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RESEARCH ARTICLE

Chemical composition and antioxidant, antibacterial and cytotoxic properties of essential oil from Teucrium polium L. from Riyadh province

Waad A. Al-Otaibi* and Sahar M. AlMotwaa

Department of Chemistry, College of Science and Humanities, Shaqra University, Saudi Arabia

ABSTRACT

This study was carried out to extract essential oil from the aerial parts of *Teucrium polium L.* (TPO) using hydro-distillation, and to determine the chemical composition of the extract with GC-MS analysis. The plant was obtained from Huraymila City in the North-Western Riyadh. Total contents of phenolics (TPC) and flavonoids (TFC) were estimated as gallic acid (GA) equivalent and catechin (CH) equivalent, respectively, whereas the antioxidant activity was measured with DPPH assay. Agar disc diffusion and micro-broth dilution methods were utilized for antibacterial evaluation, while MTT assay was used for cytotoxicity evaluation. The average yield of TPO was 0.26 ± 0.01 %(v: w), and the predominant constituents were τ-cadinol (25.58%), α-fenchene (20.09%), β-eudesmol (11.76%), β-myrcene (8.02%), γ-cadinene (5.22%), α-phellandrene (3.11%), epi-bicyclosesquiphellandrene (3.07%), and caryophyllene oxide (2.48%), all of which accounted for 79.33% of the identified compounds. The TPC and TFC of TPO were 138.33 ± 3.1 μg GA equivalents mL^{-1} and 19 ± 2.15 μg CH equivalents mL^{-1}, respectively. The oil had good radical-scavenging activity, with IC_{50} of 61.38 ± 4.33 μg mL^{-1}. It exhibited antibacterial activity against Gram-positive *S. aureus* and *MRSA* at 16.67 % (v/v), with inhibition zones of 13 ± 0.09 and 11 ± 0.13 mm, respectively, and MIC: MBC values of 0.26/0.52 and 0.5/21.04, respectively. However, it had no effect on the growth of negative strains (*S. sonnei, P. aeruginosa*, *K. pneumoniae* and *E. coli)*. Results of anti-proliferative effects of TPO on two human colon cancer cell lines (HT-29 and HCT116) and hepatic cancer HepG2 cells, showed that it had a more cytotoxic effect on HT-29 and HCT116 cells, with IC_{50} values of 0.07 ± 0.03 and 0.14 ± 0.06, respectively than on HepG2 which was markedly inhibited only at higher concentrations ($p < 0.001$). In conclusion, TPO exerted antimicrobial and antiproliferative properties. Overall, these findings are considered useful addition to biodiversity data and may be useful in food biotechnology and pharmaceutical industries.

Keywords: Herbal Medicine; Gas Chromatography-Mass Spectrometry; *Teurium Polium* L.; Antibacterial Activity; Anticancer Activity; Essential Oil.

INTRODUCTION

In recent times, there has been an increase in global interest in the discovery of natural bioactive resources for enhancing food safety and human health, while serving as replacements for synthetic compounds used as food preservative agents. Universally, medicinal plants are considered vital sources of bioactive substances with potential therapeutic effects (Aljaafari et al., 2021). *Teucrium polium* L. is one out of 300 species of the genus *Tecrium* belonging to the Lamiaceae family, and it grows mainly in Europe, North Africa and the temperate Mediterranean regions of Asia (Bukhari et al., 2015). It is a perennial shrub 20-50 cm high, with 3-cm-long linear-shaped leaves (Bahramikia and Yazdanparast, 2012). *Teucrium polium* is one of the most fragrant plants fairly distributed throughout the country of Saudi Arabia. The fragrance is related to aromatic compounds in the plant (Migahid, 1978). Over the decades, T. polium has been used worldwide in alternative medicine due to its anti-rheumatoid, anti-microbial, analgesic, anti-inflammatory, antioxidant, antispasmodic, hypolipidemic, hypoglycemic and cardioprotective properties (Khazaei et al., 2018). The biological activities of different genus of the plant are due to their chemical compositions which are affected by geographical origin, environmental conditions and the phase of the plants at the point of harvest (Assaeed et al., 2020, Abd-ElGawad et al., 2019). However, a review of existing literature showed that no studies have been done on the constituents of oil extracted from Teucrium polium L. growing in Huraymila in the North West area

***Corresponding author:**
Waad A. Al-Otaibi, Department of Chemistry, College of Science and Humanities, Shaqra University, Saudi Arabia, **E-mail:** w.otaibi@su.edu.sa

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of Riyadh (Guetata and Al-Ghamdi, 2014, Hassan et al., 1979). Therefore, the present study was carried out to investigate the composition of TPO extracted from the aerial parts of *T. polium* L. from Huraymila at the flowering stage, and to determine its antioxidant, antimicrobial and antiproliferative properties.

MATERIALS AND METHODS

A sample of the aerialparts including leaves, flowers and stems was collected on 29 May 2020 from several *Teucrium polium* L. plants growing in Huraymila at the similar period (Latitude 25° 19' 00.8" N; Longitude 46° 09' 39.9"E), situated in Northwest Riyadh. The plant was identified as *Teucrium polium* L. by Migahid (1978). Approximately, 150 g of the dried aerial parts were ground and immersed in 1L of distilled water. The TPO was obtained with hydro-distillation method at 60°C for 4 h using Clevenger-type apparatus. The % yield of TPO was calculated using the following formula:

$$\%\,\text{TPO yield} = \frac{\text{Weight of oil(g)}}{\text{The dried aerial part of plant weight(g)}} \times 100$$

PHYTOCHEMICAL ANALYSIS OF TPO

Determination of total phenolic content (TPC)

The total phenolic content of TPO was determined with the Folin-Ciocalteu reagent (FCR) and using gallic acid (GA) as standard. In essence, 800µl of distilled water and 100µl of DMSO-diluted TPO (1:10 v: v) were mixed with 100µl of FCR and incubated for 5 min in the dark at room temperature. To the reaction mixture was added 1500µl of 20% sodium bicarbonate and allowed to stand for 20 min, after which absorbance was measured at 750nm. The test was performed in triplicate.

Determination of total flavonoid content (TFC)

The total flavonoid content of TPO was estimated using catechin (CH) as standard. Approximately 1250µl of distilled water and 250µl of the DMSO-diluted TPO (1:10 v: v) were mixed with 75µl of 5% sodium nitrate solution and incubated for 60 min at room temperature. Then, 150µl of 10% aluminum trichloride ($AlCl_3$) solution, 500µl of 1 M sodium hydroxide and 275µl distilled water were added to the resultant mixture. After incubation for 5 min at room temperature, the absorbance was read at 510nm. The test was performed in triplicate.

DPPH radical scavenging activity (RSA)

Briefly, 100µl of DMSO-diluted TPO (1:10 v: v) was mixed with 900µl of 100 µM DPPH reagent and allowed to stand in the dark for 30 min. After incubation, the optical density (OD) was measured at 517 nm against methanol blank and control containing all reagents except the testedsamples. The test was performed in triplicate and the result was calculated as % of scavenging activity (RSA) relative to control as follows:

$$\text{RSA}\,(\%) = \frac{\text{Control}\,(\text{OD}) - \text{Sample}\,(\text{OD})}{\text{Control}\,(\text{OD})} \times 100$$

ANALYSIS OF CHEMICAL COMPOSITION OF TPO

The analysis of TPO was performed using a Hewlett-Packard computerized system coupled with a 7000D triple quadruple detector (Agilent Technologies, Palo, Alto, CA, USA). A non-polar HP5MS (30 m × 0.25 mm × 0.25 µm film thickness) was used as analytical column in GC-MS system, with He as carrier gas at a flow rate of 0.5 ml/min and injection volume of 0.2µl TPO diluted 1:10 (v: v) with n-hexane. The injection was performed at oven temperature of 250°C which was programmed at 60°C for 8 min, increased by 4 to 280°C/min, and kept at 280°C for 15 min. The ionization mode was maintained with electronic ionization at 70 eV over a scan range of 30 to 550 atomic mass units. The relative proportions of volatile compounds in TPO were identified through comparisons of the peak areas and retention times (RTs) with retention indices in literature and in spectral library banks (NIST).

BACTERIAL STRAINS AND CULTURES

The antibacterial property of TPO was investigated on four Gram-negative strains: *Pseudomonas aeruginosa* (*P. aeruginosa;* ATTC 27853), *Escherichia coli* (*E. coli;* ATTC 25922), *Klebsiella pneumonia* (*K. pneumonia;* ATTC 13883) and *Shigella sonnei* (*S. Sonnei;* ATCC 25931), and against two strains of Gram-positive bacteria i.e., *methicillin-resistant Staphylococcus* (MRSA*;* ATCC 43300) and *Staphylococcus aureus* (*S. aureus;* ATTC 25923). The bacteria strains were obtained from microbiology laboratory, Department of Biology at King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia.

DETERMINATION OF THE ANTIBACTERIAL EFFECT OF TPO

Agar disc diffusion assay

The antibacterial effect of TPO was determined against six bacterial strains using the agar disc diffusion method. In a brief, 100 ml of fresh inoculum suspension was spread on the surface of the agar plate, and a 6-mm diameter

sterile bank filter disc was added after the agar plate was impregnated with 10µl of TPO at concentrations of 100% and 16.67% obtained by dissolving 2µl of TPO in 8.9µl of MHB containing Tween 80 and DMSO at a ratio of 1:10. The inoculated plates were incubated at 37°C for 24h. The antibacterial activity of TPO was calculated in terms of diameter of the inhibition zone (IZ) of bacteria and the test was performed in triplicate.

Broth microdilution susceptibility test

The minimum inhibitory and bactericidal concentrations (MICs and MBCs) of TPO against the selected bacterial strains were determined using the microdilution broth method. To each well, 10µl containing 1×10^5 CFU/ml was inoculated. Diluted TPO (16.67%) was prepared by dissolving 200µl of the oil in 890µl of sterile MHB containing Tween 80 and DMSO at a volume ratio of 1:10. The bacteria strains were treated with seven serial dilutions (2-fold) of TPO ranging from 16.67 to 0.13%. For vehicle control, inoculated bacteria were treated with 100µl MHB containing Tween 80 and DMSO at a volume ratio of 1:10. After incubation at 37oC for 24h, the bacterial growth rate based on turbidity was monitored by measuring the optical density of the medium at 600 nm in a microplate reader. The MIC/MBC values were the minimum concentrations of TPO that inhibited bacterial growth/killedbacteria and the test was performed in triplicate.

MICROCULTURE TETRAZOLIUM ASSAY

The cytotoxic effect of TPO against human colon HCT116 and HT-29 cell lines, and liver HepG2 cancer cells were determined with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The cells were seeded in 96 well plates at a density of 10^4 cells/well. Diluted TPO at a concentration of 1.46 %(v/v) was prepared by dissolving 7µl of the oil in 482µl of complete medium containing 5%DMSO. After 24h, cells were exposed to serial dilutions (2-fold) of TPO ranging from 1.46 to 0.01%(v/v). For vehicle control, the cultivated cells were treated with 100µl of complete medium containing 5% DMSO, while the well containing free culture medium served as blank. Following incubation at 37oC in a 5%CO_2 incubator for 24h, 5µl of 0.5% MTT solution was added to each well, followed by incubation in the dark for 4h. Thereafter, 100µl of DMSO was added to each well and the absorbance was measured at 570 nm in a microplatereader. The test was performed in triplcate wells and the percentage (%) growth inhibition was calculated as follows:

$$\%\text{Growth inhibition rate} = 100 - \frac{\left[\begin{array}{l}\text{Absorbance sample} - \\ \text{Absorbance blank}\end{array}\right]}{[\text{Absorbance control} - \text{Absorbance blank}]} \times 100$$

Statistical analysis

Data are expressed as mean± standard deviation (Sd). Statistical analysis of data was performed using version 10.3 MegaStat. Differences between means of unpaired samples were estimated using unpaired sample *t*-test and one-factor analysis of variance (ANOVA). Differences were assumed statistically significant at $p > 0.05$.

RESULTS

Chemical profiles of TPO

A pale yellow TPO was extracted using the hydro-distillation method, with a yield of 0.26 % ± 0.01 (v: w). The chemical compositions of TPO were identified with gas chromatography-mass spectrometry according to their elution profiles on HP5-MS column. Fifty-eight compounds representing 98.7 % of the total composition of TPO were identified, as shown in Table 1 and classified in Fig. 1. Oxygenated sesquiterpenes (44.18 %) and monoterpene hydrocarbons (32.84 %) were the main compounds identified in TPO, followed by sesquiterpene hydrocarbons (17.28 %) and oxygenated monoterpenes (3.82 %). Non-terpenoid compounds accounted for only 0.55 % of compounds identified in TPO. The sesquiterpenoid alcohol, τ-cadinol was characterized as the most predominant oxygenated sesquiterpenoid compound, followed by β-eudesmol, cis-Z-alpha-bisabolene epoxide, caryophyllene oxide and ylangenal. α-Fenchene was identified as the major monoterpenoid hydrocarbon, followed by β-myrcene, α-phellandrene and o-cymene. Among the sesquiterpene hydrocarbons, the most abundant compound was γ-cadinene, followed by epi-bicyclosesquiphellandrene, α-copaene, γ-elemene, β-bourbonene, α-humulene, α-gurjunene, cis-β-farnesene, β-elemene, β-calacorene, and longiverbenone. The relative abundance of oxygenated monoterpenes was (-)-carvone, L-α-terpineol, (-)-trans-pinocarveol, longiverbenone and (-)-bornyl acetate. The other identified components were present in trace amounts (less than 0.45 %).

Phytochemical composition and free radical-scavenging properties of TPO

The total contents of phenolic and flavonoid compounds in TPO were determined. The results showed that the total phenolic contents of TPO was 138.33 ± 3.1 µg GA equivalent/mL, while the total flavonoid content was 19±2.15 µg CH mL^{-1}. The free radical scavenging capacity of the oil increased in a concentration-dependent fashion, with an IC_{50} of 61.38 ± 4.33 µg mL^{-1}.

Antibacterial activity of TPO

The agar disc diffusion and micro-broth dilution methods were used to evaluate the antibacterial activity of TPO

Table 1: Percentages compositions of *Teucrium polium* L. oil

No	RT	Compound	%	No	RT	Compound	%
1	3.6489	2,2,3,4-Tetramethylpentane [1)]	0.01	30	36.4572	Epi-bicyclosesquiphellandrene	3.07
2	8.3281	α-phellandrene	3.11	31	37.2205	α-copaene	1.53
3	11.8618	β -Myrcene	8.02	32	37.853	γ-elemene	1.36
4	12.3266	α-terpinene	0.08	33	38.0578	(-)-β-bourbonene	1.17
5	12.3598	o-cymene	0.89	34	38.5833	α-gurjunene	0.88
6	12.3911	α-fenchene	20.09	35	39.7467	β-elemene	0.58
7	16.0224	Trans-beta-ocimene	0.03	36	40.1971	β-copaene	0.29
8	16.4641	3-carene	0.24	37	40.8536	Cis-β-farnesene	0.64
9	17.9746	Terpinolene	0.11	38	40.8536	α-humulene	1.00
10	19.3664	Limonene oxide	0.10	39	43.7406	γ-cadinene	5.22
11	21.1724	Trans-sabinene hydrate	0.05	40	45.2717	Cubenene	0.09
12	21.7132	(-)-Trans-pinocarveol	0.46	41	46.9337	β -calacorene	0.45
13	22.0767	Verbenol	0.06	42	49.431	Ledol	0.36
14	22.4348	Camphor	0.14	43	50.4395	τ-cadinol	25.58
15	23.2379	Pinocarvone	0.19	44	51.3221	β-eudesmol	11.76
16	23.5298	Borneol	0.07	45	53.2567	α-calacorene	0.37
17	25.034	Umbellulone	0.11	46	53.5751	Longiverbenone	0.45
18	25.4295	L- α- terpineol	0.52	47	53.5762	Ylangenal	0.84
19	27.191	Verbenone	0.28	48	55.2904	Cis-Z-alpha-Bisabolene epoxide	2.71
20	28.0751	Geraniol	0.29	49	58.9456	3,6-Dimethylundecane	0.04
21	28.3788	(-)-Carvone	0.82	50	60.0631	Caryophyllene oxide	2.48
22	28.4588	Thymol	0.21	51	61.8595	Phytone	0.15
23	31.0731	Carvone oxide, cis-	0.06	52	63.4964	Diisobutyl phthalate	0.24
24	31.0736	Piperitenone oxide	0.11	53	64.0749	Ylangenol	0.28
25	32.1757	(-)-Bornyl acetate	0.41	54	67.5275	Dibutyl phthalate	0.19
26	33.4299	4-Terpinenyl acetate	0.11	55	75.3378	Heneicosane	0.02
27	34.8954	Germacrene B	0.06	56	83.2969	4,6-Dimethyldodecane	0.01
28	35.7283	Cadina-3,5-diene	0.12	57	86.7025	Pentacosane	0.03
29	35.9863	α -terpinyl acetate	0.03	58	93.2121	Hentriacontane	0.01
Identified compounds (%) = 98.7%							

1. Compounds are listed according to the order of their elution on a polar column (HP-5MS).
RT: Retention times in minutes

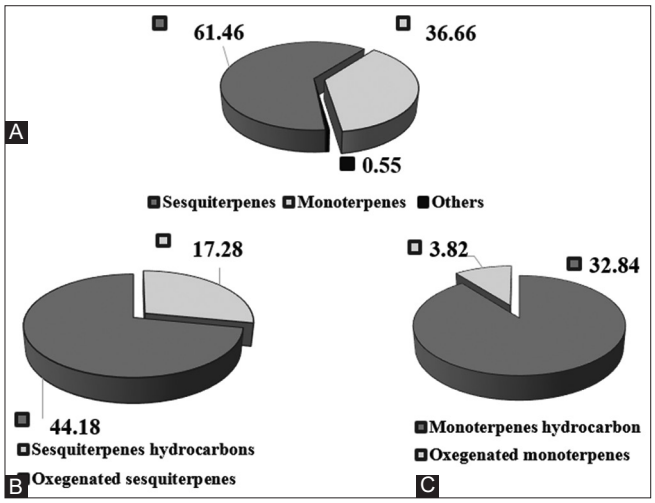

Fig 1. Bie charts showing proportions of the identified compounds in oil extracted from *Teucrium polium* L. A) general classification of sesquiterpenes, monoterpenes and other trace components, B) oxygenated and hydrocarbon sesquiterpenes, and C) oxygenated and hydrocarbon monoterpenes in oil extracted from *Teucrium polium* L. analyzed by GC-MS.

on two Gram +ve bacteria i.e, MRSA and *S. aureus,* and four Gram -ve bacteria i.e., *S. sonnei* and *P. aeruginosa, K. pneumoniae* and *E. coli.* In these tests, 10 µl of TPO was employed at concentrations of 16.67 and 100 % (v: v). As shown in Fig. 2, at 100 % TPO, bacterial growth suppression was observed in *S. aureus*, MRSA and *S. sonnei,* with inhibition zones of 20 ± 0.03, 13 ± 0.002 and 8 ±0.0.05, respectively. At 16.67 % (v: v) TPO, the growth of *S. aureus* and MRSA were reduced, with inhibition zone areas of 13± 0.09 and 11± 0.13 mm respectively, while the growth of *S. sonnei* was not affected. The oil did not produce any zone of inhibition with respect to the growth of *E. coli*, *P. aeruginosa* and *K. pneumoniae.*

The effect of TPO on the growth inhibition curves of the selected bacterial strains were evaluated with the microdilution broth method, and the results are shown in Fig. 3. The growth rates of MRSA and *S. aureus* were significantly inhibited after treatment with low and high concentrations of TPO, relative to control ($p \leq 0.001$). In

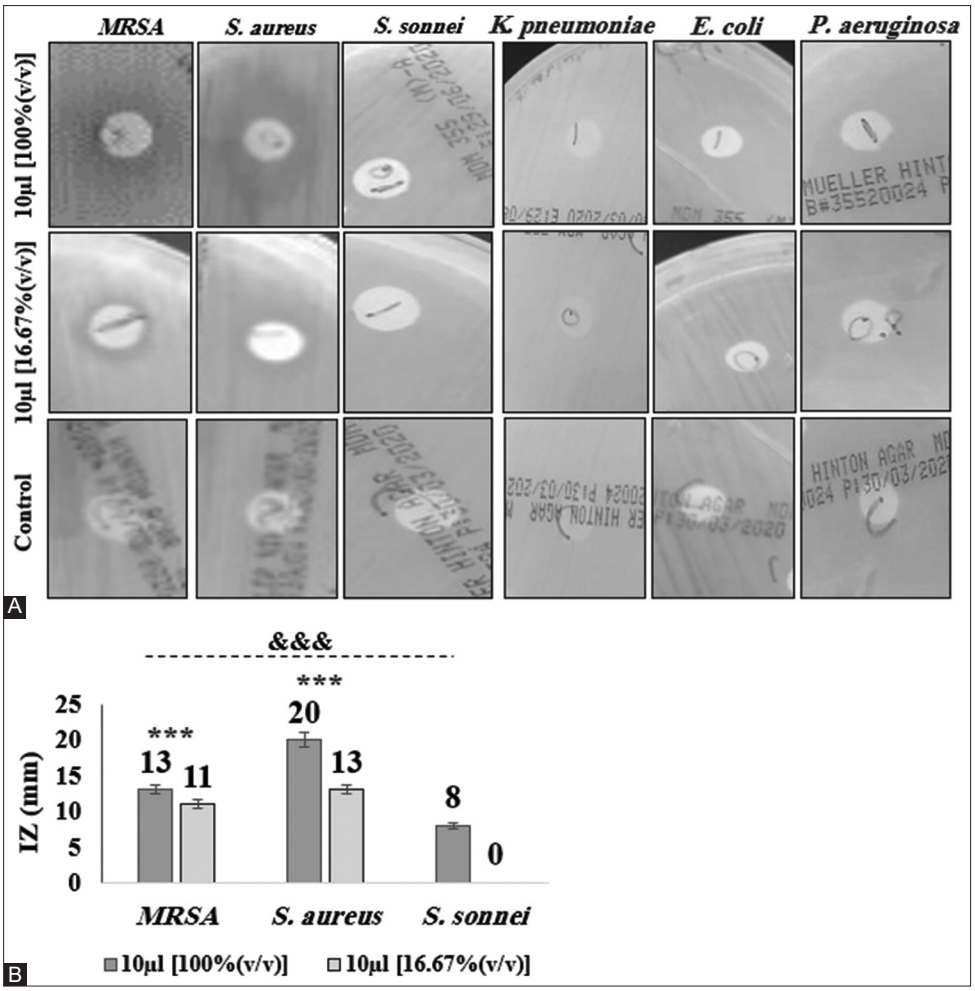

Fig 2. A) Disk diffusion assay showing the antimicrobial activity of 10µl of TPO at a concentration of 100% and 16.67%. B) Par chat showing IZ of bacteria strains MRSA, *S. aureus* and *S. sonnei*. Data are given as mean± SD. The symbols *** and &&& indicate significant differences ($p< 0.001$) between the concentrations in each strain and between strains at the same concentration, respectively. *K. pneumoniae*, *E. coli* and *P. aeruginosa* showing no inhibition zone.

particular, TPO produced strong inhibitory and bactericidal effects on MRSA and *S. aureus,* with MIC/MBC of 0.26/0.52 and 0.5/21.04, respectively. In contrast, rapid growths were observed in *K. pneumoniae*, *E. coli*, *S. sonnei* and. *P aeruginosa* after incubation with various concentrations of TPO, relative to their corresponding controls.

Anticancer activity of TPO

In the present study, the antiproliferative effects of TPO on HCT116, HT-29 and HepG2 cancer cells were determined. As shown in Fig.4, TPO inhibited the growth of these cells in a concentration-dependent manner ($p> 0.001$). The colon cancer cells HCT116 and HT-29 were more sensitive to the treatment than HepG2 liver cancer cells which were markedly inhibited only at the last two concentrations ($p< 0.001$). Similar TPO concentrations produced 30 and 90%, respectively, of growth inhibitions in the colon cancer cells (Table 2). However, HT-29 was more sensitive, since its growth was inhibited 50 % by half of concentration of oil needed for 50 % inhibition of HCT116.

DISCUSSION

In the present study, the yield of TPO (0.26%± 0.01, v: w) falls within the range of 0.14 to 0.6 % (v: w) reported in the literature (Kerbouche et al., 2015a, Cozzani et al., 2005, Bendjabeur et al., 2018a). Chromatographic data showed that the sesquiterpenes were dominant in TPO, accounting for 61.46 % of the identified compounds, which is in agreement with previous works (Kerbouche et al., 2015a, Bendjabeur et al., 2018a, El Atki et al., 2020, Saleh et al., 2020). The major components TPO in the present study were τ-cadinol, α-fenchene, β-eudesmol, β-myrcene, γ-cadinene, α-phellandrene, Epibicyclosesquiphellandrene, cis-Z-alpha-bisabolene epoxide, caryophyllene oxide, α-copaene, γ-elemene, β-bourbonene, α-humulene, o-cymene, α-gurjunene and (-)-carvone. These results are consistent with those

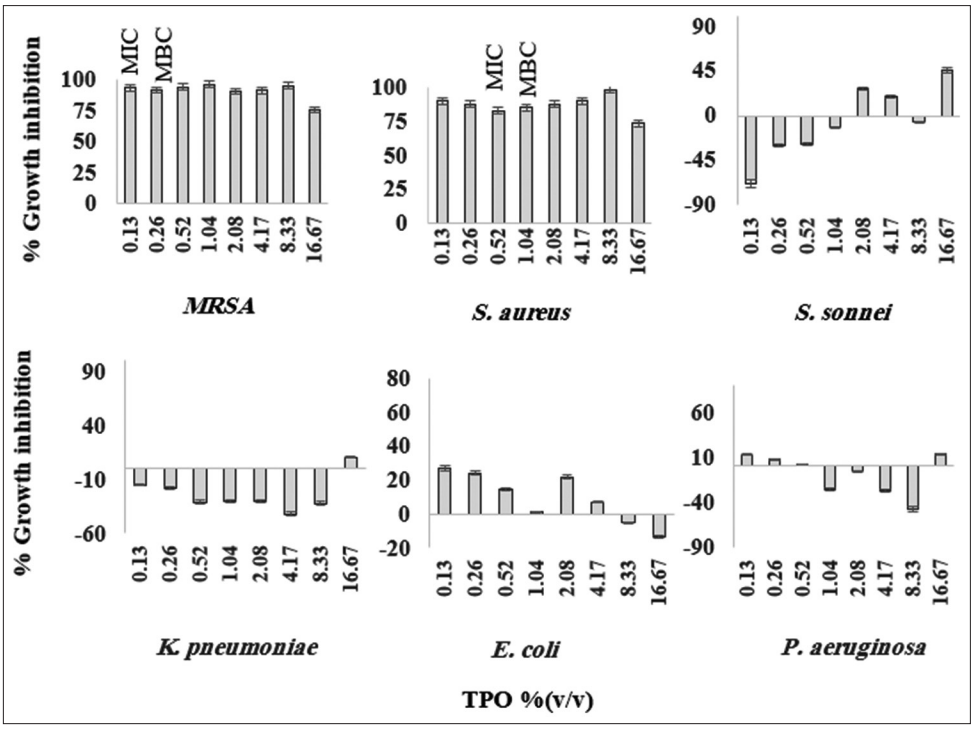

Fig 3. Effect of TPO on the growth of bacteria after treatment with two-fold serial dilutions of TPO ranging from 16.67 to 0.13%. The minimum concentration that completely restricted bacterial growth is considered as minimum inhibitory concentration (MIC). The minimum concentration that killed bacteria is the minimum bactericidal concentration (MBC). Data are presented as mean ± SD, (*n*=3).

Table 2: Concentrations of TPO (%) at 30, 50 and 90% inhibition for HCT116 and HT-29 cells after 24h treatment.

	TPO %(v/v)			Ratios	
	IC_{30}	IC_{50}	IC_{90}	IC_{50}: IC_{30}	IC_{90}: IC_{50}
HC116	0.02±0.01 (NS)	0.14±0.06 **	0.73±0.004 (NS)	2	5.21
HT-29	0.05±0.001	0.07±0.03	0.73±0.08	1.4	10.34

obtained in previous studies conducted on oil isolated from the aerial parts of *T. polium* worldwide during the flowering phase. However, the chemical profiles of TPO in the present study appeared to be somewhat different from those previously reported. Oil from *T. polium* collected from different Algerian regions contained τ-cadinol at levels of 5.5 - 18.3% (Kerbouche et al., 2015b, Bendjabeur et al., 2018b). In addition, τ-cadinol was one of the major compounds in the TPO from the aerial parts of the first and third samples of the Portuguese *T. polium*, at levels of 5.5 and 24 %, respectively (Antunes et al., 2004). Maizi et al. (2019b) showed that β-myrcene, τ-cadinol and α-phellandrene were the most predominant compounds (4.02, 3.67 and 3.45 %, respectively) in TPO isolated from *T. polium* grown in North Western Algeria. The essential oil from aerial parts of Corsican *T. polium* contained 2.9 % myrcene (Djabou et al., 2012). β-Myrcene (6.07 %) was identified as one of major compounds in TPO from Tunisian *T. polium* (Bakari et al., 2015). In addition, previous studies found that essential oil from the aerial parts of *T. polium* in Greece and Kashmar contained the major compounds caryophyllene oxide at levels of 5 and 25.9 %, respectively, in addition to other constituents (Menichini et al., 2009a, Khani and Heydarian, 2014).

In the present study, TPO produced 50 % inhibition of $DPPH^{\cdot}$ at 61.38± 4.33 μg mL^{-1}. Previous studies revealed different $DPPH^{\cdot}$ scavenging activities of TPOs extracted at the flowering phase from *Teucrium polium* L., with IC_{50} values ranging from 9200 to 16.14±0.15 mg/mL (Mahmoudi and Nosratpour, 2013, Maizi et al., 2019a). The differences in the chemical compositions of TPO obtained from the aerial parts of *T. polium* during the flowering stage, between this study and those reported in previous works, could be ascribed to the variations in climatic conditions and environmental factors, geographical locations and genetic differences (Assaeed et al., 2020, Abd-ElGawad et al., 2019). The discrepancies could also be due to differences in methods used in plant drying and oil extraction (Saleh et al., 2020). These factors greatly influence the antioxidant properties of TPO. In addition, the free radical scavenging capacity of TPO may be probably related to the qualitative and quantitative variations in sesquiterpenes and monoterpenes which

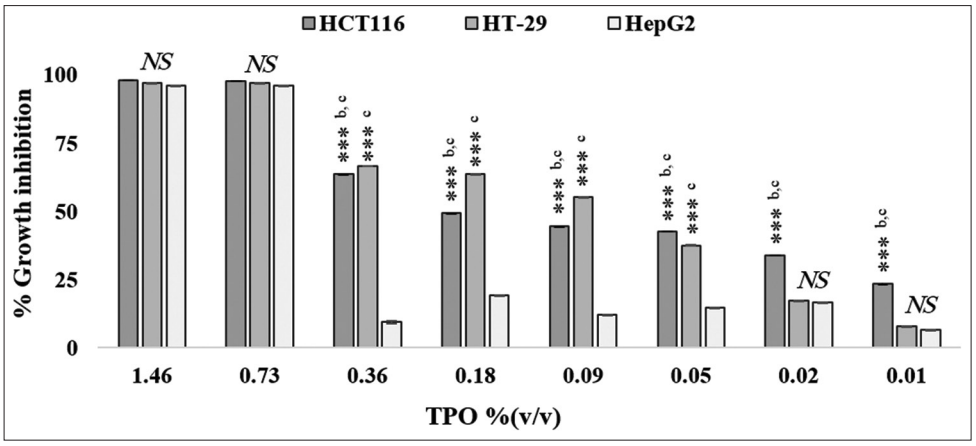

Fig 4. Percentage growth inhibition values for HCT116, HT-29 and HepG2 after 24h of treatment with eight concentrations of TPO. The symbol *** indicates significant differences (p< 0.001) between the cells at the same concentration. The symbols b and c indicate the statistical difference of HT-29 and HepG2, respectively as compared with HCT116. The symbols *NS* indicates no significance. Data are shown as mean ± SD (n=3).

have been reported to improve antioxidant network and redox balance by antagonizing oxidative stress (de Cássia Da Silveira e Sá et al., 2015, Noacco et al., 2018, Bendjabeur et al., 2018a).

The extracted TPO revealed potential antibacterial activity against Gram +ve strains (*S. aureus* and MRSA), and weak activity against Gram -ve strain *S. sonnei,* with IZ of 20± 0.03, 13± 0.002 and 8±0.05 mm after treatment with 10 ul of 100 %(v/v) TPO. With 10 ul of 16.67 % (v/v), the growths of *S. aureus* and MRSA were inhibited, with mean inhibition zones of 13±0.09 and 11±0.13 mm, with MIC/MBC values of 0.26/0.52 and 0.5/21.04, respectively, while the growth of *S. sonnei* was not affected. The disc diffusion assay was constrained by the aquaphobic nature of TPO which prevented its diffusion through the agar medium, while it produced a better effect in the liquid medium (Bilia et al., 2014). The growth of the Gram-negative strains *P. aeruginosa, K. pneumoniae* and *E. coli* was not inhibited when subjected to TPO. In general, it was previously demonstrated that Gram-positive bacteria were more susceptible to TPO than Gram-negative ones. The difference in sensitivity to essential oils could be due to the outer lipo-polysaccharide membrane in the structure of the cell envelope of Gram-ve bacteria; this effectively delineates the periplasmic space from the cytoplasmic membrane, a feature which could prevent the diffusion of hydrophobic compounds (Nazzaro et al., 2013). On the other hand, the antibacterial properties of TPO may be correlated with the presence of oxygenated sesquiterpenes (Saleh et al., 2020). τ-Cadinol (Su et al., 2015) β-eudesmol (Sellam et al., 2013) and caryophyllene oxide (Montanari et al., 2011) which previously exhibited antimicrobial properties, collectively represented 39.82 % of the major identified oxygenated sesquiterpenes in TPO. Previous studies have shown the antibacterial activities of monoterpenes (Sellam et al., 2013). It has been demonstrated that β-myrcene, one of the identified hydrocarbon monoterpenes in TPO, enhanced the membrane-fluidizing activity of oil as well as its antibacterial activity (Poleć et al., 2020). In addition, it has been reported that α-phellandrene mildly stimulated macrophage proliferation in mice (Lin et al., 2013), suggesting its ability to suppress intracellular growth of bacteria. Moreover, about 6.72% of the minor components may contribute to the antimicrobial effect of TPO, and may probably involve some type of synergism with other major active constituents (Cutillas et al., 2018).

The results of MTT assay revealed marked growth inhibition of colon cancer cells HT-29 and HCT116 by TPO, with IC_{50} values of 0.07±0.03 and 0.14±0.06%, respectively. In contrast, HepG2 cells had marked growth inhibition only at high concentrations of TPO, while the inhibitory effect was decreased as the concentration of oil decreased. The components of TPO exert anti-proliferative effects against cancer cells *in vitro*. In particular, the alcohol sesquiterpene, τ-cadinol which accounted for 25.58% of TPO in this study, was reported to be cytotoxic on human lung, liver and colon cancer cells (Su et al., 2013). Another sesquiterpene alcohol, β-eudesmol (11.76%) diminished the proliferation of HepG2 cancer cells and stimulated caspase-mediated tumor cell death pathway (Kotawong et al., 2018, Bomfim et al., 2013). Moreover, β-eudesmol triggered programmed cell death of human leukemia HL60 cells via a mitochondrial apoptotic pathway. The cytotoxic and apoptotic effects of β-eudesmol on HL60 cells were characterized by fragmentation of DNA, downregulation of Bcl-2 expression, cleavage of poly (ADP-ribose) polymerase, caspase-3 and caspase-9; release of cytochrome c from mitochondria, and decrease in mitochondrial membrane

potential (Li et al., 2013). In addition, the anticancer effects of TPO against lung carcinoma (COR-L23), colon adenocarcinoma (COCA-2) and amelanotic melanoma (C32) have been attributed to the presence of sesquiterpenes such as caryophyllene oxide in oil *T. polium* L. (Menichini et al., 2009b). Caryophyllene oxide exerted a potent anticancer effect on human osteosarcoma cancer cells (MG-63) by preventing cancer cell migration and stimulating apoptosis, as was evident in cellular shrinkage, membrane blebbing, condensation of chromatin, formation of apoptotic bodies and induction of the production of reactive oxygen species in these cells (Pan et al., 2016). Studies on the effect of β-baurbonene on human prostate PC-3M cancer cells revealed that it could induce apoptosis and G0/G1 arrest through upregulation of mRNA expression of Fas and FasL, increased Bax protein expression, decreased expression of Bcl-2 protein, and inhibition of the cellular proliferation (Karan et al., 2018).

Monoterpenes act on cancer cells through multiple chemotherapeutic and chemo-preventive mechanisms (Rodenak-Kladniew et al., 2020). An oxygenated monoterpene, myrcene produced antiproliferative effect through activation of apoptotic mechanism via mitochondrial-mediated cell death signaling, and induced oxidative stress in human non-small lung A459 cancer cells (Bai and Tang, 2020). In addition, α-phellandrene influenced a wide range of effects on expression of genes that affect DNA repair, cell cycle and apoptosis in WEHI-1 murine leukemia cells (Lin et al., 2013). It has been reported that L-carvone exerted antitumor activity on myeloma cells through the inhibition of p38 MAPK signaling pathway, induction of apoptosis and inhibition of cell invasion (Ding and Chen, 2018). Moreover, reported that L-carvone stimulated p53 and caspase-3 mediated cell death, and inhibited cell migration in breast cancer (Patel and Thakkar, 2014).

CONCLUSION

In the present study, the volatile compounds of essential oil extracted at the flowering stage from the aerial parts of *T. polium* L. grown in Huraymila, 80 km from Riyadh, are reported for the first time. The volatile compounds differed from those of the same species reported elsewhere all over the world at the same growing stage of the plant. Eighty-five compounds were identified, with sesquiterpene alcohols as predominant compounds: τ-cadinol and β-eudesmol, followed by monoterpene hydrocarbons: α-fenchene, β-myrcene and α-phellandrene; and sesquiterpene hydrocarbons: γ-cadinene, epi-bicyclosesquiphellandrene, α-copaene, γ-elemene, β-bourbonene and α-humulene. The observed differences in chemotypes in essential oils extracted from different *T. polium* L. plants species are attributed to differences in geographical origin, climate and environmental variations, in addition to differences in extractionmethods. The results of this work have demonstrated that TPO possesses antioxidant, antimicrobial and antiproliferative activities. These findings are useful data on biodiversity, and they may be beneficial in food biotechnology and pharmaceutical industries. Further identification of Saudi ecospecies of different part of *T. polium* L. plants at different growing periods could give more detailed identification at the sub-level. In addition, future studies are needed to investigate the biological importance of α-fenchene.

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Conflict of interest

No conflict of interest associated with this work.

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