Coronatine elicitation alters chemical composition and biological properties of cumin seed essential oil

Seyedeh Faezeh Taghizadeh, Ramin Rezaee, Masoumeh Mehmandoust, Fatemeh Sadat Madarshahi, Aristidis Tsatsakis, Gholamreza Karimi

ABSTRACT

The present experiment evaluated how coronatine (COR) elicitation affects chemical and biological properties of cumin (Cuminum cyminum L.) seed essential oil (CSEO). Following isolation of the EO, its chemical composition was analyzed by gas chromatography-mass spectrometry; also, its bioactivities in terms of antimicrobial/antifungal, cytotoxic (measured by MTT assay) and antioxidant effects (evaluated by DPPH, β-carotene bleaching (BCB) and TBARS methods) were evaluated. COR-elicitation significantly increased CSEO yield and the level of its chemical components, especially cumin aldehyde which is the main component of CSEO. Results showed that COR-elicitation significantly reduced the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of CSEO against 4 Gram-positive and 3 Gram-negative bacteria and 2 fungi. Moreover, elicitation markedly enhanced the antioxidant and in vitro cytotoxic activity of CSEO. Therefore, COR may be regarded as a useful biotic elicitor for improving EO chemical and biological properties.

1. Introduction

Essential oils (EOs) are natural oily liquids with diverse bioactivities used as additives, flavors and fragrance, in cosmetics, food and drugs [1,2].

Apiaceae family members (e.g. Cumin (Cuminum cyminum L.), caraway (Carum carvi L.), fennel (Foeniculum vulgare Mill.), coriander (Coriandrum sativum L.), anise (Pimpinella anisum L.), dill (Anethum graveolens L.) and parsley (Petroselinum crispum L.)) with more than 25 million tons production per year, are industrial crops from which, volatile compounds, hydrocarbons and aldehydes are isolated and used for commercial purposes [3].

Cumin is grown from northern Africa to the west and central Asia and it has been traditionally grown in Morocco, Turkey, Syria, Greece, Egypt and India [4]. Iran is one of the major producers and exporters of this plant [5]. The seeds of cumin yield an aromatic spice with medicinal properties [6]. Cumin seeds essential oil (CSEO) is commonly utilized in food and cosmetics [7].

Cumin is regarded as an important raw materials for pharmaceutical, food, cosmetic, and perfume industries. From a medicinal point of view, cumin seeds exert digestion- and appetite-enhancing properties and have been used for treatment of vomiting, fever, diarrhea, abdominal distention, edema, skin disorders (as the seeds contain high levels of vitamin E), hoarseness, epilepsy, and jaundice [6]. Since cumin are remarkable sources of iron and calcium (with > 66 mg iron and > 900 mg calcium/100 g seed powder), they are recommended for pregnant and especially lactating mothers. Cumin seeds also increase milk secretion from mammary glands and this activity was reported to be mediated by its bioactive components such as thymol. Chief chemical constituents of CSEO are cuminaldehyde (p-
isopropylbenzaldehyde and p-cuminaldehyde), aldehyde, β-pinene, γ-terpinene, p-cymene, p-mentha-1, 3-dien-7-al and p-mentha-1, 4-dien-7-al. Cuminaldehyde and thymol can act as detoxifying agents and remove the toxic substances from the body [8].

Biological/pharmacological studies showed antioxidant, anticancer, cytotoxic, anti-inflammatory, and anti-bacterial/fungal effects for CSEO [9]. Thanks to its antimicrobial activities, cumin can be used to prevent biofilm-formation to protect human, animal, food and land against Streptococcus mutans and Streptococcus pyogenes. Also, CSEO exhibits antifungal activity against food, animal and human pathogens, yeasts and mycotoxin-producers. Furthermore, cumin is widely employed in food industry as an additive to fish and meat. The EO extracted from cumin stem is used to flavor alcoholic drinks and desserts. It is also a candidate for food packaging industries to protect fresh products. Cumin oil is used in cosmetic industry and the odoriferous oil obtained from stems is used in creams, lotions and perfumes [8].

In recent years, many studies examined the effects of biological elicitors [10]. It was proven that biotic and abiotic stresses induce their effects in plants via similar mechanisms [11]. Several phytohormones which act as elicitors can combat the negative effects of abiotic stressors like extreme temperature and salinity, radiation and high humidity, or mechanical stressors [12]. Different biotic and abiotic stresses (e.g. insects, fungi, wounds, light, salinity and drought) may affect EO production [11].

Coronatine (COR) is a newly-introduced non-host-specific phytoxin derived from Pseudomonas syringae. COR has structural and functional similarities to jasmonic acid (JA) and methyl jasmonate (MeJA), but it is more active and induces its effects through other modes of action [13]. This compound contains two distinct components namely, polyketide coronafacic acid, and coronamic acid (an iso-leucine-derived ethylcyclopropyl amino acid) [14]. Following the synthesis of the two above-noted moieties, COR is formed after addition of an amide group [15]. Mechanistically, COR induces defense responses by increasing the levels of phytochemicals, chlorophyll, and secondary metabolites, and enhancing the activities of several enzymes [16].

Various factors (e.g. environmental stressor and chemical treatments) can influence the concentration and composition of plants’ secondary metabolites. COR can protect plants by enhancing the production of bioactive compounds. COR was successfully used in various plants cell suspension culture where it induced the expression of some key genes [17]. The effects of different concentrations of COR on flavonoid content of rice were reported by Tamogami and Kodama [18]. In another study done in Eschscholzia californica cell cultures, COR enhanced alkaloid production [18]. Low concentrations of COR in Corylus avellana L. cell suspension culture, induced taxane production [19].

Considering medicinal/industrial value of cumin, in the present study, the effects of COR on (1) yield and chemical composition, as well as (2) antioxidant, (3) antimicrobial and (4) cytotoxic properties of CSEO were examined.

2. Material and methods

2.1. Plant materials and treatments

The present study was carried out during 2016–2017, at an experimental farm in Mashhad (at 36°28′N and 59°54′E, 985 m above sea level), Khorasan Razavi province, Iran. Cumin seeds were randomly collected at seed ripening (dry umbels) stage, identified and authenticated by taxonomists at Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. Initially, seeds were treated with 5% (w/v) sodium hypochlorite solution for 5 min and rinsed three times with distilled water. Next, 1500 g of cumin seeds was split into two 750-mg parts: One part was soaked in 750 mL distilled water (considered control) and the other part was soaked in 750 mL of 1 mM COR solution (considered COR-elicited); both samples were kept at 24°C with relative humidity of 70%, until the distilled water or COR was completely absorbed. The concentration of the elicitor was selected based on previously published reports [20,21].

2.2. Reagents and standards

L-glutamine, penicillin/streptomycin, FBS (fetal bovine serum) and trypsin–EDTA were obtained from Gibco, MT1 (3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide), SCDA (soybean casein digest agar), MHB (Mueller-Hinton broth), TTC (2,3,5-triphenyl-terazolium chloride), SDB (dextrose broth), COR (coronatine), DPPH (1,1-diphenyl-2-picryl-hydrazil), BHT (butylhydroxytoluene), β-carotene, TBA (Thiobarbituric acid), ABAP (2′,2′-azo-bis (2-aminopropane) dihydrochloride), SDS (sodium dodecyl sulfate), DMSO (dimethyl sulfoxide) and RPMI1640 medium were procured from Sigma Aldrich (St. Louis, Missouri, USA).

2.3. Extraction equipment

2.3.1. Extraction of CSEO

Cumin seed essential oil (CSEO) was obtained from air-dried materials by hydro-distillation using a Clevenger-type apparatus for up to 3 h. CSEO was dried-out using anhydrous sodium sulfate and left in the dark at 4°C for future experiments.

2.4. CSEO characterization

2.4.1. Extraction yield (content)

The extraction yield of the CSEO was calculated using equation (1) [2]:

\[
\text{Extraction yield (%) = } \frac{\text{Volume of essential oil collected (µL)}}{\text{Initial weight of the dry seeds (g)}} \times 100
\]

2.4.2. Gas chromatography equipped with mass spectrometry (GC-MS) analysis

In this study, we used gas chromatography (GC) due to its capacity of separation of multi-component mixtures with low molecular weights that are stable at high temperatures. CSEO was dissolved in n-hexane and analyzed by GC (Agilent 5975 apparatus) with a HP-5MS column (30 m × 0.25 mm, with 0.25 μm film thicknesses) equipped with a quadruple mass detector and Wiley 7n.Library. GC-MS analysis had the following specifications: oven temperature: 50°C (for 5 min), 50–250°C (increased at 3°C/minute), and 250°C (for 10 min); injector temperature: 250°C; injector volume: 0.1 μL; split ratio: 1:50; helium was used as the carrier gas with a flow rate of 1.1 mL/min; ionization potential: 70 eV; ionization current: 150 μA; and mass range: 35–465 m/z. To identify the chemical composition of CSEO, retention indices (RI) of samples and their mass spectra were compared with the previously reported ones [22]. The relative amount of each component was calculated using the area under the curve percentage without considering the calibration factor.

2.5. Evaluation of antioxidant power

2.5.1. DPPH assay

DPPH assay is frequently employed to evaluate the antioxidant power of chemicals [23,24]. The antioxidant capacity of CSEO in terms of reduction of DPPH radicals, was assessed as previously explained by Fernández-Agulló et al. [25]. In this experiment, 2.5 mL of different concentrations of CSEO (0.625–80 mg/mL) was added to 1 mL of a methanolic DPPH solution. The mixture was rapidly shaken and maintained in the dark for 30 min. Then, samples’ absorbance was measured at 518 nm. The percentage of CSEO radical-scavenging
activity was calculated using equation (2):

Percentage of scavenging effect = \( \frac{ADPPH - As}{ADPPH} \times 100 \)  \( (2) \)

Where \( As \) and \( A_{ADPPH} \) are the absorbance of the sample and DPPH solution, respectively. CSEO concentration causing 50% reduction in DPPH free-radical levels was considered \( IC_{50} \). Ascorbic acid and DPPH solution (1.0 mL and 0.3 mM, respectively) and methanol (2.5 mL) served as positive and negative controls, respectively [25].

2.5.2. β-carotene bleaching (BCB) assay

Based on the method described by Kuliscic et al., 1 mL β-carotene solution (0.2 mg/mL in chloroform) was mixed with 20 mg linoleic acid and 100 mg Tween 40. Following evaporation of chloroform (at 50 °C for 5 min), 100 mL distilled water was added and the mixture was emulsified (emulsion A) for 1 min using sonication. Emulsion B was prepared through mixing 20 mg linoleic acid, 200 mg Tween 40 and 50 mL oxygenated water. Next, 200 μL of different concentrations of CSEO and BHT (used as positive control) was mixed with 5 mL of the emulsion A. Also, a control which lacked antioxidants, was provided by mixing 200 mL methanol with 5 mL of emulsion A. To calibrate the spectrophotometer, a mixture of 200 μL methanol and 5 mL of emulsion B was prepared. Before incubation and after 120 min of incubation, the absorbance was read at 470 nm [26]. The inhibition percentages were calculated using equation (3):

Inhibitions percentages (%) = \( \frac{[As(120) - Ac(120)]}{[Ac(0) - Ac(120)]} \times 100 \)  \( (3) \)

In equation (3), \( A_{s(120)} \), \( A_{c(120)} \) and \( A_{c(0)} \) are sample’s absorbance at 120 min, control’s absorbance at 120 min, and control’s absorbance at 0 min (i.e. before incubation), respectively [26].

2.5.3. TBARS method

Using TBARS assay, radical-scavenging capacity of CSEO was investigated [26]. Here, 0.5 mL of 10% (w/v) tissue homogenate and 0.1 mL of different concentrations of CSEO were mixed with methanol to prepare final concentrations of 0.625–80 mg/mL; of note, these samples were immediately used after preparation. Afterwards, 0.05 mL of 2, 2'-azo-bis (2-aminopropane) dihydrochloride (ABAP) solution (0.07 M in water) was added to induce lipid peroxidation. Also, 1.5 mL of 20% acetic acid (pH 3.5) was mixed with 1.5 mL 0.8% (w/v) thiobarbituric acid in 1.1% (w/v) sodium dodecyl sulfate (SDS) solution, vortexed, and heated at 95 °C for 60 min. The mixture was cooled down and 5.0 mL of butanol was added. Then, tubes were shaken and centrifuged at 2500 g for 10 min. The absorbance of the upper organic layer was read by a spectrophotometer at 532 nm. Antioxidant percentage (A%) was determined by equation (4):

Antioxidant percentages (%) = \( \frac{1 - As}{AC} \times 100 \)  \( (4) \)

In this equation, \( A_{c} \) is the control’s absorbance and \( A_{s} \) is the absorbance of each sample.

2.6. Antimicrobial activity

2.6.1. Bacteria and culture media

CSEO antimicrobial properties were examined against 4 Gram-positive (Staphylococcus aureus (PTCC (Persian Type Culture Collection) 1337), Staphylococcus epidermidis (PTCC 1435), Micrococcus luteus (ATCC (American Type Culture Collection) 9341) and Bacillus cereus (PTCC 1247) and 3 Gram-negative (Pseudomonas aeruginosa (ATCC 15442), Salmonella typhi (PTCC 1609), and Escherichia coli (ATCC 10536)) bacteria. All bacterial strains were cultured for 24 h (at 37 °C) on soybean casein digest agar (SCDA) and adjusted to 106 CFU/mL using 0.9% sterile normal saline (NS).

2.6.2. Evaluation of MIC and MBC of CSEO

In this part, using DMSO, CSEO was dissolved in Mueller-Hinton broth (MHB). The first concentration (10% v/v) of CSEO was prepared by addition of 650 μL of CSEO to 325 μL DMSO and the final volume of the mixture was adjusted to 6.5 mL using MHB. By using two-fold serial dilution method, the other concentrations of CSEO were made. Next, a mixture of 200 μL of each concentration of CSEO and 20 μL of bacterial suspension (106 CFU/mL) was added to each well of a 96-well culture plate. For each bacterium, the experiment was done in duplicates. MHB served as the negative control.

Following 24-h incubation at 37 °C, 20 μL of 2, 3, 5-triphenyltetrazolium chloride (TTC) (5 mg/mL) was added to each well for assessment of bacterial growth. The lowest concentration of the CSEO inhibiting the formation of red color in culture media was considered the minimum inhibitory concentration (MIC). In addition, the minimum bactericidal concentration (MBC) was measured by incubation of 20 μL of each well’s content (colorless content) with Mueller-Hinton agar containing media, for 24 h at 37°C [22]. For the positive control, gentamicin and vancomycin were applied against Gram-positive and Gram-negative bacteria, respectively.

2.6.3. Anti-fungal activity

The anti-fungal activity of CSEO was investigated against Candida albicans (ATCC 10231) and Aspergillus niger (ATCC 16404). Following 48-h culture on SCDA at 25 °C, a suspension of 106 CFU/mL was prepared by sterile 0.9% NS. Different concentrations of CSEO were prepared as described in the antimicrobial assay, and the volume was adjusted to 1 mL by Sabouraud’s dextrose broth (SDB). Using two-fold serial dilution technique, other concentrations of CSEO were made. Then, 200 μL of cell suspension (105 CFU/mL) was incubated for 48 h at 25 °C. Eventually, the concentration with no visible microbial growth (i.e. MIC) and the concentration with no visible fungal growth (i.e. minimum fungicidal concentration (MFC)) were determined. In this experiment, SDB and nystatin served as the negative and positive controls, respectively.

2.7. Cell culture

U-87MG (human glioblastoma cell line), MCF-7 (human breast adenocarcinoma cell line) and PC3 and DU-145 (human prostate cell lines) cells lines (Pasteur Institute, Tehran, Iran) were maintained in Dulbecco Modified Eagle Medium (DMEM, Sigma) supplemented with 10% (v/v) fetal bovine serum (FBS) (Gibco, BRL, Paisley, UK), penicillin 100 U/mL, streptomycin 100 μg/mL and 2 mm l-glutamine. Cells were cultured at 37°C in a humidified atmosphere with 5% CO2. Moreover, C-26 colon carcinoma and A2780 ovarian cancer cell lines were cultured at 37°C in a humidified atmosphere with 5% CO2 in RPMI 1640 medium containing 25 mM HEPES and 2 mM l-glutamine supplemented with 10% (v/v) heat-inactivated FCS, 100 IU/mL penicillin and 100 mg/mL streptomycin (all obtained from Gibco BRL, Paisley, UK) [27,28].

2.7.1. Cytotoxicity assay

Cytotoxicity of CSEO against six human cancer cell lines namely, U-87-MG, MCF-7, PC3 and DU-145 used, C-26 and A2780, was examined using MTT assay. Also, 3T3 was used as a normal cell line. Briefly, the cells (5 × 104 per well) were placed in a 96-well plate, each well containing 100 μL of RPMI or DMEM medium supplemented with 10% FBS. After 24 h, for adhesion, serial dilutions of the CSEO were added to wells and all experiments were done in triplicate. After 2 days, cells were incubated with 10 μL of MTT (5 mg/mL stock solution) at 37°C for 4 h. The, the medium was removed and the formazan blue crystals were dissolved by 200 μL DMSO. Since DMSO was used for dissolving for-mazan, DMSO-induced cell death was also determined. Cytotoxicity of CSEO was presented as concentration inhibiting cell growth by 50% (IC50) and the percentage of viable cells was determined using equation
Viable cell (%) = \(
\frac{(\text{Atc}-\text{Ab})}{(\text{Ac}-\text{Ab})}\) \times 100
(5)

Where Atc, Ab, Ac is the absorbance of the treated cells, blank and control, respectively [22,29,30].

2.8. Statistical analysis

All experiments were performed in triplicate and data are presented as mean ± SD. One way analysis of variance (ANOVA) and LSD test were performed to determine statistical differences among groups by JMP 8 (SAS Campus Drive, Cary, NC 27513) and Excel software. A \( p \leq 0.05 \) was considered significant. The IC\(_{50}\) values were determined by GraphPad Prism 5.0 (GraphPad software, San Diego, CA, USA).

3. Results

3.1. CSEO yield and chemical composition in control and COR-elicited groups

Based on our findings, treatment with COR significantly increased CSEO yield compared to control sample (1.89 vs. 0.87% v/w, respectively). Moreover, GC–MS analysis of CSEO revealed the presence of 31 chemicals accounting for 91.99 and 98.72% of control and COR-elicited CSEOs, respectively (Table 1). In control and COR-elicited samples, the major components were cumin aldehyde (19.54 and 21.40%, respectively), cumic alcohol (8.89 and 9.49%, respectively) and \( \alpha \)-pinene (8.20 and 8.97%, respectively).

The most abundant chemicals in CSEO obtained from COR-elicited and control groups were terpenic compounds (40.94 and 47.82%, respectively), alcohols/phenols (20.40 and 23.95%, respectively), aldehydes (20.91 and 23.42%, respectively), and esters (0.37 and 0.81%, respectively). It should be noted that COR elicitation increased the level of all components (Table 1). Interestingly, the most marked effect of COR with respect to enhancement of components levels, was observed for cumin aldehyde (increased from 19.54 to 21.40%), while it slightly increased eucaliptol, styrene and cymol contents.

3.2. COR effects on antioxidant activity

3.2.1. DPPH radical scavenging capacity

Natural antioxidant compounds can combat ROS overproduction and consequent ROS-induced diseases [31]. As a frequently used assay for measurement of the antioxidative capacity, DPPH radical scavenging method relies on reduction of DPPH free radical and evaluates the ability of the test compound for hydrogen-donation and/or radical-scavenging activity [32]; in this context, lower IC\(_{50}\) values indicate higher antioxidant activity.

According to our results, the IC\(_{50}\) value of the CSEO calculated for COR-elicited group (21.03 \( \mu \)g/mL) was significantly lower than that of the control group (29.55 \( \mu \)g/mL) (Table 2).

3.2.2. \( \beta \)-carotene bleaching assay

As a well-known spectrophotometric technique developed for assessment of antioxidant capacity, \( \beta \)-carotene bleaching (BCB) assay was used in this study. In this method, antioxidative properties of the test compound may prevent/decrease the oxidation of linoleic acid and preserve \( \beta \)-carotene's yellow/orange color [33]. Significant differences (\( P \leq 0.05 \)) in antioxidant activity were found between the control CSEO (71.22 ± 3.00 \( \mu \)g/mL) and COR-elicited CSEO (85.92 ± 2.03 \( \mu \)g/mL) (Table 2); however, BCB values calculated for both groups were lower than that of BHT (99.18 ± 2.71 \( \mu \)g/mL), at same concentrations.

3.2.3. Thiobarbituric acid reactive substances (TBARS) assay

TBARS method is usually employed to determine antioxidant potential of chemicals [34]. As noted in Table 2, TBARS assay revealed that inhibition capacity of the CSEO obtained from COR-elicited plants (72.24 ± 2.11 \( \mu \)g/mL) was significantly higher than that of the CSEO from the control sample (50.09 ± 2.38 \( \mu \)g/mL). It should be noted that, lipid oxidation preventing capacity of CSEO determined by TBARS was lower than that measured by BCB assay (Table 2).

<table>
<thead>
<tr>
<th>No</th>
<th>Compounds</th>
<th>Peak area (%) CSEO (control)</th>
<th>Peak area (%) CSEO (COR-elicited)</th>
<th>Peak area (%) BHT</th>
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<tbody>
<tr>
<td>1</td>
<td>( \beta )-farnesene</td>
<td>0.10</td>
<td>0.21</td>
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<td>2</td>
<td>( \beta )-thujene</td>
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<td>( \alpha )-Pinene</td>
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<td>Cumic alcohol</td>
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<td>2-caren-10-al</td>
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<td>Cumin aldehyde</td>
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<tr>
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<td>2-caren-10-al</td>
<td>0.78</td>
<td>0.91</td>
<td>5.03</td>
</tr>
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</table>

Table 2 Cumin (Cuminum cyminum L.) seeds essential oil composition in coronatine (COR)-elicited and control groups.

Table 2 Antioxidant activity of cumin seed essential oil.

<table>
<thead>
<tr>
<th>Assays</th>
<th>CSEO (control)</th>
<th>CSEO (COR-elicited)</th>
<th>BHT</th>
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<tbody>
<tr>
<td>DPPH (( \mu )g/mL)</td>
<td>29.55 ± 0.32</td>
<td>21.03 ± 0.32</td>
<td>9.73 ± 0.56</td>
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<td>BCB (( \mu )g/mL)</td>
<td>71.22 ± 3.00</td>
<td>85.92 ± 2.03</td>
<td>99.18 ± 2.71</td>
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<tr>
<td>TBARS (( \mu )g/mL)</td>
<td>50.09 ± 2.38</td>
<td>72.24 ± 2.11</td>
<td>94.51 ± 3.08</td>
</tr>
</tbody>
</table>

Data shown as mean ± SD.

a Retention time

b Kovats Index on the HP-5 MS column.

c TBARS: Thiobarbituric acid reactive substances.
Table 3

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>CSEO (control)</th>
<th>CSEO (COR-elicited)</th>
<th>Gentamicin</th>
<th>Vancomycin</th>
<th>Nystatin</th>
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<td>Bacterial strains</td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
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<td>S. aureus PTCC 1337</td>
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<td>E. coli ATCC 10536</td>
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<tr>
<td>Fungal strains</td>
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<td>MFC</td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
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<td>15</td>
<td>13</td>
<td>13</td>
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</tr>
<tr>
<td>A. niger ATCC 16404</td>
<td>170</td>
<td>65</td>
<td>80</td>
<td>80</td>
<td>–</td>
</tr>
</tbody>
</table>

* Essential oil obtained from control sample.
* Essential oil obtained from coronatine-treated cumin seeds.
* Minimum inhibitory concentration (μg/mL).
* Minimum bactericidal concentration (μg/mL).
* Minimum fungicidal concentration (μg/mL).

3.3. MIC and MBC values for control and COR-elicited CSEO

The influence of COR on antimicrobial activity of CSEO was examined against seven bacteria and two fungi (Table 3). According to the results, COR-elicited and control samples had similar MIC and MBC values of 8 and 16 μg/mL, respectively against S. aureus. Also, it was observed that the most sensitive strain was S. aureus. Moreover, control and COR-elicited samples had similar MIC and MFC values of 15 and 13 μg/mL, respectively against C. albicans.

In terms of antifungal activity, significant differences were observed between CSEOs from COR-elicited and control group (Table 3). Based on our findings, COR treatment had positive effects on MIC and MFC. MIC and MFC values for CSEO from COR-elicited group against C. albicans and A. niger were similarly 13 and 80 μg/mL, respectively. Furthermore, the CSEO obtained from control group had MIC and MFC values of 15 and 170 μg/mL, respectively against C. albicans; and these values against A. niger were 15 and 65 μg/mL, respectively (Table 3).

3.4. COR effect on CSEO cytotoxic properties

After 48 h of treatment, IC50 values were determined for CSEO obtained from the two groups, against different cancerous cell lines. Doxorubicin was used as a control and exhibited potent cytotoxic effects with IC50 values of 1.88 ± 0.11, 2.02 ± 0.13, 2.28 ± 0.24, 2.30 ± 0.20, 2.41 ± 0.94 and 2.62 ± 0.51 μg/mL against MCF-7, A2780, PC3, DU-145, U-87-MG and C-26, respectively. Comparison of the two groups showed that IC50 values for CSEO from COR-elicited groups were significantly lower than those of CSEO from control samples against all cell lines used (Table 4).

4. Discussion

Among natural products, EOs as complex mixtures were shown to possess promising therapeutic effects against cardiovascular and neurological diseases, diabetes and cancers. For this reason, over 250 EOs are commercialized annually [22].

Pytopathogenic organisms can manipulate plants hormones signaling, enhance plants resistance against stressors and improve the nutrients contents. Previous studies introduced MeJA and COR as bioelictors; however, COR was found to be a stronger one [16].

Our results showed significant differences between COR-treated samples and respective controls. The EO yield, levels of EO components, antioxidative activity and other biological properties were significantly improved by COR elicitation as compared to the control group. Elicitation with a red algae (Kappaphycus alvarezii) extract enhanced the extraction yield and seed nutritional qualities of green gram (Phaseolus radiate L.) [35]. Several reports showed that the levels and composition of secondary metabolites including terpenoids, flavonoids, alkaloids, anthocyanins, carotene and vitamin C, could also be influenced by biotic and abiotic elicitors [36]. For example, some abiotic elicitors like plant hormones (e.g. JA) may act as a stressor and increase the abundance of bioactive compounds in plants. It was reported that treatment with elicitors influence EOs yield and/or chemical components [37]. As shown in Table 1, significantly higher levels of cumin aldehyde, as a main component of the plant, was found in CSEO treated with COR.

Pharmacological activities of cumin have been attributed to its antioxidative potential. Data showed that stimulation of cumin seeds by COR, markedly improved free-radical scavenging potential of CSEO. Probably, EO potential in preventing linoleic acid oxidation was related to the presence of high levels of terpenic compounds (Table 2).

Alterations in the abundance of saponin were reported by previous studies following application of COR in Kalopanax septemlobores suspension cell culture [38]. It was shown that COR treatment increases the levels of hydrocinnamic acids (e.g. caffeic acid, isoleucic acid, p-cumaric acis and cinapic acid) and phytosterols (e.g. campesterol and β-sitosterol) in whole plant culture of Lemna paucicostata [39]. Another study reported that COR effectively enhances flavonoid production in cotton plants [40]. Furthermore, COR treatment enhanced the antioxidative activity in soybean and cucumber plants [41]. Consistently, taxane levels in Taxus media and Corylus avellana were increased by COR elicitor treatment [19].

Elicitors can activate various signaling pathways against microbial infection [42]. In the current experiment, antimicrobial activity of CSEO was significantly increased after stimulation with COR as a biotic elicitor. In this study, the essential oil displayed a higher activity against Gram-positive than Gram-negative strains, which is probably due to the fact that outer membrane of Gram-negative bacteria is rich in hydrophilic lipopolysaccharides (LPS) which act as a barrier against penetration of hydrophobic compounds. Owing to this structural...
property, Gram-negative bacteria show a higher degree of resistance against hydrophobic antimicrobial compounds like those found in essential oils. Nevertheless, more in-depth investigations should be conducted in future to reveal the mechanisms underlying the anti-microbial activities.

Furthermore, elicitation with COR increased cumin cytotoxicity. The IC50 values of COR-elicted CSEO against six cell lines were significantly lower than those of the control. It seems that several parameters like plant condition, as well as type and dose of elicitor may influence plant characteristics. These results indicated that COR treatment enhances biological properties of cumin EO that might be beneficial for CSEO industrial and commercial applications.

5. Conclusions

In the current study, COR elicitation significantly ameliorated yield, chemical composition and chemical and biological activities of CSEO. Elicitation seems to be a promising approach to improve the chemical/ biological quality of plants. This study provides the basis for further research on using COR elicitation to improve plants’ properties especially for industrial and commercial applications.

Conflicts of interest statement

The authors declare that they have no conflicts of interest.

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