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Evaluation of antioxidant and antifungal properties of the traditional plants against foodborne fungal pathogens

Évaluation des propriétés antioxydantes et antifongiques de plantes traditionnelles contre des champignons présents dans l'alimentation

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KEYWORDS

Foodborne pathogens;
Spoilage fungi;
Thymus vulgaris;
Essential oil;
Antifungal and
antioxidant activity

Summary

Objective. – To determine the antioxidant and antifungal activities of the essential oils from five aromatic herbs, including *Thymus vulgaris*, *Chamaemelum nobile*, *Ziziphora clinopodioides*, *Zingiber officinale* and *Cuminum cyminum*, against different *Aspergillus* and *Penicillium* species.

Methods. – The oils were subjected to screening for their possible antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The susceptibility test for the oils was carried out in terms of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) using microdilution method.

Results. – The values of the essential oils in DPPH assay were as follows: *T. vulgaris* ($450.11 \pm 5.23 \mu\text{g/mL}$), *Ch. nobile* ($602.73 \pm 4.8 \mu\text{g/mL}$), *Ziz. clinopodioides* ($1238.82 \pm 9.3 \mu\text{g/mL}$), *Cu. cyminum* ($1255.52 \pm 8.92 \mu\text{g/mL}$) and *Zin. officinale* ($5595.06 \pm 8.24 \mu\text{g/mL}$). Our findings also indicated a strong activity against tested fungi for the oil of *T. vulgaris* ($1250 \mu\text{g/mL}$), followed by *Cu. cyminum* ($1416 \mu\text{g/mL}$), *Zin. officinale* ($1833 \mu\text{g/mL}$), *Ziz. clinopodioides* ($2166 \mu\text{g/mL}$) and

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MOTS CLÉS

Pathogènes alimentaires;
Champignons dégradant
les aliments ;
Thymus vulgaris ;
Huile essentielle ;
L'activité antifongique
et antioxydante

Ch. nobile (3750 µg/mL). This study confirmed the excellent antifungal and antioxidant properties of the essential oils, especially *T. vulgaris*, against foodborne pathogenic fungi.

Conclusion. – Owing to their strong protective features, these oils could be used in ethnomedicine as preventers of lipid peroxidation and cellular damage, and in food industries as preservers of foodstuffs against spoilage fungi. Also, they could be the candidates to develop new antibiotics and disinfectants to control infective agents.

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Résumé

Objectif. – Déterminer les activités antioxydantes et antifongiques des huiles essentielles de cinq herbes aromatiques : *Thymus vulgaris*, *Chamaemelum nobile*, *Ziziphora clinopodioides*, *Zingiber officinale* et *Cuminum cyminum*, contre différentes espèces d'*Aspergillus* et de *Penicillium*.

Matériel et méthodes. – Les huiles ont été examinées pour leur activité antioxydante possible en utilisant le 2,2-diphényl-1-picrylhydrazyl (DPPH). Le test de sensibilité pour les huiles a porté sur la concentration minimale inhibitrice (MIC) et la concentration minimale fongicide (MFC) en utilisant la méthode de microdilution.

Résultats. – Les valeurs des huiles essentielles dans l'essai DPPH étaient comme suit : *T. vulgaris* (450,11 ± 5,23 µg/mL), *Ch. nobile* (602,73 ± 4,8 µg/mL), *Ziz. clinopodioides* (1238,82 ± 9,3 µg/mL), *Cu. cyminum* (1255,52 ± 8,92 µg/mL) et *Zin. officinale* (5595,06 ± 8,24 µg/mL). Nos résultats ont aussi indiqué une forte activité contre les champignons testés pour les huiles de *T. vulgaris* (1250 µg/mL), suivi par *Cu. cyminum* (1416 µg/mL), *Zin. officinale* (1833 µg/mL), *Ziz. clinopodioides* (2166 µg/mL) et *Ch. nobile* (3750 µg/mL). Cette étude a confirmé les propriétés antifongiques et antioxydantes excellentes des huiles essentielles, surtout de *T. vulgaris*, contre les champignons potentiellement pathogènes de l'alimentation.

Conclusion. – En raison de leurs fortes caractéristiques protectrices, ces huiles pourraient être utilisées dans l'ethnomédecine en prévention de la peroxydation lipidique et du dommage cellulaire et dans les industries de nourriture comme des préservateurs de denrées alimentaires contre les champignons qui les altèrent. Aussi, ils pourraient être les candidats pour développer de nouveaux antibiotiques et des désinfectants pour contrôler des agents infectieux.

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Introduction

Foods provide a suitable media for many microorganisms to grow and produce byproducts and metabolites. Spoilage and poisoning of foods by fungi is a major problem, especially in developing countries [24]. *Aspergillus*, *Fusarium* and *Penicillium* species are the most important fungi causing spoilage of foodstuffs. Their growths in food crops are also responsible for off-flavour formation and production of allergenic compounds and mycotoxins, which lead to qualitative losses [4]. Aflatoxin B₁, ochratoxin A and fumonisin B₁ produced by these fungi display carcinogenic properties in humans and in laboratory animals, leading to the appearance of hepatocarcinoma [26]. To manage post harvest losses caused by these fungi, producers usually rely on a release of chemical fungicides (benzimidazoles and aromatic hydrocarbons). Currently, there is a strong debate about the safety aspects of chemical preservatives since they are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity. For these reasons, consumers tend to be suspicious of chemical additives and thus the demand for natural and socially more acceptable preservatives has been intensified. The increase of fungal resistance to classical drugs, the treatment costs, and the fact that most available antifungal drugs have only fungistatic activity, justify the search for new strategies [28]. The exploration

of naturally occurring antimicrobials for food preservation receives increasing attention due to awareness of natural food products and a growing concern of microbial resistance towards conventional preservatives [29].

On the other hand, oxidation of lipids, which occurs during raw material storage, processing, heat-treatment and further storage of final products, is one of the basic processes causing rancidity of food products, leading to their deterioration [14]. Due to undesirable influences of oxidized lipids on the human organism, it seems to be essential to decrease contact with products of lipid oxidation in food [20]. According to toxicologists and nutritionists, the side effects of some synthetic antioxidants used in food processing, such as carcinogenic effects in living organisms have already been documented.

Herbal oils are naturally occurring terpenic mixtures isolated from various parts of plants [8,25]. Some plants from Iranian biomes, such as *T. vulgaris*, *Ch. nobile*, *Ziz. clinopodioides*, *Zin. officinale* and *Cu. cyminum* have been used as natural medicines by local populations in the treatment of infectious and non-infectious diseases (Table 1). Their antifungal properties against food spoilage and mycotoxigenic fungi have been investigated in many studies [30,33]. With the growing interest of the use of either essential oils or plant extracts in the food and pharmaceutical industries, screening of plant extracts for these properties has

Table 1 Some characteristics of the tested plants.
Caractéristiques des plantes étudiées.

Scientific name	Voucher No.	Family	Local name	Medicinal use
<i>Chamaemelum nobile</i>	1577	Asteraceae	Baboneh	Anti-inflammatory, anti-infective, sedative
<i>Cuminum cyminum</i>	1172	Apiaceae	Zireer	Carminative, anti-diarrhoeaic, anti-spasmodic
<i>Zingiber officinale</i>	1418	Zingiberaceae	Zanjebil	Digestive, carminative, nausea
<i>Ziziphora clinopodioides</i>	1680	Labitae	Kakoti	Anti-inflammatory, anti-septic
<i>Thymus vulgaris</i>	1616	Labiatae	Avishan	Anti-infective, expectorant, coughing

become of increasing importance. From this point of view, governmental authorities and consumers are concerned about the safety of their food and about the potential effects of synthetic additives on health. For these reasons, the objectives of this study were to assess *in vitro* antioxidant and antifungal activities of the essential oils from *T. vulgaris*, *Ch. nobile*, *Ziz. clinopodioides*, *Zin. officinale* and *Cu. cyminum* against six moulds, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *Penicillium citrinum* and *P. chrysogenum* for their potential use as foodstuffs preservatives.

Materials and methods

Fungal strains

The fungi used in this study were *A. niger* PTCC (Persian Type Culture Collection) 5154, *A. fumigatus* PTCC 5009, *A. flavus* PTCC 5004, *A. ochraceus* PTCC 5017, *P. citrinum* PTCC 5304 and *P. chrysogenum* PTCC 5271 which were purchased from Iranian Research Organization for Science and Technology (IROST). The fungal isolates were identified by standard mycology methods [1] and stored in Sabouraud dextrose broth (SDB) (Merck Co., Darmstadt, Germany) with glycerol at -70°C . Isolates were grown on Sabouraud dextrose agar (SDA) (Merck Co., Darmstadt, Germany) for 7 days at 35°C . The surface of the agar slants were washed over with 1 mL of sterile 0.9% saline containing 0.1% Tween 80 (C. Erba, Milan, Italy), and conidial suspensions were counted manually with a haemocytometer. The conidia were diluted in Roswell Park Memorial Institute (RPMI) 1640 medium to obtain a working suspension of 1×10^6 conidia/mL.

Plant materials

In this study, the whole aerial parts of five medicinal plants, including *T. vulgaris*, *Ch. nobile*, *Ziz. clinopodioides*, *Zin. officinale* and *Cu. cyminum*, were collected from different regions of Iran. The medicinal plants were selected on the basis of traditional information regarding the treatment of various diseases in Iran (Table 1). Botanical identification was performed at the Herbarium of Pharmacognosy Department, School of Pharmacy, Shaheed Beheshti University of Medical Sciences, Iran.

Preparation of plant essential oils

Essential oils were isolated by water distillation for three hours from air-dried materials and seeds, using a Clevenger type apparatus, according to the procedure described in the

European Pharmacopoeia [7]. The oils were stored at -4°C in sealed brown vials until use.

Measurement of reducing power

The reductive potential of plant essences was determined according to the method described by Yen and Duh [34]. The different concentrations of the essential oils were made (100–4000 $\mu\text{g}/\text{mL}$) in 0.2 M phosphate buffer pH 6.6 containing 1% ferrocyanate. The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10% w/v) was added to the mixture, which was then centrifuged at 3000 g for 10 min. The upper layer was separated and mixed with 2.5 mL of distilled water containing 0.5 mL of ferric chloride 1%. The absorbance of this mixture was measured at 700 nm using a UV-Vis spectrophotometer. The intensity in absorbance showed the antioxidant activities of the essential oils.

DPPH assay

The hydrogen atom or electron donation abilities of the corresponding essences were measured from the bleaching of the purple-colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay was done using the stable radical DPPH as a reagent, according to the method of Burits and Bucar [5]. Briefly, 50 μL of the essential oils (various concentrations) were added to 5 mL of the DPPH (Sigma-Aldrich GmbH, Steinheim, Germany) solution (0.004% methanol solution). After 30 min of incubation at room temperature, the pale pink color developed was measured spectrophotometrically at 517 nm and compared with the standard (250 $\mu\text{g}/\text{mL}$ ascorbic acid). The radical-scavenging activities of the samples were calculated as $\mu\text{g}/\text{mL}$. All tests were carried out in triplicate, and the average results and standard deviations were calculated.

Antifungal assay

Test to assess the susceptibility of various fungal isolates to the essential oils was performed based on M38-A for filamentous fungi [6]. Susceptibility was expressed as minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Stock solutions were prepared by dissolving 1 mg of each compound in DMSO 5%. The stock solutions were diluted with RPMI 1640 medium containing L-glutamine but no sodium bicarbonate (Sigma Chemical Co., St. Louis, Missouri, USA), buffered to pH 7.0 with 0.165 mol/L MOPS buffer (Sigma Chemical Co., St. Louis, Missouri, USA). One hundred microlitre aliquots of 2-fold diluted material

solutions were dispensed into each well of 96-well microtiter plates. The final concentrations of tested materials were 250, 750, 1000, 1250, 1500, 1750, 2000, 2500, 3000, 4000 and 5000 µg/mL. Subsequently, 100 µL aliquots of conidial suspensions were added to each well of a microdilution plate, which was incubated at 35 °C for 48 h. Positive control wells containing only RPMI 1640 media and fungal suspensions as well as negative control wells containing broth media and oils were prepared and incubated at the same conditions. The MICs were read visually and were defined as the lowest concentration required to arrest visible fungal growth at the end of a 48 h incubation period. The MFCs were also determined by subculturing 0.01 mL from each well without visible growth onto SDA plates. The plates were incubated at 35 °C for 48 h. MFCs were the lowest concentrations that did not permit growth on the plates.

Statistical analysis

Data were statistical analyzed by the Student's test. A $P < 0.05$ was considered significant.

Results and discussion

Recently, the scientific interest in biological properties of the essential oils has been increased. New researches about biological active compounds present in the oils of the plants have been seen as a potential way to control fungal contamination. In the reducing power assay, the more antioxidant compounds convert the oxidation form of iron (Fe^{+3}) in ferric chloride to ferrous (Fe^{+2}). Our study had assessed potential antioxidant activity of the essential oils. The highest and lowest reducing powers were observed in *T. vulgaris* and *Cu. cyminum*, respectively (Fig. 1). Thus, the decreasing order of reducing power of the samples was *T. vulgaris* > *Ch. nobile* > *Ziz. clinopodioides* > *Zin. officinale* > *Cu. cymy-cyminum* essential oils. Table 2 illustrated the comparison of the reducing powers of the essential oils at concentration, which showed absorption of 0.5. On the basis of these results, the reducing powers of all essential oils were less than *T. vulgaris* and *Ch. nobile* ($P < 0.05$). The role of reducing power, radical-scavenging ability and singlet

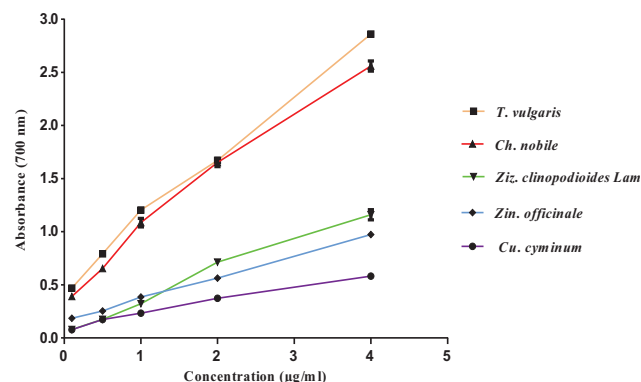


Figure 1 Reducing power of essential oils (mean ± SD of 3 samples).
Pouvoir réducteur des huiles essentielles (moyenne ± SD de 3 échantillons).

Table 2 Concentration of essential oils at absorbance 0.5 in reducing power assay (mean ± SD of 3 samples).
Concentration des huiles essentielles à l'absorbance 0,5 dans le test du pouvoir réducteur (moyenne ± SD de 3 échantillons).

Samples	Concentration (µg/mL, absorbance 0.5)
<i>Thymus vulgaris</i>	0.013 ± 0.002 ^a
<i>Chamaemelum nobile</i>	0.13 ± 0.01 ^b
<i>Ziziphora clinopodioides</i>	1.55 ± 0.05 ^c
<i>Zingiber officinale</i>	1.65 ± 0.04 ^c
<i>Cuminum cyminum</i>	3.22 ± 0.1 ^d

The same alphabetic letters implied there are not any statistical differences ($P > 0.05$) and different letters represented statistical differences ($P < 0.05$).

oxygen quenching ability in antioxidant activity of the essential oils has already been proven [11,16]. Seemingly, high percentage of phenolics, flavonoids and terpenoids constituents, in *T. vulgaris* and *Ch. nobile* essential oils could be related to their high antioxidant activities [32]. The antioxidant activity of the phenolic compounds could be due to their proton-donating effects. They can retard or stop the oxidation of other molecules by inhibiting the chain reaction of oxidation. In this regard, Luximun-Ramma et al. [17] showed a linear correlation between antioxidant activity and phenolic contents of the plant oils. Sugihara et al. [31] demonstrated that flavonoids are able to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals. In addition, Kamkar et al. [13] indicated a potent antioxidant activity for terpenoids as well. As shown in Table 3, the results of IC50 values for the essential oils in DPPH assay showed that increase in samples concentration resulted in increase in free radical-scavenging activity. In this regard, the highest and lowest radical-scavenging activities in DPPH assay were observed in *T. vulgaris* and *Zin. officinale* essential oils, respectively. The decreasing order of antiradical power of the samples was *T. vulgaris* > *Ch. nobile* > *Ziz. clinopodioides* > *Cu. cyminum* > *Zin. officinale* essential oils. These findings were similar to their reducing powers except *Zin. officinale* essential oil whose radical-scavenging activity was less than other essential oils in this

Table 3 *In vitro* antioxidant activities of essential oils in DPPH assay. Values (mean ± SD) were expressed as µg/mL.
Activités antioxydantes in vitro des huiles essentielles dans l'essai DPPH. Les valeurs (moyenne ± SD) ont été exprimées en µg/mL.

Samples	DPPH (µg/mL)
<i>Thymus vulgaris</i>	450.11 ± 5.23 ^a
<i>Chamaemelum nobile</i>	602.73 ± 4.8 ^b
<i>Ziziphora clinopodioides</i>	1238.82 ± 9.3 ^c
<i>Cuminum cyminum</i>	1255.52 ± 8.92 ^c
<i>Zingiber officinale</i>	5595.06 ± 8.24 ^e

The same alphabetic letters implied there are not any statistical differences ($P > 0.05$) and different letters represented statistical differences ($P < 0.05$).

Table 4 Antifungal susceptibility of the selected essential oils against some important foodborne fungal pathogens.
Sensibilité antifongique aux huiles essentielles choisies contre les principaux pathogènes fongiques alimentaires.

Fungal isolate	<i>Thymus vulgaris</i>		<i>Chamaemelum nobile</i>		<i>Ziziphora clinopodioides</i>		<i>Cuminum cyminum</i>		<i>Zingiber officinale</i>	
	MIC (µg/mL)	MFC (µg/mL)	MIC (µg/mL)	MFC (µg/mL)	MIC (µg/mL)	MFC (µg/mL)	MIC (µg/mL)	MFC (µg/mL)	MIC (µg/mL)	MFC (µg/mL)
<i>Aspergillus niger</i> (PTCC 5154)	1250	1750	–	–	3000	3000	1500	2000	2000	2000
<i>Aspergillus fumigates</i> (PTCC 5009)	1000	1500	3000	4000	1750	2500	1000	1500	1500	2000
<i>Aspergillus flavus</i> (PTCC 5004)	1500	2000	5000	–	2000	3000	1250	1750	1750	2000
<i>Aspergillus ochraceus</i> (PTCC 5017)	750	1250	3000	5000	1500	1750	1000	1750	1250	1500
<i>Penicillium citrinum</i> (PTCC 5304)	1250	1500	4000	5000	1750	2000	1750	2500	2000	3000
<i>Penicillium chrysogenum</i> (PTCC 5304)	1750	2000	–	–	3000	4000	2000	2500	2500	3000

test ($P < 0.05$). Although the DPPH assay is a popular method, the stable radical has no similarity to the highly reactive and transient peroxy radicals. Many antioxidants that react with peroxy radicals may react slowly or may even be inert to DPPH, due to steric inaccessibility [27]. So, the weaker antioxidant activity of *Zin. officinale* oil in DPPH assay might be related to this reason.

This study assayed antifungal characteristics of some traditional plants, including *T. vulgaris*, *Ch. nobile*, *Ziz. clinopodioides*, *Zin. officinale* and *Cu. cyminum* on foodborne *Aspergillus* and *Penicillium* species. Based on previous studies [9,12,21], the main components of the oils of these plants were thymol and carvacrol in *T. vulgaris*, α -bisabolol oxide and isopropyl hexadecanoate in *Ch. nobile*, α -pinene and cineole in *Cu. cyminum*, thymol and pulegone in *Ziz. clinopodioides* and zingiberene and curcumen in *Zin. officinale*. In our study, the MICs of *T. vulgaris* ranged from 750 to 1750 µg/mL (mean value: 1250 µg/mL) for different fungal isolates (Table 4). The addition of increased concentrations of the oil led to lower growth, showing an effect of *T. vulgaris* on inhibiting fungal development. The most and least growth inhibitions were associated with *A. ochraceus* and *P. chrysogenum*, respectively. Several studies showed that thyme oils, particularly *T. vulgaris*, possess antifungal activity, those of the thymol and carvacrol being the most active [2]. In agreement with our results, Markovic et al. [19] demonstrated thymol and carvacrol have a remarkably antifungal potential, indicating more susceptibility of *Aspergillus* spp. than that of *Penicillium* spp. The antifungal mechanism of action by which thymol or carvacrol acts is not well understood although membrane and cell wall disruption with morphological deformation, collapse and deterioration of the conidia and/or hyphae have been hypothesized [23].

As shown in Table 4, the MICs of *Cu. cyminum* ranged from 1000 to 2000 µg/mL (mean value: 1416 µg/mL) for tested fungi, and at higher concentrations, it had significantly fungicidal activity. Growth inhibition of *A. fumigates* and *A. ochraceus* was higher than that of other fungal isolates. *Ziz. clinopodioides* was also active against all tested fungi.

MIC values ranged from 1500 to 3000 µg/mL (mean value: 2166 µg/mL). The most potent inhibitory activity of *Ziz. clinopodioides* was found for *A. ochraceus*. A similar study was conducted by Khosravi et al. [15] who *Cu. cyminum* and to a lesser extent *Ziz. clinopodioides* oils exhibited the inhibitory activity against *A. fumigatus* and *A. flavus* with MIC90 ranging from 250 to 1500 µg/mL. These oils caused high vacuolation of the cytoplasm, detachment of fibrillar layer of cell wall, plasma membrane disruption and disorganization of the nuclear and mitochondrial structures. In addition, Naeini and Shokri [22] reported good inhibition of *A. nidulans*, *A. flavus*, *A. ochraceus*, *A. fumigatus*, *A. niger* by *Cu. cyminum* oil.

Data in Table 4 showed the influence of *Zin. officinale* (1250 to 2500 µg/mL; mean value: 1833 µg/mL) on the growth of all tested fungi. The incorporation of increased concentrations of *Zin. officinale* to the media led to progressive and significant reduction in growth for all fungi. *A. ochraceus* and *P. chrysogenum* showed the highest and lowest MIC values, respectively. For other fungal isolates, MIC values were to some extent similar. Our results agree with those of Gurdip et al. [10] and Bansod and Rai [3] who exhibited moderate inhibitory effect of *Zin. officinale* essential oil against *Aspergillus* and *Penicillium* species. According to these authors, *Zin. officinale* essential oil rich in sesquiterpenes such as zingiberene possessed an antifungal activity against a wide spectrum of filamentous fungi. Finally, our study showed that the oil of *Ch. nobile* showed a moderate to weak pattern of inhibition (ranging from 3000 to 5000 µg/mL; mean value: 3750 µg/mL) against foodborne fungal pathogens. To our knowledge, there is little information about *Ch. nobile* effect on the kinetics of the mycelial growth and germination of *Aspergillus* and *Penicillium* conidia. In a study by Magro et al. [18], inhibitory effect of *Ch. nobile* against *Aspergillus* and *Penicillium* species was demonstrated.

In summary, our findings indicated a strong activity against tested fungi for the oil of *T. vulgaris*, followed by *Cu. cyminum*, *Zin. officinale*, *Ziz. clinopodioides* and *Ch. nobile*. Regarding the considerable antifungal activities

and excellent protective features exhibited in antioxidant activity tests, these oils could be the candidates in ethno-medicine as preventers of lipid peroxidation and cellular damage, and in food industries as preservers of foodstuffs against spoilage fungi. The desirable flavor and odor of these essential oils at the examined concentrations was an additional benefit to their antimicrobial activities, which makes them suitable candidates to be used as food preservatives.

Disclosure of interest

The authors declare that they have no competing interest.

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References

- [1] Aissam H, Errachidi F, Penninckx MJ, Merzouki M, Benlemlih M. Production of tannase by *Aspergillus niger* HA37 growing on tannic acid and Olive Mill Waste Waters. *World J Microbiol Biotechnol* 2005;21:609–14.
- [2] Bakkali F, Averbeck S. Biological effects of essential oils. *Food Chem Toxicol* 2008;46:446–75.
- [3] Bansod S, Rai M. Antifungal activity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus fumigatus* and *A. niger*. *World J Med Sci* 2008;3:81–8.
- [4] Bennett JW, Klich M. Mycotoxins. *Clin Microbiol Rev* 2003; 16:497–516.
- [5] Burits M, Bucar F. Antioxidant activity of *Nigella sativa* essential oil. *Phytother Res* 2000;14:323–8.
- [6] CLSI/Clinical Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi; [Wayne, (Approved Standard M38-A)]; 2002.
- [7] Council of Europe. Methods of Pharmacognosy. In: European Pharmacopoeia. 3rd edition, Strasbourg: European Department for the Quality of Medicines; 1997: 121–2.
- [8] Daferera DJ, Ziogas BN, Polissiou MG. GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. *J Agric Food Chem* 2000;48: 2576–81.
- [9] Dezfooli ND, Hasanzadeh N, Rezaee MB, Ghasemi A. Antibacterial activity and chemical compositions of *Chamaemelum nobile* essential oil/extracts against *Pseudomonas tolaasii*, the causative agent of mushroom brown blotch. *Ann Biol Res* 2012;3:2602–8.
- [10] Gurdip Singh I, Kapoor IPS, Pratibha S, Carola S, Heluani D, Marina P, et al. Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale*. *J Food Chem Toxicol* 2008;46:3295–302.
- [11] Jebelli Javan A, Ghazvinian K, Mahdavi A, Javaheri Vayeghan A, Steji H, Ghaffari Khaligh S. The effect of dietary *Zataria Multiflora* Boiss essential oil supplementation on microbial growth and lipid peroxidation of Broiler Nreast filets during refrigerated storage. *J Food Process Preserv* 2012;37:881–8.
- [12] Kamaliroosta Z, Kamaliroosta L, Elhamirad AH. Isolation and identification of ginger essential oil. *J Food Biosci Technol* 2013;3:73–80.
- [13] Kamkar A, Jebelli Javan A, Asadi F, Kamalinejad M. The anti-oxidative effect of Iranian *Mentha pulegium* extracts and essential oil in sunflower oil. *Food Chem Toxicol* 2010;48: 1796–800.
- [14] Karpinska M, Browski J, Danowska-Oziewicz M. The use of natural antioxidants in ready-to-serve food. *Food Chem* 2001;72:5–9.
- [15] Khosravi AR, Minooeianhaghghi MH, Shokri H, Emami SA, Alavi SM, Asili J. The potential inhibitory effect of *Cuminum cyminum*, *Ziziphora clinopodioides* and *Nigella sativa* essential oils on the growth of *Aspergillus fumigatus* and *Aspergillus flavus*. *Braz J Microbiol* 2011;42:216–24.
- [16] Loizzo MR, Menichini F, Conforti F, Tundis R, Bonesi M, Saab AM, et al. Chemical analysis, antioxidant, antiinflammatory and anticholinesterase activities of *Origanum ehrenbergii* Boiss. and *Origanum syriacum* L. essential oils. *Food Chem* 2009;117: 174–80.
- [17] Luximun-Ramma A, Baharun T, Soobrattee MA, Aumai OI. Antioxidant activities of phenolic, proanthocyanidin, and flavonoid components in extracts of *Cassia fistula*. *J Agric Food Chem* 2002;50:5042–7.
- [18] Magro A, Carolino M, Bastos M, Mexia A. Efficacy of plant extracts against stored-products fungi. *Rev Iberoam Micol* 2006;23:176–8.
- [19] Markovic T, Chatzopoulou P, Siljegovic J, Nikolic M, Glamoclija J, Ciric A. Chemical analysis and antimicrobial activities of the essential oils of *Satureja thymbra* L. and *Thymbra spicata* L. and their main components. *Arch Biol Sci* 2011;63:457–64.
- [20] Mccord JM. The evolution if free radicals and oxidative stress. *Am J Med* 2000;108:652–9.
- [21] Naeini A, Khosravi AR, Chitsaz M, Shokri H, Kamalnejad M. Anti-*Candida albicans* activity of some Iranian herbs used in traditional medicine. *J Mycol Med* 2009;19:168–72.
- [22] Naeini A, Shokri H. Chemical composition and *in vitro* antifungal activity of the essential oil from *Cuminum cyminum* against various *Aspergillus* strains. *J Med Plants Res* 2012;6:1702–6.
- [23] Neri F, Mari M, Brigati S. Control of *Penicillium expansum* by plant volatile compounds. *Plant Pathol* 2005;55:1–6.
- [24] Nielsen PV, Rios R. Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. *Int J Food Microbiol* 2000;60:219–29.
- [25] Paranagama PA, Abeysekera KHT, Abeywickrama K, Nugaliyadde L. Fungicidal and anti-aflatoxicogenic effects of the essential oil of *Cymbopogon citratus* (DC.) Stapf. (lemongrass) against *Aspergillus flavus* Link. isolated from stored rice. *Lett Appl Microbiol* 2003;37:86–90.
- [26] Pfohl-Leskowicz A, Manderville RA. Ochratoxin A toxicity: an overview on toxicity and carcinogenicity in animals and humans. *Mol Nut Food Res* 2007;51:61–9.
- [27] Prior RL, Wu XL, Schatich KJ. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem* 2005;53: 4290–302.
- [28] Rapp RP. Changing strategies for the management of invasive fungal infections. *Pharmacother* 2004;24:4–28.
- [29] Schuenzel KM, Harrison MA. Microbial antagonists of foodborne pathogens on fresh minimally processed vegetables. *J Food Protect* 2002;65:1909–15.
- [30] Singh G, Maurya S, Lampasona MP, Catalan C. Chemical constituent, antifungal and antioxidative potential of *Foeniculum vulgare* volatile and its acetone extract. *Food Control* 2006;17:745–52.
- [31] Sugihara N, Arakawa T, Ohnishi M, Furuno K. Anti- and pro-oxidative effects of flavonoids on metal-induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes loaded with alpha-linolenic acid. *Free Radical Biol Med* 1999;27:1313–23.
- [32] Thompson J, Chalcha J, Michet A, Linhart Y, Ehlers B. Qualitative and quantitative variation in monoterpene co-occurrence

- and composition in the essential oil of *Thymus vulgaris* chemotypes. *J Chem Ecol* 2003;29:859–80.
- [33] Velluti A, Sanchis V, Ramos AJ, Egido J, Marin S. Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B1 production by *Fusarium proliferatum* in maize grain. *Int J Food Microbiol* 2003;89:145–54.
- [34] Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen species. *J Agric Food Chem* 1994;42:629–32.