International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 11 (5): 496-501, 2015
ISSN 1811-7775

ansinet
Asian Network for Scientific Information

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/ijp.2015.496.501

In vitro Susceptibility of Clinical *Aspergillus* Species to Some Antifungal Agents

[1]Ali H. Bahkali, [1,2]Abdallah M. Elgorban, [1]Abd El-Rahim M.A. El-Samawaty, [1]Huda-Mogren A. Almogren, [2]Mohamed A. El-Metwally and [1]Naif S. Al-Harbi
[1]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia
[2]Plant Pathology Institute, Agricultural Research Center, Giza, Egypt

ARTICLE INFO
Article History:
Received: February 27, 2015
Accepted: April 21, 2015

Corresponding Author:
Abdallah M. Elgorban,
Department of Botany and Microbiology,
College of Science,
King Saud University, Riyadh,
Saudi Arabia

ABSTRACT
Aspergillus species are regularly involved in human broncho-pulmonary diseases, mainly in immunocompromised patients. The essential oils extracted from three different plants were tested for their inhibitory effect on the growth of five pathogenic Aspergilli including *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus versicolor* and *Aspergillus terreus* which are pathogens with clinical importance. The results of the antimicrobial tests showed that the isolated essential oils inhibited the growth of *Aspergillus* species to varying degrees. The *Ocimum basilicum* oil completely inhibited the mycelial growth and spore germination of *A. terreus* and *A. fumigatus* at 200 and 250 ppm. In addition, this oil showed a very strong antifungal activity against the mycelial growth and spore germination of *A. flavus* and *A. versicolor* with 97.7, 97.5, 91.2 and 93.5%, respectively, when compared with control. The essential oil extracted from *Eucalyptus globulus* also exhibited significant antifungal activity against the *Aspergillus* species tested. This oil completely inhibited the mycelial growth and spore germination of *A. fumigatus*. Conversely, *Nigella sativa* oil exhibited moderate inhibitory activity against all the tested *Aspergillus* species. The present study supports the proposition that plant essential oils may have a role in both pharmaceutical and agricultural practices.

Key words: *Nigella sativa*, *Aspergillus niger*, essential oils, spore germination

INTRODUCTION

Aspergilli cause a wide range of diseases, involve otomycosis, cutaneous manifestations and invasive infections such as endocarditis and pulmonary aspergillosis (Balajee *et al*., 2007). Aspergillosis includes a wide range of diseases caused by the members of the genus *Aspergillus*. *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus* and *Aspergillus fumigatus* are the most common species responsible for infections. *Aspergillus* species are one of the main causes of hospital-acquired infections which patients may contract by direct insemination, inhalation in a contaminated air-conditioned environment, exposure to contaminated surgical tools and filters or mechanical ventilation (Haiduven, 2009). Furthermore, incidences of these fungi developing resistance to voriconazole, itraconazole and posaconazole have been reported (Arikan *et al*., 2008). Moreover, *A. fumigatus* has showed alterable susceptibility to amphotericin B (Snelders *et al*., 2008; Qiao *et al*., 2008).

The development of fungal drug resistance has encouraged the search for new natural product-based alternatives (Cavaleiro *et al*., 2006). Plants are used in conventional medicine as antimicrobial agents and their extracts and essential oils have been known to possess antifungal and antibacterial properties (Hussain *et al*., 2013; El-Solh *et al*., 2009; Pereira *et al*., 2014). Previous studies have reported that numerous extracts and essential oils show important antifungal activity against many fungi and have potential therapeutic effects mainly against fungal diseases of the respiratory tract as well as cutaneous and mucosal infections (Bachir and Benali, 2012; Pereira *et al*., 2014). Several *in vitro* investigations have confirmed the effects of plant essential oils and their major compounds on human pathogenic fungi (Sienkiewicz *et al*., 2012). Recently, the antimicrobial

potential of essential oils has been of considerable interest to the pharmaceutical industry. In addition, their use as alternative anti-infective agents has emerged from a growing trend to reduce the utilization of antibiotics against human pathogenic fungi (Abadio *et al*., 2011; Celikel and Kavas, 2008; Mohammadpour *et al*., 2012). The aim of this study was to evaluate the antifungal activity of some plant essential oils against five *Aspergillus* species. The median lethal dose (LD_{50}) values of the antifungal activity against these pathogens are available.

MATERIALS AND METHODS

Tested microorganisms: In this study, five clinical *Aspergillus* species responsible for human cutaneous infection and representative of medically relevant was provided by the Microbiology and Parasitology Department, Riyadh Military Hospital, Riyadh, Saudi Arabia. Isolates were cryopreserved in liquid nitrogen until the time of use. Before the experiments, the fungi were transferred to Potato dextrose agar media and incubated for 7 days in duplicate.

Extracts and essential oils extraction: The essential oils were extracted from *Nigella sativa* (seeds), *Eucalyptus globulus* (dried leaves) and *Ocimum basilicum* (dried leaves) using microwave-assisted hydrodistillation (30 min, 250 mL water) with a Clevenger-type distillation device and a Dean-Stark distillation trap in a domestic microwave oven (Stashenko *et al*., 2004). The extracts were obtained by soaking 50 g of the dried leaves or seeds in 200 mL of ethanol for 7 days at 28°C. The mixture was then filtered and concentrated using a rotary evaporator (Hei-VAP, Heidolph, Germany). Stock solutions of 40 and 20 mg mL^{-1} of the extracts and oils, respectively, were prepared in dimethyl sulphoxide for subsequent bioassays.

Inhibitory effect of plant essential oils on the radial growth of fungal pathogens: The antifungal activities of the essential oils against the test fungi were studied using a dual culture assay. Potato Dextrose Agar (PDA) was autoclaved and cooled to near 45°C. The plant essential oils were mixed with the sterile PDA to obtain final concentrations of 0, 100, 150, 200 and 250 ppm. Tween 80 (0.5%) was used as a surfactant to disperse the oil in the PDA which was poured in petri plates. Mycelial disks of 3 mm diameter cut out from the periphery of 7 days old cultures of the tested fungi, were aseptically inoculated upside down on the PDA. The plates were incubated at 27±2°C and there were four replicates per treatment. The growth of the tested fungi was recorded for 7 days and the percentage inhibition of the mycelial growth was computed by comparison with the control. The values were calculated using the following equation:

$$\text{Inhibition}\,(\%) = \left(\frac{\text{Md} - \text{F}}{\text{Md}}\right) \times 100$$

where, Md and F are the mean diameters of the mycelial growth of the control and the treatment groups, respectively. The median effective dose (ED_{50}) and ED_{95} were also determined.

Inhibitory effect of plant essential oils on conidial germination of fungal pathogens: The essential oils tested were dissolved in 0.1% Tween 80 in a 20 mL sterile glass tube containing 5 mL potato dextrose broth to obtain the final concentrations (100, 150, 200 and 250) and 0.1% Tween 80 (v/v) without essential oil was used as the control (Elgorban *et al*., 2015). A total of 100 µL of conidia (1×10^6 spores mL^{-1}) fungal pathogens were added to each tube. Following a 24 h incubation at 27±2°C on a shaker (200 rpm), 100 conidia spores per replicate were observed microscopically to determine the germination rate. Four replicates were evaluated for every treatment and experiments were performed three times. The ED_{50} and ED_{95} were also determined.

Statistical analysis: Antifungal experiments were performed in triplicate and the data was analyzed using the statistics for the social sciences (SPSS) software and are presented as the Mean±Standard error of the mean (SEM)**.** The ED_{50}, ED_{95} and slope of the activity of the essential oils against *Aspergillus* species were obtained using a probit analysis.

RESULTS

Inhibitory effect of essential oils against *A. terreus*: The data shown in Table 1 revealed that the *O. basilicum* oil completely inhibited the linear growth of *A. terreus* at concentrations of 200 and 250 ppm (ED_{50} 111.8 ppm L^{-1}, ED_{95} 232.3 $ppmL^{-1}$ and slope 5.19±0.46). The oils of *N. sativa* and *E. globulus* significantly inhibited the radial growth of *A. terreus*. The *N. sativa* oil was more effective as an antifungal agent than the eucalyptus oil against the pathogen at a concentration of 250 ppm. The *O. basilicum* oil completely suppressed spore germination at concentrations of 150, 200 and 250 ppm (ED_{50} 86.5 ppm L^{-1}, ED_{95} 129.0 ppm L^{-1} and slope, 9.46±4.01). The efficacy of *E. globulus* and *N. sativa* followed with a 95.0 and 90.2% inhibition of spore germination, respectively, at 250 ppm.

Inhibitory effect of essential oils against *A. versicolor*: The oil of *O. basilicum* highly repressed the mycelial growth and spore germination of *A. versicolor* at 92.1% (ED_{50} 39.0 ppm L^{-1}, ED_{95} 455.8 ppm L^{-1} and slope, 2.38±0.21) and 93.5% (ED_{50} 110.6 ppm L^{-1}, ED_{95} 262.6 ppm L^{-1} and slope 4.38±0.26), respectively and this was significantly compared with the control. The oil extract of *E. globulus* significantly inhibited the mycelial growth of *A. versicolor* and showed a moderate effect on sporulation (76.7 and 74.8%, respectively), compared with the control (Table 2). *Nigella sativa* oil produced the lowest inhibition of the mycelial growth of *A. versicolor* and moderately reduced the spore germination with 59.9 and 67.8% inhibition, respectively compared with the control.

Table 1: Inhibitory influence of plant essential oils against the mycelial growth and spore germination of *Aspergillus terreus*

Treatments	Cont. R.G.	100 (ppm) R.G.	100 (ppm) Inh. (%)	150 (ppm) R.G.	150 (ppm) Inh. (%)	200 (ppm) R.G.	200 (ppm) Inh. (%)	250 (ppm) R.G.	250 (ppm) Inh. (%)	ED_{50} (ppm L^{-1})	ED_{95} (ppm L^{-1})	Slope±SE
Radial growth												
N. sativa	56.5±5.8	41.0±0.8	27.4	36.5±1.7	35.4	22.5±1.3	60.2	12.3±2.1	78.2	164.9	489.8	3.48±0.21
E. globulus	65.5±5.8	41.0±0.8	37.4	39.3±1.0	40.1	36.5±1.0	44.3	23.8±1.0	63.7	191.1	2428.9	1.48±0.18
O. basilicum	56.5±5.8	29.3±0.5	48.2	25.0±0.8	55.8	0.0±0.0	100.0	0.0±0.0	100.0	111.8	232.3	5.19±0.46
Spore germination												
N. sativa	100.0±0.0	61.5±1.3	38.5	51.8±1.3	48.3	16.5±5.1	83.5	9.8±1.7	90.2	125.3	319.0	4.16±0.22
E. globulus	100.0±0.0	80.8±1.0	19.3	57.5±1.3	42.5	15.3±0.5	84.8	5.0±0.8	95.0	145.6	262.8	6.40±0.31
O. basilicum	100.0±0.0	27.8±2.5	72.3	0.0±3.2	100.0	0.0±0.0	100.0	0.0±0.0	100.0	86.5	129.0	9.46±4.01

ED_{50}: Median effective dose to kill 50% of the fungus, ED_{95}: Dose required for desired effect in 95% of the fungus, R.G: Radial growth, Inh. (%): Inhibition %

Table 2: Inhibitory influence of plant essential oils against the mycelial growth and spore germination of *Aspergillus vesciolar*

Treatments	Cont. R.G.	100 (ppm) R.G.	100 (ppm) Inh. (%)	150 (ppm) R.G.	150 (ppm) Inh. (%)	200 (ppm) R.G.	200 (ppm) Inh. (%)	250 (ppm) R.G.	250 (ppm) Inh. (%)	ED_{50} (ppm L^{-1})	ED_{95} (ppm L^{-1})	Slope±SE
Radial growth												
N. sativa	77.3±1.7	50.5±0.6	34.6	47.8±1.0	38.2	43.8±2.1	43.4	31.0±0.8	59.9	210.2	2695.0	1.48±0.18
E. globulus	77.3±1.7	47.5±0.6	38.5	35.5±5.4	54.0	31.0±5.4	59.9	18.0±4.1	76.7	136.9	685.9	2.35±0.19
O. basilicum	77.3±1.7	31.8±1.2	58.9	30.3±0.5	60.8	20.0±1.8	74.1	6.8±1.3	91.2	39.0	455.8	2.38±0.21
Spore germination												
N. sativa	99.3±1.0	87.3±0.5	12.1	48.3±1.0	51.4	40.3±1.0	59.4	32.0±0.8	67.8	174.0	466.6	3.84±0.22
E. globulus	99.3±1.0	72.5±1.7	27.0	50.8±1.0	48.9	36.0±0.8	63.7	25.0±0.8	74.8	154.6	504.5	3.20±0.20
O. basilicum	99.3±1.0	59.3±1.3	40.3	23.5±1.9	76.3	14.5±5.9	85.4	6.5±2.4	93.5	110.6	262.6	4.38±0.26

ED_{50}: Median effective dose to kill 50% of the fungus, ED_{95}: Dose required for desired effect in 95%of the fungus, R.G: Radial growth, Inh. (%): Inhibition %

Table 3: Inhibitory influence of plant essential oils against the mycelial growth and spore germination of *Aspergillus fumigatus*

Treatments	Cont. R.G.	100 (ppm) R.G.	100 (ppm) Inh. (%)	150 (ppm) R.G.	150 (ppm) Inh. (%)	200 (ppm) R.G.	200 (ppm) Inh. (%)	250 (ppm) R.G.	250 (ppm) Inh. (%)	ED_{50} (ppm L^{-1})	ED_{95} (ppm L^{-1})	Slope±SE
Radial growth												
N. sativa	59.5±0.6	41.3±2.5	30.7	31.5±2.4	47.1	14.3±2.2	76.1	12.8±2.0	78.5	142.9	414.60	3.56±0.21
E. globulus	59.5±0.6	51.0±0.8	14.3	48.8±2.2	18.1	12.3±2.6	79.4	0.0±0.0	100.0	160.6	289.90	7.87±0.41
O. basilicum	59.5±0.6	21.0±0.8	64.7	12.0±2.9	79.8	0.0±0.0	100.0	0.0±0.0	100.0	87.8	188.86	4.94±0.62
Spore germination												
N. sativa	99.5±1.0	59.3±0.5	40.5	37.5±1.9	62.3	10.8±1.0	89.2	0.0±0.0	100.0	117.7	234.6	5.49±0.31
E. globulus	99.5±1.0	41.3±1.0	58.5	31.0±0.8	68.8	2.8±1.7	97.2	0.0±0.0	100.0	98.4	209.9	4.99±0.36
O. basilicum	99.5±1.0	21.3±0.5	78.6	7.5±6.1	92.5	0.0±0.0	100.0	0.0±0.0	100.0	70.3	151.8	4.92±1.03

ED_{50}: Median effective dose to kill 50% of the fungus, ED_{95}: Dose required for desired effect in 95% of the fungus, R.G: Radial growth, Inh. (%): Inhibition %

Inhibitory effects of the essential oils against *A. fumigatus*: The oil extracted from *O. basilicum* showed fungicidal activity against the mycotoxigenic *A. fumigatus* (Table 3). This oil completely suppressed the radial growth and spore germination of *A. fumigatus* at concentrations of 200 and 250 ppm (ED_{50} 87.8 ppm L^{-1}, ED_{95} 188.8 ppm L^{-1} and slope 4.94±0.62). This efficacy was followed by that of *E. globulus* with 100% inhibition of mycelial growth (ED_{50} 160.6 ppm L^{-1}, ED_{95} 289.9 ppm L^{-1} and slope 7.87±0.41) and spore germination of the pathogen at a concentration of 250 ppm compared to the control. The oil of *N. sativa* significantly reduced the mycelial growth of *A. fumigatus* (18.5%) and completely inhibited the spore germination of the fungus at a concentration of 250 ppm.

Inhibitory effect of the essential oils against *A. flavus*: The *E. globulus* oil at concentrations of 200 and 250 ppm prevented the mycelial growth and spore germination of *A. flavus* that is particularly harmful to humans. The values for these concentrations were higher at ED_{50} 100.7 ppm L^{-1}, ED_{95} 215.5 ppm L^{-1} and slope 4.97±0.51 and ED_{50} 85.2 ppm L^{-1}, ED_{95} 178.5 ppm L^{-1} and slope 5.11±0.69, respectively, than the control (Table 4). The oil extract of *O. basilicum* significantly inhibited the mycelial growth of *A. flavus* with 97.7 and 97.5% inhibition of the sporulation of the fungus. These levels of efficacy were followed by the *N. sativa* oil with a 72.6 and 86.0% reduction in the mycelial growth and spore germination of *A. flavus*, respectively.

Inhibitory effect of essential oils against *A. niger*: The data shown in Table 5 revealed that *O. basilicum* oil exhibited strong antifungal properties against *A. niger* with a 73.3 and 89.0% inhibition of the mycelial growth (ED_{50} 92.0 ppm L^{-1}, ED_{95} 1243.6 ppm L^{-1} and slope 1.45±0.18) and spore germination (ED_{50} 136.8 ppm L^{-1}, ED_{95} 277.0 ppm L^{-1} and slope 5.36±0.26), respectively compared to the control. This efficacy level was followed by that of the *E. globulus* oil with a 72.5% reduction of the radial growth and 84.5% inhibition of the spore germination of the fungus. The lowest mycelial growth (21.5 mm) was recorded for *A. niger* and was caused by the *N. sativa* oil which was more effective against the germination of the fungal spores with 83.2% inhibition.

Table 4: Inhibitory influence of plant essential oils against the mycelial growth and spore germination of *Aspergillus flavus*

		100 (ppm)		150 (ppm)		200 (ppm)		250 (ppm)				
Treatments	Cont. R.G.	R.G.	Inh. (%)	R.G.	Inh. (%)	R.G.	Inh. (%)	R.G.	Inh. (%)	ED_{50} (ppm L^{-1})	ED_{95} (ppm L^{-1})	Slope±SE
Radial growth												
N. sativa	56.5±1.7	28.3±1.9	50.0	24.3±2.6	57.1	19.5±5.9	65.5	15.5±1.3	72.6	105.1	1333.1	1.49±0.19
E. globulus	56.5±1.7	25.3±3.5	55.3	18.3±0.5	67.7	0.0±0.0	100.0	0.0±0.0	100.0	100.7	215.5	4.97±0.51
O. basilicum	56.5±1.7	25.0±1.4	55.8	23.3±1.0	58.8	20.3±1.0	64.1	1.3±2.5	97.7	104.7	419.9	2.72±0.21
Spore germination												
N. sativa	100.0±0.0	53.5±2.9	46.5	30.8±1.0	69.3	17.3±2.1	82.8	14.0±1.8	86.0	104.7	357.7	3.08±0.22
E. globulus	100.0±0.0	33.3±0.5	66.8	16.3±1.0	83.8	0.0±0.0	100.0	0.0±0.0	100.0	85.2	178.5	5.11±0.69
O. basilicum	100.0±0.0	72.8±1.7	27.3	21.8±2.2	78.3	10.8±1.3	89.3	2.5±1.3	97.5	121.0	219.4	6.36±0.35

ED_{50}: Median effective dose to kill 50% of the fungus, ED_{95}: Dose required for desired effect in 95% of the fungus, R.G: Radial growth, Inh. (%): Inhibition %

Table 5: Inhibitory influence of plant essential oils against the mycelial growth and spore germination of *Aspergillus niger*

		100 (ppm)		150 (ppm)		200 (ppm)		250 (ppm)				
Treatments	Cont. R.G.	R.G.	Inh. (%)	R.G.	Inh. (%)	R.G.	Inh. (%)	R.G.	Inh. (%)	ED_{50} (ppm L^{-1})	ED_{95} (ppm L^{-1})	Slope±SE
Radial growth												
N. sativa	63.0±1.6	30.0±1.2	52.4	27.0±0.8	57.1	23.5±1.3	62.7	21.5±1.3	65.9	88.5	6122.3	0.89±0.18
E. globulus	63.0±1.6	37.5±2.1	40.5	35.5±0.6	43.7	32.0±2.2	49.2	17.3±1.7	72.5	157.6	1255.7	1.83±0.19
O. basilicum	63.0±1.6	30.5±0.6	51.6	23.3±1.7	63.1	19.8±2.8	68.7	16.8±1.0	73.3	92.0	1243.6	1.45±0.18
Spore germination												
N. sativa	99.8±0.5	47.0±1.4	52.9	40.3±1.3	59.6	20.8±1.0	79.2	16.8±1.3	83.2	100.1	494.0	2.37±0.20
E. globulus	99.8±0.5	59.8±2.6	40.1	37.8±1.0	62.2	22.8±1.3	77.2	15.5±1.3	84.5	121.0	419.7	3.05±0.20
O. basilicum	99.8±0.5	79.8±1.0	20.1	36.3±1.0	63.7	17.0±1.4	83.0	11.0±0.8	89.0	136.8	277.0	5.36±0.26

ED_{50}: Median effective dose to kill 50% of the fungus, ED_{95}: Dose required for desired effect in 95% of the fungus, R.G: Radial growth, Inh. (%): Inhibition %

DISCUSSION

This present study assessed the inhibitory activity of three essential oils against the growth of five *Aspergillus* species *in vitro*. The essential oils of *O. basilicum* had a strong fungicidal effect against all the *Aspergillus* species. This oil completely inhibited the mycelial growth and spore germination of both *A. terreus* and *A. fumigatus* at concentrations of 200 and 250 ppm. In addition, this oil exhibited strong antifungal activity against the mycelial growth and spore germination of *A. flavus* (97.7 and 97.5% inhibition, respectively) and *A. niger* (91.2 and 93.5% inhibition, respectively). These results are in agreement with those of Singh *et al*. (2011) who reported that *O. basilicum* oil exhibited strong fungitoxicity against some aflatoxigenic fungi which contaminated food including *Fusarium oxysporum*, *A. flavus*, *Alternaria alternata*, *A. fumigatus*, *Curvularia lunata A. niger*, *Penicillium italicum* and *F. nivale*. *O. basilicum* oil showed great antimicrobial potential against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Escherichia coli* and *Listeria monocytogenes* (Hossain *et al*., 2010). Al-Hajj *et al*. (2014) reported the antimicrobial activity of *O. basillicum* oil against *S. aureus*, *Streptococcus pneumonia, E. coli*, *Candida albicans* and *B. subtilis*. The previous results reported the excellent antimicrobial activity of *Ocimum* oil against the toxigenic strain of *A. flavus* (Kumar *et al*., 2011) and the multidrug-resistant bacterial strains of *Enterococcus*, *Pseudomonas* and *Staphylococcus* (Opalchenova and Obreshkova, 2003). The high antimicrobial activity of the *Ocimum* oil is probably attributable to methyl eugenol which was found to be the main constitutive followed by eugenol, 1,8-cineole and b-caryophyllene (Kumar *et al*., 2011).

Furthermore, Yavari *et al*. (2011) identified 21 ingredients in the oil extracted from the purple type of *O. basilicum,* including *trans*-∞-bergamotene, 1, 8-cineole and linalool as the main components which represent about 92.1% of the oil content. A number of other components that were present in significant amounts in the *Ocimum* oil included bicyclogermacrene, fenchone, β-caryophyllene (E)-ß-farnesene and germacrene. All these components play an important role in the inhibition of microbial growth and spread shown by the oil. In addition, the essential oil of *Ocimum* contains some antioxidant components such as tocopherols, polyphenols and fatty acids (Moreira *et al*., 2010; Puupponen-Pimia *et al*., 2001).

The *E. globulus* oil showed bioactivity against the *Aspergillus* species. This oil completely inhibited the mycelial growth and spore germination of *A. flavus* at concentrations of 200 and 250 ppm. In addition, it strongly inhibited the radial growth and spore germination of *A. fumigatus* by 100%. Similar results have been previously reported (Bakkali *et al*., 2008; Elgorban *et al*., 2015; Hatamleh *et al*., 2014; Tyagi and Malik, 2011). Vilela *et al*. (2009) reported that *E. globulus* oil and 1,8-cineole completely inhibited the growth of both *A. flavus* and *Aspergillus parasiticus* which also showed a reduction in aflatoxin B1 production. The results revealed extreme reduction in the growth of *A. flavus* and *A. fumigatus* by the methanolic extract and essential oil of *E. globulus* (Javed *et al*., 2012). In addition, *E. globulus* extract was highly effective against 16 isolates of *Pseudomonas aeruginosa* (Pereira *et al*., 2014).

The chemical groups such as ketones, phenols, alcohols and terpenes of components found in plant extracts and essential oils are linked to their antimicrobial characteristics (Sartorelli *et al*., 2006). The antimicrobial efficacy of

E. globulus extract and oil has been due to the components such as 1,8-cineole, ρ-cymene, citronellol, eucomalol, citronella, citronellyl acetate, β-pinene, ρ-cymene and alloocimene (Tyagi and Malik, 2011; Nezhad *et al*., 2009), ∞-terpinol and aromadendrene (Bachir and Benali, 2012).

CONCLUSION

The present study suggests that *O. basilicum* and *E. globulus* oils could be sources of natural antifungal agents for use in the pharmaceutical and food manufacturing industries against pathogenic microbes. Additional research is required to obtain information on the practical efficiency of plant essential oils or extracts, in preventing the growth of food-borne and contaminating microbes, for specific applications and conditions.

ACKNOWLEDGMENT

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding this Research group No (RG-1436-025).

REFERENCES

Abadio, A.K.R., E.S. Kioshima, M.M. Teixeira, N.F. Martins, B. Maigret and M.S.S. Felipe, 2011. Comparative genomics allowed the identification of drug targets against human fungal pathogens. BMC Genom., Vol. 12. 10.1186/1471-2164-12-75

Al-Hajj, N.Q.M., H.X. Wang, C. Ma, Z. Lou, M. Bashari and R. Thabit, 2014. Antimicrobial and antioxidant activities of the essential oils of some aromatic medicinal plants (*Pulicaria inuloides*-Asteraceae and ocimum forskolei-Lamiaceae). Trop. J. Pharm. Res., 13: 1287-1293.

Arikan, S., B. Sancak, S. Alp, G. Hascelik and P. Mcnicholas, 2008. Comparative *in vitro* activities of posaconazole, voriconazole, itraconazole and amphotericin B against *Aspergillus* and *Rhizopus* and synergy testing for Rhizopus. Med. Mycol., 46: 567-573.

Bachir, R.G. and M. Benali, 2012. Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*. Asian Pac. J. Trop. Biomed., 2: 739-742.

Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar, 2008. Biological effects of essential oils: A review. Food Chem. Toxicol., 46: 446-475.

Balajee, S.A., J. Houbraken, P.E. Verweij, S.B. Hong, T. Yaghuchi, J. Varga and R. A. Samson, 2007. *Aspergillus* species identification in the clinical setting. Stud. Mycol., 59: 39-46.

Cavaleiro, C., E. Pinto, M.J. Goncalves and L. Salgueiro, 2006. Antifungal activity of *Juniperus* essential oils against dermatophyte, *Aspergillus* and *Candida* strains. J. Applied Microbiol., 100: 1333-1338.

Celikel, N. and G. Kavas, 2008. Antimicrobial properties of some essential oils against some pathogenic microorganisms. Czech J. Food Sci., 26: 174-181.

El-Solh, A.A., D. Amsterdam, A. Alhajhusain, M.E. Akinnusi, R.G. Saliba, S.V. Lynch and J.P. Wiener-Kronish, 2009. Matrix metalloproteases in bronchoalveolar lavage fluid of patients with type III *Pseudomonas aeruginosa* pneumonia. J. Infect., 59: 49-55.

Elgorban, A.M., M. Elsheshtawi, M.A. Abdel-Wahab, A.H. Bahkali and M.A. El-Metwally, 2015. *In vitro* antifungal activity of some plant essential oils. Int. J. Pharmacol., 11: 56-61.

Haiduven, D., 2009. Nosocomial aspergillosis and building construction. Med. Mycol., 47: S210-S216.

Hatamleh, A.A., A.H. Bahkali, M. El-Sheshtawi, M.A. Elmetwally and A.M. Elgorban, 2014. Inhibitory influence of plant extracts on soil borne fungi infecting Muskmelon (*Cucumis melo* L.). Int. J. Pharmacol., 10: 322-327.

Hossain, M.A., M.J. Kabir, S.M. Salehuddin, S.M.M. Rahman and A.K. Das, *et al*., 2010. Antibacterial properties of essential oils and methanol extracts of sweet basil *Ocimum basilicum* occurring in Bangladesh. Pharm. Biolo., 48: 504-511.

Hussain, A., A. Shrivastav and S.K. Jain, 2013. Antifungal activity of essential oils against local wood degrading cellulolytic filamentous fungi. Adv. Bioresearch, 4: 161-167.

Javed, S., A. Shoaib, Z. Mahmood, S. Mushtaq and S. Iftikhar, 2012. Analysis of phytochemical constituents of *Eucalyptus citriodora* L. responsible for antifungal activity against post-harvest fungi. Nat. Prod. Res., 26: 1732-1736.

Kumar, A., R. Shukla, P. Singh, B. Prakash and N.K. Dubey, 2011. Chemical composition of *Ocimum basilicum* L. essential oil and its efficacy as a preservative against fungal and aflatoxin contamination of dry fruits. Int. J. Food Sci. Technol., 46: 1840-1846.

Mohammadpour, H., E. Moghimipour, I. Rasooli, M.H. Fakoor, S.A. Astaneh, S.S. Moosaie and Z. Jalili, 2012. Chemical composition and antifungal activity of *Cuminum cyminum* L. essential oil from alborz mountain against *Aspergillus* species. Jundishapur J. Nat. Pharm. Prod., 7: 50-55.

Moreira, A.C.P., E.O. de Lima, P.A. Wanderley, E.S. Carmo and E.L. de Souza, 2010. Chemical composition and antifungal activity of *Hyptis suaveolens* (L.) poit leaves essential oil against *Aspergillus* species. Braz. J. Microbiol., 41: 28-33.

Nezhad, F.M., H. Zeigham, A. Mota, M. Sattari and A. Yadegar, 2009. Antibacterial activity of Eucalyptus extracts on methicillin resistance Staphylococcus aureus. Res. J. Biol. Sci., 4: 905-908.

Opalchenova, G. and D. Obreshkova, 2003. Comparative studies on the activity of basil, an essential oil from *Ocimum basilicum* L. against multi-drug resistant clinical isolates of the genera *Staphylococcus*, *Enterococcus* and *Pseudomonas* by using different test methods. J. Microbiol. Meth., 54: 105-110.

Pereira, V., C. Dias, M.C. Vasconcelos, E. Rosa and M.J. Saavedra, 2014. Antibacterial activity and synergistic effects between *Eucalyptus globulus* leaf residues (essential oils and extracts) and antibiotics against several isolates of respiratory tract infections (*Pseudomonas aeruginosa*). Ind. Crops Prod., 52: 1-7.

Puupponen-Pimia, R., L. Nohynek, C. Meier, M. Kahkonen, M. Heinonen, A. Hopia and K.M. Oksman-Caldentey, 2001. Antimicrobial properties of phenolic compounds from berries. J. Applied Microbiol., 90: 494-507.

Qiao, J., W. Liu and R. Li, 2008. Antifungal resistance mechanisms of *Aspergillus*. Nippon Ishinkin Gakkai Zasshi, 49: 157-163.

Sartorelli, D.S. and M.A. Cardoso, 2006. Association between dietary carbohydrates and type 2 diabetes mellitus: Epidemiological evidences. Arquivos Brasileiros Endocrinologia Metabologia, 50: 415-426.

Sienkiewicz, M., E. Kowalczyk and M. Wasiela, 2012. Recent patents regarding essential oils and the significance of their constituents in human health and treatment. Recent Patents Anti-Infect. Drug Discovery, 7: 133-140.

Singh, A.K., S. Singh, S.S. Lodhi, S. Saxena and A. Gaur, 2011. *Essential oil* of Ocimum basilicum as a safe plant based antimicrobial in checking post harvest microbial infestations and aflatoxin contamination of food commodities. J. Pharm. Res., 4: 526-529.

Snelders, E., H.A.L. van der Lee, J. Kuijpers, A.J.M.M. Rijs and J. Varga *et al*., 2008. Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. PLoS Med., Vol. 5, No. 11. 10.1371/journal.pmed.0050219

Stashenko, E.E., B.E. Jaramillo and J.R. Martinez, 2004. Comparison of different extraction methods for the analysis of volatile secondary metabolites of *Lippia alba* (Mill.) N.E. Brown, grown in Columbia and evaluation of its *in vitro* antioxidant activity. J. Chromatogr. A, 1025: 93-103.

Tyagi, A.K. and A. Malik, 2011. Antimicrobial potential and chemical composition of *Eucalyptusglobulus oil* in liquid and vapour phase against food spoilage microorganisms. Food Chem., 126: 228-235.

Vilela, G.R., G.S. de Almeida, M.A.B.R. D'Arce, M.H.D. Moraes and J.O. Brito *et al*., 2009. Activity of essential oil and its major compound, 1, 8-cineole, from *Eucalyptus globulus* Labill., against the storage fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. J. Stored Prod. Res., 45: 108-111.

Yavari, M., S. Mirdamadi, S. Masoudi, M. Tabatabaei-Anaraki, K. Larijani and A. Rustaiyan, 2011. Composition and antibacterial activity of the essential oil of a green type and a purple type of *Ocimum basilicum* L. from Iran. J. Essential Oil Res., 23: 1-4.