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Antifungal Activities of five commercial extracts against *Alternaria alternate*

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Abstract
The inhibitory effects of five commercial extracts (*Allium sativum, Chamaemelum nobile, Thymus vulgaris, Zingiber officinale* and *Ricinus communis*) against *Alternaria alternata* were tested three concentrations (2.5, 5, 12.5 µl/ml) *in vitro*. *T. vulgaris* and *R.communis* extracts both exhibited the most effective antifungal activity against *A. alternate* with diameter of inhibition zones of 54 mm. The *C. nobile* extract exhibited a lower degree of inhibition 24.5 to 45 mm at three concentrations. The antifungal indices of *T. vulgaris* and *R.communis* extracts at three concentrations against *A. alternata* were all 98.14%, while this of *C. nobile* extract was 43.52% at 12.5 µl/ml. The results show that the five commercial extracts have potential for the development of natural antifungal agents, which could be an alternative to chemicals for control of phytopathogenic fungi on fruits or vegetables.

Keywords: commercial extracts; antifungal activities; *Alternaria alternata*

Introduction

Plant pathogenic fungi negatively affect a large number of important fruits and vegetables, and limit crop production worldwide, especially in developing countries (Dellavalle et al., 2011; Shuping and Eloff, 2017). The chemical control of these pathogens is responsible for the increase in the productivity and quality of the crop but it is inappropriate and nondiscriminatory use has put human and animal health at risk, as well as contaminating the environment (Al-Rahmah et al., 2013; Xie et al., 2017). The search of new fungicides effective, biodegradable and with greater selectivity is necessary to face chemicals' related problems (Askarne et al., 2012). Within this context is the utilization of plant extracts which are natural sources of antimicrobial substances, regarded as safe and degraded by natural soil microbes; they do not pose any health residual or environmental problems at any concentration which they are used(Kim et al., 2004;Yang et al., 2010). Antifungal potential of plant extracts has been demonstrated in several works (Curir et al., 2005; Kumar et al., 2008; Dellavalle et al., 2011). For example, there are studies evaluating the inhibitory activity of extract on plant pathogenic fungi; Arora and Kaushik (2003) investigated ginger with 40 different plant extracts for their activity against soybean fungal pathogens as *F. oxysporum* and they published that ginger inhibit their mycelial growth. Saha et al. (2005) tested ethanol and aqueous extracts of 30 plants toward the development of ecofriendly antifungal compounds for controlling pathogens responsible fungal diseases of tea (*Pestalotiopsis theae*. (Saw.) Stey., *Colletotrichum camelliae*. Mess., *Curvularia eragrostidis*. (P. Hennings) Meyer, and *Botryodiplodia theobromae*). Results of this study showed that ethanol and aqueous extracts of *Allium sativum*. L., *Datura metel*. L., *Dryopteris filix-mas*. (L.) Schott, *Zingiber officinale*. Rosc., *Smilax zeylanica*. L., *Azadirachta indica*., A. Joss. and *Curcuma longa*. L. recorded 100% inhibition of spore germination. Al-Rahmah et al. (2013) evaluated fungal activity of five methanolic plant extracts from *Lantana camara*, *Salvadora persica*, *T. vulgaris*, *Z. officinale* and *Ziziphus spina-christi* on tomato phytopathogenic fungi, *F. oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani*. They found that methanolic extracts from *T.vulgaris* and Z. *officinales* were strongly active on these phytopathogenic fungi. Alemu et al. (2014) tested antifungal effects of 20 plants against *Colletotrichum gloeosporioides*. They showed that *Datura stramonium* L. methanol extract exhibited a very good antifungal effect on the tested fungus, *Datura stramonium* L. and *E. globulus* Labill. of leaf extracts inhibited the pathogenic spore germination more than the other extracts (14.7% and 15.7%, respectively).

Alternaria alternata is one of the most common saprophytes found throughout the world (Mohammadi and Bahramikia, 2019). *A. alternata* cause a range of diseases with economic impact on a large variety of important agronomic host plants and fruits including potatoes, pomegranate, almond, kiwi, aloe vera, tomato, ginseng, citrus, banana, pepper , water hyacinth, *Lantana camara*, and *Amaranthus* spp. (Dube, 2014). This species has been clinically associated with asthma, allergic rhinosinusitis, hypersensitivity, oculomycosis, onychomycosis, skin infections, and allergic bronchopulmonary mycosis (Mirhendi et al., 2013). *Alternaria alternata* is also one of the most important species of *Alternia* that produces AAL toxins, causing many problems for humans and animals and endangering their health (Barkai-Golan and Paster, 2011). In addition, Resistance to fungicide has been shown in this species (Dry et al., 2004; Avenot and Michailides, 2007; Ozkilinc et al., 2017;Yang et al., 2019).The aim of this work was to evaluate *in vitro* the potential antifungal activity of five commercial extracts (*Allium sativum*, *Chamaemelum nobile*, *Thymus vulgaris*, *Zingiber officinale* and *Ricinus communis*) against *Alternaria alternata*, in order to verify possible inhibition activity.

Materials and methods

Commercial extracts

The following five tested extracts were produced by the Egyptian company El Capitaine (CAPPHARM) for the extraction of oils from natural and cosmetic plants: *Allium sativum*, *Chamaemelum nobile*, *Thymus vulgaris*, *Zingiber officinale* and *Ricinus communis*.

Fungal isolates

The fungi *Alternaria alternata* was obtained from experimental farm of nature and life sciences faculty of Mascara University, which isolated from lentil seed of Syria R3 and was cultured on to Sabouraud Dextrose Agar at 28° C for 7 days.

Anti-fungal assay

The method of Luan et al. (2018) and Hasnaoui et al. (2014) with modifications was employed for antifungal evaluation of the selected extracts, which were tested at 2,5 ; 5 et

12,5 µl/ml concentrations against *A. alternata* added to 20 ml of sterilized potato dextrose agar in 9 cm Petri dishes. After the mixture was cooled in the plate (6.0 cm diameter), 5.0 mm diameter of fungi mycelium was transferred to the test plate and incubated at 28 ± 4°C for 3–7 days. When fungi mycelium reached the edges of control plate (without the presence of extracts), the antifungal index was calculated as follows:

Antifungal index (%) = $(1 - D_a/D_b) \times 100$
Where, D_a =the diameter of growth zone in the experimental plate (mm), D_b= the diameter of growth zone in the control plate (mm).

Results and discussion

Antifungal activity

The antifungal activity of the selected extracts by direct contact against *A. alternata* was qualitatively assessed by the presence or absence of the inhibition zone. The antifungal activity is summarized in Table 1.

Table 1. Diameter of inhibition zones (mm) of five extracts against *A. alternata* by direct contact method

Extracts	Extract concentrations			
	2.5µl/ml	5µl/ml	12.5µl/ml	control
A. sativum	52	50	47	54
C. nobile	45	42	24.5	54
T. vulgaris	54	54	54	54
Z. officinale	48	42	30	54
R.communis	54	54	54	54

The results revealed that the selected extracts showed a higher antifungal activity with diameter of inhibition zones ranged between 24.5 to 54 mm. Among extracts, *T. vulgaris* and *R.communis* exhibited the most effective antifungal activity with diameter of inhibition zones of 54 mm in the three concentrations of extracts, followed by *A. sativum* (47 to 52 mm), *Z. officinale* (30 to 48 mm) and *C. nobile* (24.5 to 45 mm) which is the weakly active on *A. alternata* in comparison to other extracts.

The antifungal indexes of the five selected extracts against *A. alternata* were shown in Table 2. Results showed that *T. vulgaris* and *R.communis* extract induced 98.14% antifungal index of *A. alternata* with three concentrations (2.5, 5 and 12.5 µl/ml). Similarly, *A. sativum* extract exhibited strong antifungal action with antifungal indices of 94.44 % at concentration of 2.5µl/ml and decrease to 90.74 and 85.18 at concentration of 5 and 12.5µl/ml, respectively.
C. nobile extract which exhibited the lowest antifungal activity induced 81.48 % antifungal index at concentration of 2.5µl/ml and decrease to 75.93 and 43.52 at concentration of 5 and 12.5 µl/ml respectively.

Table 2. Antifungal index of five extracts against *A. alternata* (%)

Extracts	Extract concentrations		
	2.5µl/ml	5µl/ml	12.5µl/ml
A. sativum	94.44	90.74	85.18
C. nobile	81.48	75.93	43.52
T. vulgaris	98.14	98.14	98.14
Z. officinale	87.04	75.93	53.70
R.communis	98.14	98.14	98.14

In this study, extracts of the five plants (*A. sativum*, *C. nobile*, *T. vulgaris*, *Z. officinale* and *R. communis*) were evaluated for their antifungal activities on *A. alternata*. *T. vulgaris* and *R. communis* extract were most active against this phytopathogenic fungi. Thyme oil is one of the 10 most commercial oils worldwide, since it is used as a natural food preservative, has considerable antioxidant, antibacterial, and antifungal effects, and is used as an aromatic additive to a variety of foods and drinks, as well as in personal care products (perfumes, cosmetics, soaps, oral solutions) (Sakkas and Papadopoulou, 2017). Several works reported the antifungal effects of *Thymus vulgaris* extracts against *A.alternata*. Indeed, Segvić Klarić et al. (2007) compare the antifungal activities of essential oil of thyme (*Thymus vulgaris* L.) and pure thymol on different mould species (*Aspergillus*, *Penicillium*, *Alternaria*(include *A.alternata*) , *Ulocladium*, *Absidia* and *Mucor*, *Cladosporium*, *Trichoderma* and *Rhizopus*, and *Chaetomium*) isolated from damp dwellings and found that Thymol exhibited approximately three-times stronger inhibition than essential oil of thyme. The MIC and MFC of thyme oil on *A.alternata* were 4.70 µg /ml and 9.40 µg /ml, respectively. In other study, Hadizadeh et al. (2009) investigated the antifungal effect of essential oils obtained from some medicinal plants of Iran (*Urtica dioica* L., *Thymus vulgaris* L., *Eucalyptus* spp., *Ruta graveolens* L. and *Achillea millefolium* L.) against *A. alternata*. Both *U. dioica* and *T. vulgaris* oils exhibited antifungal activity against *A. alternata*. The thyme oil exhibited a lower degree of inhibition 68.5 and 74.8% at 1500 and 2000 ppm, respectively. In similarly to Segvić Klarić et al. (2007), Perina et al. (2015) reported that thyme essential oil from leaves and its major compound thymol had minimum inhibitory concentrations (MIC_s) of 500 and 250 µg mL^{-1} respectively against *A. alternata*.

In other study carried out by Puškárová et al. (2017) on antimicrobial activity of Six essential oils (from oregano, thyme, clove, lavender, clary sage, and arborvitae) against pathogenic (*E. coli*, *S. typhimurium*, *Y. enterocolitica*, *S. aureus*, *L. monocytogenes*, and *E. faecalis*) and environmental bacteria (*B. cereus*, *A. protophormiae*, *P. fragi*) and fungi (*C. globosum*, *P. chrysogenum*, *C. cladosporoides*, *A. alternata*, and *A. fumigatus*). The MIC and MFC of thyme oil on *A.alternata* were 0.025 and 0.05 (% w/v), respectively. Recently, In vitro antifungal activities of vapours of four plant essential oils, cinnamon oil, fennel oil, origanum oil and thyme oil, were investigated by Hong et al. (2018) during in vitro conidial germination and mycelial growth of *A. alternata* causing the tomato leaf spots to find eco-friendly alternatives for chemical fungicides. The four plant essential oils showed different antifungal activities against in vitro conidial germination of *A. alternata* in dose-dependent manners. One µl/disc of thyme oil slightly decreased the mycelial growth by ca. 84.3% and increasing to 2 µl/disc led to more decrease in the mycelial growth showing ca. 39.1% compared to that of untreated control. The antimicrobial activities of *Thymus vulgaris* extract is mostly believed to be related to the thymol and carvacrol contents of the oil (Fani and Kohanteb, 2017, Gedikoğlu et al., 2019). Several studies were reported antimicrobial effects of thymol alone or in combination with carvacrol(Lambert et al., 2001; Trombetta et al., 2005; Botelho et al., 2007; Du et al., 2015). They cause structural and functional disturbances in the cellular membrane (Lopez-Romero et al., 2015). Thymol is lipophilic compound, that alone or with carvacrol, can change the cell membrane fluidity and permeability (Yanishlieva et al., 1999). In addition to this, the compound can changes the cell membrane in fungi by the affect the function of the cell membrane enzymes that catalyzes the synthesis of the cell wall polysaccharide compounds such as β -glucan and inhibit the growth of cells (Vasconcelos et al., 2014; Bennis et al., 2004). *R.communis* extracts exhibited also similar and stronger antifungal effect on *A. alternata* as *T. vulgaris* extracts. Comparing our results with other researchers, they are in good agreement with those of Jassim (2017) who tested antifungal activity of *Conocarpus lancifolius*, *Ricinus communis*, *Nerium oleandra* and *Clerodendron inerme* extracts on the fungus *A. alternata* the causal agent of leaves spots of Date Palm. Regarding the treatment of *R. communis* extract 10 and 15% concentrations the inhibition percentage of mycelial growth were 72 and 74.44% respectively. The antifungal activity of *R. communis* extracts is mainly attributed to the presence of camphor (Zarai et al., 2012), which reported to have antimicrobial properties (Mahboubi et al., 2013; Swamy et al., 2016).

A. sativum extracts exhibited also remarkable antifungal effects on *A. alternata* near to those of two precedent extracts (*T. vulgaris* and *R. communis*). The results obtained in our screen are in agreement with published results for Taskeen et al. (2010), Şesan et al. (2016), Alseeni et al. (2019) which reported that the extract of *A. sativum* possessed antifungal activity on *A. alternata*. The antimicrobial activity of garlic is believed to be due to the effect of allicin (diallyl thiosulfinate), ajoene, and other sulfite compounds (Yin and Cheng, 1998).

For *Z. officinale* extracts, it exhibited less antifungal activity than the precedent tested extracts. The results of our study are in line with the report by many researchers (Fawzi et al., (2009); Sharma and Tiwari (2013); Osman et al., (2016); Ahmad Mir and Qureshi (2017); Alseeni et al., (2019), which showed the extracts from ginger possess antibacterial activity against *A. alternata*. The gingerol and shogaol are the major components of ginger, which could be responsible agents for the antimicrobial properties of ginger. They are phenolic compounds causing rupture of the bacterial cell membrane and loss of their properties (Masniari., 2011) and greater loss of cell contents or critical output of molecules and ions can lead to cell death (Ali et al., 2015 and Fei et al., 2017).

Comparatively, *A. alternata* were less sensitive to the inhibitory activity of the *C. nobile* extracts than other four tested extracts. To our best knowledge, therefore, this is the first study to determine the antifungal activity of *C. nobile* extract on *A. alternata*. In contrary to our find, Kloucek et al (2012) tested sixty nine for its antimicrobial activity against three bacteria (*S. aureus*, *S. enteritidis*, *P. aeruginosa*) and three fungi (*A. alternata*, *A. niger*, *P.digitatum*), and among these tested oils, *Anthemis nobilis* (currently *C. nobile*) don't has activity on *A. alternata* . Some studies have been performed concerning the antifungal activity of essential oils or extracts of other *Chamaemelum* species or on *Alternaria* sp. Rizwana et al.(2016) have screened *in-vitro* the antimicrobial activity of five organic solvents extracts(Chloroform, ethyl acetate, acetone, ethanol and methanol) of *Matricaria aurea* (golden chamomile) on *S. aureus*, *B. subtilis*, *S.pyogenes* , *E. faecalis*, *E. coli*, *P.aeruginosa*, *K. pneumonia*, *F. oxysporum*, *F. solani*, *A. alternata*, *A. niger*, *A. flavus* and *C. gleosporoides* and found that all extracts inhibit the mycelial growth of *A. alternata* in a percentage ranged between 50.74-100%. In other study, the volatile oil of *Anthemis nobilis* (currently *C. nobile*) inhibited the growth of dermatophytons, *Alternaria* sp., *Aspergillus fumigatus* and *A. parasiticus* (Hänsel et al., 2016). The antimicrobial properties of chamomile have been well documented in some studies (Móricz et al., 2012; Al-Kuraishy et al., 2015; Mahmoud et al., 2016). Compounds in the essential oil of chamomile were effective against *Staphylococcus* and *Candida* (Aggag and Yousef, 1972). Of chamomile's essential oil components, α-bisabolol had the strongest activity against Gram-positive and Gram-negative bacteria. Chamazulene also had strong antimicrobial activity. Spiroethers had weak activity against Gram-positive bacteria but were inactive against Gram-negative bacteria (Kedzia, 1991). German chamomile esters and lactones showed activity against *Mycobacterium tuberculosis* and *M. avium* (Lu et al., 1998). Chamazulene, α-bisabolol, flavonoids and umbelliferone displayed antifungal properties against *Trichophyton mentagrophytes*, *T. rubrum* and *Candida albicans* (Kedzia, 1991; Szalontai et al., 1976, 1977; Ahmed et al., 1994).

Conclusion

In this study, we investigated the antifungal activities of five plant extracts (*A. sativum, C. nobile, T. vulgaris, Z. officinale* and *R. communis*) against *A. alternata*. Our study demonstrated that the five plant extracts showed good antifungal activities against this fungi. In particular, *T. vulgaris* and *R.communi* offer effective inhibition activity to the growth of the *A.alternata*. Even at low concentrations, these extracts showed strong antifungal activity. Thus, the results show that the five plant extracts have potential for the development of natural preservatives as alternative to antibiotics and artificial preservatives both of which can be toxic at certain concentrations.

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