Zulu *et al. Chem. Biol. Technol. Agric.* (2023) 10:82
https://doi.org/10.1186/s40538-023-00434-3

Chemical and Biological Technologies in Agriculture

RESEARCH

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Antifungal effects of seven plant essential oils against *Penicillium digitatum*

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Abstract

Background Research interest in plant essential oils has increased significantly due to their natural properties and consumer demand for safer methods of food preservation. Plants produce large amounts of secondary metabolites, which have potential activity against fungal pathogens. This study aimed at screening essential oils for their antifungal effects on citrus against *Penicillium digitatum*, morphological effect and finally determine which essential oils are the most effective.

Results The EC_{50} of seven selected cinnamon (0.424 µL/mL), patchouli (0.513 µL/mL), vetiver (0.612 µL/mL), dill (1.597 µL/mL), origanum (1.971 µL/mL) and ylang (2.214 µL/mL) was determined. In addition, cinnamon substantially reduced sporulation (100%) followed by patchouli (86.02%), vetiver (82.73%), and chamomile (79.04%), respectively. Our GC–MS result determined variance in concentration of essential oils compound composition. The total compound composition in all seven essential oils > 1% was found to be 3 in cinnamon, 5 in dill, 10 in origanum, 13 in ylang, 11 in patchouli, 9 in chamomile and 16 in vetiver. Addition of essential oils significantly altered fungal morphology by scanning electron cryomicroscopy. Patchouli and origanum showed broken hyphae while there was an indication of severe deformation and collapse of spores in cinnamon and chamomile.

Conclusion Based on our findings, we report that these essential oils could potentially be applicable in controlling *P. digitatum* with reduced concern for human health, environmental contamination and possibly replacement of synthetic treatments.

Keywords *Penicillium digitatum*, Essential oil, Antifungal, Sporulation, Inhibition, Cryo-SEM

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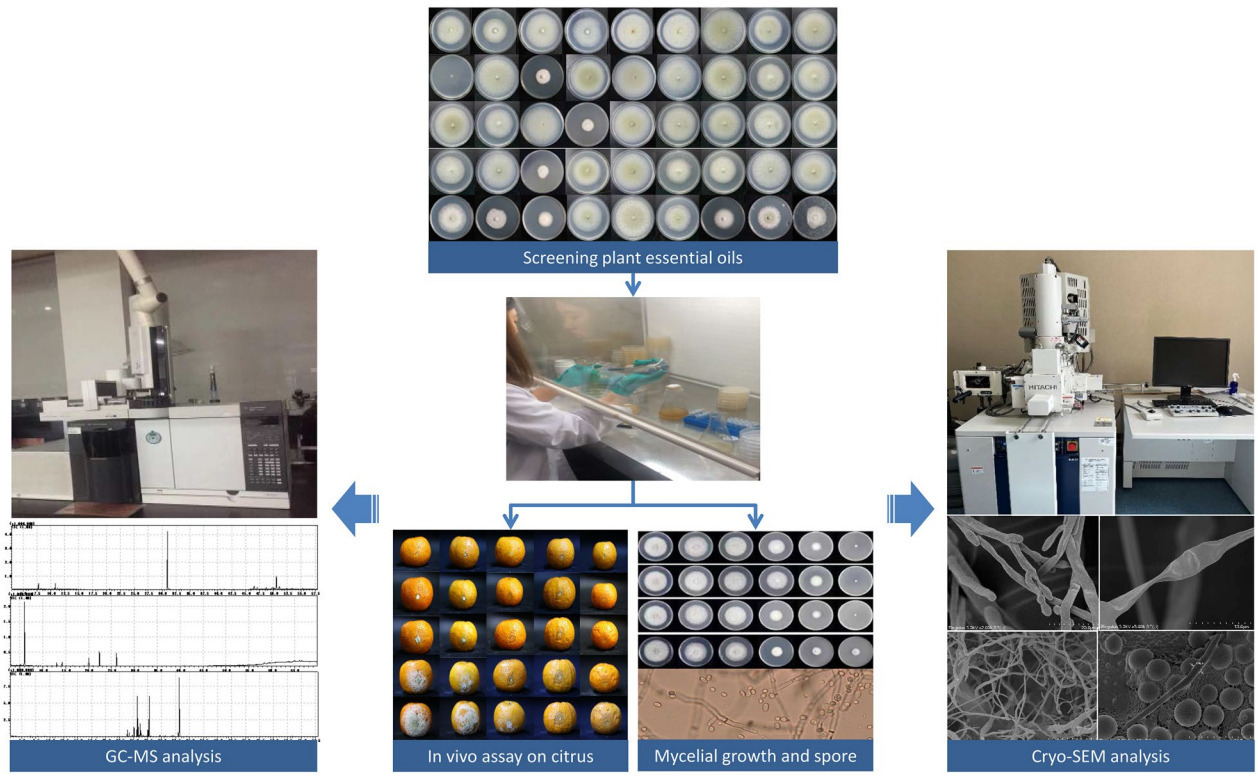
Graphical abstract



Introduction

Citrus is a member of the Rutaceae family and is one of the earliest cultivated plants. Citrus fruits were first documented as being cultivated as early as 2100 BC [1]. Due to their high nutritional and market value, citrus fruits are cultivated all over the world. Citrus fruits have a delicate pericarp and abundant nutrition. However, they are susceptible to infection by fungal pathogens during post-harvest which can result in significant economic losses [2]. Synthetic fungicides are the most commonly used and efficient method of managing post-harvest diseases in citrus [3, 4]. The majority of all citrus losses can occur during shipping or storage due to green mold induced by *Penicillium digitatum* estimated as high as 90% [5]. Although the use of chemical fungicides is strictly controlled in many countries, fungicide-resistant pathogens are emerging and the public concern over the chemical residues impact on human health and the environment has increased [6]. Thus, current microbiological technology has revealed two crucial factors: first, consumers are more aware of the health concerns of synthetic preservatives and the advantages of natural antibacterial, antifungal, and antimicrobial qualities over fungicides [7]. Second, since most essential oils are safe, they are one of the most promising approaches for controlling post-harvest rot. Research interest in essential oils has increased significantly due to their natural properties and consumer demand for safer methods of food preservation. Several studies have shown that EOs from clove, cinnamon, lemon grass, origanum, palm, fennel, thyme and tea tree oil are potent in treating fungal development, controlling fungal pathogens such as *Penicillium* and *Botryti* [8–10]. Plants produce large amounts of secondary metabolites, which have potential activity against fungal pathogens [11]. Of these volatile compounds, more than 3000 EOs are present in blends containing identified secondary metabolites [12]. The properties and applications of these EOs vary, particularly their antifungal, antimicrobial, antibacterial, antidepressant, anti-inflammatory, antiviral and antispasmodic properties. The presence of monoterpenes (hydrocarbon and oxygenated monoterpene) in essential oils, as well as sesquiterpenes (hydrocarbon, oxygenated sesquiterpenes), is thought to produce strong antioxidant and antibacterial properties [13]. The antifungal, antimicrobial

and antibacterial properties of Eos such as *Achillea millefolium*, *cumin seeds* and *fennel* have been reported by researchers [14–16]. The effect of volatile oils on different fungi is also different because of their effect on organelles and biosynthesis, not only on the semipermeable membrane [17]. Several previous studies have shown that natural and artificial antimicrobial agents can substantially reduce the amount of ergosterol [18]. This important sterol component helps to maintain cell function and integrity within the semipermeable membrane of fungi [19]. EOs is highly effective at preventing the development of fungi in citrus [20]. To inhibit fungi, antifungal drugs may interfere with membrane development and function, alter organelles, and/or target the cell's nucleus and protein synthesis [21].

Despite extensive research on EOs and their antifungal effects, there are gaps in our knowledge, and more research is needed to fill the gaps in the use of EOs in food preservation systems. EOs has not been widely studied on *P. digitatum* for EOs characterization nor its antifungal deployment in food systems. Therefore, there is a need for more research and obtaining results on the influence of EOs from different plant species that still need to be explored and to have plants with more potential for use in the food and active food packaging industry. The assessment of the most effective EOs in different foods in terms of shelf-life extension and EOs that have less impact on the original sensory properties of foods are other areas for future research. The lack of results in warehouse applications and antifungal, antimicrobial and antibacterial studies of EOs preservatives needs to be addressed in future research prospects. Accordingly, although few reports have shown the antifungal mode of action of EOs and their effects, a relatively more appropriate underlying mechanism can be developed and proposed for better understanding by readers and researchers.

This investigation aimed at screening 15 EOs for their antifungal effects on citrus and to determine which EOs were the most effective. Studies have reported EOs to be effective against *P. digitatum* in the treatment of fungal infections. Therefore, we investigated the morphological and antifungal effects of these EOs on *P. digitatum*. Most research on EOs against *P. digitatum* has been done in vitro (malt agar plate assay) and in vivo study on fruit itself has received little attention. We investigated and characterized the antifungal activities of EOs in vitro and in vivo against *P. digitatum*. The present study elucidates GC–MS composition of EO compounds providing a foundation for future studies in understanding single compound effects on plant fungal pathogens. Scanning electron cryomicroscopy (cryo-SEM) a low-temperature technique was used to observe EOs deleterious effect on fungal morphology. This research illuminates the full scope of EOs and their substantial activity against *P. digitatum*.

Materials and methods

Pathogen

The *P. digitatum* fungal strain was used in this investigation. It was isolated from diseased citrus (*Citrus reticulata*) collected from an orchard, Zhejiang, China, and identified as *P. digitatum* [22]. Identification of fungal isolates was performed by morphological and molecular biological methods based on appropriate taxonomic keys and descriptions. The fungus was grown at 25 °C on potato dextrose agar (PDA) plates for approximately 7 days. The fresh spores *P. digitatum* were harvested by adding 3 μL/mL of sterile deionized water onto the PDA plate and scrapped with sterile rod [23]. In addition, we strained the mycelium solution through two layers of cheesecloth to obtain a spore solution.

Citrus reticulata

Citrus fruits were purchased from Guangxi Province in southern China. Ripe, fresh fruits free of visible disease or damage were selected for in vivo experiments and kept at 4 °C until use. All citrus fruits were sterilized by immersion in 1.5% (v/v) of sodium hypochlorite followed by rinsing three times in sterile water. Citrus were dried at room temperature for 1 h after being rinsed. 3-mm-deep incisions were made in the pericarp of the fruits, which were then promptly inoculated with a 6-mm agar disk of 7-day-old *P. digitatum*. Seven days were spent incubating infected fruits with a single incision in the equatorial region at 20 °C and 90% relative humidity [24].

Essential oils

The test was carried out using 100% pure EOs derived from patchouli (*Pogostemon cablin*), chamomile (*Matricaria chamomilla*), cedrus (*Cedrus deodara*), rose (*Rosa damascene*), crown daisy (*Glebionis coronaria*), dill (*Anethum graveolens*), vetiver (*Chrysopogon zizanioides*), melissa (*Melissa officinalis*), niaouli (*Melaleuca quinquenervia*), origanum (*Origanum vulgare*), tea tree (*Melaleuca alternifolia*), thyme (*Thymus vulgaris*), vanilla (*Vanilla fragrans*), ylang (*Cananga odorata*) and cinnamon (*Cinnamomum verum*) purchased from a manufacturer (Huien International Business Co. Ltd. Shanghai, China). EOs dilution followed a modification to methods described by Jiang et al. [25] with slight modifications.

In vitro antifungal assay of 15 essential oils (EOs)

To test the antifungal properties of EOs in vitro, we used the agar dilution method to spread the EOs on petri dishes [26]. Pre-test screening involved placing a hyphal

agar disk (6 mm) cut from the edge of a newly emerging *P. digitatum* colony on the middle of a PDA plate with EOs (1.0% v/v) [27]. After the screening test, EOs patchouli, chamomile, dill, vetiver, origanum and ylang were diluted into five final concentrations 5, 2.5, 1.25, 0.625 and 0.313 μL/mL, respectively. Cinnamon exhibited a higher inhibition rate than the other essential oils used in the experiment. As a result, it was diluted to lower concentrations of 1.25, 0.625, 0.313, 0.156 and 0.078 μL/mL, respectively. Tween-80 (0.1% v/v) and 50% ethyl alcohol were used as solvents for the dilution of EOs [28], and results were expressed as the mean ± S.E. of disk growth. Cultures without any EOs were used as control.

The antifungal study was repeated three times, and the average values were calculated. Parafilm-covered Petri dishes were inverted and cultivated at 25 °C. Fungal growth and inhibition rates were calculated after 5 days of culturing the fungi, and the inhibition rate was estimated using a variation of the methodology reported by Messgo-Moumene et al. [29].

Spores were harvested in 3 mL sterile water and examined under a light microscope Nikon eclipse 80i, Japan 40×/0.75 magnification to see the impact of the EOs on the inhibition of *P. digitatum*.

The antifungal effects of all the investigated EOs were evaluated using an analytical approach (agar plate disk fungal growth) in triplicate. Strain purification methods refer to Arrebola et al. [30] and pesticide indoor bioassay test criteria.

In vivo antifungal essay

Referring to the published fruit inoculation method illustrated by Ouyang et al. [31], citrus fruits were artificially inoculated and then treated with patchouli, chamomile, dill, vetiver, origanum, ylang and cinnamon. For this test, only fruits with uniform size and no blemishes were selected. The citrus fruits were sterilized and then infected with a sterile tip at a central location, where the infection was left for 2 h to set in. A sample with no EOs was used as a control, and the EOs were introduced to a 15-mL plastic container. For a period of 120 h, citrus fruits were kept in an incubator at 20 °C, 80 to 90% humidity, and air circulation. Each treatment was replicated three times. Results were recorded daily and disease suppression was determined by lesion diameter.

Constituent identification using GC–MS for essential oils

The essential oils were analyzed using GCMS-QP2010 instrument (Shimadzu, Japan). For this study, we employed an Rtx-5MScapillary column (30 m × 0.25 mm × 0.25 μm). The following were the operating parameters for the column. The oven temperature program was set as follows: 50℃ for 3 min, 50 °C to 100 °C (3 min hold) at 5 °C/min, from 100 to 150 °C (3 min hold) at 5.0 °C/min, from 150 to 200 °C (3 min hold) at 5.0 °C/min, and from 200 to 230 °C 10 min hold) at 5.0 °C/min. The following conditions were used: an injector temperature of 250 °C, a sample injection volume of 1 μL, a pressure of 53.5 kPa, a total flow rate of 22.0 mL/min, a column flow rate of 1.21 mL/min, a linear velocity of 36.3 cm/s, a purge flow rate of 1.0 mL/min, scan mass range is *m/z* 35–650; ion source temperature is 230 °C; interface line temperature is 220 °C. Compounds were identified by matching their chemical components to information already in the public domain on the NCBI PubChem database (National Center for Biotechnology Information).

Cryogenic scanning electron microscopy (cryo-SEM)

Cryo-SEM instrument model: Hitachi Regulus 8100 scanning electron microscope and Quorum PP 3010T frozen transmission device. The experimental methodology involved the following steps: firstly, vacuum the instrument, purge and clean the pipeline with nitrogen, and pre-cool to −175 °C. Next, prepare liquid nitrogen slush and pre-cool the sample in supercooled liquid nitrogen (−210 °C). The appropriate coating procedure was selected to spray the samples. Finally, observations and image acquisition were carried out under a scanning electron microscope.

Statistical analysis

After measuring six separate samples, the experimental data were averaged and represented as a mean S.E. The SPSS 28.0 statistical software package was used to run the Duncan test to compare the experimental and control groups (SPS Inc., Chicago, Illinois, USA). If the probability of the difference was less than $P < 0.05$, it was deemed to be statistically significant.

Results

Screening antifungal effect of EOs on *Penicillium digitatum*

The effects of EOs on fungal disk growth were determined using in vitro analysis. For *P. digitatum*, the Eos' trial test revealed variations in conidia growth and sporulation. According to our research, *P. digitatum* was significantly inhibited by each EO under in vitro conditions; however, the suppression was strongest at high dosages. The most insignificant antifungal effects are exhibited by cedrus, rose, niaouli, and thyme, which, in low quantities, can even encourage the growth of *P. digitatum*. The antifungal effect of these EOs was less potent when compared to the control of mycelium development and spore germination. It was observed that the disease progression of *P. digitatum* was faster after 72 h.

Treatment of cinnamon exhibited the most potent antifungal activity, with complete suppression of mycelial growth of *P. digitatum* (Fig. 1). In addition, the inhibition rate on mycelial growth was relatively higher in patchouli, vetiver, origanum, dill, ylang, and chamomile, and being able to inhibit mycelial growth of *P. digitatum* significantly. As shown in Fig. 2, sporulation inhibition was highest in EOs of cinnamon, patchouli, vetiver, origanum, dill, ylang and chamomile. The inhibition rates were 100.00%, 98.61%, 96.60%, 92.26%, 73.87% and 86.93%, respectively. However, differences were statistically significant in spore and mycelia inhibition of the pathogen among the EOs. For instance, cedrus, rose and niaouli EOs (inhibition rates 10.93%, 34.27%, and 52.27%), spore and mycelial inhibition (1.95%, 18.29%, and 25.04%) exhibited varying spore and fungal efficacy. Our findings elucidated a relationship between fungal mycelia and spore germination, low inhibition of mycelia led to minimal inhibition of *P. digitatum* spore germination. Most EOs showed significantly higher antifungal activity against *P. digitatum* than the untreated control.

Antifungal activity of seven selected essential oils

The pre-test result indicated that seven EOs; cinnamon, dill, origanum, ylang, patchouli, vetiver, and chamomile EOs impact on *P. digitatum* was significant. To further evaluate their potency, the EC_{50} of the EOs was bioassayed and analyzed (Table 1). Of all the seven EOs we tested; cinnamon had the lowest EC_{50}. Therefore, EOs were tested and investigated for their antifungal activities against *P. digitatum*. When tested in vitro against *P. digitatum* mycelium, these EOs showed varying degrees of hyphae growth suppression after 5 days. Therefore, these concentrations are consistent with the minimal inhibitory concentration, which has a constant impact on *P. digitatum*. The result of these EOs on the fungus demonstrated an increase in citrus resistance to green mold.

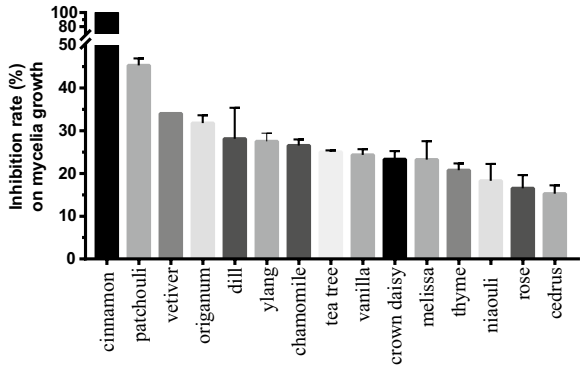


Fig. 1 Inhibition rate (%) on mycelia growth of essential oils (EOs) incorporated into potato dextrose agar on the growth of *P. digitatum* after 5 days of incubation at 25 °C

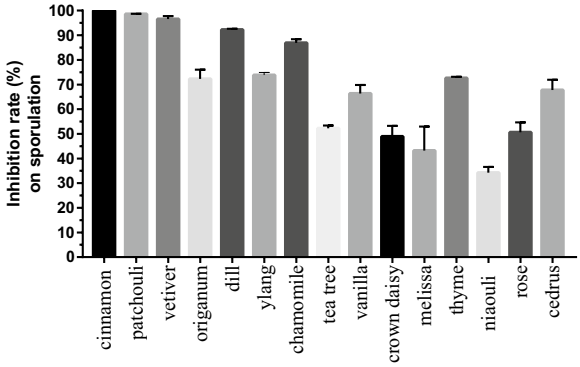


Fig. 2 Inhibition rate of essential oils (EOs) on fungal sporulation incorporated into PDA plates on the growth of *P. digitatum* after 5 days of incubation at 25 °C

After 5 days, an inhibition rate of 100.0% was observed from a concentration of 5 µL/mL of origanum, ylang, and dill EOs. In addition, it was discovered that higher concentrations of EOs had a greater inhibitory effect. Dill, ylang, and origanum showed statistically significant differences between the 0.313 and 5 µL/mL treatments, with the higher concentration inhibiting fungal growth and the lower concentrations displaying only weak antifungal activity. Additionally, 1.25 µL/mL of cinnamon oil completely stopped the growth of *P. digitatum*, hence its antifungal action was highly effective across all treatments. Moreover, even at the highest dose, chamomile and vetiver showed significantly less robust mycelial development compared to the other EOs.

Effects of essential oils on spore germination

Patchouli, cinnamon, dill, ylang and origanum oil had the highest antifungal activity against *P. digitatum*, while 1.25 µL/mL treatment of cinnamon inhibited spore germination. Spore germination rates were significantly

Table 1 Confidence intervals and half-maximal effective concentration (EC_{50}) for seven EOs evaluated against *P. digitatum*

EOs	EC_{50} µL/mL	95% confidence interval	Toxicity regression equation	r^2
Cinnamon	0.424	0.357–0.510	$y = 2.991 + 1.114$	0.891
Dill	1.597	1.431–1.772	$y = 3.862x - 0.785$	0.843
Origanum	1.971	1.746–2.209	$y = 4.238x - 1.249$	0.735
Ylang	2.216	1.730–2.761	$y = 4.138x - 1.430$	0.668
Patchouli	0.513	0.427–0.624	$y = 0.677x + 0.196$	0.947
Vetiver	0.612	0.473–0.816	$y = 0.492x + 0.105$	0.923
Chamomile	1.849	1.318–2.764	$y = 0.479x - 0.128$	0.953

lower when treated with all seven EOs compared to the control test. The spore germination of *P. digitatum* decreased slightly with the increase of seven EOs concentrations. Furthermore, the most effective EOs was origanum, cinnamon, dill, and ylang, since the concentration of cinnamon oil at 1.25 μL/mL inhibited spore germination. In contrast, origanum, cinnamon, dill and ylang oils completely inhibited the germination of spores (Table 2).

The results reported highlight a significant inhibition of sporulation by all the EOs. It was noticed that colonies with green vegetation were found to have the highest number of spores. A varying spore rate with an increase in EO concentration was observed; this could be attributed to cell disruption by EO action on the colony inhibiting spore production. The present results demonstrate that the presence of the tested EOs positively affected *P. digitatum* spore germination. Although EOs had an increased spore production in low concentration, it was noticed that there was a more significant inhibition of spores with an increase in concentration.

Antifungal activity of essential oils on mycelial growth under in vivo conditions

The disease resistance of untreated fruits was significantly higher than those treated with EOs. Our study showed that inoculated citrus fruits started to decay after 48 h of storage at 80–90% relative humidity and 20 °C storage temperature. Dill and origanum had significant antifungal activity, and they could effectively inhibit green mold with treatments of 1.25, 2.5 and 5 μL/mL. Furthermore, EOs of patchouli, vetiver and chamomile reduced the severity of the disease (85.9%, 85.26%, and 85.27%) and inhibited disease progression in the inoculated fruit (Fig. 3). It can be determined that these EOs significantly influence fungal hyphae growth and conidia germination, affecting different stages of disease progression in citrus.

Similarly, we found that as storage time went up from 3 to 5 days, the rate at which fruit deterioration progressed also accelerated. The degradation area was nearly twice as large as in untreated fruit when the storage period ended. Other EOs also had an effective impact than the control on the decaying area, although not as much as origanum and dill oil. We hypothesize that depending on their chemical composition and biological interactions, different EOs have different effects on fungal decay caused by *P. digitatum*.

Chemical composition of seven essential oils by GC/MS analysis

Antifungal results on the inhibition of *P. digitatum* by EOs showed a high mycelia and spore inhibition. However, following obtained GC–MS analysis results, it was determined that the test reagents have a different concentration of compounds, leading to a potential variance in performance on the fungi. For instance, patchouli had patchouli alcohol compound (28.22%) with the highest concentration from the total compounds present (Table 3).

Interestingly, compound diversity in cinnamon, dill and chamomile was low compared to other EOs. Benzyl benzoate, carvacrol (20.43 and 60.96%) in ylang and origanum had the highest compound concentration of the two EOs while *cis*-2-methoxycinnamic acid (51.21%) was the highest in cinnamon. Chamomile and vetiver the two less potent in vitro among the seven EOs reported the highest compound variance. The main components in the EOs detected by GC–MS are *cis*-2-methoxycinnamic acid, (−) carvone, carvacrol, benzyl benzoate, patchouli alcohol, phenethyl isobutyrate and isolongifolene, respectively. Total compound composition in all seven EOs > 1% by GC–MS composition was; chamomile 88.6%, vetiver 62.03%, patchouli 89.62%, ylang 86.36%, origanum 91.96%, dill 84.79% and the highest constituent composition was recorded in cinnamon 93.59%.

Table 2 The mean (± SE) inhibition effects of seven EOs on the spore germination of *P. digitatum*

EOs	Concentration (μL/mL)						
	0.078	0.157	0.313	0.625	1.25	2.5	5
Cinnamon	53.47 ± 5.12	71.84 ± 2.51	74.88 ± 1.37	81.45 ± 1.11	100.00 ± 0.00	–	–
Dill	–	–	79.83 ± 1.08	87.35 ± 0.07	92.77 ± 1.57	97.05 ± 0.34	100.00 ± 0.00
Origanum	–	–	69.84 ± 0.24	76.40 ± 1.76	86.96 ± 1.40	88.58 ± 1.96	100.00 ± 0.00
Ylang	–	–	68.22 ± 3.90	71.55 ± 2.38	87.63 ± 0.27	93.43 ± 0.71	100.00 ± 0.00
Patchouli	–	–	52.74 ± 0.73	60.96 ± 2.20	69.04 ± 0.98	79.45 ± 0.73	83.70 ± 1.50
Vetiver	–	–	50.14 ± 0.70	58.90 ± 1.37	73.70 ± 0.58	75.48 ± 0.54	80.96 ± 0.68
Chamomile	–	–	34.25 ± 0.73	45.48 ± 3.05	65.48 ± 0.60	80.96 ± 1.84	81.78 ± 1.40

Data representing the inhibitory effect of different plant EOs at specific categorical concentrations are significantly different according to Duncan's test (ANOVA, $P < 0.05$)

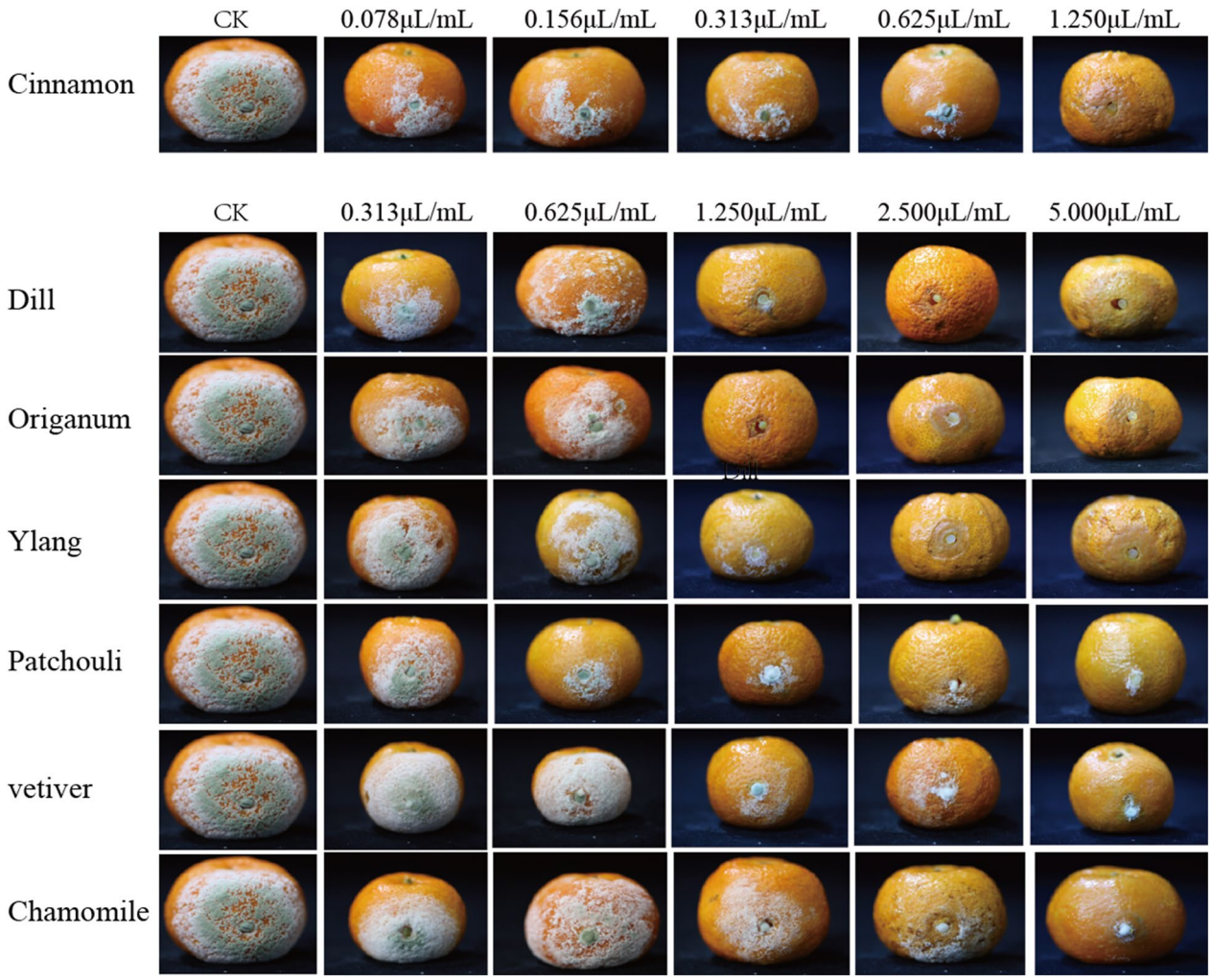


Fig. 3 In vivo antifungal effect of seven essential oils on fungal growth of *P. digitatum* on citrus after 5 days of incubation at 25 °C

The antifungal properties of these compounds remain largely unexplored, but remain a possible source for antifungal properties in EOs. Understanding individual EOs antifungal effect on fungal pathogens can enhance our already existing knowledge on EO application. In the present study, single or blended synergy treatment of these compounds is beyond the scope of this paper.

Effects of EOs on fungal morphology

Cryogenic scanning electron cryomicroscope (cryo-SEM) findings of the control group showed that the hyphae walls were normal and linear. Further, the apical hyphae spores were not damaged (Figs. 4-CK, 5-CK). After the treatment of EOs, we observed the mycelial morphology changes, folding of hyphae (Fig. 4-ch), constricted hyphae in vetiver and patchouli treatments (Fig. 4-Ve, Pa) and no branching (Fig. 4-Di). Additionally, spores of *P. digitatum* did not germinate (Figs. 4-Ci, 5-B) showing a spore inhibition. However, spore observation indicated severe deformation and collapse of spores in cinnamon and chamomile treatments (Fig. 5A, B). As a result, the addition of any EOs may significantly alter the roughness of the fungal surface. The morphology of the hyphae in *P. digitatum* was altered as a result of the origanum treatment. It is known that fungal mycelial growth takes place near the end of the mycelium. The lack of vesicle directionality caused the release of membrane exudation in areas unique to exocytosis in the vetiver, chamomile, and dill treatments. The alignment and branching of the terminal hyphae were lost as a result of this alteration. When mycelia were exposed to ylang, the mycelium thinned and twisted, which caused the hyphae to fall apart. Inhibited hyphae growth of cinnamon following cryo-SEM analysis showed single hyphae on the medium with no elongation. Although broken hyphae were observed in patchouli and origanum treatments, no

Table 3 EOs compound composition by GC/MS

EOs	RT (tr/min)	Compound name	Cas	Compound best match	Total concentration (%)
Cinnamon	30.834	*cis*-2-Methoxycinnamic acid	14737-91-8	$C_{10}H_{10}O_3$	51.21
	31.244	Cinnamaldehyde, (E)-	14371-10-9	C_9H_8O	39.78
	36.519	Isopropyl myristate	110-27-0	$C_{17}H_{34}O_2$	2.60
Dill	5.998	Limonene	138-86-3	$C_{10}H_{16}$	33.46
	16.740	(+)-Dihydrocarvone	7764-50-3	$C_{10}H_{16}O$	2.16
	17.247	(E)-Dihydrocarvone	5948/4/9	$C_{10}H_{16}O$	1.63
	21.780	L(−)-Carvone	6485-40-1	$C_{10}H_{14}O$	39.43
	42.673	Apiol	523-80-8	$C_{12}H_{14}O_4$	8.11
Origanum	7.775	DL-α-Pinene	2437-95-8	$C_{10}H_{16}$	1.62
	9.123	β-Pinene	127-91-3	$C_{10}H_{16}$	1.30
	9.595	β-Myrcene	123-35-3	$C_{10}H_{16}$	1.26
	10.666	*p*-Cymene	99-87-6	$C_{10}H_{14}$	12.61
	10.803	(±)-Limonene	138-86-3	$C_{10}H_{16}$	1.36
	11.789	γ-Terpinene	99-85-4	$C_{10}H_{16}$	2.17
	13.102	Linalool	78-70-6	$C_{10}H_{18}O$	4.15
	20.822	Thymol	89-83-8	$C_{10}H_{14}O$	5.02
	21.203	Carvacrol	499-75-2	$C_{10}H_{14}O$	60.96
	25.066	(−)-Isocaryophyllene	118-65-0	$C_{15}H_{24}$	1.51
Ylang	10.537	4-Methylanisole	104-93-8	$C_8H_{10}O$	2.90
	11.049	Benzyl alcohol	100-51-6	C_7H_8O	2.74
	12.968	Methyl benzoate	93-58-3	$C_8H_8O_2$	3.31
	13.116	Linalool	78-70-6	$C_{10}H_{18}O$	19.37
	15.559	Benzyl acetate	140-11-4	$C_9H_{10}O_2$	6.35
	18.340	Nerol	106-25-2	$C_{10}H_{18}O$	2.38
	19.377	Geraniol	106-24-1	$C_{10}H_{18}O$	6.53
	19.523	Benzyl propionate	122-63-4	$C_{10}H_{12}O_2$	5.10
	23.245	Neryl acetate	141-12-8	$C_{12}H_{20}O_2$	4.35
	23.864	Geranyl acetate	105-87-3	$C_{12}H_{20}O_2$	3.80
	36.114	Benzyl benzoate	120-51-4	$C_{14}H_{12}O_2$	20.43
	37.570	Isopropyl myristate	110-27-0	$C_{17}H_{34}O_2$	2.20
	38.773	Benzyl salicylate	118-58-1	$C_{14}H_{12}O_3$	7.08
Patchouli	23.881	β-Patchoulene	514-51-2	$C_{15}H_{24}$	2.66
	24.191	(−)-β-Elemene	515-13-9	$C_{15}H_{24}$	1.53
	25.072	(−)-Isocaryophyllene	118-65-0	$C_{15}H_{24}$	3.66
	25.636	α-Guaiene	3691/12/1	$C_{15}H_{24}$	14.97
	25.775	(−)-α-Panasinsen	56633-28-4	$C_{15}H_{24}$	6.88
	26.188	α-Patchoulene	560-32-7	$C_{15}H_{24}$	4.96
	26.291	Seychellene	20085-93-2	$C_{15}H_{24}$	2.83
	27.540	(+)-Valencene	4630/7/3	$C_{15}H_{24}$	3.30
	27.791	δ-Guaiene	3691-11-0	$C_{15}H_{24}$	18.42
	32.962	Epiglobulol	0-00-0	$C_{15}H_{26}O$	2.19
	33.170	Patchouli alcohol	5986-55-0	$C_{15}H_{26}O$	28.22

Table 3 (continued)

EOs	RT (tr/min)	Compound name	Cas	Compound best match	Total concentration (%)
Chamomile	7.209	3-Methoxy-3-methyl-1-butanol	56539-66-3	$C_6H_{14}O_2$	8.23
	10.809	(*S*)-(−)-Limonene	5989-54-8	$C_{10}H_{16}$	1.68
	13.111	Linalool	78-70-6	$C_{10}H_{18}O$	2.91
	13.317	(−)-α-Thujone	546-80-5	$C_{10}H_{16}O$	1.52
	18.339	Citronellol	106-22-9	$C_{10}H_{20}O$	2.77
	19.433	Linalyl acetate	115-95-7	$C_{12}H_{20}O_2$	22.94
	20.161	Citronellyl formate	105-85-1	$C_{11}H_{20}O_2$	1.96
	22.781	Terpinyl acetate	80-26-2	$C_{12}H_{20}O_2$	4.12
	24.288	Phenethyl isobutyrate	103-48-0	$C_{12}H_{16}O_2$	42.47
Vetiver	16.330	Isolongifolene	1135-66-6	$C_{15}H_{24}$	11.05
	18.030	(+)-α-Funebrene	50894-66-1	$C_{15}H_{24}$	1.53
	25.263	1,4-Methanonaphthalene, 6,7-diethyldecahydro-, *cis*-	16539-02-9	$C_{15}H_{26}$	1.60
	33.171	Isolongifolone	23787-90-8	$C_{15}H_{24}O$	3.92
	35.637	(+)-Cedrol	77-53-2	$C_{15}H_{26}O$	4.70
	41.961	(−)-Isolongifolol	1139-17-9	$C_{15}H_{26}O$	2.14
	43.374	2-Octylcyclopropene-1-heptanol	54467-85-5	$C_{18}H_{34}O$	4.45
	43.848	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	7220-78-2	$C_{20}H_{34}O_2$	2.25
	44.833	9-Isopropylidenebicyclo[6.1.0]nonane	56666-90-1	$C_{12}H_{20}$	5.91
	45.637	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	301643-32-3	$C_{19}H_{34}$	1.58
	45.835	Cyclopentanecarboxylic acid, 3-isopropylidene-, bornyl ester	0-00-0	$C_{19}H_{30}O_2$	2.87
	46.798	(1*R*)-1β-[(1*R*,2Z)-1,5-Dimethyl-2-hexenyl]-2,3,3aα,4,5,6,7,7a-octahydro-7aβ-methyl-1*H*-indene	54411-95-9	$C_{18}H_{32}$	5.80
	47.402	*cis*-m-Menth-8-ene	24399-15-3	$C_{10}H_{18}$	1.02
	47.710	8α,13-Epoxylabd-14-en-3-one	26729-54-4	$C_{20}H_{32}O_2$	10.41
	47.912	*cis*-m-Menth-8-ene	24399-15-3	$C_{10}H_{18}$	1.65
	59.267	Palmitic acid	1957/10/3	$C_{16}H_{32}O_2$	1.15

Present compounds likelihood best match (CBM) was analyzed using the NCBI system PubChem for all compounds obtained through GC–MS. Compounds in tables are arranged according to the total concentration percentage value (> 1%)

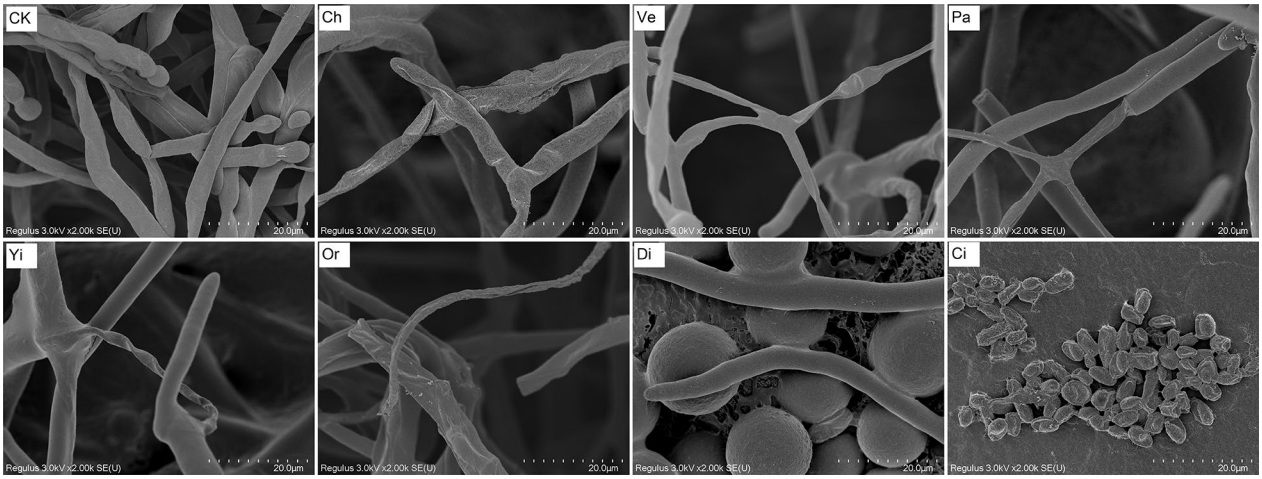


Fig. 4 *P. digitatum* treated with seven essential oils under a scanning electron microscope (×2000). CK was the control; chamomile (Ch), vetiver (Ve), patchouli (Pa), ylang (Yi), origanum (Or), dill (Di) and cinnamon (Ci), respectively

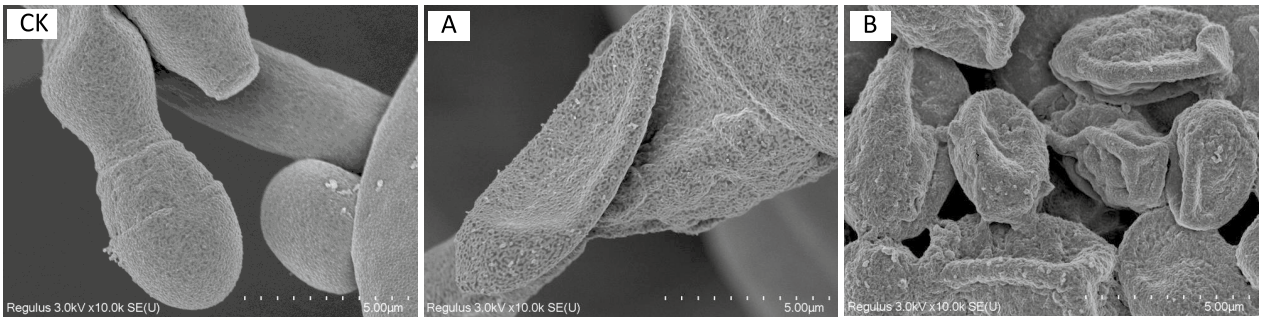


Fig. 5 Spore morphology of *P. digitatum* under a scanning electron microscope (×10,000). CK: was the control for 5 days. **A**: spore malformation (Ch treatment); **B**: spore did not germinate (Ci treatment)

severe collapse and squashing of hyphae were observed in the patchouli treatment. This change caused by the presence of EOs may be related to the enzymatic reaction between EO components and wall structure, which affects the morphogenesis and growth of fungi.

Discussion

Our study found that seven EOs significantly affected the growth and pigment accumulation of *P. digitatum*. In vitro, green fungal pigmentation was found to have low sporulation. Since there was no colouration after high-dose treatments, there was little or no sporulation observed. In particular, EO treatment in vitro could lead to smaller colony sizes, decreased spore germination, and a high rate of *P. digitatum* growth suppression [32]. Treatment of EOs may reduce the virulence of the pathogen, and further reduce the prevalence of the disease [33]. To corroborate previous studies, our study strongly suggested that EOs are promising antifungal agents with good inhibition of *P. digitatum* [33]. Finally, we demonstrated that a variety of essential oils, including patchouli, vetiver, chamomile, origanum, ylang, cinnamon, and dill, have a similar antifungal effect. The EOs tested here had a considerable inhibitory effect on spore germination when applied to *P. digitatum*. Statistical differences between treatments were observed elucidating a dose-dependent manner EOs antifungal effect on the pathogen. Similar to previous findings [34, 35], EOs like origanum, ylang, cinnamon, and dill strongly inhibit green mold. They found that 600 mL of *Trichoderma vulgaris* EO completely inhibited the germination of *Botrytis cinerea* spores. In a separate study, 1000 mL of *T. vulgaris* EO was found to effectively suppress spore germination, complete inhibition was reported at 400 μL [36]. Both oregano and clove have been demonstrated to have antibacterial properties that are effective against a wide variety of bacteria, viruses, and fungi [37]. Our results suggest that the inhibition rate of *P. digitatum* can be increased owing to the vapor activity of EOs. Our findings showed that EOs improved the viability of *P. digitatum*, suggesting their potential use in citrus storage as an alternative to chemical fungicides.

Following in vitro test, seven EOs were chosen and their antifungal inhibitory effects on the infected citrus were confirmed in the in vivo analysis. These EOs also considerably decreased the occurrence of green mold in citrus fruits when grown in semi-commercial settings, proving their efficacy against *P. digitatum*. Elshafie et al. examined the in vivo antifungal efficacy of *T. vulgaris* against brown rot produced by *Monilinia fructicola* and *Monilinia laxa* and found that 500 ppm EO considerably decreased brown rot damage [38]. Our findings are similar to those of previous studies that have looked at the effects of *Botrytis cinerea* and other apple fruit diseases on common apple fruits in a semi-commercial set-up. We hypothesize that the EOs we chose to manage citrus green mold after harvest work by lowering ergosterol levels. Fungal membranes contain high concentrations of ergosterol, which has a role in controlling membrane permeability and fluidity as well as other cellular processes [39]. Further research is required to elucidate the role of EO synergy in investigations of yeast antifungal and antimicrobial control. Brochot et al. claims that a combination of EOs from *Cinnamomum zeylanicum*, *Daucus carota*, *Eucalyptus globulus*, and *Rosmarinus officinalis* has antibacterial, antifungal, and antiviral effects [40].

This study confirmed the deleterious effects of EOs through scanning electron cryomicroscopic observation. It has been reported that lemon grass oil alters *R. stolonifera* [41]. Alterations to the fungal hyphae include changes in shape, surface damage including cracking and shrinking, and the development of a sporangium devoid of spores. Yahyazadeh et al. [42] found a connection between clove oil and the morphological collapse and flattening of *P. digitatum* hyphae, and they also suggested that this was due to erroneous hyphal

separation. We present findings that are consistent with those from other investigations, demonstrating that seven of the EOs we examined changed the fungal morphology of *P. digitatum*, leading to significant hyphal atrophy and collapse. Patchouli, origanum, and chamomile oil all contributed to the hyphae becoming thinner and more easily broken, based on what we could observe under the microscope. Cinnamon and chamomile, on the other hand, shrank and disrupted the plasma membrane.

Cryo-SEM analysis is consistent with in vitro studies, which found that cinnamon, for example, significantly slowed the growth of the pathogen *P. digitatum*. Cinnamon's strong inhibitory effect led to the development of single hyphae by stopping their elongation. Essential oils like patchouli, cinnamon, and origanum exhibit strong antifungal activity, which may be attributed to the abundance of phenolic chemicals in these oils. We concur with the findings of the studies conducted by Charai et al. [43], which indicate a well-established consistency between the antifungal activity of EOs and the presence of the phenolic system. According to the findings [43], the following is the order of the most active compounds in terms of inhibiting the growth of microorganisms: phenols (alcohol, aldehyde, ketone, ether, and hydrocarbon). Compared with untreated citrus, membrane damage was much less severe in the treated group. Therefore, the treated controls consistently exhibited an effect on fungal mycelium through plasma damage, thereby limiting growth.

Conclusion

Results obtained show that EOs namely cinnamon, dill, vetiver, chamomile, origanum, patchouli and ylang which were tested against *P. digitatum* in vitro and in vivo were able to effectively inhibit mycelia growth and alter fungal morphology. Cinnamon, origanum, dill and ylang EOs were evaluated as the most effective in vitro in this study. In vivo experiments confirmed EOs inhibitory effect against *P. digitatum* on citrus. Our results thus provide an alternative strategy for using EOs to protect citrus from post-harvest fungal decay problems, making EOs a promising alternative in disease control of citrus. Future research should focus on establishing the mode of action of EOs in a manner that provides significant indications for their application. EO blends provide an interesting study for future research. Moreover, commercial implementation of EOs that have demonstrated efficacy in in vitro and in vivo studies should be considered.

Acknowledgements
The authors would like to thank Dr. Philip Kea and Mr. Amosi Leonard Mbuji for their help towards proofreading of this manuscript.

Author contributions
QR, YX and HY conceived and designed the experimental plan; LZ, HG and YZ performed experiments. LZ, YZ and HW analyzed the data. LZ, HY, XL and QR drafted the manuscript.

Funding
This work was supported by Key Research and Development Program of Zhejiang Province (2020C02027); "812" talent "Unveiling" Leading Key Project, Hangzhou Lin'an, China (Grant Number 2022-014); National College Students' Innovation and Entrepreneurship Training Program, China (Grant Number 202110341004).

Availability of data and materials
The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

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Received: 15 April 2023 Accepted: 26 June 2023
Published online: 22 August 2023

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