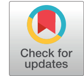
Biotechnology Reports 27 (2020) e00490

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Inhibitory effect of *C. zeylanicum*, *C. longa*, *O. basilicum*, *Z. officinale*, and *C. martini* essential oils on growth and ochratoxin A content of *A. ochraceous* and *P. verrucosum* in maize grains



Naveen Kumar Kalagatur[a], Selvakumar Gurunathan[b], Jalarama Reddy Kamasani[c], Lokanadhan Gunti[d], Krishna Kadirvelu[a], Chakrabhavi Dhananjaya Mohan[e], Shobith Rangappa[f], Ram Prasad[g,h], Fausto Almeida[i], Venkataramana Mudili[j,*], Chandranayaka Siddaiah[k,*]

[a] DRDO-BU-Centre for Life Sciences, Bharathiar University Campus, Coimbatore, 641046, India
[b] Center for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, 641003, India
[c] Freeze Drying and Animal Products Technology, Defence Food Research Laboratory, Mysuru, 570011, India
[d] Department of Microbiology, Pondicherry University, Pondicherry, 605014, India
[e] Department of Studies in Molecular Biology, University of Mysore, Mysuru, 570005, India
[f] Adichunchanagiri Institute for Molecular Medicine, Mandya, 571448, India
[g] School of Environmental Science and Engineering, Sun Yat-Sen University, Guangzhou, 510006, China
[h] Department of Botany, School of Life Sciences, Mahatma Gandhi Central University, Motihari, 845401, India
[i] Department of Biochemistry and Immunology, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, SP, 14049-900, Brazil
[j] Lorven Biologics Pvt. Ltd., Hyderabad, 500 100, India
[k] Department of Biotechnology, University of Mysore, Mysuru, 570 005, India

ARTICLE INFO

Article history:
Received 10 December 2019
Received in revised form 23 April 2020
Accepted 15 June 2020

Keywords:
Fungi
Mycotoxins
Ochratoxin A
Essential oils
Maize grains

ABSTRACT

In the study, antifungal and ochratoxin A (OTA) production inhibitory activities of essential oils (EOs) of *Cinnamomum zeylanicum*, *Curcuma longa*, *Ocimum basilicum*, *Zingiber officinale*, and *Cymbopogon martini* were reported on *Aspergillus ochraceus and Penicillium verrucosum*. EOs were obtained by hydro-distillation and GC–MS technique was chosen to deduce their chemical profile. Major chemical compounds in EOs of *C. zeylanicum*, *C. longa*, *O. basilicum*, *Z. officinale*, and *C. martini* were (E)-cinnamaldehyde (35.81 %), ar-turmerone (46.13 %), eugenol (36.58 %), geranyl proprionate (18.93 %), and geranyl acetate (14.88 %), respectively. The EOs shown potent antioxidant activity by DPPH and ABTS assays. The EOs presented superlative antifungal activity against *P. verrucosum* related to *A. ochraceus*. The *C. zeylanicum* and *C. martini* EOs shown superlative antifungal activity related to other EOs. The *C. zeylanicum* and C. *martini* EOs completely inhibited the growth and OTA production of *P. verrucosum* and *A. ochraceous* at 1500 and 2500 μg/g in maize grains, respectively.

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1. Introduction

Mycotoxins are poisonous substances and belong to metabolites of fungi and that are naturally produced on food matrices such as cereal products, various fruit juices, vegetables, spices, etc [1]. It has been known that almost one-fourth of cereals consumed worldwide are contaminated with mycotoxins and pose serious health risk to human society [2]. Until the date, about 400 mycotoxins were known, and their number will be raised with advancement of the novel analytical techniques in the forthcoming years [3]. Fungal infestation and mycotoxin production can occur during the pre-harvest and post-harvest seasons, and on/in the food itself often at an opportune environmental milieu [4]. The mycotoxin occurrence in food matrices principally relies on diversity of fungal strain, fungal vulnerability of the plant in the field, microbial population, moisture content, nutrient composition, temperature, aeration, and stress factors [5]. Most of the mycotoxins are thermally stable and conventional food-processing techniques with temperatures up to 100 °C have little or no effect on detoxification of mycotoxins [6].

The toxic effects of mycotoxins range from mild disturbance to be acute in humans and farm animals [7,8]. The long-term

* Corresponding authors.
E-mail addresses: ramana.micro@gmail.com (V. Mudili), moonnayak@gmail.com (C. Siddaiah).

https://doi.org/10.1016/j.btre.2020.e00490
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consumption of mycotoxins contaminated food cause immune deficiency and cancer [9]. The human exposure to mycotoxins can occur either directly by consumption of mycotoxin contaminated food or indirectly by consuming the animal products (milk, egg, and meat) from animals that fed with mycotoxin contaminated feed [10]. The major and most commonly encountered mycotoxins in food with concern to the toxic effects on humans and farm animals are aflatoxins, ochratoxin A (OTA), fumonisins, trichothecenes, and zearalenone [11].

Among the major mycotoxins, OTA is one of the frequently encountered mycotoxins in variety of food sources such as cereals, coffee beans, fruits, spices, wine, beer, animal products, etc [12–15]. OTA has shown carcinogenic properties in laboratory animals and therefore, International Agency for Research on Cancer (IARC) has categorized into Group 2B, which means possible to cause cancer in humans [16]. Moreover, OTA has been shown to be fetal nephrotoxic and immunosuppression in laboratory animals and considered as most toxic [17]. In consequence, some nations and regulatory bodies such as European Union (EU) and Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) have evaluated the risk assessment of OTA and recommended regulatory level to control or prevent OTA contamination. The allowable concentrations for OTA in unprocessed cereals, processed cereals for human consumption, dried vine fruits, roasted coffee beans and ground roasted coffee, instant coffee, wine, and grape juice are 5.0, 3.0, 10.0, 5.0, 10.0, 2.0 and 2.0 μg/kg, respectively [18]. Therefore, OTA has received utmost special attention from professionals of the microbiology, toxicology, and food technology.

Ochratoxin A is produced as the secondary metabolite by certain fungal species of *Aspergillus* and *Penicillium,* and these were regular contaminants of various food commodities such as cereals, fruits, vegetables, spices, animal products, etc [12]. The exposure to OTA needs to be maintained as low as likely to protect the human society. Mycotoxins not only pose a risk to both human and animal health, but also impact food security and nutrition by reducing people's access to healthy food [12,18].

Therefore, microbiologists and food technologists have a great concern over minimization of OTA in food and feed matrices. At present, various physical, chemical, and biological methods were available to minimize the mycotoxins in food. The physical methods such as irradiation, high-pressure processing, microwave, and cold plasma were found effective in minimizing the fungal growth and mycotoxin contamination, and nevertheless; these have certain limitations, i.e. high-cost, unsafe, and pre-requisite of skilled expertise [19–24]. The chemical treatments such as ammonia, citric acid, salicylic acid, etc. partially degrades mycotoxins and their by-products were found still toxic and usage of these chemicals in food is in no way acceptable [25].

Captivatingly, researchers revealed that oxidative stress has been extensively involved in the secondary metabolism of fungi. These secondary metabolites can counteract or, on the other hand, augment the harmful effects of oxidative stress, and thus, fungi may well use reactive oxygen species as messengers that induce/modulate the biosynthesis of defensive chemical agents like mycotoxins [26]. In this context, usage of antioxidants could overwhelm the oxidative stress and decline the biosynthesis of mycotoxins [27]. Further, antioxidant substances with potential antifungal activity could be highly appropriate for minimizing the fungal growth as well as mycotoxin production in food sources [28]. Consequently, researchers, consumers, and government are highly opted in usage of natural antioxidants that have potent antifungal activity for minimizing the fungal growth and mycotoxin contamination owed to their non-toxicity and eco-friendly [29–32]. In the scenario, essential oils (EOs) have received great consideration as fungicides and antioxidants for controlling the fungal growth and mycotoxin contamination in food [32].

In the present study, EOs of *C. zeylanicum*, *C. longa*, *O. basilicum*, *Z. officinale*, and *C. martini* were obtained by hydrodistillation and GC–MS analysis was chosen to deduce their chemical profile. The antioxidant action of EOs was measured by radical scavenging assays. Antifungal activity of EOs on selected fungi *A. ochraceus* and *P. verrucosum* was tested by micro-well dilution technique. Finally, inhibitory activity of EOs on the growth and OTA content of *A. ochraceus* and *P. verrucosum* was established in maize grains.

2. Materials and methods

2.1. Fungi cultural conditions

Fungal cultures capable to produce OTA such as *A. ochraceus* – ITCC 1456 and *P. verrucosum* – ITCC 2986 were from The Indian Type Culture Collection (ITCC), India, and cultured for period of 7 days at 28 °C on Sabouraud dextrose agar Petri plates (HiMedia, Mumbai, India) [33]. Succeeding, fungal spores were obtained by gentle scarping in peptone water containing 0.001 % Tween 80 (HiMedia, Mumbai, India) and their total count was set to 1×10^6 spores per mL using hemocytometer.

2.2. Plant materials collection and EOs extraction

The bark of *C. zeylanicum*, rhizome of *C. longa*, leaves of *O. basilicum*, rhizome of *Z. officinale*, and leaves of *C. martini* were collected during January to July of 2019 from Ooty, India. Vouchers were identified and safeguarded in Department of Biotechnology, University of Mysore, Mysuru, India. They were subjected to drying in an ambient temperature for duration of couple of week under dark and made into fine powder using blender. About, 250 g of fine powder of each sample was distinctly subjected to hydrodistillation practice to extract EO using apparatus of Clevenger-type (Teknik, Ambala, India). The practice was in consistent with the way of European Pharmacopoeia [34] and followed our previous reported technique of Kalagatur et al. [22,24].

2.3. Chemical profile of EOs

The constituents of EOs were determined by PerkinElmer Clarus 600 C GC–MS, which is connected with DB-5MS column (30 × 0.25 mm; 0.25 μm) and analysis was done as per methodology of Kalagatur et al. and Adams [3536].

2.4. Antioxidant activity of EOs

Antioxidant activity of EOs was determined by DPPH and ABTS radical scavenging assays (HiMedia, Mumbai, India) and as per technique of Kalagatur et al. [35] and Lokanadhan et al. [37]. Quercetin was chosen as standard antioxidant compound.

2.5. Antifungal activity of EOs

Antifungal activity of EOs on *A. ochraceus and P. verrucosum* were determined in minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) by micro-well dilution method [38].

Various dose of EOs (up to 3000 μg/mL) containing 0.001 % of Tween 80 were added to 10 μL of fungal spores (1×10^6 per mL) in 96-well plate (Corning Inc. Technology Company, USA) and total volume was adjusted to 100 μL with Sabouraud dextrose broth (HiMedia, Mumbai, India). Following, plates were allowed for incubation at 28 °C, and concentration at which fungal growth was not observed after 3 days of incubation was determined as MIC.

Succeeding, 10 μL was recovered from the wells of microtiter plate and plated on Sabouraud dextrose agar Petri plates and allowed for incubation yet again at 28 °C for 3 days. Quantity of EOs at which fungal viability was not noticed measured as MFC. The standard antifungal agent in the study was nystatin.

2.6. Antifungal and OTA inhibitory activities of EOs in maize

Antifungal and OTA inhibitory activities of EOs on *A. ochraceus* and *P. verrucosum* in maize grains were studied as per methodology of Velluti et al. [39]. From local agriculture market, fresh and dried maize grains were collected and decontaminated by autoclave and subjected to drying in hot air oven under sterile conditions at 60 °C. The oven, incubation chamber, and premises were thoroughly fumigated and maintained strict aseptic conditions. The water activity of thoroughly dried maize grains was determined as 0.70. Following, different concentration of EOs (up to 3500 μg/g) containing 0.001 % of Tween 80 and 10 μL of fungal spores (1×10^6 per mL) of *A. ochraceus* and *P. verrucosum* were distinctly treated to 100 g of maize in 500 mL of Erlenmeyer flask (Borosil, India) and vigorously shaken to distribute homogeneously and incubated for 14 days at 28 °C. Maize samples distinctively inoculated with spores of *A. ochraceus* and *P. verrucosum* were considered as control in their respective study.

Following incubation phase, maize samples were crushed into fine powder under sterile condition and 1 g was subjected to serial dilution and plated on Sabouraud dextrose agar Petri plates. The fungal growth was determined in log CFU/g. Another, 10 g of fine powder was suspended in acetonitrile (HiMedia, Mumbai, India) and used for immunoaffinity clean-up of OTA as per instructions of manufacturer (Vicam, USA).

The eluent was used for determination of OTA in HPLC (Shimadzu Corp., Kyoto, Kyoto Prefecture, Japan) equipped with RP-C18 column (5 μm thickness, 250 mm × 4.6 mm length, 100 Å pore size). The HPLC was performed with following conditions; mobile phase: isocratic solution of acetonitrile, water and acetic acid (45:54:1, v/v/v), flow rate: 1 mL/min, detector: fluorescence, injecting volume: 25 μL, phase: reverse phase, excitation and emission wavelength: 365/455 nm, and run time: 25 min.

Prior to OTA determination in test samples, stock solution of OTA standard (98 % HPLC pure, Sigma-Aldrich, Bengaluru, India) was made in acetonitrile (1 mg/mL) and following lower OTA concentrated dilutions were made in HPLC evaluation water (Merck Millipore Pvt Ltd, Bengaluru, India). The LOD (limit of detection) and LOQ (limit of quantification) for OTA was estimated. The LOD was determined as the threefold the signal of the blank and LOQ was determined as three folds of LOD and it was determined as 21 ng/mL and 84 ng/mL, respectively. Curve of calibration for OTA was constructed with different concentration of OTA and peak area. The linearity of calibration curve was judged from regression coefficient (R^2) and noticed as 0.9891. The recovery of the technique was noticed as 92.31 % and found as satisfactory.

2.7. Statistical analysis

The experiments were executed in replicates (n = 6) and scrutinized by one-way ANOVA and results were stated as mean ± standard deviation. The significant ($p \leq 0.05$) difference was judged by Dunnett's test and denoted as '*'. Whereas, $p > 0.05$ was measured as non-significant and denoted as '#'. The statistical examination was performed using trial version 8 of GraphPad Prism, CA 92108, United States.

3. Results

3.1. Extraction and chemical profile of EOs

In this study essential oils were extracted by hydrodistillation technique and their yield was tabulated in Table 1. The extracted EOs were subjected to chemical profile assessment by GC–MS analysis. Total chemical composition of 98.80 %, 98.22 %, 94.58 %, 97.19 %, and 96.27 % were identified in *C. zeylanicum*, *C. longa*, *O. basilicum*, *Z. officinale*, and *C. martini* EOs, respectively (Table 2). Total of 38, 32, 33, 41, and 42 chemical compounds were identified in EOs of *C. zeylanicum*, *C. longa*, *O. basilicum*, *Z. officinale*, and *C. martini*, respectively. The major chemical compounds in EOs of *C. zeylanicum* were limonene (10.54 %), (E)-cinnamaldehyde (35.81 %), and eugenol (12.41 %), and in *C. longa* were ar-turmerone (46.13 %) and ar-curcumene (8.33 %), and in *O. basilicum* were eugenol (36.58 %) and linalool (10.83 %), and in *Z. officinale* were geraniol isobutanoate (10.41 %), geranyl acetone (11.05 %), geranyl acetate (14.59 %), geranyl proprionate (18.93 %), thymol (10.86 %), and limonene (10.88 %), and in *C. martini* were terpinen-4-ol (11.52 %) and geranyl acetate (14.88 %). The results settled that EOs of *C. zeylanicum*, *C. longa*, *O. basilicum*, *Z. officinale*, and *C. martini* were belonged to the chemotypes of linalool, ar-turmerone, eugenol, geranyl proprionate, and geranyl acetate, respectively.

3.2. Antioxidant activity of EOs

The functional property of EOs such as antioxidant activity was determined by DPPH and ABTS radical scavenging assays and IC50 results (effective concentration required to inhibit 50 % of free radicals) were shown in Table 3. Best antioxidant potential in DPPH assay was noticed in EOs of *C. longa* and *C. zeylanicum*, and their IC50 values were noticed as 21.56 ± 0.77 μg/mL and 22.14 ± 1.72 μg/mL, respectively. The lowermost antioxidant potential (IC50 value) in DPPH assay was noticed in EO of *Z. officinale* and it was 29.11 ± 1.60 μg/mL. On the other hand, *C. martini* showed best antioxidant potential in ABTS assay, and its IC50 was noticed as

Table 1
Yield of essential oils (EOs).

Essential oil	Yield (w/w)	Antioxidant compounds	Antifungal compounds
C. zeylanicum	2.11 %	α-Phellandrene, limonene, β-phellandrene, γ-terpinene, linalool, (E)-cinnamaldehyde, and eugenol	Limonene, linalool, eugenol, and (E)-cinnamaldehyde
C. longa	1.59 %	α-Phellandrene, β-phellandrene, terpinen-4-ol, ar-curcumene, α-turmerone and ar-turmerone	ar-Turmerone and ar-curcumene
O. basilicum	2.71 %	o-Ocimene, limonene, (Z)-β-ocimene, (E)-β-ocimene, linalool, thymol, eugenol, β-Caryophyllene, and germacrene D	Eugenol, linalool, and linalool
Z. officinale	1.44 %	Limonene, γ-terpinene, linalool, terpinen-4-ol, α-terpineol, thymol, geranyl acetate, geranyl acetone, geranyl proprionate, and geraniol isobutanoate	Geranyl proprionate, geraniol isobutanoate, geranyl acetone, geranyl acetate, linalool, thymol, and limonene
C. martini	1.93 %	α-Phellandrene, limonene, β-phellandrene, γ-terpinene, linalool, terpinen-4-ol, α-terpineol, citronellal, thymol, eugenol, geranyl acetate, β-caryophyllene, and geranyl proprionate	Terpinen-4-ol, limonene, thymol, eugenol and geranyl acetate

Table 2
Chemical profile of essential oils (EOs) determined by GC–MS analysis.

Chemical compound	Essential oils									
	C. zeylanicum		*C. longa*		*O. basilicum*		*Z. officinale*		*C. martini*	
	RI[a]	%[b]	RI[a]	%[b]	RI[a]	%[b]	RI[a]	%[b]	RI[a]	%[b]
α-Thujene	921	0.17	922	0.05	921	0.03	923	0.02	921	0.02
α-Pinene	934	0.60	931	0.36	931	0.12	931	0.08	930	0.09
Camphene	947	0.19	945	0.02	944	0.41	947	0.02	947	0.12
Sabinene	971	0.71	971	0.16	970	0.09	971	0.14	971	0.15
β-Pinene	973	0.44	973	0.09	976	0.04	976	0.81	976	1.21
Myrcene	985	0.09	989	0.18	989	0.02	989	0.07	989	0.02
α-Phellandrene	1004	5.92	1000	5.81	1004	1.31	1003	0.23	1004	2.41
δ-3-Carene	–	–	1009	1.21	–	–	1010	0.42	1009	0.11
α-Terpinene	–	–	1012	2.82	1016	1.48	1012	0.02	1015	1.21
p-Cymene	1019	1.21	–	–	1021	0.82	–	–	1021	0.18
o-Ocimene	–	–	–	–	1023	6.01	–	–	–	–
Limonene	1023	10.54	–	–	1024	9.44	1023	10.88	1024	8.46
β-Phellandrene	1026	8.21	1026	6.77	1026	2.57	1025	1.19	1026	6.92
(Z)-β-Ocimene	–	–	–	–	1034	2.92	–	–	–	–
(E) -β-Ocimene	–	–	–	–	1042	5.05	–	–	–	–
(3Z)-Octen-1-ol	1049	1.28	1048	0.79	–	–	–	–	1046	0.51
γ-Terpinene	1057	2.71	1055	1.31	1055	0.41	1051	1.56	1055	2.83
Acetophenone	1061	0.51	–	–	–	–	–	–	–	–
p-Mentha-2,4(8)-diene	1083	1.37	–	–	–	–	–	–	1086	0.14
Linalool	1092	3.01	–	–	1096	10.83	1093	2.09	1097	9.59
n-Nonanal	1102	0.21	–	–	–	–	1101	0.06	1103	0.32
exo-Fenchol	–	–	1119	0.15	–	–	–	–	1119	0.01
trans-Pinocarveol	–	–	–	–	–	–	–	–	1140	0.01
Camphor	1142	0.70	–	–	1142	0.45	1142	0.14	–	–
p-Menth-8-en-3-ol	–	–	–	–	–	–	–	–	–	–
Isoborneol	1157	0.16	1157	0.82	1157	0.66	–	–	–	–
Pinocarvone	–	–	1165	3.01	–	–	–	–	–	–
Borneol	–	–	–	–	1166	0.04	1166	0.42	1166	0.70
Terpinen-4-ol	–	–	1175	3.24	1175	1.41	1176	4.91	1179	11.52
Isomenthol	1181	0.83	1185	0.81	–	–	1184	0.16	1182	0.03
α-Terpineol	–	–	1186	0.99	1188	1.39	1187	2.19	1188	7.19
trans-Carveol	1217	1.31	–	–	1217	0.46	1216	0.81	1217	0.81
Citronellal	1224	0.57	–	–	–	–	1224	0.07	1224	2.37
Pulegone	–	–	1239	0.08	–	–	–	–	1239	0.05
Linalyl acetate	–	–	–	–	–	–	1249	0.77	–	–
(E)-cinnamaldehyde	1279	35.81	–	–	–	–	–	–	–	–
Bornyl acetate	1288	0.57	–	–	1288	0.05	–	–	–	–
Thymol	–	–	1290	0.12	1290	3.59	1290	10.86	1291	6.19
Carvacrol	1297	0.70	–	–	–	–	1299	0.04	1299	0.56
Isomenthyl acetate	1303	0.46	–	–	–	–	–	–	1306	1.29
δ-Elemene	1336	1.31	–	–	–	–	–	–	–	–
α-Cubebene	1346	0.16	–	–	1346	0.21	1346	0.42	–	–
Eugenol	1357	12.41	–	–	1358	36.58	–	–	1357	2.81
Cyclosativene	1371	0.37	–	–	–	–	1371	0.03	–	–
α-Ylangene	–	–	–	–	–	–	–	–	1374	0.08
α-Copaene	1377	0.19	–	–	1377	0.05	–	–	–	–
Geranyl acetate	–	–	–		–	–	1381	14.59	1381	14.88
β-Elemene	1388	0.22	–	–	1390	0.06	1390	0.11	–	–
β-Caryophyllene	–	–	1419	2.08	1416	4.13	1418	1.78	1419	3.91
β-Copaene	–	–	–	–	–	–	1431	0.16	–	–
α-Guaiene	1436	0.82	–	–	–	–	–	–	1439	0.18
α-Humulene	1453	1.09	1453	0.02	1450	0.02	1451	0.09	1453	0.29
Geranyl acetone	–	–	–	–	–	–	1454	11.05	–	–
Allo-Aromadendrene	1457	0.06	–	–	–	–	1459	0.09	1460	0.11
Ishwarane	1467	0.61	–	–	–	–	–	–	–	–
Geranyl proprionate	–	–	–	–	–	–	1477	18.93	1477	6.40
ar-Curcumene	–	–	1480	8.33	–	–	–	–	–	–
Germacrene D	–	–	–	–	1483	3.82	1485	1.19	1485	1.29
β-Selinene	1490	1.88	–	–	–	–	–	–	–	–
Viridiflorene	–	–	–	–	–	–	–	–	–	–
α-Muurolene	1502	1.29	–	–	–	–	–	–	–	–
γ-Cadinene	–	–	–	–	1515	0.03	1512	0.11	1515	0.72
Geraniol isobutanoate	–	–	–	–	–	–	1516	10.41	–	–
δ-Cadinene	1523	0.11	1523	0.51	1523	0.08	–	–	–	–
Elemol	–	–	1550	0.05	–	–	–	–	–	–
Elemicin	–	–	–	–	–	–	1557	0.02	1559	0.11
Carotol	–	–	1596	0.42	–	–	1596	0.09	1596	0.19
Cubenol	–	–	–	–	–	–	1640	0.12	–	–
Klusinone	–	–	–	–	–	–	–	–	–	–
α-Turmerone	–	–	1624	7.91	–	–	–	–	–	–
β-Turmerone	–		1635	3.04	–	–	–	–	–	–
ar-Turmerone	–	–	1640	46.13	–	–	–	–	–	–
Bulnesol	–	–	1671	0.14	–	–	1671	0.04	1671	0.28

Table 2 (*Continued*)

Chemical compound	Essential oils									
	C. zeylanicum		*C. longa*		*O. basilicum*		*Z. officinale*		*C. martini*	
	RI[a]	%[b]	RI[a]	%[b]	RI[a]	%[b]	RI[a]	%[b]	RI[a]	%[b]
Cyclocolorenone	–	–	1761	0.39	–	–	–	–	–	–
Laurenene	–	–	1880	0.41	–	–	–	–	–	–
Total (%)		98.80		98.22		94.58		97.19		96.27

[a] Retention indices of compounds determined on DB-5MS column.
[b] Quantity of individual chemical compounds in percentage.

Table 3
Antioxidant activity of essential oils (EOs).

Essential oil	DPPH assay (IC50)	ABTS assay (IC50)
C. zeylanicum	22.14 ± 1.72 μg/mL	38.41 ± 1.21 μg/mL
C. longa	21.56 ± 0.77 μg/mL	35.29 ± 1.06 μg/mL
O. basilicum	27.32 ± 2.11 μg/mL	40.31 ± 1.07 μg/mL
Z. officinale	29.11 ± 1.60 μg/mL	38.18 ± 1.68 μg/mL
C. martini	24.14 ± 1.03 μg/mL	33.47 ± 1.71 μg/mL
Quercetin	21.19 ± 0.62 μg/mL	38.92 ± 0.96 μg/mL

The experiments were executed in independent replicates (n = 6) and results were expressed as mean ± standard deviation.

33.47 ± 1.71 μg/mL, and lowermost antioxidant potential was noticed in EO of *O. basilicum* (40.31 ± 1.07 μg/mL).

Overall, all EOs showed potent antioxidant potentials and found competitive with IC50 value of standard antioxidant quercetin (21.19 ± 0.62 μg/mL). The diversity of biological applications of the plant materials mainly depends on chemical constituents as well as antioxidant potential. The study showed that EOs have potent antioxidant activity and thus, highly appropriate for their usage in various biological applications.

3.3. Antifungal activity of EOs

Antifungal activity of EOs on OTA producing fungi (*A. ochraceus* and *P. verrucosum*) was tested by micro-well dilution technique. The MIC and MFC values of the EOs were shown in Table 4. The EOs were shown potent antifungal activity on both the tested fungi, but superlative results were noticed on *P. verrucosum* related to *A. ochraceus*. The EOs of *C. zeylanicum* and *C. martini* were shown best antifungal activity in *A. ochraceus* and *P. verrucosum* related to standard antifungal agent nystatin as well as other tested EOs. While, lowest antifungal activity was noticed in EO of *Z. officinale* against *A. ochraceus* and EO of *C. longa* against *P. verrucosum*. The study exhibited that tested EOs were potential fungicidal agents and might found their role in food and feed matrices as bio-fungicides. To conclude, antifungal efficacy of EOs was evaluated in in-vitro food source maize grains under laboratory conditions.

3.4. Antifungal and antimycotoxin activities of EOs in maize grains

All EOs were shown potent antifungal and antimycotoxin activities in in-vitro food sample; maize grains and results were shown in Table 5 and Table 6. Standard calibration curve was constructed with area of peak and different concentration of OTA and used to determine the unknown concentration of OTA in maize grains. Among the tested EOs, *C. zeylanicum* and *C. martini* were shown potent inhibitory action on growth as well mycotoxin production of *A. ochraceous* and *P. verrucosum*. The EOs of *C. zeylanicum* and C. *martini* were completely inhibited the growth and OTA production of *P. verrucosum* and *A. ochraceous* at 1500 and 2500 μg/g, respectively. The best antifungal and OTA inhibitory activities was noticed against *P. verrucosum* related to *A. ochraceous*. These results were well in agreement with the conclusion of micro-well dilution technique. While, EOs of *C. longa*, *O. basilicum*, and *Z. officinale* were exhibited lowest antifungal and OTA inhibitory activities against *A. ochraceous* and it was 3500 μg/g. The EOs of *O. basilicum* and *Z. officinale* were presented lowest antifungal and OTA inhibitory activities against *P. verrucosum* at 3500 μg/g.

4. Discussion

In the middle ages onwards, EOs have been widely utilized as antimicrobial, antiparasitic, and insecticidal agents. Exclusively these days widely used in pharmaceutical, cosmetic, sanitary, food industries, etc. These EOs were mostly extracted by distillation from various parts of plants such as buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and EOs comprise of complex of volatile chemical compounds, which include terpenes and terpenoids, phenolic and aliphatic compounds. They exhibit the characteristic properties of antioxidants and found non-genotoxic. Therefore, Food and Drug Administration (FDA) of federal agency acknowledged EOs as "GRAS", which states that **G**enerally **R**ecognized **A**s **S**afe under sections 201(s) and 409 of the Federal Food, Drug, and Cosmetic Act of FDA [40].

In our study, we identified more than 30 different compounds in EOs obtained from bark of *C. zeylanicum*, rhizome of *C. longa*, leaves of *O. basilicum*, rhizome of *Z. officinale*, and leaves of *C.*

Table 4
Antifungal activity of essential oils (EOs).

EOs/standard antifungal agent	*A. ochraceus*		*P. verrucosum*	
	MIC (μg/mL)	MFC (μg/mL)	MIC (μg/mL)	MFC (μg/mL)
C. zeylanicum	1106 ± 31.11	1430 ± 47.78	837 ± 23.62	1141 ± 28.32
C. longa	1608 ± 12.81	2140 ± 32.21	1329 ± 15.77	1771 ± 43.48
O. basilicum	1791 ± 26.59	2355 ± 62.11	1006 ± 32.19	1512 ± 56.63
Z. officinale	1898 ± 33.41	2621 ± 37.72	1255 ± 18.30	1442 ± 37.81
C. martini	1308 ± 16.69	1756 ± 26.93	964 ± 18.72	1221 ± 39.32
Nystatin	1446 ± 63.58	1791 ± 19.41	1121 ± 36.91	1359 ± 16.78

The experiments were executed in independent replicates (n = 6) and results were expressed as mean ± standard deviation.

Table 5
Effect of different concentration of essential oils (EOs) on fungal growth (log CFU/g) in maize grains.

Essential oil	*A. ochraceus* (log CFU/g)					*P. verrucosum* (log CFU/g)				
	0 (control)	500 μg/g	1500 μg/g	2500 μg/g	3500 μg/g	0 (control)	500 μg/g	1500 μg/g	2500 μg/g	3500 μg/g
C. zeylanicum	8.22 ± 1.21	4.70 ± 0.88*	1.21 ± 0.09*	Nil*	Nil*	7.78 ± 1.39	2.66 ± 0.88*	Nil*	Nil*	Nil*
C. longa	8.22 ± 1.21	5.43 ± 0.65*	3.84 ± 0.73*	1.66 ± 0.68*	Nil*	7.78 ± 1.39	5.32 ± 0.59*	1.77 ± 0.62*	Nil*	Nil*
O. basilicum	8.22 ± 1.21	6.12 ± 0.33*	4.05 ± 0.68*	1.59 ± 0.23*	Nil*	7.78 ± 1.39	5.91 ± 1.32*	3.67 ± 0.84*	1.09 ± 0.46*	Nil*
Z. officinale	8.22 ± 1.21	6.51 ± 0.82*	3.03 ± 0.19*	1.22 ± 0.04*	Nil*	7.78 ± 1.39	6.01 ± 1.57#	4.93 ± 0.44*	1.62 ± 0.59*	Nil*
C. martini	8.22 ± 1.21	3.69 ± 0.51*	1.81 ± 0.02*	Nil*	Nil*	7.78 ± 1.39	3.64 ± 1.01*	Nil*	Nil*	Nil*

The experiments were executed in independent replicates (n = 6) and results were expressed as mean ± standard deviation. The significant ($p \leq 0.05$) difference between control and test samples was judged by Dunnett's test and denoted as '*'. Whereas, $p > 0.05$ was measured as non-significant and denoted as '#'.

Table 6
Effect of different concentration of essential oils (EOs) on OTA production of fungi in maize grains.

Essential oil	*A. ochraceus* (OTA μg/g)					*P. verrucosum* (OTA μg/g)				
	0 (control)	500 μg/g	1500 μg/g	2500 μg/g	3500 μg/g	0 (control)	500 μg/g	1500 μg/g	2500 μg/g	3500 μg/g
C. zeylanicum	10.42 ± 1.59	6.11 ± 1.47*	2.38 ± 0.61*	Nil*	Nil*	8.30 ± 0.48	2.06 ± 0.06*	Nil*	Nil*	Nil*
C. longa	10.42 ± 1.59	8.96 ± 1.77#	6.08 ± 0.78*	1.52 ± 0.70*	Nil*	8.30 ± 0.48	5.17 ± 1.09*	1.81 ± 0.18*	Nil*	Nil*
O. basilicum	10.42 ± 1.59	8.33 ± 1.30#	5.12 ± 0.33*	1.88 ± 0.39*	Nil*	8.30 ± 0.48	6.91 ± 1.83*	4.81 ± 0.94*	1.55 ± 0.02*	Nil*
Z. officinale	10.42 ± 1.59	7.81 ± 0.91*	3.77 ± 0.26*	1.91 ± 0.58*	Nil*	8.30 ± 0.48	7.14 ± 1.09#	3.88 ± 0.20*	2.09 ± 0.07*	Nil*
C. martini	10.42 ± 1.59	6.37 ± 0.83*	2.03 ± 0.44*	Nil*	Nil*	8.30 ± 0.48	2.12 ± 0.15*	Nil*	Nil*	Nil*

The experiments were executed in independent replicates (n = 6) and results were expressed as mean ± standard deviation. The significant ($p \leq 0.05$) difference between control and test samples was judged by Dunnett's test and denoted as '*'. Whereas, $p > 0.05$ was measured as non-significant and denoted as '#'.

martini. Most of the identified chemical compounds were in accordance to earlier literature. Pawar et al. [41] Simić et al. [42] and Ranasinghe et al. [43] documented that (E)-cinnamaldehyde as major compound and followed by eugenol, linalool, limonene, and benzaldehyde occupy major composition in EO of *C. zeylanicum*. Though, Ranasinghe et al. found that eugenol and cinnamaldehyde as major compound in leaf and bark *C. zeylanicum* EO, respectively [43]. In our study, EO of *C. zeylanicum* have (E)-cinnamaldehyde, eugenol, and limonene as major compounds and thus, belong to the aforementioned chemotype. Further, EO of *C. longa* belong to the chemotype of α-turmerone, β-turmerone, and ar-turmerone as per past descriptions of Avanço et al. [44] and Kumar et al. [38]. In our study, EO of *C. longa* was found as chemotype of ar-turmerone and well in accord with earlier reports. In case of *O. basilicum* EO, previous reports of Snoussi et al. [45] de Almeida et al. [46] and Grayer et al. [47] were determined that EO of *O. basilicum* belong to the chemotype of eugenol and linalool. In accordance to these reports, EO of *O. basilicum* in our study belong to chemotype of eugenol and linalool. Similarly, EO of *Z. officinale* in our study belong to the chemotype of geranyl proprionate, geranyl acetate, thymol, and limonene as per past descriptions of Yamamoto-Ribeiro et al. [48] and Singh et al. [49]. Also, in our study, EO of *C. martini* was found chemotype of geranyl acetate and terpinene-4-ol and found in accord with prior information of Kalagatur et al. [50], Kakaraparthi et al. [51] and Nirmal et al. [52]. However, quantity of EOs composites was quite wide-ranging in interrelated to that past information. Quantity and quality of chemical composites of EOs depends on the part of the plant used for EO extraction, genome of the plant, nutrient availability and final extraction technique as well [51,53–57]. Therefore, quantity of chemical contents was relatively diverse in our study compared to previous reports.

The EOs are regarded as potential antioxidants, and their applications as natural antioxidants is growing interest in food over synthetic antioxidants owed to toxic effects on human health. The antioxidant potential of EOs highly useful in food products, either by direct mixing or in active packaging and package coatings, thereby represent the safe strategies to avoid autoxidation of food and lengthen the shelf life of food. Consequently, assessment of the antioxidant potential of EOs is most vital in food science [58]. Moreover, many researchers determined that EOs normalize the oxidative stress of mycotoxigenic fungi and decline the biosynthesis of mycotoxins [35,59,60]. Therefore, in our study, antioxidant potential of EOs was considered and EOs exhibited potent antioxidant potential in scavenging the radicals of DPPH and ABTS. In present study, assortments of potential antioxidants were noticed in EOs (Table 1). Therefore, EOs in our study exhibited potent antioxidant activity. In support of our results, several reports were documented the antioxidant potential of EOs of *C. zeylanicum*, *C. longa*, *O. basilicum*, *Z. officinale*, and *C. martini* [44,49,55,58,61]. The antioxidant study and GRAS safety assessment of FDA accomplish that EOs of *C. zeylanicum*, *C. longa*, *O. basilicum*, *Z. officinale*, and *C. martini* could find the potential representation in food industry as natural antioxidants and mycotoxin inhibitors through regulation of oxidative stress in fungi.

In last two decades, EOs have given away exceptional protection against foodborne fungi and their associated mycotoxins and successfully substituted the usage of synthetic fungicides offering safer and eco-friendly tactic. Now, EO based bio-preservatives i.e. Sporan^{TM}, Promax^{TM}, 'DMC Base Natural', EcoPCOR, and EcoTrol are listed under GRAS by FDA and commercially available [62]. Therefore, in our study, an attempt was made to control the fungal growth and OTA content in food using EOs of *C. zeylanicum*, *C. longa*, *O. basilicum*, *Z. officinale*, and *C. martini* for the reason that these were eco-friendly and safer and attained the status of GRAS from FDA.

In our study, *C. zeylanicum*, *C. longa*, *O. basilicum*, *Z. officinale*, and *C. martini* EOs were exhibited potent antifungal and antimycotoxin activities on *A. ochraceous* and *P. verrucosum* in maize grains. In support of our study, Sumalan et al. conveyed the antifungal and mycotoxin inhibitory activities of *C. zeylanicum* on *Fusarium* and *Aspergillus* fungi in wheat grains [63]. Ferreira et al. described the antifungal and antimycotoxin activities of *C. longa* EO and curcumin against *A. flavus* [64]. Dambolena et al. documented the antifungal and antimycotoxin activities of EO of *O. basilicum* L [65]. Similarly, Císarová et al. reported the inhibitory effect of *Z. officinale* EO on the growth and mycotoxin production of *Aspergillus*

species [66]. Further, antifungal and antimycotoxin activity of *C. martini* was reported against Fusarium toxins in maize grains by Kalagatur et al. [50].

In the present study, EOs contain variety of terpenes and terpenoids, phenolic and aliphatic antifungal compounds (Table 1). These lipophilic and low molecular weight compounds easily cross through the fungal plasma membrane and disrupts the integrity and permeability of membrane. These volatile compounds of EOs negatively interact with the cellular membrane by countering with enzymes and cellular ions, and cause leakage of ions (Ca^{2+}, Mg^{2+}, H^{+}, and K^{+}) and osmotic imbalance, thereby depletes the ATP synthesis and activate the apoptotic death. Moreover, EOs depletes the level of ergosterol content in fungal cell membrane and thereby facilitates the easy entry of antimicrobial components through cell membrane [62,67].

Recently, some researchers disclosed that EOs independently act on inhibition of mycotoxin biosynthesis without/mild reducing effect on the fungal growth. The researchers reported that EOs cease the formation of precursors for mycotoxin biosynthesis (acetyl-CoA, the main precursor of aflatoxin biosynthesis) [62] and in other way, down-regulate the mycotoxin biosynthesis transcription factors, viz. *PKS13* and *PKS4* in zearalenone biosynthesis [35], *acOTApks,acOTAnrps* and *acpks* in OTA biosynthesis [59], and *aflR*, *aflT*, *aflD*, *aflM*, and *aflP* in aflatoxin B_1 biosynthesis [68].

In our study, *C. zeylanicum*, *C. longa*, *O. basilicum*, *Z. officinale*, and *C. martini* EOs withdrawn the viability of fungi and OTA content in maize. Present study and as well as earlier studies showed that EOs from bark of *C. zeylanicum*, rhizome of *C. longa*, leaves of *O. basilicum*, rhizome of *Z. officinale*, and leaves of *C. martini may perhaps considerably* appropriate for minimizing the fungal growth and mycotoxins in food sources. Though, mechanism of action involved in the antifungal and OTA inhibitory activities need to be disclose in detail. However, several studies reported that EOs are volatile and highly unstable to light, temperature, oxygen, pH and other environmental conditions. Therefore, stability and activity of EOs in food matrices need to be enhanced by modern techniques, i.e. nanotechnology [50,69].

5. Conclusions

In this study, EOs were extracted from bark of *C. zeylanicum*, rhizome of *C. longa*, leaves of *O. basilicum*, rhizome of *Z. officinale*, and leaves of *C. martini* by hydrodistillation technique. The GC–MS chemical profile settled that EOs of *C. zeylanicum*, *C. longa*, *O. basilicum*, *Z. officinale*, and *C. martini* were belonged to the chemotype of linalool, ar-turmerone, eugenol, geranyl proprionate, and geranyl acetate, respectively. The EOs presented potent antioxidant activity in DPPH and ABTS radical scavenging assays and thereby EOs could find their potential representation in food industry as natural antioxidants. The EOs exhibited potential MIC and MFC values against *P. verrucosum* and *A. ochraceous* in micro-well dilution technique. Further, EOs showed excellent antifungal and OTA inhibitory activities against *P. verrucosum* and *A. ochraceous* in in-vitro food sample, maize grains. The investigation clinched that EOs could be considerably appropriate for keeping safe of food and feed matrices from fungal infestation and mycotoxin contamination, especially during the period of storage.

CRediT authorship contribution statement

Naveen Kumar Kalagatur: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Selvakumar Gurunathan:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing - original draft, Writing - review & editing. **Jalarama Reddy Kamasani:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Lokanadhan Gunti:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Krishna Kadirvelu:** Writing - original draft, Writing - review & editing. **Chakrabhavi Dhananjaya Mohan:** Writing - original draft, Writing - review & editing. **Shobith Rangappa:** Writing - original draft, Writing - review & editing. **Ram Prasad:** Writing - original draft, Writing - review & editing. **Fausto Almeida:** Writing - original draft, Writing - review & editing. **Venkataramana Mudili:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Chandranayaka Siddaiah:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

First author Naveen Kumar Kalagatur was thankful to CSIR for providing CSIR-RA [File no. 09/1202(001)/2018-EMR-I]. The corresponding author Chandranayaka Siddaiah thanks the Institution of Excellence, University of Mysore, for providing infrastructure, financial and other research facilities. The authors would also like to acknowledge the facilities provided by DST-PURSE program, DST, New Delhi to the University of Mysore, Mysuru, 570005, India.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2020.e00490.

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