Chemical composition and antimicrobial activity of essential oil ofwild and cultivated Origanum syriacum plants grown in Sinai, Egypt

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Abstract tThe main constituents of essential oil (EO) of Origanum syriacum L. collected from cultivated and growingwild in Sinai, Egypt were identified and determined by GC–MS. The antimicrobial activity of these essen-tial oils against Gram positive and Gram negative bacterial strains was studied. The antimycotic activityof these EOs was also evaluated against eight fungal strains isolated from different sources. Forty-sixcompounds were identified in the EO of both samples, dominated by carvacrol in cultivated type. Thy-mol, _-terpinene, linalool and 4-terpineol were the most represented constituents in O. syriacum plantscollected from wild populations. Both EOs showed antibacterial activity with varying magnitudes, whileEO from cultivated O. syriacum showed high antibacterial activity against Staphylococcus aureus with aninhibition zone of 32 ± _4.0 mm. Both EOs showed good antifungal activity against all fungal strains. O.syriacum EO from cultivated plants showed the lowest MIC 0.25 mg/L with Aspergillus fumigates clinicalstrain isolated either with Aspergillus flavus.

1. IntroductionMedicinal plants and culinary herbs have long been the basis oftraditional medicine in many countries. Some important medici-nal wild plants suffered from unwise human manipulation whichresulted in the extinction of some. Projects to recover the geneticdiversity of wild plants are carried out in Egypt as well as scientificresearch of their use based on traditional medicine in Bedouin com-munities. Indeed, there are a great number of rural jobs dependenton this sector (Viuda-Martos et al., 2010, 2011). Cultivation of thesespecies is considered a mean to conserve these species within ouroutside their habitats (in-sito or ex-sito conservation). Cultivationof medicinal and aromatic plants in Egypt is of great socioeconomicinterest as they are mainly produced for export.Essential oils of herbs and their components, products of a sec-ondary metabolism of the plant, have many applications in folkmedicine, food flavoring and preservation as well as in cosmeticand pharmaceutical industries. The antimicrobial and antioxidant* properties of essential oils have been known for a long time, anda number of investigations have been conducted on antimicro-bial activities using various bacteria, viruses and fungi (Alma et al.,2003).Oregano is the common name for the general aroma and fla-vor of the plants derived from the Lamiaceae family (Ibrahim et al.,2012). The genus Origanum is well known for its volatile oil andconstituents and is characterised by a large morphological andchemical diversity. Forty-nine taxa divided into 10 sections belongto this genus, most of them having a very local distribution aroundthe Mediterranean basin (Loizzo et al., 2009). In folk medicine, Ori-ganum species are used as powerful disinfectants, flavoring agents,in perfumes and in scenting soaps (Kamel et al., 2001). They arealso useful as a source of antimicrobial compounds (Sokovic et al.,2007). Due to these properties, spices and herbs have been addedto food since ancient times, not only as flavoring agents but also aspreservatives (Kalemba and Kunicka, 2003).Origanum syriacum L. is an aromatic, herbaceous and peren-nial plant growing wild in the Sinai Desert of Egypt (Tackholm,1974). Oregano is also used as a stimulant, analgesic, antitussive,expectorant, sedative, antiparasitisc and antihelminthic, butmostly for gastrointestinal complaints (Baser et al., 2003).
2. The essential oil of oregano is composed of carvacrol and/orthymol as its dominant components, followed by _-terpinene,pcymene, linalool, terpinen-4-ol and sabinene hydrate (Skoula andHarborne, 2002). Sarer et al. (1982) reported that thymol and car-vacrol represent the major constituents of the essential oils ofOriganum species. The most abundant chemotypes in these essen-tial oils were thymol and carvacrol (Loizzo et al., 2009; Zein et al.,2011). There is another chemotype between thymol and carvacrolbut not as widespread. This chemotype contains a high con-tent of two monoterpene hydrocarbons, _-terpinene or p-cymene,whereas some species were found with high values of linalool, phe-nols, alcohols, ethers, aldehydes and ketones (Russo et al., 1998). Oilquality is determined by its composition, which varies with geno-type, climate, soil type, orientation and plant development (Russoet al., 1998; Baydar et al., 2004).The spread of drug resistant microbial pathogens is one ofthe most serious threats to the successful treatment of infec-tious diseases (Owlia et al., 2009). Fungal and bacterial pathogensare serious problems in agricultural, food and medical practices.Treating infected plants, human diseases or food-borne microbeswith synthetic fungicides, antibiotics or similar have never beensatisfactory. Furthermore, these substances usually cause resis-tance, health risks or environmental pollutions and thus are oftenforbidden in many countries. Concerns over side-effects of theconventional methods used for controlling these pathogens led toincreasing interest in finding safer and environmentally friendlieralternatives (Ibrahim et al., 2012).Aspergillus species are common environmental molds con-taminant of various substrates. Several of them can act bothas human/animal pathogens and as producers of toxic metabo-lites. The presence in food stuffs of mycotoxins, fungal secondarymetabolites, is of extreme importance, because these can lead to oncogenesis and several other pathologies (Queiroz et al., 2013). Fur-thermore these fungi can also determine the unwanted mouldingof comestibles.This work aimed to (i) determine the main constituents of theessential oil of O. syriacum L. plants collected from wild populationsand cultivated one in Sinai, Egypt; (ii) study the antibacterial activ-ity of resulted essential oils against four reference bacterial strains(two Gram positive and two Gram negative); and (iii) determinethe effectiveness of the isolated essential oil against eight fungalstrains isolated from different sources.2. Methods and materials2.1. Plant materialSamples of O. syriacum L. plants were collected before flower-ing period from populations growing wild in Wadi-Firan and from cultivated plants grown in El-Naby Danyal, Saint Katherine in theSouth Sinai, Egypt. The geographical conditions and air temperatureand relative humidity and average of precipitation (mm) duringthe investigation period are presented in Table 1 and Fig. 1. Thesoil composition from cultivated and wild O. syriacum also is pre-sented in Table 1. The plants were identified by Prof. Dr. KamalZayed Professor of plant taxonomy, Plant Department, Faculty ofScience, Cairo University and according to a voucher specimen wasdeposited in the herbarium (No. 1022) of the National ResearchCentre (Cairo).2.2. Cultivation practicesThe seeds of wild O. syriacum plants were collected from thewild populations grown in Saint Katherine and were planted innursery beds in green houses in the second week of October 2010.When the seedlings reached a height of 15 cm at the beginning ofMarch 2011, they were transplanted to a uniform soil located inEl-Naby Danyal, Saint Katherine, (Sinai, Egypt) in lines containingtwo irrigation dropping lines with 30 cm between the hoses. Aftersowing, the seeds received a rich irrigation and later as required tomaintain vigorous growth.The soil was prepared for cultivation by adding 20 m^3com-post, 200 Kg rock phosphate, 100 Kg feldspar per acre (4200 m^2)as organic fertilizer and then irrigated. The harvest processes were conducted by hand in the morning between October and November2011, and then moved to a clean drying location in the shade forseven to ten days until dry. The air dried samples were kept inplastic bags at room temperature until analysis for essential oil.2.3. Extraction of essential oil (EO)The EO of O. syriacum from the two different sources wasobtained from entire plants by hydro-

distillation using a Clevenger-type apparatus for 3 h. The oily layer obtained on top of the aqueousdistillate was separated and dried with anhydrous sodium sulfate(0.5 g). The extracted EOs were kept in sealed air-tight glass vialsand covered with aluminum foil at 4◦C until further analysis.The yields of the EOs were 5.5%, (5.39 g Oil/100 g herb) and 4.63%,(4.34 g Oil/100 g herb) for cultivated and wild O. syriacum plants,respectively.2.4. Gas chromatography–mass spectrometryThe essential oil compounds were isolated, identified, quanti-fied and analyzed by GC/MS with a CP-3800 gas chromatographequipped with HP-5 capillary column (30 m × _0.25 mm; coatingthickness, 0.25 mm) and Varian Saturn 2000 ion trap mass detec-tor. Analytical conditions were as follows: injector and transfer linetemperature, 220 and 240◦C, respectively; oven temperature, pro-grammed from 60 to 240◦C at 3◦C per min; carrier gas, helium at1 ml min−1; injection, 2 _l (10% hexane solution); split ratio, 1:30.The constituents were identified by comparing the retention timewith the authentic samples, on the basis of their linear indicesrelative to a series of n-alkanes (C_8–C_{23}). Further identificationswere carried out using a homemade library of mass spectra cre-ated from pure substances and components of known oils, and theMS literature data (NIST 2000, ADAMS).2.5. Antimicrobial activity assay2.5.1. Antibacterial activity2.5.1.1. Microbial strains. The essentials oils were individuallytested against Staphylococcus aureus ATCC 6538 and Enterococcusfaecalis ATCC 70080 2D-5 (strain V 583) as Gram positive bacteria,and Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC25422 as Gram negative bacteria. All the strains belonging to theAmerican Type of Culture Collection were obtained from Depart-ment of Veterinary Sciences, Laboratory of Bacteriology, Universityof Pisa, Italy.2.5.1.2. Agar disc diffusion method. The agar disc diffusion method(Kirby-Bauer) described by Clinical and Laboratory Standards Insti-tute (CLSI) 2012, with some modifications, was used to determinethe antibacterial activity of essential oils, as follows:- Active cultures for experiments were prepared by transferring aloopful of bacterial cells from the stock cultures to tubes contain-ing brain hearth infusion broth (BHIB, Oxoid Ltd., Basingstoke,Hampshire, England) that were incubated for 24 h at 37◦C.- The cultures were suspended in sterile saline solution to obtain aturbidity equivalent to a 0.5 McFarland standard approximately1–2 × 10^7CFU/mL.- The microbial suspension was streaked over the surface of theMueller Hinton agar (MHA, Oxoid) plates using a sterile cottonswab in order to get a uniform microbial growth on test plates.Under aseptic conditions, the discs (diameter 6 mm, Whatmanpaper No.1, Oxoid) were placed on the agar plates (one filter paperdisc was placed per Petri dish in order to avoid a possible additiveactivity) and then 10 _l from each of the essential oils dilutions(dimethyl sulfoxide (DMSO) stock solution of EOs of four differentconcentrations (4, 2, 1 and 0.5 _l EOs per disc) was put on thediscs.- The plates were then incubated at optimum temperature 37◦Cfor 24 h, followed by the measurement of the diameter of growthinhibition zone expressed in millimetres (mm). All tests wereperformed in triplicate.- The reference bacterial strains were tested by Kirby-Bauermethod to evaluate their in vitro sensitivity to 5 Antibiotics:Tetracycline (30 _g), Ceftazidime (30 _g), Rifampicin (30 _g),Cephalexin (30 _g), and Cefotaxime (30 _g). The results wereinterpreted on the basis of the indications suggested by theNational Committee for Clinical Laboratory Standards, 2002.2.5.1.3. Determination of concentration effect. The concentrationeffect (CE) was studied to ascertain which dose of EOs had aninhibitory effect on bacterial growth in the disc diffusion assay. Theculture techniques used were those described in the previous para-graph (Agar disc diffusion method), but adding 4, 2, 1 and 0.5 _l ofeach EO, which mean doses of 100, 50, 25 and 12.5% of the ini-tial volume (Viuda-Martos et al., 2010). All tests were performed intriplicate.2.5.2. Antifungal activity2.5.2.1. Fungal isolates. Eight fungal isolates belonging to 3Aspergillus species (Aspergillus fumigatus, Aspergillus flavus andAspergillus niger) were examined. More in detail 3 isolates of A. fumi-gatus were cultured from birds died from invasive aspergillosis, theother was obtained from soil, 2 isolates of A. flavus were isolatedfrom Pinus pinea and bovine litter, respectively, and 2 of A. niger iso-lated from crops and soil (one isolate each). All fungal strains wereisolated in the Mycology Laboratory of Department of VeterinarySciences, University of Pisa, Italy.2.5.2.2. In vitro antifungal testing. EOs were diluted in sweetalmond oil (Prunus dulcis, Mill) to obtain a 20% stock solution.Serial dilutions in RPMI were made to achieve final concentra-tions ranging from 10% to 0.25%. To avoid bias due to differingamounts of sweet almond oil, each test was carried out usinga concentration equal to 40% of this oil. This concentration wasalso added to the medium in control cultures. Before testing, eachisolate was subcultured at least twice on potato dextrose agar(PDA) to ensure viability and purity. EUCAST broth microdilutionmethod testing (BMD) was performed as detailed by EUCAST withslight modification (2008 EUCAST technical note on the methodfor the determination of broth dilution minimum inhibitoryconcentrations of antifungal agents for conidia-forming mouldshttp://www.eucast.org/documents/technical notes) by using RPMI1640 medium with 2.0% glucose, flat-bottom microdilution trays,inocula of 2 × $10^5$to 5 × 10^5CFU/ml, and incubation at 25◦C(EUCAST, 2008). The MIC values were determine visually as the low-est concentration of EO that cause complete inhibition of growth(first clear well) relative to that of the growth control after 48 h ofincubation. A conventional drug (itraconazole) was also tested, fol-lowing the above described procedure. To assess MFC value inoculawhich did not grow were washed with distilled water, subculturedonto PDA and incubated at 25◦C for 4 weeks, to evaluate if the lackof growth was due to a fungistatic or fungicidal effect of EOs.2.6. Statistical analysisConventional statistical methods were used to calculate meansand standard deviations of three simultaneous assays carriedout with the different methods. Statistical analysis (ANOVA) wasapplied to the data to determine differences ($P < 0.05$). To discoverwhere there were significant differences between the levels of the main factor, contrasts (Tukey test) between means were made (Afifiand Azen, 1979).

3. Results and discussion

3.1. Chemical composition of the essential oils

The y_i_e_l_d_ _o_f_ _t_h_e_ _e_s_s_e_n_t_i_a_l_ _o_i_l_ _f_r_o_m_ _c_u_l_t_i_v_a_t_e_d_ _a_n_d_ _w_i_l_d_ _O. syr-iacum p_l_a_n_t_s_ _w_a_s_ _5_._5_%_,_ _a_n_d_ _4_._6_3_%_,_ _r_e_s_p_e_c_t_i_v_e_l_y_._ _T_h_e_ _c_h_e_m_i_c_a_l_c_o_m_p_o_s_i_t_i_o_n_ _o_f_ _e_s_s_e_n_t_i_a_l_ _o_i_l_s_ _o_b_t_a_i_n_e_d_ _f_r_o_m_ _c_u_l_t_i_v_a_t_e_d_ _a_n_d_ _w_i_l_d_O. syriacum L._ _p_l_a_n_t_s_ _c_o_l_l_e_c_t_e_d_ _f_r_o_m_ _p_o_p_u_l_a_t_i_o_n_s_ _g_r_o_w_i_n_g_ _i_n_ _S_a_i_n_t_K_a_t_h_e_r_i_n_e_,_ _S_o_u_t_h_ _S_i_n_a_i_,_ _E_g_y_p_t_ _a_r_e_ _p_r_e_s_e_n_t_e_d_ _i_n_ _T_a_b_l_e_ _2_._ _G_C–M_S_a_n_a_l_y_s_i_s_ _o_f_ _t_h_e_ _v_o_l_a_t_i_l_e_ _o_i_l_s_ _i_n_ _b_o_t_h_ _s_a_m_p_l_e_s_ _i_n_d_i_c_a_t_e_d_ _t_h_a_t_ _a_l_l_i_d_e_n_t_i_f_i_e_d_ _c_o_m_p_o_u_n_d_s_ _w_e_r_e_ _d_e_t_e_c_t_e_d_ _i_n_ _b_o_t_h_ _o_i_l_s_ _w_i_t_h_ _d_i_f_f_e_r_e_n_t_ _

percentages. Forty six compounds were similarly identified in thesetwo EOs from each cultivated and wild O. syriacum plant mate-rial which represented 98.93% and 99.42% of the total detectedconstituents in EOs of cultivated and wild plants, respectively.Nevertheless there is a difference in the chemical compositionbetween cultivated and wild O. syriacum essential oil. Carvacrol wasthe major compound in the essential oil of cultivated O. syriacumplants (81.38%), followed by a low amount of _-cymene (8.48%), _-terpinene (1.98%), _-myrcene (1.32%), _-terpinene (1.24%), 4-terpineol (0.89%), _-pinene (0.72%) and thymol (0.35%). On theother hand, thymol was the main compound in the essential oil ofWos plants (31.73%), together with _-terpinene (14.32%), linalool(9.44%), 4-terpineol (7.68%), _-terpineol (5.51%), while, carvacrolwas present in a very low concentration (3.90%). These data pre-sented in Table 2 indicated that the oxygenated monoterpeneswere abundant with 83.83% in cultivated plants and 62.61% for wildplants, followed by 14.68 and 33.07% of monoterpene hydrocar-bons, respectively.These differences in the major constituents in EO between cul-tivated and wild O. syriacum plants confirmed the presence oftwo chemotypes (carvacrol types and thymol types) of O. syriacumplants since chemotypes influence the plant biosynthetic pathwaysand consequently the relative proportion of the main characteris-tic compounds

(Viuda-Martos et al., 2011) and these results wereagreement with a previous work on Lebanese O. syriacum plants(Zein et al., 2011, 2012). In fact, in the present study, the cultivatedplants were characterized by the dominant presence of carvacrol(81.38%), while the wild samples revealed predominance of thy-mol (31.73%) even though one third lower than carvacrol. However,the prevailing chemotypes would depend on many factors such asgeographical origin (Alma et al., 2003; Baser et al., 2003; Tonceret al., 2010; Fatima et al., 2012), climate, soil composition, seasonvariation and harvesting time (Zein et al., 2011) and cultivationconditions may also influence the essential oil content of oreganoleaves and the composition of the essential oil (Omer, 1999 andAzizi et al., 2009).In our work the differences of major constituents of EO may beattributed to fertilization, since the wild populations do not havefertilization or irrigation, the wild plant depends on the rain anddewy in their water requirements and elevation about sea level(altitude m). Since the highest thymol obtained at lowest altitudein wild plants (Wadi-Firan 1362 m) while the highest carvacrolobtained at highest altitude in cultivated plants (Saint Katherine1574 m) and this may be due to climatic factors and since thymoland carvacrol are isomers, this may reveal a genetic correlation asreported in the study done by Toncer et al., 2010 and Fatima et al.2012. The opposite occur in study done in Turkey in which altitudehas positive effect on thymol (69%) and negative effect on carvacrol(3.08%) and this also may reveal climatic and genetic factors (Avci,2011). Some studies proved that chemical composition change indifferent regions is due to the environmental factors that affect thebiosynthesis pathway, in which this pathway begin by autooxida-tive conversion of _-terpinene to _-cymene and then hydroxylationof _-cymene to thymol or carvacrol and this pathway over activatedduring flowering stage (De Martino et al., 2009).Similar results were found by many authors (Loizzo et al., 2009,Zein et al., 2011; Ibrahim et al., 2012) who reported that the mostabundant compounds in O. syriacum essential oils were carvacroland thymol. Zein et al. 2010 found that, the main constituents ofOriganum essential oils were determined as carvacrol in cultivatedplant and thymol in wild plant. Also Farhat et al. (2012) found thatthe two dominant constituents identified in O. syriacum leaves werecarvacrol (78.4%) and thymol (17.9%). Arnold et al. (2000) foundthat O. syriacum contained carvacrol (88.3%) as the predominantconstituent and was also the main component of the Greek oreganoessential oil (Kokkini and Vokou, 1989).

Since both cultivated and wild O. syriacum EOs were rich in oxy-genated monoterpenes and their precursors, they were tested forantimicrobial activities against fungal and bacterial pathogens.3.2. Antibacterial activityThe diameter of inhibition zones of the tested essential oils fromboth cultivated and wild O. syriacum measured by disk agar dif-fusion method is presented in Table 3. The results revealed thatboth essential oils showed significance antibacterial activity withvarying magnitudes, depending on the essential oil concentra-tion. The inhibition zone of EO from cultivated plant was moresignificant than EO from wild plants. Inhibition zone of EO fromcultivated plant ranged from 9 ± _0.01 to 32 ± _4.0 mm. The largestinhibition zone was obtained against S. aureus (32 ± _4.0 mm) with4 _l/disc (100%) and the lowest against E. faecalis (9 ± _0.01 mm)with 0.5 _l/disc (12.5%) concentration. Similarly, the antibacterialactivity of EO from wild plant varied from 8 ± _0.01 to 24 ± _4.0 mm,where the largest zone of inhibition was obtained against S. aureus(24.5 ± _2.5 mm) with 4 _l/disc (100%) concentration, while the low-est against E. faecalis and E. coli (8 ± _0.01 mm) with 2 and 0.5 _l/disc(50 and 12.5%) concentration, respectively.Either cultivated or wild plant essential oils had activity againstP. aeruginosa in all tested concentrations. Furthermore wild planthad no activity against E. faecalis with lower concentrations (25 and12.5%).Cultivated O. syriacum EO showed a good activity against thebacterial strains tested, in particular against S. aureus, where theinhibition zone was ranged between 32 ± _4.0 and 22 ± _4.0 mm with4 and 0.5 _l/disc, respectively. A good inhibition was also obtainedagainst E. coli with a concentration of 4 _l/disc, while wild O. syri-acum EO resulted less effective than cultivated EO.These results are very similar to those reported by otherresearchers on the antimicrobial activity of O. syriacum EOs as wellas for other origanum species. Viuda-Martos et al. (2010), Lakis et al.(2012), and Ibrahim et al. (2012) reported that the essential oils of O.syriacum can be considered as effective antistaphylococcal na Some authors reported that Gram-negative micro-organismsare slightly more sensitive to essential oils when compared toGram-positive (Ravikumar et al., 2012; Bachir and Benali, 2012).The difference in sensitivity of the bacteria to EOs is thought toarise as a result of the differences in their cell wall structure.The cell envelopes of Gram-negative bacteria are more complexthan the cell wall of Gram-positive bacteria. Gram-negative bac-teria are composed of two layers that protect the cell and providerigidity: thin peptidoglycan layer and outer membrane containinglipopolysaccharide (LPS) in its outer leaflet and phospholipids inthe inner leaflet. Gram-positive bacteria have a thicker peptidogly-can layer but lack the outer membrane. That could therefore be thereason why they would be more susceptible to the action of phe-nolic components of EOs (Angienda et al., 2010). Indeed, the LPSof Gram-negative bacteria makes their surfaces highly hydropho-bic, whereas the lipophilic ends of the lipoteichoic acids of the cellmembrane of Gram-positive bacteria may facilitate penetration byhydrophobic compounds (Cox et al., 2000). Bachir and Benali (2012)reported that there is a relationship between the chemical struc-tures of the most abundant compounds in the tested EOs and theirantimicrobial activity. Strong antibacterial action of this oil couldbe attributed to the activities of carvacrol by interacting with thebacterial wall (Ibrahim et al., 2012).Due to the large number of different classes of chemical com-pounds present in EOs, their antibacterial activity is not attributableto one specific mechanism, but there are several targets in the cell(Burt, 2004). Different modes of action have been suggested, andit can act over membrane/wall or cytoplasm. EOs pass throughthe cell wall and cytoplasm membrane, disrupt the structure oftheir different layers of polysaccharides, fatty acids and phospho-lipids, and permeabilize them. Membrane dysfunction depends oninterference with the energy (ATP) generation system and enzymeinhibition preventing substrate utilization for energy production(Speranza and Corbo, 2010). Prindle and Wright (1997) and Viuda-Martos et al. (2011). They reported that the antimicrobial activity ofphenolic compounds was concentration dependent, affecting enzy-matic activity related to energy production at low concentrationsand causing protein precipitation at high concentrations. Thymol has been shown to cause disruption of the cellular membrane, inhi-bition of ATPase activity, and release of intracellular ATP and otherconstituents (Viuda-Martos et al., 2010, 2011). Both EOs showeda good activity against the bacterial strains tested, compared tostandard antibiotics as shown in Table 4

4.3.3. Antifungal activity

The minimum inhibitory concentration MIC values of both cul-tivated plants and wild plants EOs against several fungal strainsare shown in Table 5. The MICs of both EOs were corresponding forA. fumigatus clinical strain (isolated from lung of Psittacus eritha-cus) and A. flavus (isolated from P. pinea). The MICs of cultivated O.syriacum EOs ranged from 0.25 to 2.5 mg/L and those of wild O. syr-iacum ranged from 0.25 to 5 mg/L. All tested isolates were sensitiveto itraconazole.Our results are similar to those reported by different authors onthe antifungal activity of EO of O. syriacum as well as those of simi-lar species (Ibrahim et al., 2012; Turkolmez and Soylu 2014). Someresearchers reported antifungal activity of the plant oil against dif-ferent phytopathogenic and medically important fungi (Tolooeeet al., 2010). The antifungal activity of essential oils is mainlyattributable to their major components although the possibilityof other phenomena, such as synergy or antagonism with minorcomponents, must also consider (Daferera et al., 2003).In general, the antifungal activity of oregano EOs is mostly due tothe presence of phenols such as thymol and carvacrol (Portillo-Ruizet al., 2012). Possible action mechanisms by which mycelia growthmay be reduced or totally inhibited have been proposed. The effectof phenolic antioxidants on microbial growth and toxin productioncould be the result of the ability of phenolic compounds to altermicrobial cell permeability, permitting the loss of macromoleculesfrom the interior. They could also interact with membrane proteins,causing a deformation in their structure and functionality (Funget al., 1977).Conner and Beuchat (1984) suggested that the antimicrobialactivity of the essential oils of herbs and spices or their constituentssuch as thymol, carvacrol, and eugenol, etc., could be the resultof damage to

enzymatic cell systems, including those associatedwith energy production and synthesis of structural compounds.Davidson (2001) reported that the exact cause effect relation forthe mode of action of phenolic compounds, such as thymol, eugenoland carvacrol, has not been determined, although it seems that theymay inactivate essential enzymes, react with the cell membrane ordisturb genetic material functionality.

4Conclusions

The main constituents of Origanum essential oils were deter-mined as carvacrol in cultivated plant and thymol in wild plant.Both EOs showed antibacterial activity with varying magnitudes, The tested EOs were active against all tested fungi, in particularcultivated plants appeared to show lower MIC values, probablydue to its high carvacrol content. These products could thereforebe considered as promising candidates to control the overgrowthof Aspergillus spp. in food stuffs. These EOs could be used as naturalfood ingredients and could represent a useful alternative for thefood industry to reduce the quantity of synthetic additives. Theseanalyses need to be extended to other Egyptian populations of O.syriacum, in order to obtain more possibility for clinical, nutritionalor pharmacological applications.

References

Afifi, A.A., Azen, S.P., 1979. Statistical analysis. In: A Computer Oriented Approach.Academic, London, pp. 18. Alma, M.H., Mavi, A., Yildirim, A., Digrak, M., Hirata, T., 2003. Screening chemicalcomposition and in vitro antioxidant and antimicrobial activities of theessential oils from Origanum syriacum L. growing in Turkey. Biol. Pharm. Bull.26, 1725–1729.Angienda, P.O., Onyango, D.M., Hill, D.J., 2010. Potential application of plantessential oils at sub-lethal concentrations under extrinsic conditions thatenhance their antimicrobial effectiveness against pathogenic bacteria. Afr. J.Microbiol. Res. 4 (16), 1678–1684.Arnold, N., Bellomaria, B., Valentini, G., 2000. Composition of the essential oil ofthree different species of Origanum in the eastern Mediterranean. J. Essent. OilRes. 12 (2), 192–196.Avci, A.B., 2011. Chemical variation on the essential oil of Thymus praecox ssp.Scorpilii var. laniger. Int. J. Agric. Biol. 13, 607–610.Azizi, A., Yan, F., Honermeier, B., 2009. Herbage yield, essential oil content andcomposition of three oregano (Origanum vulgare L.) populations as affected bysoil moisture regimes and nitrogen supply. Ind. Crops Prod. 29, 554–561.Bachir, R.G., Benali, M., 2012. Antibacterial activity of the essential oils from theleaves of Eucalyptus globulus against Escherichia coli and Staphylococcus aureus.Asian Pac. J. Trop. Biomed. 2, 739–742.Baser, K.H.C., Kurcuolu, M., Demirci, B., Ozek, T., 2003. The essential oil of Origanumsyriacum L. van sinaicum (Boiss.) letswaart. Flavour Fragance J. 18, 98–99.Baydar, H., Sagdis, O., Ozkan, G., Karadogan, T., 2004. Antibacterial activity andcomposition of essential oils from Origanum, Thymbra and Satureja specieswith commercial importance in Turkey. Food Control 15, 169–172.Burt, S., 2004. Essential oils: their antibacterial properties and potentialapplications in food – a review. Int. J. Food Microbiol. 94, 223–253.Conner, D.E., Beuchat, L.R., 1984. Effects of essential oils from plants on growth offood spoilage yeasts. J. Food Sci. 49, 429–434.Cox, S.D., Mann, C.M., Markham, J.L., Bell, H.C., Gustafson, J.E., Warmington, J.R.,2000. The mode of antimicrobial action of the essential oil from Malaleucaalternifolia (tea tree oil). J. Appl. Bacteriol. 88, 170–175.Daferera, D.J., Ziogas, B.N., Polissiou, M.G., 2003. The effectiveness of plant essentialoils on the growth of Botrytis cinerea, Fusarium sp. and Clavibactermichiganensis subsp. Michiganensis. Crop Prot. 22, 39–44.Davidson, P.M., 2001. Chemical preservatives and naturally antimicrobialcompounds. In: Beuchat, M.P., Montville, L.R. (Eds.), Food Microbiology.Fundamentals and Frontiers. , 2nd ed. ASM Press, Washington, DC, pp.593–628.De Martino, L., De Feo, V., Formisano, C., Mignola, E., Senatore, F., 2009. Chemicalcomposition and antimicrobial activity of the essential oils from threechemotypes of Origanum vulgare L. ssp. hirtum (Link) Ietswaart growing wild inCampania (Southern Italy). Molecules 14, 2735–2746.EUCAST (2008): technical note on the method for the determination of brothdilution minimum inhibitory concentrations of antifungal agents forconidia-forming moulds http://www.eucast.org/documents/technical notesFarhat, M., Toth, J., Hethelyi, B.E., Szarka, Sz., Czigle, Sz., 2012. Analysis of theessential oil compounds of Origanum syriacum L. Acta facultatispharmaceuticae universitatis. Comenianae 59 (2), 6–14.Fatima, A., Ahmad, K., Ali Chokr, H., 2012. Factors affecting quantitative andqualitative variation of thyme (Origanum syriacum L.) essential oil in Lebanon.Adv. Environ. Biol. 6 (4), 1509–1514.Fung, D.Y.C., Taylor, S., Kahan, J., 1977. Effects of butylated hydroxyanisole (BHA)and butylated hydroxytoluene (BHT) on growth and aflatoxin production ofAspergillus flavus. J. Food Saf. 1, 39–51.Ibrahim, L., Karaky, M., Ayoub, P., El Ajouz, N., Ibrahim, S., 2012. Chemicalcomposition and antimicrobial activities of essential oil and its componentsfrom Lebanese Origanum syriacum L. J. Essent. Oil Res. vol. 24 (4), 339–345.Kalemba, D., Kunicka, A., 2003. Antibacterial and antifungal properties of essentialoils. Current Med. Chem. 10, 813–829.Kamel, M.S., Assaf, M.H., Hasanean, H.A., Ohtani, K., Kasai, R., Yamasaki, K., 2001.Monoterpene glucosides from Origanum syriacum. Phytochemistry 58,1149–1152.Kokkini, S., Vokou, D., 1989. Carvacrol-rich plants in Greece. Flavour Fragrance J. 4,1–7.Lakis, Z., Mihele, D., Nicorescu, I., Vulturescu, V., Udeanu, D.I., 2012. TheAntimicrobial Activity of Thymus Vulgaris and Origanum syriacum EssOils on Staphylococcus aureus, Streptococcus pneumoniae and Candida albicans.Farmacia (Bucharest Romania) 60 (6), 857–865.Loizzo, M.R., Menichini, F., Conforti, F., Tundis, R., Bonesi, M., Saab, A.M., Statti, G.A.,Cindio, B., Houghton, P.J., Menichini, F.R., Frega, N.G., 2009. Chemical analysis,antioxidant, anti-inflammatory and anticholinesterase activities of Origanumehrenbergii Boiss and Origanum syriacum L. essential oils. Food Chem. 117,174–180.National Committee for Clinical Laboratory Standards, 2002. PerformanceStandards for Antimicrobial Susceptibility Testing. Twelfth InternationalSupplement. NCCS, Wayne, PA, pp. M100–M112.Omer, E.A., 1999. Response of wild Egyptian oregano to nitrogen fertilization in asandy soil. J. Plant Nutr. 22,103–114.Owlia, P., Saderi, H., Rasooli, I., Sefidkon, F., 2009. Antimicrobial characteristics ofsome herbal oils on Pseudomonas aeruginosa with special reference to theirchemical compositions. Indian J. Pharm. Res. 8 (2), 107–114.Portillo-Ruiz, M.C., Sánchez, R.A., Ramos, S.V., Mu˜noz, J.V., Nevárez-Moorillón, G.V.,2012. Antifungal effect of Mexican oregano (Lippia berlandieri Schauer)essential oil on a wheat flour-based medium. J. Food Sci. 77 (August (8)),M441–M445.Prindle, R.F., Wright, E.S., 1997. Phenolic compounds. In: Block, S.S. (Ed.),Disinfection, Sterilization and Preservation. Lea & Febiger, Philadelfia.Queiroz, B., Pereyra, C.M., Keller, K.M., Almeida, T., Cavaglieri, L.R., Magnoli, C.E.,Rocha Rosa, C.A., 2013. Fungal contamination and determination of fumonisinsand aflatoxins in commercial feeds intended for ornamental birds in Rio deJaneiro, Brazil. Lett. Appl. Microbiol. 57 (5), 405–411.Ravikumar, S., Gokulakrishnan, R., Boomi, P., 2012. In vitro antibacterial activity ofthe metal oxide nanoparticles against urinary tract infections bacterialpathogens. Asian Pac. J. Dis. 2 (2),85–89.Russo, M., Galletti, G.C., Bocchini, P., Carnacini, A., 1998. Essential oil chemicalcomposition of wild populations of Italian Oregano spice (Origanum vulgaressp. hirtum (Link) Ietswaart): a preliminary evaluation of their useinchemotaxonomy by cluster analysis 1. Inflorescences. J. Agric. Food Chem.46, 3741–3746.Sarer, E., Schefler, J., Baerheim, S., 1982. Monoterpenes of the essential oil ofOriganum majorana. Planta Med. 46, 236–239.Skoula, M., Harborne, J.B., 2002. Taxonomy and chemistry. In: Kintzios, S.E. (Ed.),Oregano: The Genera Origanum and Lippia. Medicinal and AromaticPlants-Industrial Profiles, 25. Taylor & Francis/CRC Press, USA, pp. 67–108.Sokovic, M., Marin, P.D., Brkic, D., van Griensven, L.J.L.D., 2007. Chemicalcomposition and antibacterial activity of essential oils often aromatic plantsagainst human pathogenic bacteria. Food 1, 220–226.Speranza, B., Corbo, M.R., 2010. Essential oils for preserving perishable foods:possibilities and limitations. In: Bevilacqua, A., Corbo, M.R., Sinigaglia, M.(Eds.), Application of Alternative Food-Preservation Technologies to EnhanceFood Safety and Stability, 23. Bentham, Oak Park, IL, pp. 35–37.Tackholm, V., 1974. Students Flora of Egypt, 2nd ed. Cooperative Printing Co.,Beirut, pp. 562.Tolouee, M., Alinezhad, S., Saberi, R., Eslamifar, A., Zad, S.J., Jaimand, K., 2010. Effectof Matricaria chamomilla L. flower essential oil on the growth andultrastructure of Aspergillus niger van Tieghem. Int. J. Food Microbiol. 139,127–133.Toncer, O., Karaman, S., Diraz, E., 2010. An annual variation in essential oilcomposition

of Origanum syriacum from southeast Anatolia of Turkey. J. Med.Plant Res. 4 (11), 1059–1064.Turkolmez, S., Soylu, E.M., 2014. Antifungal efficacies of plant essential oils andmain constituents against soil-borne fungal disease agents of bean. J. Essent.Oil Bear. Plants 17, 1–9.Viuda-Martos, M., Abd El Nasser, G., El, G., Sendra, E., Fernandez-Lopez, J., El Razik,K.A.A., Omer, E.A., 2010. Chemical composition, antioxidant and anti-Listeriaactivity of essential oils obtained from some Egyptian plants. J. Agric. FoodChem. 58, 9063–9070.Viuda-Martos, M., Mohamady, M.A., Fernandez-Lopez, J., Abd El Razik, K.A., Omer,E.A., Perez-Alvarez, J.A., 2011. In vitro antioxidant and antibacterial activities ofessential oils obtained from Egyptian aromatic plants. Food Control 22,1715–1722.Zein, S., Awada, S., Rachidi, S., Hajj, A., Krivoruschko, E., Kanaan, H., 2011. Chemicalanalysis of essential oil from Lebanese wild and cultivated Origanum syriacum(Lamiaceae) before and after flowering. J. Med. Plants Res. 5,379–387.Zein, S., Awada, S., Al-Hajje, A., Rachidi, S., Salameh, P., Kanaan, H., 2012. Variationof thymol, carvacrol and thymoquinone production from wild and cultivatedOriganum syriacum of South Lebanon. J. M. Plants Res. 6, 1692–1696.

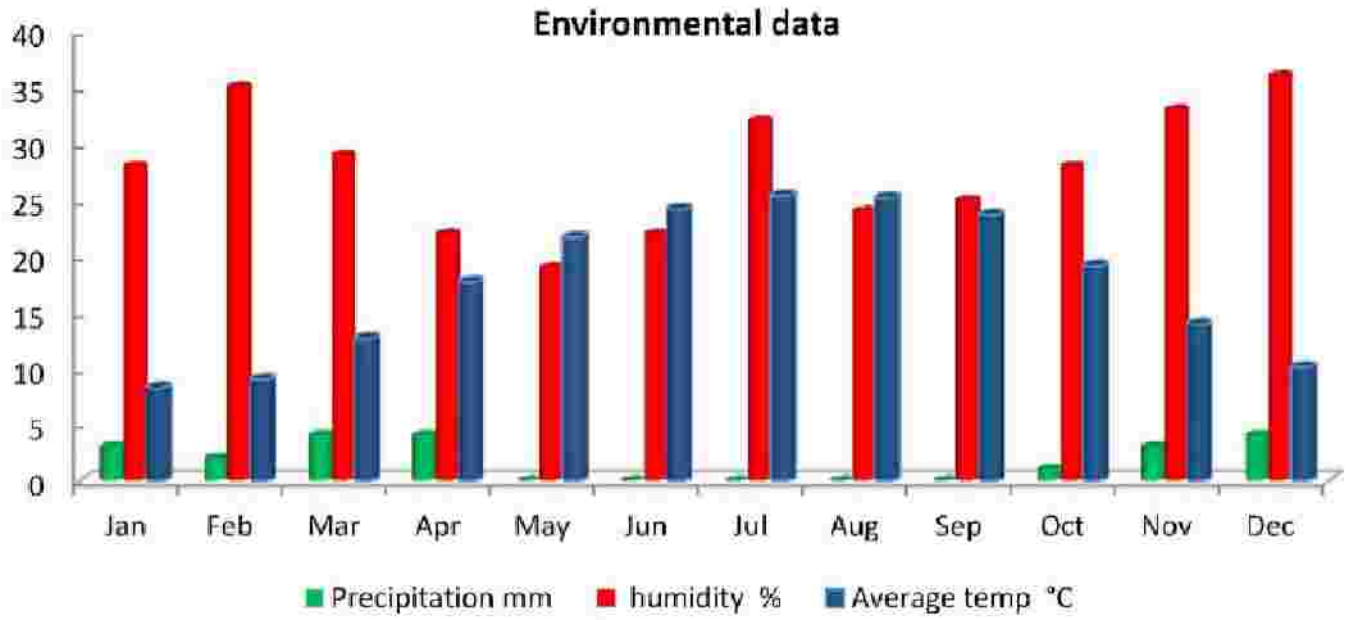

Fig 1. Environmental data during the investigation period 2012–2013.

Table 1
Geographical and environmental conditions and some soil composition from cultivated and wild *O. syriacum*.

	Saint Katherine cultivated *O. syriacum*	Wadi-Firan wild *O. syriacum*
Latitude (N)	38°33'19.2	28°42'42.5
Longtude (E)	33°56'52.9	33°39'46.9
Altitude (*m*)	1574	1362
Distance (km)[a]	450	400
Direction[a]	North–East	North–East
Max. temperature[b]	23.8	23.8
Min. temperature[b]	10.38	10.38
Max.–Min. temp.[b]	13.42	13.42
Average temperature[b]	18.68	18.68
Relative humidity (%)[b]	27.67	27.67
Average precipitation mm	21	21
Soil type	Sandy	Sandy
EC (1:5)	7.6	8.3
pH (1:2.5)	7.2	7.7
TSS %	2.12	2.66
O.M %	0.50	0.15
$CaCO_{3\%}$	2.20	2.34

N direction from north, E direction from east.

[a] From Cairo.

[b] Year average.

Table 2
The main constituents of the essential oil of cultivated and wild *O. syriacum* plants grown in Sinai Peninsula, Egypt.

Compound	RT	KI	Cultivated	Wild
α-Thujene	4.625	930	0.06 ± 0.01	2.1 ± 0.15
α-Pinene	4.792	939	0.72 ± 0.01	0.65 ± 0.03
Camphene	5.153	953	0.12 ± 0.02	0.05 ± 0.01
Sabinene	5.772	978	tr	1.24 ± 0.06
1-Octen-3-ol	5.877	979	0.22 ± 0.01	0.31 ± 0.02
β-Myrcene	6.226	991	1.32 ± 0.01	1.76 ± 0.06
α-Phellandrene	6.63	1003	0.17 ± 0.02	0.3 ± 0.01
δ-3-Carene	6.816	1011	0.09 ± 0.01	0.04 ± 0.01
α-Terpinene	7.012	1017	1.24 ± 0.01	5.5 ± 0.19
ρ-Cymene	7.269	1025	8.48 ± 0.05	3.88 ± 0.16
Limonene	7.402	1031	0.37 ± 0.02	1.77 ± 0.03
β-(*Z*)-ocimene	7.687	1041	tr	0.03 ± 0.02
β-(*E*)-ocimene	8.04	1050	tr	0.06 ± 0.01
γ-Terpinene	8.41	1064	1.98 ± 0.02	14.32 ± 0.75
cis sabinene hydrate	8.76	1072	0.11 ± 0.02	2.34 ± 0.05
Terpinolene	9.475	1088	0.21 ± 0.01	1.37 ± 0.03
Linalool	9.905	1101	0.22 ± 0.01	9.44 ± 0.33
β-Thujone	10.463	1114	tr	0.20 ± 0.01
cis-*p*-Menth-2-en-1-ol	10.736	1122	tr	0.69 ± 0.03
trans-*p*-Menth-2-en-1-ol	11.456	1141	tr	0.37 ± 0.02
Menthone	12.044	1153	tr	0.05 ± 0.01
Borneol	12.511	1169	0.19 ± 0.01	0.09 ± 0.01
4-Terpineol	12.996	1177	0.89 ± 0.01	7.68 ± 0.11
p-Cymen-8-ol	13.344	1183	tr	tr
α-Terpineol	13.556	1189	0.15 ± 0.01	5.51 ± 0.11
cis-Dihydrocarvone	13.835	1193	0.18 ± 0.03	0.14 ± 0.01
trans-Dihydrocarvone	14.154	1201	0.06 ± 0.03	tr
iso-Dihydrocarveol	14.24	1215	tr	0.18 ± 0.01
cis-Carveol	15.155	1229	tr	tr
Thymol methyl ether	15.853	1235	tr	tr
Carvone	16.031	1243	tr	tr
Carvacrol methyl ether	16.19	1245	0.11 ± 0.01	tr
Carvenone	16.524	1258	tr	0.19 ± 0.01
Bornyl acetate	16.871	1263	tr	0.1 ± 0.02
Thymol	18.001	1290	0.35 ± 0.02	31.73 ± 0.93
Carvacrol	18.672	1299	81.38 ± 0.08	3.9 ± 0.18
Eugenol	20.777	1359	tr	0.04 ± 0
β-Caryophyllene	23.355	1419	0.11 ± 0.01	1.92 ± 0.11
Aromadendrene	24.169	1441	tr	0.22 ± 0.01
α-Humulene	24.78	1456	tr	0.09 ± 0
Bicyclogermacrene	26.608	1495	tr	0.52 ± 0.03
γ-Cadinene	27.332	1513	tr	0.06 ± 0
Spathulenol	29.87	1578	tr	0.17 ± 0.02
Caryophyllene oxide	30.071	1581	0.09 ± 0.01	0.14 ± 0.02
1,10-di-epi-Cubenol	31.365	1614	tr	tr
τ-Cadinol	32.372	1640	0.12 ± 0.03	0.28 ± 0.03
Monoterpene hydrocarbons			14.68 ± 0.08	33.07 ± 1.51
Oxyg. Monoterpenes			83.83 ± 0.14	62.61 ± 1.83
Sesquiterpene Hydroc.			0.11 ± 0.01	2.81 ± 0.16
Oxig. Sesquiterpenes			0.09 ± 0.04	0.58 ± 0.06
Others			0.22 ± 0	0.35 ± 0.02
Total			98.93 ± 0.26	99.42 ± 3.59

KI[b] = Kovats indices in reference to *n*-alkanes (C_8–C_{23}) confirmed by comparison on DB-5MS.
Tr = relative percentage less than 0.025%.

Table 3
Antibacterial activity (inhibition zone, mm) of cultivated and wild *O. syriacum* EOs against the selected bacterial strains tested by disc diffusion method.

Strains	Cultivated							
	4 µl/disc 100%		2 µl/disc 50%		1 µl/disc 25%		0.5 µl/disc 12.5%	
	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
S. aureus	32 a	±4.0	27.5 ab	±0.5	23 bc	±5.0	22 bc	4.0
E. faecalis	19 a	±1.0	13.5 b	±1.5	10.5 c	±0.5	9 cd	0.0
E. coli	25 a	±1.0	18.5 b	±0.5	18 b	±1.0	12 c	0.0
P. aeruginosa	NA		NA		NA		NA	
	Wild							
S. aureus	24.5 abc	2.5	18 cd	1.0	13.0 de	±2.0	9.0 e	±0.0
E. faecalis	11.0 c	1.0	8 d	1.0	NA		NA	
E. coli	17.0 b	3.0	11 cd	1.0	8.5 cd	±0.5	8.0 d	±0.0
P. aeruginosa	NA		NA		NA		NA	

Values followed by the same small letter within the same line have not significant differences ($P > 0.05$) according to Tukey's multiple range test. N.A. – non-active.

Table 4
The inhibition zones resulted from the application of different antibiotics against bacteria strains.

	Tetracycline (30 µg/disc)	Ceftazidime (30 µg/disc)	Rifampicin (30 µg/disc)	Cephalexin (30 µg/disc)	Cefotaxime (30 µg/disc)
S. aureus	21 (S)	16 (S)	32 (S)	32 (S)	24 (S)
E. faecalis	22 (S)	NA	21 (S)	NA	18 (I)
E. coli	19 (S)	23 (S)	15 (R)	18 (S)	30 (S)
P. aeruginosa	11 (R)	7 (R)	11 (R)	NA	NA

Legend: S – susceptible; R – resistant; I – intermediate; NA – no activity.

Table 5
Minimum inhibitory concentration (MIC) values of the essential oil of cultivated and wild *Origanum syriacum* against some fungal strains.

Strain	Source	Cultivated (%)	Wild (%)	Itraconazole (mg/L)
A. fumigatus	Clinical isolate[a]	0.25	0.25	1.0
A. fumigates	Clinical isolate[b]	2.5	5.0	1.0
A. fumigatus	Clinical isolate[c]	1.25	5.0	1.0
Aspergillus	Soil	1.25	2.5	1.0
A. flavus	*P. pinea*	0.25	0.25	0.25
A. flavus	Bovine litter	2.5	5.0	0.5
A. niger	Food	1.25	5.0	1.0
A. niger	Soil	1.25	5.0	0.5

[a] Clinical isolated from *Pneumonid Psittaeus* Erithaeus.
[b] Clinical isolated from *Larus ridibundus.*
[c] Clinical isolated from *Trachea Psittaeus* Erithaeus.