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Investigating the chemical composition and antifungal effect of *Cinnamomum cassia* essential oil against *Saccharomyces cerevisiae* and *Acremonium* sp

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Essential oils are promising, safe, and eco-friendly alternatives to chemical fungicides. This study aimed to develop an effective biological control agent using *Cinnamomum cassia* essential oil (CCEO) as potential fungicidal agent against *Saccharomyces cerevisiae* and *Acremonium* sp, both isolated from natural orange juice. The yield, chemical composition and antifungal activity of CCEO were evaluated. The essential oil was extracted via hydro-distillation, and its composition was analyzed using gas chromatography-mass spectrometry (GC-MS). The antifungal activity was assessed using the disk diffusion agar method. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined using microdilution methods. The extraction yield was 2.8%. (E)-cinnamaldehyde was identified as the major compound (37.72%). Inhibition zones ranged from 51 mm to 80 mm against *Saccharomyces cerevisiae* and from 75 mm to 90 mm against *Acremonium* sp. Equal MIC and MFC values were recorded for both fungal strains: MIC = MFC = 6.25% against *Saccharomyces cerevisiae* and MIC = MFC = 3.125% against *Acremonium* sp. These findings demonstrate for the first time that CCEO could be a promising antifungal agent against the two primary fungal contaminants of fruit products, *Saccharomyces cerevisiae* and *Acremonium* sp.

Keywords Chemical composition, Antifungal activity, *Cinnamomum cassia* essential oil, Orange juice, *Saccharomyces cerevisiae*, *Acremonium* sp

Abbreviations

CCEO	Cinnamomum cassia essential oil
CEO	Cinnamomum essential oil
GC-MS	Gas chromatography–mass spectrometry
GRAS	Generally Recognized as Safe
RT	Retention time
PDA	Potato dextrose agar
PDB	Potato dextrose broth
DMSO	Dimethyl sulfoxide
MIC	Minimum inhibitory concentration
MFC	Minimum fungicidal concentration

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Plant pathogenic fungi and foodborne fungi cause various post-harvest diseases, leading to significant losses in agricultural products and posing threats to both animal and human health. In recent years, protecting food and agricultural products has become a global priority, focusing on the control of both pre- and post-harvest diseases in fresh fruits and vegetables[1,2]. For over a century, biocontrol agents have been used to manage target plant diseases because of their effectiveness and associated health benefits[3]. Moreover, biocontrol agents can positively impact food production, nutrient availability, and environmental health, which in turn contribute to economic development and ecological sustainability. These benefits are largely attributed to their eco-friendly properties and durability[4].

Orange juice is popular worldwide for its refreshing taste and nutritional benefits. It is rich in essential nutrients, including vitamin C, potassium, folate, and antioxidants such as flavonoids, which support overall health and well-being[5]. Orange juice is primarily produced in countries where citrus fruits are cultivated. Global orange juice production has increased significantly in recent years, reaching a total of 1.5 million tons in 2023. Brazil is the world's leading orange juice producer, with 1.1 million tons, followed by Mexico with 155,000 tons, the United States with 110,000 tons, and the European Union with 47,000 tons[6]. Juice manufacturers worldwide face significant challenges due to microbial contamination naturally present in raw fruits. Few microorganisms can survive pasteurization and continue to grow, leading to substantial nutritional and economic losses in the final product. Additionally, the risk of contamination increases with the use of low-quality raw materials[7].

Among the yeasts that cause spoilage in orange juice, *Saccharomyces cerevisiae* is one of the most common. It can spoil fruit products by fermenting sugars to produce CO_2 and ethanol[8]. On the other hand, among the molds that cause spoilage of fruit products, *Acremonium* sp is responsible for white cottony rot in orange fruits before harvest. These fungi affect the sensory and nutritional qualities of orange juice by producing unpleasant odors, causing color loss through pectin methyl esterase activity, and generating carbon dioxide during the decomposition of vitamin C[9].

In recent times, significant efforts have been made to improve production technology, distribution, hygiene, and consumption standards. However, various foodborne microorganisms with biofilm-forming abilities continue to cause serious contamination in the food industry, resulting in substantial economic losses. Controlling microbial growth in food products has become a top priority to ensure both food safety and reduce food waste. The use of plant essential oils (EOs) as natural antifungals to replace chemical antifungal agents has recently gained interest in the food industry. These natural preservatives are considered healthier than synthetic ones and offer added nutritional and biological value[10].

Essential oils (EOs) are important aromatic compounds found in herbs and spices, and their biological activities have been recognized and utilized since ancient times in perfumery, food preservation, flavoring, and medicine. The antimicrobial properties of essential oils clearly demonstrate that they are highly valued for their unique antibacterial, antifungal, and antiviral effects[11], which makes them an ideal alternative to synthetic food additives for preserving food products[12]. Furthermore, EOs are Generally Recognized as Safe (GRAS), making them a viable alternative to synthetic preservatives for extending the shelf life of highly perishable food products by impeding the proliferation of foodborne pathogens[10]. In 2008, the European Commission published a list of approved compounds, which is regularly updated. Among the aromatic compounds recognized as safe for human health are limonene, linalool, β-caryophyllene, pinene, thymol, carvacrol, carvone, eugenol, isoeugenol, cinnamaldehyde, vanillin, citral, citronellal, menthol and lavandulol[13].

Since EOs contain volatile components with potentially strong antifungal activity, selecting an appropriate in vitro antifungal screening assay is crucial and should be tailored to food applications[14]. According to a 2018 literature review, there have been 2,473 studies on the antimicrobial effects of EOs on food products since 1990. These include 97 studies on bread and baked goods, 216 on milk and dairy products, 410 on meat products, 415 on fish products, 403 on vegetables, and 657 on fruits[15,16]. Several publications have investigated the potential of EOs to extend shelf life and prevent the growth of pathogens in food. Dwivedy et al.[17] revealed that *Mentha cardiaca* EO could be used as a natural food alternative to prevent fungal contamination in stored dry fruits. Almeida et al.[18] reported that *Mentha piperita* L. EO was effective in inactivating *Candida albicans*, *Candida tropicalis*, *Pichia anomala* and *Saccharomyces cerevisiae* in cashew, guava, mango and pineapple juices during 72 h of refrigerated storage. Kumar et al.[19] proved the efficacy of *Artemisia nilagirica* EO to enhance the shelf life of stored millets by preventing fungal contamination. Similarly, Hamada Saoud et al.[20] showed that wild fennel leaf EO exhibited a high level of antifungal activity and could be a very promising natural option for use in food preservation. Also, Aminifard and Bayat[21]. reported that anise and black caraway EOs can be used as bio-fungicides against the food-borne fungi *Penicillium digitatum* on blood orange fruit. Valková et al.[22] indicated that lemongrass EO exhibited strong antifungal activity and could serve as a promising natural antimicrobial agent. It could be applied in innovative packaging for bakery products and various types of vegetables by combining commonly used packaging materials with the addition of lemongrass EO.

Cinnamomum cassia is a tropical medicinal and aromatic plant from the *Lauraceae* family, commonly used as a natural spice in food preparation and in traditional medicine to treat respiratory and digestive disorders[23]. The US Environmental Protection Agency has exempted *C. cassia* essential oil from toxicity reporting requirements, and the US Food and Drug Administration has recognized it as safe[24]. *Cinnamomum cassia* essential oils contain many functional components with antimicrobial properties, making them a good alternative for use as a natural antifungal[25]. The aromatic, non-toxic and broad-spectrum antimicrobial properties of *C. cassia* EO and cinnamaldehyde can give *C. cassia* bark versatile for use in food, veterinary applications, and alternative medicine. This aligns with the centuries-old traditional use of cinnamon bark in Chinese herbal medicine[26]. The objectives of the present study were to control spoilage of orange juice caused by *Saccharomyces cerevisiae* and *Acremonium* sp. using *Cinnamomum cassia* essential oil (CCEO), and to identify the chemical compounds responsible for its

antifungal activity. This aims to support its future use by food manufacturers and in agricultural products as a natural antifungal agent, reducing the harmful side effects of chemical antifungal products.

Materials and methods

Plant material and chemicals

C. cassia barks were purchased from a local herbalist in Bordj Bou-Arreridj, Algeria. The plant material was verified to ensure its identity by comparison with a reference herbarium sample in the Department of Biology, Faculty of Science, University of Saida, Algeria, identified by taxonomist Pr. Sitayeb under the code P-200,986. Dimethyl sulfoxide (DMSO), anhydrous sodium sulfate, hexane, Potato Dextrose Agar (PDA), and Potato Dextrose Broth (PDB) were obtained from Sigma-Aldrich.

Essential oil extraction

EO extraction was carried out by hydro-distillation in a Clevenger-type apparatus. Briefly, 100 g of crushed *C. cassia* barks were submitted to hydro-distillation for 3 h with 500 ml of distilled water. After extraction, the EO was dried with anhydrous sodium sulfate, filtered and then stored in an opaque bottle at 4 °C until analysis[27]. The extraction yield was calculated as the ratio between the amount of EO obtained and the amount of plant material used for extraction (100 g)[28] and calculated using the following formula (1):

$$\mathrm{YEO}(\%) = (\mathrm{MHE}/\mathrm{MS}) \times 100 \quad (1)$$

Y_{EO}: essential oil yield (%); M_{EO}: mass of essential oil extracted (g); M_S: mass of plant material used (g).

Chemical composition by GC-MS analysis

Gas chromatography-mass spectrometry (GC–MS) equipment was used to analyze the chemical composition of CCEO. GC–MS analysis was performed using a gas chromatograph (Shimadzu GC 2010; Columbia, MD) equipped with a split-splitless injector system and an autosampler. The system was coupled to a mass spectrometer (Shimadzu QP-2010) operating in electron impact (EI) mode. A Restek Rtx-5MS capillary column was used for separation (30 m × 0.25 mm inner diameter × 0.25 µm film thickness). The volume of injection was 1µL with a split of 1 min and the injector temperature was 290 °C. Helium (99.9% purity) served as the carrier gas. Oven temperature was held at 60 °C for 2 min and increased to 270 °C for 20 min, to 300 °C for 5 min. MS operated simultaneously in full scan (SCAN) and the parameters were set to the following conditions: ionization energy 70 eV, ion source temperature 250 °C, mass range 40 to 510 m/z and total analysis time 2–80 min. Compound identification was performed using computer matching by the NIST mass spectrum database (NIST 2011 version) and home-made library mass spectra, based on the data in the MS literature.

Antifungal effect

Preparation of natural orange juice samples

Fresh orange fruits were purchased from an Algerian local market. The fruits were cleaned to remove any residual contaminants or physical damage. The oranges were rinsed with water and cut into half. Orange juice was extracted using a home extractor (Condor JC115, Algeria), then purified by filter (filter pore size 10 µm) to obtain 10 ml of orange juice without any treatments.

Fungal isolation and identification

Fungal isolation was performed according to Samson et al.[29]. 1 mL of orange juice sample was diluted in 9 mL of 0.1% sterilized peptone water, then serially diluted to 10^{-4}. An aliquot of 0.1 mL of each dilution was surface-plated on 20 mL PDA plates and incubated for 7 days at 25 °C. The isolated pure colonies were again sub-cultured on PDA and incubated in the same conditions to obtain pure monosporic strains. Fungal strains were identified using the macroscopic and microscopic aspects.

Preparation and standardization of fungal inoculum

Fungal strains were maintained on PDA. They were inoculated at 37 °C for 24–48 h then, 3 to 5 of isolated colonies from the overnight culture were suspended in 0.9% of sterile saline. The turbidity of the suspension was measured using the spectrophotometer to obtain the absorbance of 0.08–0.10 which is comparable to 0.5 McFarland standards equivalent to 1×10^6 CFU/mL[30].

Antifungal activity of CCEO

Disc diffusion method

Antifungal activity was evaluated by disc diffusion method against *Saccharomyces cerevisiae and Acremonium* sp. Briefly, 200 µL of spore suspension (10^6 CFU/mL) was spread on a PDA plate. Five concentrations of CCEO (100, 50, 25, 12.5 and 6.25%) were prepared using a series of two-fold dilutions in DMSO. Sterile Whatman paper discs (no. 1, 6 mm in diameter) were impregnated with 20 µL of CCEO and aseptically placed on the surface of the PDA plates. A plate containing PDA and fungal suspension without EO was used as a control, while the plate containing PDA and fungal suspension with impregnated disk of DMSO was used as negative control. The plates were placed in a refrigerator at 4 °C for 2 h to allow the essential oil to diffuse into the agar then incubated at 28 °C for five days for mold and three days for yeast[31]. After incubation, the diameter (mm) of the inhibition zone was measured to evaluate the antifungal activity[32].

Determination of MIC

Minimum inhibitory concentration (MIC) was determined using the microdilution method in a 96-well microtiter plate. Essential oil was dissolved in 1% DMSO, and then 100 µL of EO solution diluted in 100 µL of PDB (Potato Dextrose Broth). Serial dilutions of EO (concentrations ranging from 100% (1/1) to 0.00195% (1/512) v/v %) were prepared in a 96-well microtiter plate. Each dilution of essential oils was inoculated with 100 µL of the fungal inoculum (10^6 CFU/mL). The experimental group was the inoculum with culture medium and the tested concentrations of CCEO in the columns from 1 to 10. Columns 11 and 12 contained controls (inoculum and medium) and negative controls (DMSO and medium), respectively. The microplates were incubated for 24 h at 35 °C, and the MIC was determined by visual observation. The formation of cell clumps ("buds") at the bottoms of the wells was considered, and the MIC was defined as the lowest concentration of EO in the first non-turbid well[33].

Determination of MFC

The minimum fungicidal concentration (MFC) was determined by subculturing 10 µL aliquots of the concentration corresponding to the MIC and the two immediately higher concentrations onto a PDA plate, followed by incubation at 35 °C for 48 h. The minimum concentration of the essential oil where no visible growth was observed after 48 h of incubation was defined as the MFC. The MFC/MIC ratio was calculated to determine whether CCEO exhibits fungistatic (MFC/MIC ≥ 4) or fungicidal activity (MFC/MIC < 4)[34].

Statistical data analysis

Statistical data analysis was performed using SPSS IBM Statistics version 29.0.10. A one-way ANOVA test followed by Tukey's post hoc multiple comparison test was used to determine significant differences ($P < 0.05$). All experiments were conducted in triplicate and the results from the three measurements were reported as the mean ± SD ($n = 3$).

Results

Extraction yield and chemical composition of *C. cassia*

The hydro-distillation of *C. cassia* bark gave clear yellow wax oil and the yield obtained was 2.8%; which was relatively high. Chemical compositions of CCEO are presented in Fig. 1 and the data are given in Table 1. The chemical analysis of *C. cassia* EO oil revealed 25 compounds, along with other components (≤ 0.05%), totaling 99.61%. These compounds belonged to two main classes: aromatic organic compounds and sesquiterpenes. The major compounds were (E)-cinnamaldehyde (37.72%), δ-cadinene (5.02%), α-copaene (4.23%), α-murolene (3.90%) and γ-cadinene (3.43%). Other components were present in amounts less than 2%.

Antifungal activity of CCEO

The macroscopic and microscopic characteristics of the isolated strains allowed us to identify two fungal strains *Saccharomyces cerevisiae* (yeast) and *Acremonium* sp (mold). As shown in Table 2, CCEO possessed a significant

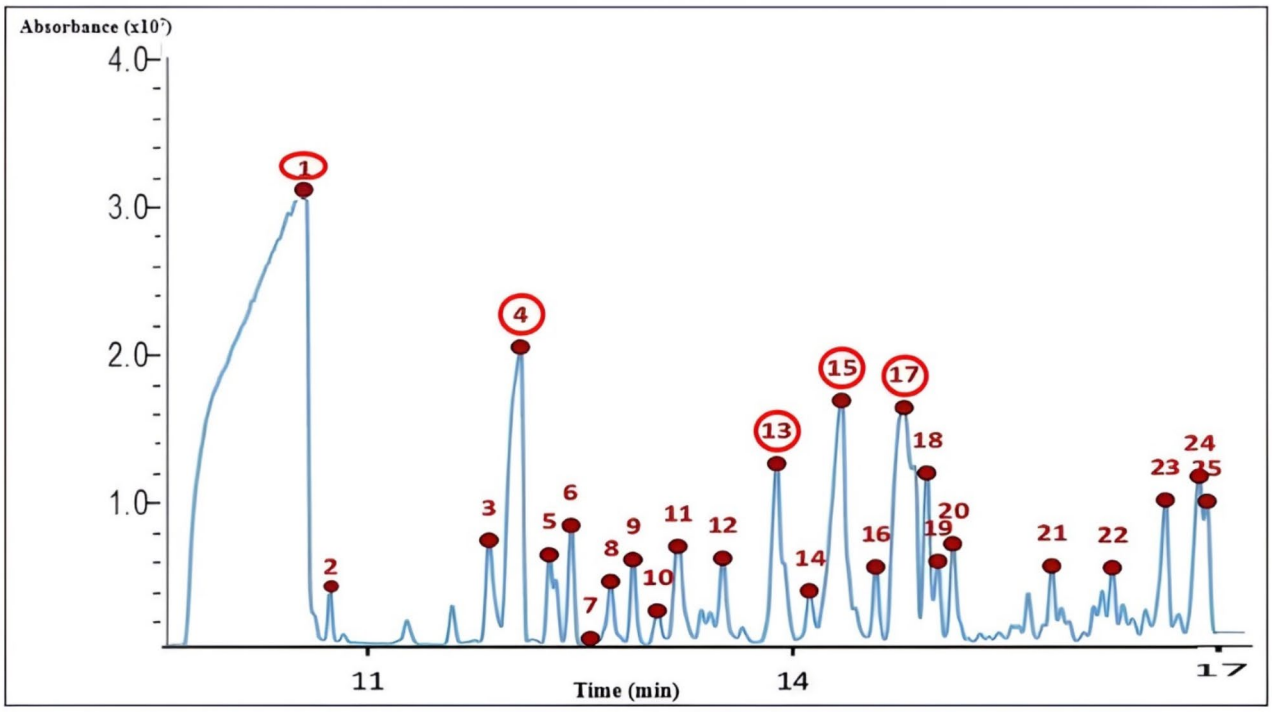

Fig. 1. GC–MS chromatogram of CCEO. The major compounds are circled in red.

Peak	Compound	Formula	Peak RT	Area %	Height%
1	Cinnamaldehyde, (E)-	C_9H_8O	8,79	37,72	15,67
2	Cuminaldehyde	$C_{10}H_{12}O$	9,24	0,99	0,53
3	Cyclosativene	$C_{15}H_{24}$	11,85	0,98	1,78
4	α-Copaene	$C_{15}H_{24}$	12,08	4,23	5,00
5	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1 S-(1α,2β,4β)]-	$C_{15}H_{22}O$	12,28	0,59	1,54
6	Sativene	$C_{15}H_{24}$	12,43	0,79	2,03
7	Benzene, (1-propyl-1-nonenyl)-	$C_{16}H_{26}$	12,57	0,09	0,14
8	Isosativene	$C_{15}H_{24}$	12,71	0,56	1,09
9	Caryophyllene	$C_{15}H_{24}$	12,87	0,57	1,46
10	Bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)-	$C_{16}H_{26}$	13,04	0,31	0,61
11	2 H-1-Benzopyran-2-one	$C_{11}H_{10}O$	13,19	1,12	1,68
12	α-Humulene	$C_{15}H_{24}$	13,50	0,76	1,48
13	γ-Cadinene	$C_{15}H_{24}$	13,89	2,84	3,06
14	β-Selinene	$C_{15}H_{24}$	14,12	0,50	0,94
15	α-Muurolene	$C_{15}H_{24}$	14,34	3,90	4,11
16	γ-Cadinene	$C_{15}H_{24}$	14,59	0,59	1,34
17	δ-Cadinene	$C_{15}H_{24}$	14,78	5,02	3,99
18	Naphthalene,1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	$C_{15}H_{24}$	14,95	1,27	2,90
19	4,5,9,10-Dehydro-isolongifolene	$C_{15}H_{20}$	15,02	0,55	1,43
20	α-Calacorene	$C_{15}H_{20}$	15,13	0,85	1,72
21	Gleenol	$C_{15}H_{26}O$	15,83	0,56	1,35
22	Viridiflorol	$C_{15}H_{26}O$	16,26	0,55	1,33
23	Di-epi-α-cedrene-(I)	$C_{15}H_{24}$	16,63	1,37	2,45
24	t-Muurolol	$C_{15}H_{26}O$	16,87	1,70	2,85
25	Torreyol	$C_{15}H_{26}O$	16,92	0,89	2,43
	Total compound (%)			65,93	62,16
	Other components < 0.05%			33,68	36,95
	Total			99,61	99,11
	Total identification classes			99,61	99,11
	Aromatic organic compounds			55,28	54,52
	Sesquiterpenoid			44,33	44,59

Table 1. Chemical composition of CCEO by GC–MS analysis. *RT* retention time (min).

	Concentrations (v/v)					
	Inhibition zone diameters (mm)					
Fungi	100%	50%	25%	12.5%	6.25%	DMSO
Saccharomyces cerevisiae	80 ± 0.83 a	78 ± 0.32 b	63 ± 0.44 c	58 ± 0.36 d	51 ± 0.71 e	0 ± 0.00 f
Acremonium sp	90 ± 0.55 a	90 ± 0.90 a	90 ± 0.22 a	80 ± 0.12 b	75 ± 0.55 c	0 ± 0.00 d

Table 2. Inhibition zone diameters of CCEO against tested fungi. Values are given as mean ± SD ($n = 3$). Different capital letters represent significant variations ($P < 0.05$) according to the ANOVA test followed by Tukey's comparison tests.

antifungal activity on both fungi tested at all concentration with diameters of the inhibition zone ranging from 51 mm to 80 mm and from 75 mm to 90 mm for *Saccharomyces cerevisiae* ($P < 0.05$) and *Acremonium* sp, respectively. The growth of *Acremonium* sp was completely inhibited by CCEO at 100, 50 and 25% (v/v) ($P > 0.05$) (Fig. 2). The results obtained indicate that The diameters of the inhibition zone increased proportionally with CCEO concentration, a dose-dependent effect. In general, fungal growth was significantly ($P < 0.05$) slowed down by increasing CCEO concentrations. No inhibition of fungal growth by negative control (DMSO 1%). On the other hand, both fungi tested were sensitive to CCEO, with *Acremonium* sp. being the most sensitive. As a result, the inhibitory effect depended on the concentration of CCEO and the sensitivity of the fungal strains tested.

The results of MIC and MFC are presented in Table 3 and the aspects of the microplates are shown in Fig. 3. Equal values of MIC and MFC were obtained against the two fungal strains tested (MIC = MFC = 6.25% against *Saccharomyces cerevisiae* and MIC = MFC = 3.125% against *Acremonium* sp). It is notable that the MIC and MFC

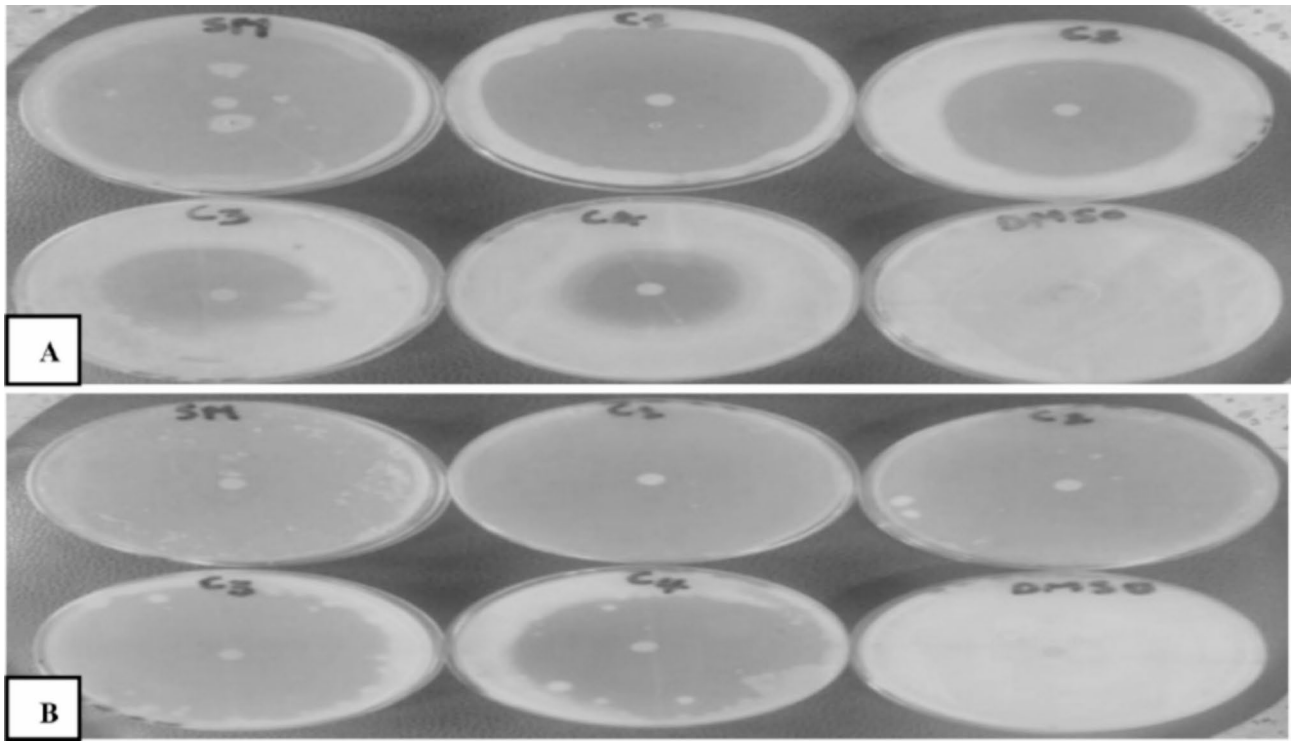

Fig. 2. Effect of CCEO on (**A**) *Saccharomyces cerevisiae* and (**B**) *Acremonium* sp respectively.

Fungi	MIC %	MFC %	MIC/MFC ratio
Saccharomyces cerevisiae	6.25 ± 0.00	6.25 ± 0.00	1
Acremonium sp.	3.125 ± 0.00	3.125 ± 0.00	1
Classification of CCEO	Fungicidal	Fungicidal	/

Table 3. MIC and MFC of CCEO against tested fungi. Fungicidal: MFC/MIC < 4; Fungistatic MFC/MIC ≥ 4.

values were the same, and the MIC/MFC ratio was 1 (< 4), indicating that CCEO exhibited both inhibitory and fungicidal effects at a single concentration for both fungal strains.

Discussion

The hydro-distillation of *C. cassia* bark yielded 2.8%, which is relatively high compared to some plants that are industrially exploited for their essential oils. Various studies on *C. cassia* essential oil have been reviewed to compare the yield obtained. The extraction yield of *C. cassia* EO was similar to that previously reported by Vu and Ho[35] (2.55%), Benmoussa et al.[36] (2.67%) and Jadhav et al.[37] (2.98%) respectively. However, this yield was higher than those obtained by Chen et al.[38] (1.68%) and Jeyaratnam et al.[39] (1.89%). Jilali et al.[40] linked this difference in EO yield to the various extraction techniques and the extraction period. Additionally, Rezouki et al.[41] found that the harvest period, the variation of the phenological stages and the drying conditions influence also the yields and the contents of EOs.

According to the GC-MS results, the major compounds of *C. cassia* EO were identified as (E)-cinnamaldehyde (37.72%), δ-cadinene (5.02%), α-copaene (4.23%), α-murolene (3.90%) and γ-cadinene (3.43%). Similarly, the major compound of *C. cassia* EO with the highest amount was (E)-cinnamaldehyde with a total percentage of 37.40%, 61.57%, 73.2%, 85.77% and 86.64% [25; 42,43; 44; 45]. A previous study revealed that E-cinnamaldehyde (73.23%), α-copaene (6.44%), and eugenol (4.23%) were the major compounds in *C. cassia* essential oil, which closely aligns with the results obtained in this research[43]. Similarly, another conducted study demonstrated that (E)-cinnamaldehyde (86.64%), cinnamyl acetate (6.66), α-copaene (0.95) and bornyl acetate (0.65) were the major compounds of *C. cassia* EO[45]. Also, Kačániová et al.[25] found that the main compounds of CCEO were cinnamaldehyde (61.57%), trans-4-methoxycinnamaldehyde (13.78%), cinnamyl acetate (5.35%), and o-hydroxy-cinnamic acid (4.12%). However, the chemical composition of C. *cassia* EO differed from that reported by Vu and Ho[35]; Yang et al.[46] and Ma et al.[47] where the major compound was the trans-cinnamaldehyde with a total percentage of 99.24%, 74.60% and 75.65%, respectively. Recent research conducted by Li et al.[48] reported that the variation in climate conditions has a significant influence on the phytochemical profile and the percentage of *C. cassia* EO (the percentage of trans-cinnamaldehyde varied in 31 samples collected from different

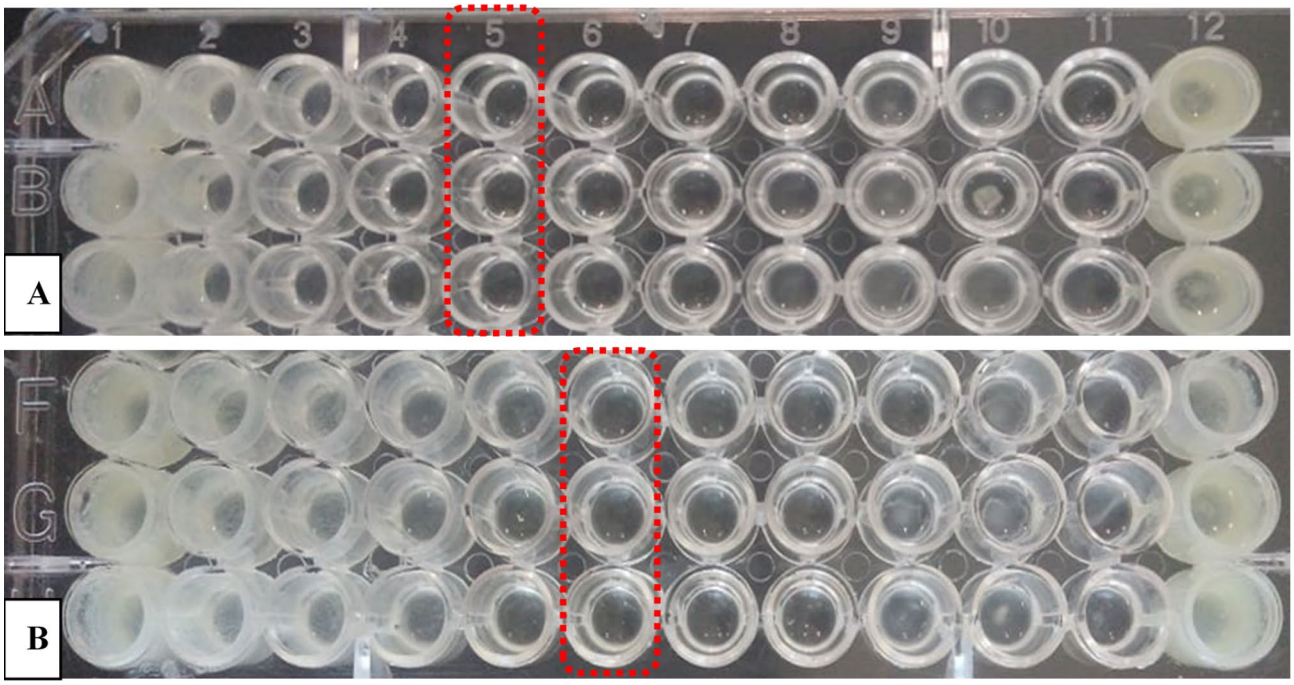

Fig. 3. MIC microplate photo for (**A**) *Saccharomyces cerevisiae* (MIC=6.25%) and (**B**) *Acremonium* sp (MIC=3.125%), respectively. MIC rectangles in red.

habitat in the same area from China). Also, Thinh et al.[49] revealed that the various extraction techniques such as hydro-distillation, steam distillation, and microwave-assisted hydro-distillation has a significant influence the phytochemical profile of EO. Furthermore, there was also a difference among the compounds extracted from each specific plant material, meaning that each plant had a distinct EO with different components. As a result, the variations observed in previous research are justified.

Oranges are an important crop for the fresh market and for producing orange juice. Fungal diseases that damage orange crops and orange juice have raised concerns about human health and the risks associated with the extensive use of chemical fungicides. This investigation is the first to demonstrate the inhibitory effect of *C. cassia* EO against the two primary contaminants: *Saccharomyces cerevisiae* and *Acremonium* sp of fruit products. The result showed that *C. cassia* EO possessed a fungicidal action with MIC = MFC of 6.25% and MIC = MFC of 3.125% against *Saccharomyces cerevisiae* and *Acremonium* sp, respectively. However, several previous studies have investigated the inhibitory effect of *C. cassia* EO on other food-borne strains. Similarly, previous research found that *C. cassia* EO exhibited a strong antifungal action with different MIC values. Kocevski et al.[50] reported that *C. cassia* EO had a strong inhibitory effect against food-borne *Aspergillus* species with a MIC of only 75 ppm. Zamindar et al.[51] mentioned that cinnamon bark EO inhibited the growth of *Byssochlamys fulva* in a tomato sauce with an MIC of 400 ppm. More so, Singh et al.[52] revealed a strong antifungal activity of CCEO against aflatoxigenic food borne *Aspergillus flavus* which completely inhibited the fungal growth and aflatoxin B1 secretion at a MIC of 0.06 µL/mL. Interestingly, Minozzo et al.[53] suggested that CCEO can be used as natural alternatives in maize flour to control *Penicillium crustosum*, *Alternaria alternata* and *Aspergillus flavus* with a low MIC value of 0.5%. In addition, *C. cassia* EO inhibited the growth of *Botrytis cinerea* in strawberries with an MIC of 400–800 ppm[54]. Similarly, Valková et al.[55] found that *C. cassia* EO exhibited a strong antifungal activity with an MIC of 500 µL/L against wheat bread contamination by *Penicillium crustosum*. Kulkarni et al.[56] revealed that *C. cassia* EO oil at 6 µL per plate completely inhibited the fungal growth of *Colletotrichum musae* and *Lasiodiplodia theobromae*, which cause crown-rot postharvest diseases in banana fruit. Kačániová et al.[25] recorded that CCEO possessed a high antifungal activity against *Penicillium citrinum*, *Penicillium crustosum*, *Penicillium expansum* on carrot at a concentration of 125 µL/L, on potato at a concentration of 250 µL/L and on sweet potato at a concentration of 500 µL/L. Another study reported that cinnamon essential oil (CEO) inhibited the germination growth of *Aspergillus. flavus* in coix seed with an MIC of 0.5 µL/mL[57]. Similarly, CEO completely inhibited the spore germination of *Penicillium oxalicum* isolated from rice noodles with an MIC of 0.025% (v/v)[58]. Also, CEO inhibited the growth of *Rhizopus stolonifer*, which is the major cause of soft rot in peaches with an MIC of 0.8 mL/L[59]. The qualitative and quantitative variation in the phytochemical profile of EOs may affect its antimicrobial activity[40]. Furthermore, the antimicrobial activity may be influenced by differences in the concentrations of EOs used and the target foodborne strains[60]. In our opinion, the variation in MIC values across the literature could be related to the methods used to determine the MIC, the concentrations and volumes of EO used, the target strains, and the lack of literature recommendations on the minimum volume required for MIC determination.

Generally, it is often challenging to associate the inhibitory effect of EO with a specific component due to their complexity and variability. However, some previous studies have indicated a correlation between the chemical composition of the predominant constituents of the EO and its antimicrobial effect[60]. In the present study, *C. cassia* studied proved that (E) – cinnamaldehyde is the main compound. It has been previously reported that (E)-cinnamaldehyde was responsible for the effective antimicrobial activity of *C. cassia* EO[26,36,38]. On the other hand, the antifungal mechanisms of CCEO are not well established. However, several recent studies have been conducted in order to investigate the antifungal mechanisms of CEO. Zhao et al.[57] reported that CEO disrupted cell membrane integrity, suppressed aflatoxin production, and increased mycelial oxidative damage of *Aspergillus flavus*. Similarly, Zhao et al.[59] found that CEO destroyed the cell membrane structure and caused oxidative damage, which led to the ions and protein leakage and disrupted the metabolic equilibrium of *Rhizopus stolonifer*. Meanwhile, Liu et al.[58] revealed that CEO caused the disruption of the fungal plasma membrane by the changing of plasma membrane permeability and the leakage of cellular components of *Penicillium oxalicum*. Furthermore, the mechanism of action of most EOs can be primarily linked to their hydrophobic properties, which allow them to penetrate the lipids present in the microbial cell membrane. This penetration disrupts the structural integrity of the membranes, increasing their permeability and leading to cell death[61]. The antimicrobial activity of EOs is also largely linked to their composition, and the mechanism of action of terpene is typically associated with its interaction with membrane cells[62]. In this context, terpenes such as the hydrophobic cinnamaldehyde alter the lipid components of microbial cell membranes, increasing membrane permeability and causing the breakdown of the cell envelope. Also, the aldehyde groups have the ability to cross-link through amine groups with fungal DNA and proteins, thereby disrupting fungal reproduction[63]. Hu et al.[64] reported that cinnamaldehyde disrupts calcium homeostasis, which in turn affects fungal metabolism and inhibits growth. A recent study also revealed that cinnamaldehyde damages the integrity of the fungal cell wall and cell membrane, leading to growth inhibition[65]. Furthermore, cinnamaldehyde alters the mycelial morphology, damage the plasma membrane and hinder the biosynthesis of ergosterol and induced the generation of reactive oxygen species[66]. Additionally, the synergistic effect of various compounds of CCEO may be involved. Boniface et al.[67] attributed the significant antifungal activity of CEO against food-borne pathogens to the synergistic effect of its main compounds cinnamaldehyde, cinnamyl acetate and cinnamyl benzoate. Jiang et al.[68] tested the effects of three compounds of CCEO on the growth of *Sclerotinia sclerotiorum* and found that cinnamaldehyde exhibited the highest antifungal activity with 2' methoxycinnamaldehyde and coniferyl aldehyde. Similarly, Ma et al.[47] recorded that the synergistic action of the CCEO with its main active components trans-cinnamaldehyde, cinnamaldehyde and (E)-2 methoxycinnamaldehyde exhibited significant antifungal activity against the pathogenic fungi in *Panax notoginseng*. Also, Kulkarni et al.[56] revealed that the synergistic effect of various compounds of CCEO (aryophyllene, eugenol, cinnamyl acetate, trans-calamenene and humuleneis) responsible for the strong antifungal activity against the growth crown-rot postharvest diseases in banana fruits.

Conclusion

In conclusion, the present study demonstrates that *C. cassia* essential oil exhibits a strong fungicidal activity against two primary contaminants: *Saccharomyces cerevisiae* and *Acremonium* sp. Therefore, it could be used as a natural fungicide to control diseases in orange crops and orange juice. The antifungal effect of CCEO may be due to the synergistic action of various compounds and/or the presence of (E)-cinnamaldehyde as the major compound. However, the mechanism of the fungicidal action of CCEO remains unclear. Furthermore, additional in vivo studies are needed to better understand the mechanisms of action of all the compounds, both separately and in combination.

Data availability

All data supporting the findings of this study are available within the paper.

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Author contributions

Conceptualization, F.B., T.B., N.B.; methodology, F.B.; software, F.B., T.B.; validation, T.B., Y.B. and N.Y.R.; formal analysis, F.B., T.B. and A.O.U.; investigation, F.B., Y.B.and O.D.K.; resources, T.B.; data curation, F.B.; writing the original draft manuscript: F.B.; writing—review and editing, Y.B., A.A. and N.Y.R.; visualization, F.B., T.B., N.B.; supervision, T.B., D.E.K.; performed the chemical characterization of CCEO by GC-MS analysis: AA.; project administration, D.E.K. All authors have agreed to the published of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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