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REVIEW ARTICLE

Antioxidant properties and anti-quorum sensing potential of *Carum copticum* essential oil and phenolics against *Chromobacterium violaceum*

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Abstract The chemical composition, antimicrobial and antioxidant properties of *Carum copticum* essential oil and its methanolic extract were investigated. Thirteen compounds were identified representing 99.3% of the total oil composition. Oxygenated monoterpenes (53.0%) dominated the *C. copticum* essential oil with high contents of thymol (51.7 ± 1.51%), *p*-cymene (26.9 ± 1.11%), γ-terpinene (16.7 ± 0.76%), and β-pinene (1.6 ± 0.15%). In the methanolic extract, the caffeic, gallic, chlorogenic, coumaric and ferulic acids, flavan-3-ols (catechin), flavone (hyperoside), and the flavonol quercetin were identified. Antimicrobial activity of essential oil and the organic extract was tested by disk diffusion and broth microdilution method. The essential oil was effective against the tested bacteria and yeast strains with the highest activity and the MICs and MBCs values were lower as compared to the methanolic extract. The essential oil showed anti-quorum sensing activity against *Chromobacterium violaceum*, and the IC_{50} value for violacein inhibition was 0.23 mg/ml. Both the essential oil and the methanolic extract also showed antioxidant activities. The results obtained highlight the potential use of *C. copticum* as a possible source of antimicrobial and antioxidant compounds to be used both as food flavor and as a broad spectrum antibiotic.

Keywords *Carum copticum* · Essential oil · Antimicrobial activity · Antioxidant activity · Anti-quorum sensing

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Introduction

Medicinal plants represent a natural source of phyto-compounds with antioxidant and antimicrobial properties, and have extensive application in the food industry. To prevent the spoilage due to foodborne pathogens, the use of plant-derived components are the safe alternative as compared to synthetically prepared antibiotics (Ríos and Recio 2005). The antibiotic production, biofilm formation and virulence properties of many pathogenic bacteria are controlled by a cell-to-cell signaling process mediated by low molecular weight diffusible signal molecules. This system is known as quorum sensing (QS) or cell-to-cell communication (Bhardwaj et al. 2013) and bacteria use QS system to sense and respond to their population by regulating the expression of a set of genes required for the bacterial physiology. Targeting QS system has attracted a significant attention as it does not impose the selection pressure that the conventional antibiotics exert. Plant metabolites including the

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essential oils display QS modulatory properties (Nazzaro et al. 2013).

Carum copticum (*Family*: *Apiaceae*) is an annual herbaceous flowering plant grown in arid, and semiarid regions including central Europe, Asia, India (in the states of Rajasthan Gujarat and West Bengal), Iran (eastern regions of Baluchistan), Iraq Afghanistan, Pakistan, and Egypt (Zahin et al. 2010). The fruit has an aromatic odor and a spicy taste, and is widely used as a spice in the curry powder (curry). The fruits are also used in the folk medicine due to their antibacterial, antifungal, anti-inflammatory, anti-vomiting, antiasthma, anti-spasmodic, antitussive, anti-flatulent, anti-arthritic, anti-hypertensive, bronchodilator and analgesic properties (Gilania et al. 2005). The composition of its essential oil is largely dependent on the geographic origin of the plant. The major constituents are carvacrol, thymol, *p*-cymene, or γ-terpinene (Mohagheghzadeh et al. 2007). Moreover, the fruit acetone extract has thymol, oleic acid, linoleic acid, γ-terpinene, *p*-cymene, palmitic acid, and xylene as main compounds. The methanol extract of the fruit contain saponins, flavonoids, tannins, phenols, alkaloids, anthraquinones, monoterpenoids, and thymol (Singh et al. 2004).

In the present work, we report the phytochemical compositions of the essential oil and the methanol extract obtained from *C. copticum* seeds and their biologically important activities such as antioxidant, antimicrobial and anti-QS potential.

Materials and methods

Plant material and samples preparation

The dried seeds of *C. copticum* were purchased from a local market from Jeddah (KSA) in 2014. The plant was identified by Pr. Al-Sieni Abdulbasit, and a voucher specimen (SM-CC-01) was deposited in the CERTE (Technopark of Borj Cedria Tunisia). The volatile oil was obtained by hydrodistillation by using a Clevenger-type apparatus as previously described by Snoussi et al. (2015).

Analysis of the volatile oil

GC/EIMS analyses were performed as previously described (Flamini et al. 2007) with a Varian CP-3800 GC equipped with a HP-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector.

Identification of polyphenols in the methanolic extract

Ultra-performance liquid chromatography (UPLC) analyses were carried out using an ACQUITY Ultra Performance LCTM system (Waters Milford MA, USA) linked simultaneously to a PDA 2996 photodiode array detector (Waters) as previously described by Fratianni et al. (2013).

Analysis of phenolic compounds and flavanoids in the methanol extract

Phenolic content was assayed using the Folin–Ciocalteu (FC) reagent following the slightly modified Singleton's method (Dewanto et al. 2002). Total phenolic content was calculated from the calibration curve with gallic acid (0–400 μg/ml) and expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g DW). Total flavonoids were measured according to Dewanto et al. (2002). Total flavonoids were estimated from the calibration curve using (+)-catechin (0–400 μg/ml) and expressed as mg (+)-catechin/g DW (mg CE/g DW). Proanthocyanidins were measured using the modified vanillin assay (Sun et al. 1998). The amount of total condensed tannins is expressed as mg (+)-catechin/g DW.

Antioxidant activities of the essential oil and the methanolic extract

Total antioxidant capacity was evaluated using the assay described by Prieto et al. (1999). This is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acidic pH. Gallic acid in the range of 0–500 μg/ml concentration was used for generating the calibration curve. The DPPH radical scavenging activity of all the test samples was measured according to the method described by Hanato et al. (1998). The radical scavenging activity was expressed as IC_{50} (μg/ml). The ability to scavenge the DPPH radical was calculated using the following Eq. (1):

$$\text{DPPH scavenging effect}\,(\%) = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

where A_0 and A_1 are respectively the absorbance of the control and sample at 30 min.

β-Carotene bleaching test was also used to estimate the antioxidant activity according to a previously described method (Koleva et al. (2002). The antioxidant activity (AA) of the extracts was evaluated in terms of β-carotene bleaching using the following formula (2):

$$AA\% = (A_0 - A_1/A_0) \times 100 \quad (2)$$

where A_0 is the absorbance of the control at 0 min and A_1 is the absorbance of the sample at 120 min. The results are expressed as IC_{50} values (μg/ml).

Screening for antimicrobial and antifungal activities

Disk diffusion assay was used for primary screening of antimicrobial activity, and a microdilution method for the determination of the minimal inhibitory concentration (MIC) and the minimal bactericidal/fungicidal concentration were used in the present study. Twenty four important microorganisms were used. These included nine Gram positive, sixteen Gram negative bacteria, and five *Candida* spp. strains. Pure bacterial colonies were used to prepare 0.5 McFarland turbidity standards in API suspension medium. The fungal suspensions in API medium were adjusted to 2 McFarland turbidity standards. The bacterial/yeast/fungal lawns were prepared using sterile swabs on freshly prepared agar plates. Sterile filter-paper disks (diameter 6 mm Biolife, Italy) impregnated with 10 μl of the crude methanolic extract (at 10, 30, and 50 mg/ml) or the essential oil (10 mg/disk), were placed on the agar plates and these Petri dishes were kept for 1 h at 4 °C. The antimicorbial activity was evaluated by measuring the diameter of growth inhibition zone around the disks after incubating at 37 °C for 24 h (48 h for the yeasts). The scheme proposed by Parveen et al. (2010) was used to interpret the antimicrobial activity based on the mean diameter of zone of inhibition (GIZ) obtained. The activity was interpreted as: low (GIZ: 1–6 mm), moderate (GIZ: 7–10 mm), high (GIZ: 11–15 mm), very high (GIZ: 16–20 mm), and no activity (GIZ = 0 mm). For comparison of the activity Ampicillin (10 mg/ml) and Amphotericin B (10 mg/ml) were used as positive controls.

To estimate the minimum inhibitory concentrations (MICs), broth microdilution method was used. Twofold serial dilution starting from 25 mg/ml using 10% stock solution of the essential oil in DMSO was prepared in 96-well plates. The MIC was defined as the lowest concentration of compound capable of inhibiting the growth of the microorganisms. To determine the MBC and MFC values, 10 μl cultures from the wells with no visible growth were inoculated to appropriate agar medium to assess the growth if any and the concentration at which no growth was recorded as the MBC or the MFC. Ampicillin and Amphotericin B were used as reference. Interpretation of the activity based on the MIC was made according to Aligiannis et al. (2001): MIC 0.05–0.5 mg/ml (strong activity), 0.6–1.5 mg/ml (moderate activity), and MIC > 1.5 mg/ml (weak activity).

Screening for anti-quorum sensing (anti-QS) activities

Anti-QS activities were tested using two strains; *Chromobacterium violaceum* ATCC 12472 and *C. violaceum* CV026. The bacteria were grown in Luria–Bertani (LB) medium at 32 °C for 24 h. For the *C. violaceum* CV026 agar plate bioassay, LB medium was supplemented with C_6-HSL (Sigma). Inoculum was prepared in 10 ml LB broth incubated at 32 °C for 24 h. The culture turbidity was measured by recording the OD_{600} spectrophotometrically (UV-1800 Shimadzu Japan).

Evaluation of QS inhibition was tested by using the CV026 reporter strain. For this, 2 μl of essential oil or thymol was loaded to the sterile disks and placed on the surface of CV026 inoculated LB agar plates supplemented with C_6-HSL (50 μl of 1 μg/ml stock). The plates were incubated upright for 24 h at 32 °C and zone of inhibition of QS was indicated detected by the presence of colourless but viable cells around the disks and the zone of growth inhibition was also recorded by clear zone around the disks.

For quantification of violacein inhibition, twofold serial dilutions of the essential oil were prepared in 96-well plates starting from 5 mg/ml in LB broth and inoculated with *C. violaceum* ATCC 12472. After incubation for 24 h at 32 °C, the growth in each well was recorded using a multimode plate reader. The MIC was calculated based on the OD_{600} readings of the treatments with respect to control. For quantification of violacein the contents of the wells were aspirated into Eppendorf tubes and centrifuged (8000 rpm 6 min) to collect cells. Violacein was extracted from the cells using water saturated n-butanol. The extracted violacein was separated from the cell debris by centrifugation and quantified by recording OD_{585} readings spectrophotometrically. Percentage inhibition of violacein by the essential oil was calculated with respect to control and 50% inhibition concentration (IC_{50}) was recorded.

Statistical analysis

All the experiments were carried out in triplicates and results were presented as the mean values ± standard deviations. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used for evaluating the significance of the difference between means. Differences at $p < 0.05$ were considered statistically significant.

Results

Essential oil composition

The phytochemical composition of the tested essential oil is summarized in Table 1. Altogether, 13 compounds were identified representing 99.3% of the total oil chemical composition. Oxygenated monoterpenes (53.0%) dominated the *C. copticum* essential oils with thymol (51.7 ± 1.51%) as the main representative. On the contrary, *p*-cymene (26.9 ± 1.11%), γ-terpinene (16.7 ± 0.76%), and β-pinene (1.6 ± 0.15%) were the most abundant monoterpene hydrocarbons identified.

Phytochemical analysis of the methanolic extract

The major compounds in the *C. copticum* methanolic extract are presented in the Fig. 1. The phenolic acids identified using the UPLC-DAD technique were; caffeic, gallic, chlorogenic, coumaric, and ferulic acids, the flavan-3-olcatechin, the flavone hyperoside, and the flavonol quercetin. Overall phenolic acids represented 40.88% of the total polyphenols identified by UPLC technique. The flavonoid hyperoside (11.034 mg/g) followed by quercetin (10.004 mg/g) were the two dominant phenolic compounds (26.25 and 23.80% of the total composition, respectively). While, gallic and chlorogenic acids represented 13.14 and 11.98%, respectively (Table 2).

Table 1 Chemical composition of *Carum copticum* essential oil

Constituents	l.r.i.	% Mean ± SD
Monoterpene hydrocarbons (46.3%)		
α-Thujene	933	0.2 ± 0.06
α-Pinene	941	0.2 ± 0.00
β-Pinene	982	1.6 ± 0.15
Myrcene	993	0.4 ± 0.10
p-Cymene	1028	26.9 ± 1.11
Limonene	1032	0.3 ± 0.10
γ-Terpinene	1063	16.7 ± 0.76
Oxygenated monoterpenes (53.0%)		
Linalool	1101	0.1 ± 0.10
4-Terpineol	1179	0.3 ± 0.06
α-Terpineol	1191	0.1 ± 0.06
Cumin aldehyde	1241	0.3 ± 0.15
Thymol	1292	51.7 ± 1.51
Carvacrol	1301	0.5 ± 0.10
Total identified		99.3

The data are expressed as mean ± SD (n = 3)

l.r.i. Linear Retention Index, *SD* standard deviation

Polyphenols in the methanol extract

Colorimetric quantification of total polyphenols showed the presence of 23.42 ± 2.5 mg of GAE/g DW of total polyphenols in the methanol extract. High concentrations of flavonoids (364.41 ± 6.74 mg EC/g DW) and tannins (336.33 ± 2.88 mg EC/g DW) were also found in *C. copticum* methanolic extract.

Antioxidant activity

The methanolic extract showed higher antioxidant capacities compared to the essential oil (Table 3). The methanol extract exhibited antioxidant activity of 34.45 ± 4.33 mg GAE/g DR. The The IC_{50} for DPPH radical scavenging by the methanolic extract was 30.33 ± 0.57 µg/ml, while the IC_{50} was 4.7-fold higher for the essential oil (143.33 ± 2.88 µg/ml). The EC_{50} for the reducing power were 466.66 ± 14.43 µg/ml and 1800 ± 115.47 µg/ml for the methanolic extract and essential oil respectively. The EC_{50} for ascorbic acid was 37.53 ± 0.39 µg/ml.

Antibacterial and antifungal activities

Data reported in Table 4 shows the results of antibacterial and antifungal activities of *C. copticum* essential oil and the methanolic extract as compared to the standard antimicrobial drugs (Ampicillin and Amphotericin B). For the essential oil, the GIZ varied significantly from 11.33 mm for *K. pneumoniae* to 52.33 mm for *S. aureus* ATCC 6816. These diameters were significantly higher as compared to the antibacterial drug tested (Ampicillin) and with respect to those obtained using the methanol extract. Overall, the activity of the essential oil was higher against Gram-positive bacteria (GIZ ranging between 35 and 52.33 mm) compared to Gram-negative strains (except *E. coli* ATCC 25922). Interestingly, the methanol extract exhibited a different sensitivity towards two different strains of *V. alginolyticus*, *V. vulnificus* and *V. parahaemolyticus*. Such variation was also shown for the two strains of *E. coli* with respect to the essential oil. Moreover, the two *V. vulnificus* strains showed a completely opposite response with the essential oil and the methanol extract.

The essential oil exhibited high activity also against the yeast strains tested with GIZ ranging from 32.33 to 51.67 mm. These diameters were significantly higher than those obtained for the methanol extract (9.67–12.67 mm) and the positive control (Amphotericin B: 6–14.67 mm). The MIC ranged from 0.024 to 0.048 mg/ml and the MFCs of the essential oil ranged from 1.56 to 3.125 mg/ml.

In this study, we also demonstrated the QS inhibitory potential *C. copticum* essential oil against the QS

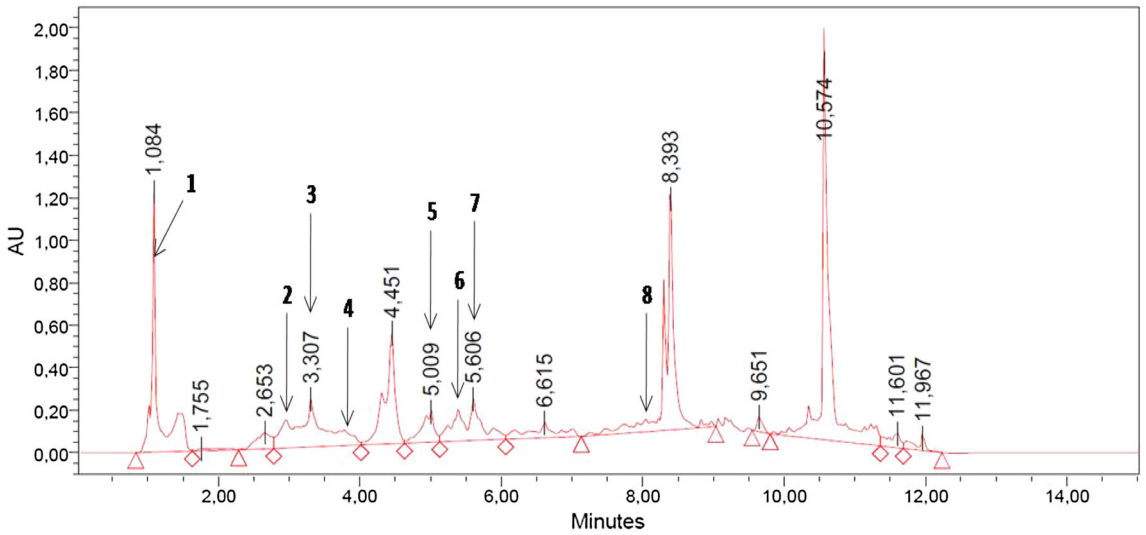

Fig. 1 Ulra-performance liquid chromatogram of methanolic extract from seeds of *Carum copticum*. *AU* arbitrary units. 1, Gallic acid; 2, chlorogenic acid; 3, catechin; 4, caffeic acid; 5, coumaric acid; 6, hyperoside; 7: ferulic acid; 8, quercitin

Table 2 Quali-quantitative analysis of polyphenols in the *C. copticum* methanolic extract by using the ultrahigh-performance liquid chromatography

Polyphenols	Retention time (min)	Concentration (mg/g)	%
Gallic acid	1.084	5.034	11.98
Chlorogenic acid	2.953	5.525	13.14
Catechin	3.307	3.814	9.07
Caffeic acid	3.833	1.496	3.56
Coumaric acid	5.009	1.419	3.38
Hyperoside	5.391	11.034	26.25
Ferulic acid	5.606	3.709	8.82
Quercetin	8.044	10.004	23.80

% percentage

Table 3 Phenolic contents and antioxidant activities of the essential oil and the methanolic extract of *C. copticum* seeds

Antioxidant activities tested	Essential oil	Methanolic extract	Ascorbic acid	BHA
TAA: (mg GAE/g DR)	–	34.45 ± 4.33	–	–
Total polyphenols: (mg GAE/g DR)	–	23.42 ± 2.5	–	–
Flavonoids: (mg EC/g DR)	–	364.41 ± 6.74	–	–
Tannins: (mg EC/g DR)	–	336.33 ± 2.88	–	–
DPPH: IC_{50} (µg/ml)	143.33 ± 2.88	30.33 ± 0.57	–	–
Reducing power: EC_{50} (µg/ml)	1800 ± 115.47	466.66 ± 14.43	37.53 ± 0.39	–
β-Carotene: PI (%)	–	113.96 ± 0.39	–	48.00 ± 0.50

TAA: total antioxidant activity is expressed as mg gallic acid equivalents per gram of dry residue; total polyphenols is expressed as mg gallic acid equivalents per gram of dry residue; tannins is expressed as mg (+)-catechin/g of dry residue; DPPH radical-scavenging activity is expressed as IC_{50} values (µg/ml); Reducing Power activity is expressed an EC_{50} values (µg/ml). β-carotene bleaching test is expressed as PI (%)

dependent phenotypic expression of violacein in *C. violaceum*. The anti-QS activity of the essential oil is shown in Fig. 2. The essential oil at 2 µl/disk showed anti-QS activity, while, the major component thymol did not show anti-QS activity in *C. violaceum*. However, the minor compounds such as linalool and cuminaldehyde showed anti-QS activity. Quantitative estimation on the effect of different concentration of essential oil on the growth of *C.*

Table 4 Growth inhibitions zone, MIC and MBC/MFC values of *C. copticum* essentail oil and methanolic extract tested against Gram+ and Gram− bacteria, and yeasts

Microorganisms	Essential oil			Methanolic extract	Ampicillin		
	GIZ ± SD[a]	MIC[b]	MBC[c]	GIZ ± SD[a]	GIZ ± SD*	MIC	MBC
Bacteria							
A. viridans	43.33 ± 1.15^{g}	0.024	0.39	11.67 ± 0.57^{h}	14.67 ± 0.57	0.011	1.5
E. coli	23.33 ± 0.57^{m}	0.048	6.25	12.33 ± 0.57^{fgh}	27.33 ± 0.57	0.011	1.5
E. coli ATCC 25922	50.33 ± 0.57^{c}	0.024	3.125	10.33 ± 0.57^{i}	11.67 ± 0.57	0.023	3
Shewanella putrefaciens	32.33 ± 0.57^{k}	0.048	3.125	13.67 ± 0.57^{bcd}	7.00 ± 0	0.023	0.75
S. flexenerii ATCC 12022	42.67 ± 0.57^{f}	0.024	3.125	14.00 ± 0^{bc}	10.67 ± 0.57	0.023	0.093
S. typhimirium ATCC 14028	24.33 ± 0.57^{m}	0.096	3.125	12.33 ± 0.57^{fgh}	17.67 ± 1.15	0.023	0.093
E. faecalis ATCC 29212	35.00 ± 1^{i}	0.048	6.25	12.33 ± 0.57^{fgh}	13.67 ± 0.57	0.023	0.093
V. parahaemolyticus ATCC 17802	34.67 ± 0.57^{ij}	0.048	0.39	7.33 ± 0.57^{j}	13.33 ± 0.57	0.011	3
V. alginolyticus ATCC 17749	37.00 ± 1^{h}	0.048	0.78	15.67 ± 0.57^{a}	12.33 ± 0.57	0.011	3
V. vulnificus ATCC 27562	42.67 ± 1.15^{f}	0.024	0.78	12.00 ± 0.57^{gh}	30.33 ± 0.57	0.023	6
V. alginolyticus ATCC 33787	37.67 ± 0.57^{h}	0.048	0.78	13.33 ± 0.57^{cde}	13.33 ± 0.57	0.023	6
V. vulnificus ATCC 33149	37.67 ± 0.57^{h}	0.048	3.125	13.33 ± 0.57^{cde}	12.33 ± 0.57	0.046	1.5
V. parahaemolyticus ATCC 43996	43.33 ± 0.57^{f}	0.024	3.125	13.00 ± 0^{def}	12.00 ± 0	0.011	6
V. cholerae ATCC 9459	26.00 ± 1^{l}	0.048	3.125	12.33 ± 0.57^{fgh}	7.00 ± 0	0.011	12
P. aeruginosa ATCC 27853	20.00 ± 0^{n}	0.048	6.25	12.67 ± 0.57^{efg}	22.67 ± 0.57	0.011	1.5
Serratia marscecens	32.00 ± 0^{k}	0.048	3.125	13.00 ± 0^{def}	13.67 ± 0.57	0.011	1.5
M. luteus NCIMB 8166	50.33 ± 0.57^{c}	0.024	3.125	12.67 ± 0.57^{efg}	30.33 ± 0.57	0.023	0.375
L. monocytogenes ATCC 19115	45.67 ± 0.57^{e}	0.024	3.125	13.33 ± 0.57^{cde}	12.33 ± 0.57	0.023	0.093
B. subtilis ATCC 6633	52.00 ± 0^{ab}	0.024	6.25	12.00 ± 0^{gh}	11.33 ± 0.57	0.011	3
S. aureus MR (B2)	50.33 ± 0^{c}	0.048	0.39	12.00 ± 0^{gh}	16.33 ± 0.57	0.011	1.5
S. aureus ATCC 6816	52.33 ± 0.57^{a}	0.024	6.25	11.67 ± 0.57^{h}	24.33 ± 0.57	0.011	0.093
S. epidermidis ATCC 12228	41.00 ± 1^{g}	0.048	3.125	13.33 ± 0.57^{cde}	12.33 ± 0.57	0.011	0.187
B. cereus	35.33 ± 0.57^{i}	0.048	1.56	14.33 ± 0.57^{b}	14.67 ± 0.57	0.023	1.5
K. pneumoniae	11.33 ± 0.57^{o}	0.096	6.25	12.33 ± 0.57^{fgh}	17.33 ± 0.57	0.011	1.5
P. mirabils	41.33 ± 0.57^{g}	0.024	6.25	12.00 ± 0^{gh}	25.67 ± 0.57	0.023	0.375
Yeasts					*Amphotericin B*		
					*GIZ ± SD**	*MIC*	*MFC*
C. tropicalis 06-085	51.67 ± 0.57^{abc}	0.024	1.56	12.33 ± 0.57^{a}	6 ± 0	0.39	6.25
C. parapsilosis ATCC 22019	50.33 ± 0.57^{c}	0.024	1.56	12.00 ± 0^{a}	10.33 ± 0.57	0.195	0.39
C. krusei ATCC 6258	32.33 ± 0.57^{k}	0.048	3.125	12.67 ± 0.57^{a}	12 ± 0	0.097	0.195
C. glabrata ATCC 90030	50.33 ± 0.57^{c}	0.024	3.125	13.00 ± 1^{a}	14.33 ± 0.57	0.195	1.562
C. guilliermondi 06-018	51.00 ± 1^{bc}	0.024	1.56	9.67 ± 0.57^{b}	21 ± 1	0.024	0.781
C. albicans ATCC 2019	33.67 ± 0.57^{j}	0.048	3.125	12.33 ± 0.57^{a}	14.67 ± 0.57	0.024	0.781
S. cerevisiae 11-161	48.67 ± 1.15^{d}	0.024	3.125	12.33 ± 0.57^{a}	8.67 ± 0.57	0.39	3.125

SD standard deviation

*Inhibition zone around the discs impregnated with Ampicillin or Amphotericin B (10 mg/ml) expressed as mean of three replicates (mm ± SD)

[a, b, c, d, e, f, g, h, i, j, k, l, m, n, o] Means followed by the same letters are not significantly different at $P = 0.05$ based on Duncan's multiple range tests

violaceum showed a MIC of 0.6 mg/ml and the IC_{50} value for violacein inhibition was 0.23 mg/ml.

Discussion

It is clear from the results that the chemical composition of *C. copticum* volatile oil varies depending on the source of raw material. Thymol was identified as the main component in the essential oil from Iran (Kazemi et al. 2011). The

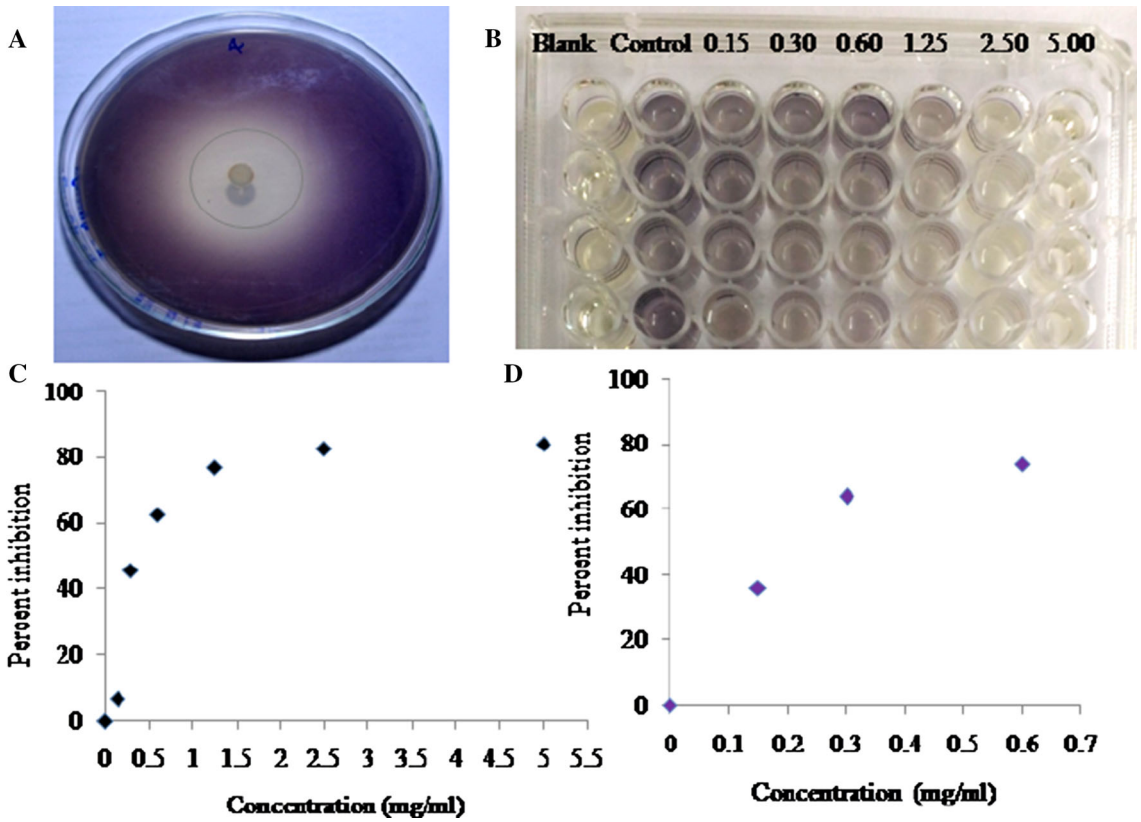

Fig. 2 Anti-quorum sensing activity of *C. copticum* essential oil tested on LB agar (**a**), microplate assay (**b**) and determination of MIC (**c**) and IC_{50} value for the violacein inhibition (**d**)

main components of the oil of African and Iranian *C. copticum* oil are γ-terpinene and *p*-cymene. Kazemi et al. (2011) reported the identification of thymol (72.3%), terpinolene (13.12%), and *o*-cymene (11.97%) as the mains constituents of *C. copticum*. Additionally, Mahboubi and Kazempour (2011) reported varying concentrations of thymol (45.9%), γ-terpinene (20.6%), and *o*-cymene (19%) in the oil of *C. copticum* from Iran. According to Srivastava et al. (1999), the main constituents of the fruits oil of this species were *p*-cymene (41.98%), carvacrol (45.20%), and thymol (0.48%). These differences in essential oil profiles are the result of different geographical locations and cultivation techniques and also depend on the extracting methods.

The UPLC-DAD technique has been already applied to analyze other types of vegetables and herbs. Previous phytochemical studies of *C. copticum* fruits described many phyto-constituents like steroptin, cumene, thymine, tannins, and essential oils components such as thymol, γ-terpinene, *p*-cymene, and other bioactive molecules (Mohagheghzadeh et al. 2007). In 2010, Zahin and colleagues reported the identification of four components in the methanol extract of *C. copticum* seeds extract by using the GC–MS technique in particular thymol (95.14%), methyl ester (1.54%), *cis*, *cis*-linoleic acid (2.55%), and 3-nitrophthalic acid (0.77%) were characterized.

Our results on the polyphenols in the methanolic extract are in good agreement with those reported by Kazemi (2015), who reported a total phenolic content of 200.07 ± 37 mg GA/g of dry material and total flavonoid content of 94.07 ± 29 mg of rutin/g of dry aqueous extract of *C. copticum* plants at early flowering stage. These phenolic compounds are responsible for the taste, color, aroma, odor, and health-beneficial effects.

As reported by Kavoosi et al. (2013), the Carum oil (thymol chemotype) has a high radical scavenging capacity for ROS, RNS, H_2O_2, and TBARS with an IC_{50} values about 8.6 ± 1.4, 8.1 ± 1.5, 6.1 ± 0.95, and 6.8 ± 1.1 mg ascorbic acid per gram of carum oil respectively. The *C. copticum* essential oil also has broad range activity including the ability to reduce the stable DPPH and H_2O_2 radicals (Samojlik et al. 2010). The antioxidant and antiradical properties are attributed to the presence of compounds such as monoterpenes and oxygenated sesquiterpenes. The monoterpenes such as thymol, *p*-cymene, γ-terpinene, and β-pinene can significantly add to the antioxidant activity of the *C. copticum* essential oil. Further, thymol has been reported to elicit several biological activities including the radical scavenging activity.

The antimicrobial potency of the extracts from Carum seeds differs among Gram- positive and Gram-negative and type of microorganism tested (bacteria or yeast). This let us to hypothesize that the action of the essential oil could be influenced among other things from the different structure of the microbial cell wall (Nazzaro et al. 2013). Likewise, the antibacterial activity of methanol extract differed depending on the strains tested. This suggests that the antimicrobial and antifungal activity tests must be carried out using two or more strains within each species (bacterial or fungal). *C. copticum* essential oil from different origins has been established with antibacterial activity against a large set of bacteria (Singh et al. 2002; Zomorodian et al. 2015) The antimicrobial activity of *C. copticum* essential oil can be associated to the high amount of the oxygenated monoterpene constituents especially thymol and its monoterpenes hydrocarbon precursors: *p*-cymene and γ-terpinene.

The hydrophobic molecules such as γ-terpinene and *p*-cymene thymol induce their antimicrobial activity by interacting with the lipid fraction of the microbial membrane leading to leakage of intracellular constituents (Trombetta et al. 2005). In combination with other oxygenated monoterpenes, *p*-cymene incorporated by the bacterial lipid bilayer facilitates the transport of the phenolic compounds across the bacteria cytoplasmic membrane (Juliano et al. 2000). This synergism between the major and the minor compounds can explain the stronger antibacterial activity of the whole essential oil as compared to the individual major components.

The essential oil also exhibited high activity against *Candida* spp. and *S. cerevisiae* strains. These activities were significantly higher than those obtained for the methanol extract and the antifungal compound tested (Amphotericin B). To inhibit the fungal growth, very low concentrations of the essential oil was sufficient. The MFCs s values of the essential oil was lower as compared to Amphotericin B. Kavoosi et al. (2013) demonstrated similar high antifungal activity of the thymol-rich *C. copticum* essential oil from Iran with MFCs of 5.6 ± 1.3 µg/ml against *A. niger*, and 8.8 ± 2.2 µg/ml against *C. albicans* strains.

Many plant essential oils have ability to modulate virulence properties controlled by the quorum sensing mechanisms in both Gram positive and Gram negative bacteria. The anti-quorum sensing activity of essential oils extracted from *Cuminum cyminum*, *Zingiber officinale*, *Myristica fragrans*, *Syzygium aromaticum*, *Cinnamomum verum*, *Rosmarinus officinalis*, and *Curcuma longa* against *C. violaceum* ATCC 12472 have been reported by Ganesh and Rai (2015) investigated. They also showed that all the tested essential oils exhibited antimicrobial activity and essential oils from *C. cyminum*, *S. aromaticum*, *C. verum*, *R. officinalis*, and *C. longa* inhibited the violacein production at different concentrations. Due to complex composition of the essential oils, they offer a large specter of QS inhibitors. The mechanisms of QS inhibition can be explained by (1) the competition between the essential oil components and the signal molecules to be bind to their respective receptors on the bacterial cell wall, (2) the degradation of the cell to cell signal molecules, and (3) the inhibition of reception of signal molecules. It is possible that plant essential oils exhibiting anti-QS activity might influence bacterial QS-controlled phenotypes by inhibiting AHL synthesis or through binding the AHL receptors. The ani-quorum sensing and antimicrobial activities of the *C. copticum* essential oil may have multiple benefits if used as valuable therapeutic entities.

Taken together, the results obtained in the present work suggest that the essential oil and the methanol extract of *C. copticum* exhibit a broad spectrum and a high antibacterial and antifungal activities along with antioxidant effect. Hence, *C. copticum* could represent a good source for food and drug preparations. The essential oil and the metabolites make *C. copticum* a versatile food and medicinal commodity. It can be used to develop new generation medicinal products with high efficacy and less adverse effects.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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