{-1}$ to 19.86 $\mu\text{g mL}^{-1}$ (Table 3). A much lower amount of viable cells (less than 10%) were detected at this range of concentrations, which showed the maximum inhibition concentration. These results were in accordance with previous studies performed on *T. erecta* conducted by Gupta and Gupta [6], Vallisuta and Nukoolkarn [25], and Ayyadurai and Valarmathy [26]. Interestingly, the study conducted by [7] for determining the cytotoxic activity of the essential oils of four plants (*T. erecta* L., *Tetradenia riparia*, *Bidens sulphurea*, and *Foeniculum vulgare*) against six tumor cell lines murine melanoma (B16F10), human colon carcinoma (HT29), human breast adenocarcinoma (MCF-7), human cervical adenocarcinoma (HeLa), human hepatocellular liver carcinoma (HepG2), and human glioblastoma (MO59J, U343, and U251), showed that cancer cells have higher sensitivity to the oil of *T. erecta*.

This would suggest that, as claimed for the antimicrobial effect, the cytotoxicity of *T. erecta* EO could be ascribed to the lipophilic nature and low molecular weights of the constituents of essential oils that allow them to cross cell membranes, altering the phospholipid layers, increasing membrane fluidity, and leading to leakage of ions and of cytoplasmic content [27]. Accordingly, we can conclude that

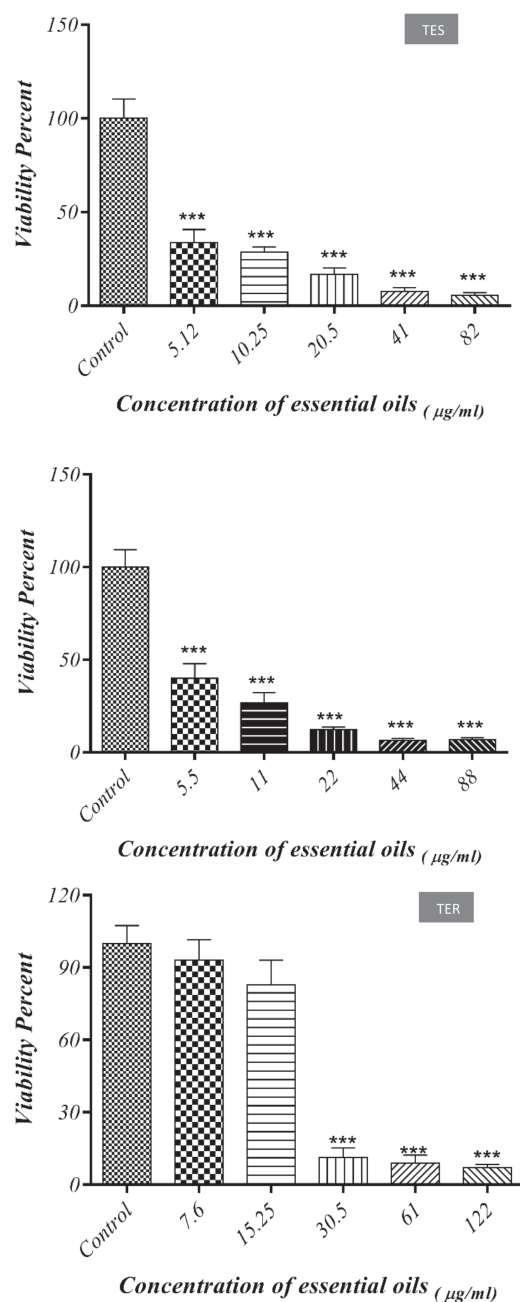


Fig. 2. Inhibitory effect of *T. erecta* EO on HepG2 cell growth. The number of viable cells is expressed as a percentage of vehicle control. Mean \pm standard deviation (SD) of 5 independent experiments.

Table 3. Cytotoxicity of the essential oil of *T. erecta* on HepG2 cells.

#	Plant part EO	IC ₅₀ (µg/mL)
1.	TES	17.46
2.	TEF	11.58
3.	TER	19.86

the EOs of *T. erecta* were found to be highly effective against HepG2 cells without affecting normal cells.

Conclusions

Today an approach has been made to use EOs as a source of new antibiotics. However, the EO components of *T. erecta* and their biological activities are widely documented worldwide. Thus, the role of this plant cannot be neglected as is evident with the present results. Generally, 111 compounds were screened; out of these, more than 60 new EOs were extracted for the first time in this plant, including iso-bergapten, bergapten, (3)-thujanol acetate, sylvestrene, α -vetivone, β -bisabolene, tridecenol acetate, (E)-phytol acetate, β -atlantol, and p-cymene. The results showed that the chemical composition of *T. erecta* EO varies when compared with the literature chart, depending on the geographical situation, extraction method, environmental factors, and plant organ. The above findings suggest that the EOs of this plant are a good source of antifungal and anticancer agents due to the high concentration of the lipophilic oxygenated terpenoids.

Abbreviations

GC: Gas chromatography
 GC-MS: Gas chromatography-mass spectrometry
 GC-FID: Gas chromatography-flame-ionization detection
 EO: Essential oil
 TES: Shoot of *Tagetes erecta*
 TES-EO: Essential oil of *Tagetes erecta* shoot
 TEF: Flowers of *Tagetes erecta*
 TEF-EO: Essential oil of *Tagetes erecta* flower
 TER: Root of *Tagetes erecta*
 TER-EO: Essential oil of *Tagetes erecta* root
 KI: Kovats' retention indices
 RT: Retention time
 SDB: Sabouraud dextrose broth
 Nys: Nystatin
 CFU: Colony-forming unit

Conflict of Interest

The authors declare no conflict of interest.

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