Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy

Composition, antioxidant and chemotherapeutic properties of the essential oils from two *Origanum* species growing in Pakistan

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Aop14911

Received 18 Oct 2010
Accepted 27 Dec 2010

Keywords:
antibacterial
antimalarial
antioxidant
cytotoxicity
Lamiaceae
Origanum

ISSN 0102-695X

Abstract: The GC-MS analyses of *Origanum majorana* L. (OME) and *Origanum vulgare* L. (OVE), Lamiaceae, essential oils helped identification of 39 (96.4% of the total oils) and 43 (92.9% of the total oils) components, respectively. The major constituents of OME were terpinene-4-ol (20.9%), linalool (15.7%), linalyl-acetate (13.9%), limonene (13.4%) and α-terpineol (8.57%), whereas, thymol (21.6%), carvacrol (18.8%), *o*-cymene (13.5%) and α-terpineol (8.57%) were the main components of OVE. In the disc diffusion and the resazurin microtitre assays, OME showed better antibacterial activity than OVE with larger zones of inhibition (16.5-27.0 mm) and smaller MIC (40.9-1250.3 µg/mL) against the tested bacterial strains. Only OVE displayed anti-heme biocrystallization activity with an IC50 at 0.04 mg/mL. In the DPPH assay, OVE showed better radical-scavenging activity than OME (IC50=65.5 versus 89.2 µg/mL) and both OME and OVE inhibited lionleic acid oxidation. However, in the bleaching β-carotene assay, OVE exhibited better antioxidant activity than OME. In the MTT assay, OME was more cytotoxic than OVE against different cancer cell types, such as MCF-7, LNCaP and NIH-3T3, with IC50s of 70.0, 85.3 and 300.5 µg/mL, respectively. Overall, some components of OME and OVE may have antiparasitic and chemotherapeutic activity.

Introduction

The genus *Origanum* L., Lamiaceae, comprises *ca*. 38 species of annual, perennial and shrubby herbs, most of which are native to or restricted to the eastern part of the Mediterranean area, Europe, Asia and North Africa (GRIN Taxonomy Database, 2010). This genus includes some important culinary herbs and medicinal plants, including *Origanum vulgare* L. (common name 'oregano') and *Origanum majorana* L. (common name 'sweet marjoram'). The antioxidant and other biological properties of the *Origanum* essential oils and extracts have recently been of great interest in both academia and food industries because of their antioxidant and antimicrobial potentials. Even though a few reports on the antimicrobial and antioxidant activities of the *Origanum* essential oils are available to date (Daferera et al., 2000; Esen et al., 2007; Busatta et al., 2008), the potential antimalarial activity of *O. vulgare* and *O. majorana* essential oils have never been investigated before. In continuation of our phytochemical and bioactivity studies on plants of Pakistani flora (Anwar et al., 2009), we now report on the potential antibacterial, antimalarial, antioxidant activities and cytotoxicities of the essential oils of *O. vulgare* and *O. majorana* using various *in vitro* assays. Composition of these essential oils determined by GC and GC-MS has also been reported here.

Material and Methods

Plant material

The aerial parts of cultivated *Origanum majorana* L. and *Origanum vulgare* L. (Lamiaceae) at the flowering stage were harvested in July-August 2008 from the Botanical Garden, University of Agriculture, Faisalabad, Pakistan. The plant specimens were further

identified and authenticated by Dr Mansoor Hameed (Taxonomist), Department of Botany, University of Agriculture, Faisalabad, Pakistan, where the voucher specimens (*O. majorana* voucher no. 10473 and *O. vulgare* voucher no. 10476) were deposited. The plant materials were dried at the temperature not exceeding 35 °C for essential oil isolation.

Extraction of essential oils

The dried and finely ground (80 mesh) plant materials were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus (Hussain et al., 2008). Distillates of essential oils were dried over anhydrous sodium sulfate (Merck, Darmstadt, Germany), filtered and stored at -4 °C until analyzed.

Gas chromatography (GC) analysis

The essential oils were analyzed using a Perkin-Elmer gas chromatograph model 8700, comprising flame ionization detector (FID) and HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm). The injector and detector temperatures were set at 220 and 290 °C, respectively. The column oven temperature was programmed from 80 °C to 220 °C at the rate of 4 °C/min; initial and final temperatures were held for 3 and 10 min, respectively. Helium was used as carrier gas with a flow of 1.5 mL/min. A sample of 1.0 µL was injected, using the slit mode (split ratio, 1:100). All quantifications were done by a built-in data-handling program of the equipment used (Perkin-Elmer, Norwalk, CT, USA). The composition was reported as a relative percentage of the total peak area.

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis of the essential oils was performed on an Agilent-Technologies (Little Falls, California, USA) 6890N Network gas chromatographic (GC) system, equipped with an Agilent-Technologies 5975 inert XL Mass selective detector and Agilent-Technologies 7683B series auto-injector. Compounds were separated on HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm; Little Falls, CA, USA). A sample of 1.0 µL was injected in the split mode with split ratio 1:100. For GC/MS detection, an electron ionization system, with ionization energy of 70 eV, was used. The column oven temperature program was the same as in the GC analysis. Helium was used as a carrier gas at a flow rate of 1.5 mL/min. Mass scanning range was 50-550 *m/z* while the injector and MS transfer line temperatures were set at 220 and 290 °C, respectively.

Identification of compounds

The components of the essential oils were identified by comparison of their retention indices relative to (C_9-C_{24}) *n*-alkanes either with those of published data or with authentic compounds (Massada, 1976; Adams, 2004). Compounds were further identified using their MS data compared with those from the NIST02.L and WILEY7n.L mass spectral libraries and published mass spectra and, wherever possible, by co-injection with authentic standards (Mimica-Dukic et al., 2003; Vagionas et al., 2007; Anwar et al., 2009).

Antibacterial assays

The essential oils of *O. majorana* and *O. vulgare* were individually tested against a panel of pathogenic and clinically isolated bacterial strains, including: *Staphylococcus aureus* NCTC 6571, *Bacillus cereus* NCTC 7464, *B. subtilis* NCTC 10400, *Pseudomonas aeruginosa* NCTC 1662, *Salmonella poona* NCTC 4840, *Escherichia coli* ATCC 8739 and ampicillin-resistant *E. coli* NCTC 10418. The bacterial strains were obtained from Northern Ireland Public Health Laboratory, Belfast City Hospital, Belfast, and Microbiology Laboratory, School of Biomedical Sciences, University of Ulster, Coleraine, Northern Ireland, UK.

Disc diffusion assay

The antibacterial activity of the essential oils was assessed by the disc diffusion method (Kelen & Tepe, 2008). Briefly, 100 µL of suspension containing approx 5×10^5 colony-forming units (CFU)/mL of bacteria cells on nutrient agar was used. The sterile filter discs (6 mm in diameter) were separately impregnated with 10 µL of essential oils and placed on the agar which had previously been inoculated with the test microorganism. Ciprofloxacin (25 µg/disc) was used as positive control, while discs without oil were used as a negative control. The plates were incubated at 37 °C for 24 h. Antibacterial activity was assessed by measuring the diameter of the zone of inhibition in millimeters (including disc diameter of 6 mm) for the test organisms, compared to the controls.

Resazurin microtitre-plate assay

For the measurement of minimum inhibitory concentration (MIC) of essential oils of *O. majorana* and *O. vulgare*, a modified resazurin microtitre-plate assay, as reported by Sarker et al. (2007), was used. Briefly, a volume of 100 µL essential oils solutions (2.5 mg/mL, w/v in 10% DMSO), pure components (2.0 mg/

mL in 10% DMSO) and standard antibiotic (1.0 mg/mL in 10% DMSO) was pipetted into the first row of the 96 well plates. To all other wells 50 µL of nutrient broth was added. Two fold serial dilutions were performed using a multichannel pipette such that each well had 50 µL of the test material in serially descending concentrations. A volume of 30 µL of 3.3x strength isosensitized broth and 10 µL of resazurin indicator solution (prepared by dissolving 270 mg tablet in 40 mL of sterile distilled water) were added in each well. Finally, 10 µL of bacterial suspension was added to each well to achieve a concentration of approx 5×105 CFU/mL. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a set of controls: a column with a ciprofloxacin as positive control, a column with all solutions with the exception of the test compound, a column with all solutions with the exception of the bacterial solution adding 10 µL of nutrient broth instead and a column with 10% DMSO (v/v) solution as a negative control. The plates were prepared in triplicate, and incubated at 37 °C for 24 h. The color change was then assessed visually. The growth was indicated by color changes from purple to pink or colorless. The lowest concentration at which color change occurred was taken as the MIC value.

Heme biocrystallization and inhibition assay for potential antimalarial activity

The potential antimalarial activity of plant extracts was evaluated by the method described by Fitch et al. (1999) with some modifications (Tripathi et al., 2004). Briefly, 100 µL of essential oils at a concentration of 0.01-10 mg/mL in 10% DMSO were incubated with 100 µL of 3 mM hematin (freshly dissolved in 0.1 M NaOH), 10 mM oleic acid, 10 µL of 1M HCl. After adding the test samples at varying concentrations, the reaction volume was adjusted to 1000 µL using 500 mM sodium acetate buffer of pH 5. Chloroquine diphosphate was used as a positive control with the negative control containing buffer without test compounds. The samples were incubated for 4 h with gradual shaking/inverting of each tube. After incubation, samples were centrifuged (14,000 rpm, 10 min, at 21 °C) and the hemozoin pellets were repeatedly washed with 2% (w/v) sodium dodecyl sulfate (SDS) in 0.1 M sodium bicarbonate, pH 9.0, with sonication (30 min, at 21°C; FS100 bath sonicator; Decon Ultrasonics Ltd.) until the supernatant was clear (usually 3-5 times). After the final wash, the supernatant was removed and the pellets were re-suspended in 1 mL of 0.1 M NaOH and incubated for an additional hour at r.t. Thereafter, the samples were vortexed and the hemozoin content was determined by measuring the absorbance at 400 nm (Beckmann DU640 spectrophotometer) using a 1 cm quartz cuvette. The concentration of drug required to produce 50% inhibition of polymerization (IC50) was determined graphically (Baelmans et al., 2000).

Antioxidant activity

The following assays were employed to assess the antioxidant properties of the essential oils.

The DPPH radical scavenging assay

The free radical scavenging activity of the *Origanum* essential oils was assessed by measuring their ability to scavenge 2,2′-diphenyl-1-picrylhydrazyl stable radicals (DPPH). The DPPH assay was performed as described by Mimica-Dukic et al. (2003). The samples (from 0.5 to 500.0 µg/mL) were mixed with 1 mL of 90 µM DPPH solution and made up with 95% methanol, to a final volume of 4 mL. Synthetic antioxidant, butylated hydroxytoluene (BHT) was used as control. After 1 h incubation period at room temperature, the absorbance was recorded at 515 nm. Percent radical scavenging concentration was calculated using the following formula:

$$\text{Radical scavenging (\%)} = 100 \times (A_{blank} - A_{sample}/A_{blank})$$

where A_{blank} is the absorbance of the control (containing all reagents except the test essential oils), and A_{sample} is the absorbance of the test essential oils/compounds. IC50 values, which represented the concentration of essential oil that caused 50% scavenging, were calculated from the plot of percent scavenging against concentration.

Percent inhibition in linoleic acid system

The antioxidant activity was also determined in terms of measurement of percent inhibition of peroxidation in linoleic acid system following the method described by Iqbal et al. (2005) with slight modification. Essential oils (5 mg) were added to a solution mixture of linoleic acid (0.13 mL), 99.8% ethanol (10 mL) and 10 mL of 0.2 M sodium phosphate buffer (pH 7). Total mixture was diluted to 25 mL with distilled water. The solution was incubated at 40 °C for 175 h. The extent of oxidation was measured by peroxide value using the colorimetric method. To the 0.2 mL sample solution, 10 mL of ethanol (75%), 0.2 mL of an aqueous solution of ammonium thiocyanate (30%) and 0.2 mL of ferrous chloride solution (20 mM in 3.5% HCl) were added sequentially. After 3 min of stirring, the absorbance was read at 500 nm. A control was performed with linoleic acid but without essential oils. BHT was used as a positive control. Percent

inhibition of linoleic acid oxidation was calculated as follows:

Percent inhibition of linoleic acid oxidation = 100 – [(increase in absorbance of sample at 175 h/increase in absorbance of control at 175 h) x 100]

Bleachability of β-carotene in linoleic acid system

The antioxidant activity of the essential oils was further assessed by the bleaching of β-carotene/ linoleic acid emulsion system as reported by Hussain et al. (2008). A stock solution of β-carotene-linoleic acid mixture was prepared by dissolving 0.1 mg β-carotene, 20 mg linoleic acid and 100 mg Tween 40 in 1.0 mL of chloroform (HPLC grade). The chloroform was removed under vacuum in a rotary evaporator at 50 °C. Then, 50 mL of distilled water saturated with oxygen (30 min, 100 mL min^{-1}) were added with vigorous shaking. An aliquot (5.0 mL) of this reaction mixture was dispensed to test tubes with 200 µL of the essential oils or main components solution, prepared at 4.0 g/L concentrations and the absorbance immediately (t = 0) measured at 490 nm against a blank, consisting of an emulsion without β-carotene. Then emulsion was incubated for 50 h at room temperature and the absorbance recorded at different time intervals. The same procedure was repeated with BHT and blank.

Anticancer assay: cytotoxicity

The human breast cancer cell line MCF-7 was maintained in Dulbecco's Minimum Essential Medium (DMEM), while hormone dependent prostate carcinoma LNCaP was cultured in RPMI 1640 medium. Both media were supplemented with 10% heat-inactivated fetal calf serum, 1% L-glutamine, and 1% penicillin/ streptomycin. Cells of MCF-7 (10^4/well) and LNCaP (10^5/well) were cultivated in 96 well plates for 24 h before the *Origanum* essential oils were added. Essential oils were solubilized in DMSO then diluted in culture media for use. The essential oils dilutions (0 to 0.50 mg/mL) were added to triplicate wells and cells incubated for further 24 h. DMSO was tested as solvent control while doxorubicin was used as a reference standard. Cell viability was assessed by MTT assay and the percent inhibition of cell viability was calculated using cells treated with DMSO as control (Mosmann, 1983). The IC50 values (concentrations at which 50% of cells were killed) were calculated from % inhibition of cell viability versus drug concentration graph.

Statistical analysis

All the experiments were conducted in three replicate and the data are presented as mean values±standard deviation of triplicate determinations. Statistical analysis of the data was performed by Analysis of Variance (ANOVA) using Statistica 5.5 (Stat Soft Inc, Tulsa, Ok, USA) software and a probability value of $p \leq 0.05$ was considered to represent a statistical significance difference among mean values.

Results and Discussion

The yields of the essential oils of *O. majorana* and *O. vulgare* were 17.2 and 22.0 g/kg, respectively (Table 1). It can be noted that Busatta et al. (2008) reported 1.20 % essential oils yield from *O. majorana*. However, variations in the essential oils content and profiles within a single species growing in different geographical locations are not uncommon. In the *Origanum* oils, 43 compounds in all were identified (Table 1): a total of 39 individual compounds were identified from the essential oils of *O. majorana* (96.4 % of the total oil), and 43 were identified from the essential oils for *O. vulgare* (92.9 % of the total oil). The retention indices, percentage composition and identification methods are presented in Table 1, where the compositions are listed in order of elusion from HP-5MS column. The major components in *O. majorana* essential oils were identified as terpiene-4-ol (20.9%), linalool (15.7%), limonene (13.4%), linalyl acetate (13.9%) and α-terpineol (10.8%), and the most abundant constituents (>5%) in the essential oils of *O. vulgare* were found to be thymol (21.6%), carvacrol (18.8%), *o*-cymene (13.5%) and α-terpineol (8.57%). Essential oils of these two *Origanum* species mainly consisted of oxygenated monoterpenes and monoterpene hydrocarbons. Terpiene-4-ol and thymol were the main oxygenated monoterpenes identified from the essential oils of *O. majorana* and *O. vulgare*, respectively. These findings were in good agreement with reports published previously (Vagi et al., 2005; Busatta et al., 2008), where the reported main constituent of *O. majorana* essential oils was terpinen-4-ol. The oils from *O. vulgare*, collected in Turkey, showed varied composition (Esen et al., 2007). According to Vagi et al. (2005), terpinen-4-ol was the main component of *O. majorana* essential oil, collected from Hungary, with contribution of 30.3%.

The antibacterial activity of the essential oils of *O. majorana* and *O. vulgare* were assessed against a panel of human and plant pathogenic and food-borne bacterial strains. The essential oils exhibited antimicrobial activity against all bacterial strains tested (Table 2). In the disc diffusion assay as well as in the resazurin microtitre assay, the essential oils of *O. majorana* showed slightly better antimicrobial activity than that of *O. vulgare* with larger

Table 1. Yield and composition of the essential oils of *O. majorana* and *O. vulgare*.

Components[a]	RI[b]	Composition (%)[c]		Mode of Identification[d]
		O. vulgare	*O. majorana*	
Monoterpene hydrocarbons		33.0	22.4	
α-Pinene	939	3.72±0.13	0.96±0.08	RT, RI, MS
Camphene	954	0.74±0.06	0.12±0.05	RT, RI, MS
Sabinene	968	0.30±0.02	---	RI, MS
β-Pinene	979	0.53±0.02	0.32±0.09	RT, RI, MS
β-Myrecene	991	0.39±0.03	0.62±0.07	RT, RI, MS
α-Phellandrene	999	0.10±0.01	0.25±0.08	RT, RI, MS
2-Carene	1003	2.05±0.10	2.53 ±0.09	RI, MS
α-Terpinene	1018	0.08±0.01	---	RT, RI, MS
o-Cymene	1024	13.5±0.32	---	RT, RI, MS
p-Cymene	1025	0.10±0.02	---	RT, RI, MS
Limonene	1029	3.48±0.13	13.4±0.44	RT, RI, MS
β-Cymene	1031	2.82±0.19	---	RI, MS
β-Phellandrene	1032	---	1.86±0.12	RT, RI, MS
β-Ocimene	1037	0.04±0.01	0.03±0.01	RT, RI, MS
γ-Terpinene	1060	1.36±0.13	1.42±0.05	RT, RI, MS
α-Terpinolene	1089	1.76±0.13	0.94±0.09	RT, RI, MS
δ-Terpinene	1090	2.03±0.12	---	RT, RI, MS
Oxygenated monoterpenes		55.1	67.1	
1,8-Cineol	1031	---	0.86±0.10	RT, RI, MS
cis-Sabinene hydrate	1070	---	2.11±0.11	RI, MS
p-Mentha-3,8-diene	1072	0.29 ± 0.08	---	RI, MS
Linalool oxide	1088	0.02±0.01	---	RI, MS
Linalool	1097	3.02±0.08	15.7±0.45	RT, RI, MS
Fenchyl alcohol	1109	0.09±0.01	0.10±0.02	RI, MS
Isoborneol	1146	0.38±0.03	---	RI, MS
Borneol	1169	1.17±0.03	---	RT, RI, MS
Terpiene-4-ol	1177	0.45±0.02	20.9±0.40	RT, RI, MS
α-Terpineol	1189	8.57±0.24	10.8±0.50	RT, RI, MS
Nerol	1207	0.19±0.02	---	RI, MS
Piperitol	1208	---	0.48±0.02	RI, MS
trans-Carveol	1217	---	0.38±0.03	RI, MS
β-Citronellol	1228	0.12±0.01	---	RI, MS
cis-Carveol	1229	---	1.61±0.03	RT, RI, MS
Linalyl acetate	1257	---	13.9±0.30	RT, RI, MS
Anethole	1288	0.18±0.03	0.25±0.08	RI, MS
Thymol	1290	21.6±0.84	---	RT, RI, MS
Carvacrol	1328	18.8±0.42	---	RT, RI, MS
Geranyl acetate	1380	0.20±0.02	0.06±0.02	RI, MS
Sesquiterpene hydrocarbons		2.34	6.63	
α-Cubebene	1340	0.38±0.03	0.41±0.02	RI, MS
Longicyclene	1373	1.17±0.03	0.15±0.03	RI, MS
Copaene	1377	0.05±0.01	0.04±0.01	RI, MS
β-Longipinene	1398	0.04±0.01	3.46±0.08	RI, MS

Table 1. (cont.)

Longifolene	1409	0.07±0.02	---	RI, MS
β-Caryophyllene	1421	0.45±0.03	1.64±0.03	RT, RI, MS
Aromadendrene	1430	---	0.07±0.02	RI, MS
α-Humulene	1450	0.08±0.02	0.09±0.02	RI, MS
β-Farnesene	1458	0.02±0.01	0.05±0.01	RI, MS
Alloaromadendrene	1461	---	0.06±0.01	RI, MS
α-Selinene	1470	---	0.06±0.02	RI, MS
ar-Curcumene	1475	0.08±0.01	0.03±0.01	RI, MS
Germacrene D	1480	---	0.27±0.02	RI, MS
Valencene	1482	---	0.18±0.02	RI, MS
α-Muurolene	1499	---	0.06±0.02	RI, MS
α-Farnesene	1500	---	0.06±0.01	RI, MS
Oxygenated sesquiterpenes		2.50	0.19	
Caryophyllene oxide	1583	0.21±0.02	0.19±0.02	RT, RI, MS
Spathulenol	1586	0.14±0.02	---	RI, MS
Cadrol	1596	1.04±0.04	---	RI, MS
α-Cadinol	1654	1.11±0.02	---	RI, MS
Total		92.9	96.4	
Yield (g/kg)		22.0±1.5	17.2±0.90	

[a]Compounds are listed in order of elution from a HP-5MS column; [b]Retention indices relative to C9-C24 *n*-alkanes on the HP-5MS column; [c]Values are mean±standard deviation of three different samples of essential oils from each *Origanum* species, analyzed individually in triplicate. Mean followed by different superscript letters in the same row represent significant difference ($p<0.05$). Compounds present in trace amounts (<0.1%) were not registered; [d]RT, identification based on retention time; RI, Identification based on retention index; MS, identification based on comparison of mass spectra.

zones of inhibition (16.5-27.0 mm) and smaller MIC values (40.9-1250.3 µg/mL) against test bacterial strains. However, the antibacterial activity of the essential oils of *O. vulgare* was also good (>15 mm zones of inhibition) with the zones of inhibition within the range of 15.3-25.4 mm and the MIC values in the range of 70.0-2081.1 µg/mL. Both essential oils were active, although at relatively high concentrations, against ampicillin-resistant *E. coli.* The greater resistance of Gram-negative bacteria to essential oils, as observed in this study (Table 2) might be owing to the great complexity of the double membrane-containing cell envelope of these bacterial in contrast to the single membrane structures of Gram-positive bacteria (Kalemba & Kunicka, 2003; Bagamboula et al., 2004). Positive control, ciprofloxacin, showed much better activity than essential oils as expected. The findings of this study were in line with the antibacterial properties of several *Origanum* species reported previously (Bouchra et al., 2003; Bendahou et al., 2008; Ozcan & Chalchat, 2009; Al-Kalaldeh et al., 2010; Eng & Norman, 2010; Gonzalez & Marioili, 2010). Essential oils of *Origanum* species generally contain monoterpenes, carvacrol, thymol, terpinene-4-ol and linalool. The biological activity of these oils is often attributed to the occurrence of such bioactive compounds (Soylu et al., 2006; Esen et al., 2007).

There are many methods reported in literature to evaluate the antimalarial activity of crude plant extracts (Kurosawa et al., 2000; Ncokazi & Egan, 2005; Huy et al., 2006; Busatta et al., 2008). Almost all of them use live malaria parasites and or parasite extracts. Although the tests give an indication of the direct effects of the agents against the parasites, specialist care is needed to grow the parasites and the evaluation process may be costly and time consuming. On the other hand, the haem biocrystallisation assay is less costly and easy to perform. It is based on observation that during the intraerythrocytic development, malaria parasites degrade large amounts of hemoglobin within a specialized organelle, the digestive vacuole (Goldberg & Slater, 1992) to supply amino acids for growth and development. A toxic by-product, ferriprotoporphyrin IX (FPIX), is produced during the catabolism of hemoglobin. The parasite detoxifies this FPIX through the formation of insoluble hemozoin crystals. Thus, agents that inhibit the conversion of FPIX to hemozoin may cause death from the lytic effects of haem or the haem drug complex may mediate parasite death (Egan & Marques, 1999). Moreover, *in vitro*, hematin at acidic pH, leads to β-hematin, a compound presumed to be identical to hemozoin. Hence, bioactive compounds or plant extracts able to inhibit the biocrystallization of hematin at this pH, may possess antiplasmodial properties. This assay was employed to assess the potential

Table 2. Antibacterial activity of *O. majorana* and *O. vulgare* essential oils[a].

Bacterial strains	*O. majorana*		*O. vulgare*		Ciprofloxacin	
	Zone of inhibition in mm[b]	MIC (µg/mL)[c]	Zone of inhibition in mm[b]	MIC (µg/mL)[c]	Zone of inhibition in mm[b]	MIC (µg/mL)[c]
Staphylococcus aureus (NCTC 6571)	24.0±1.2	80.6±3.4	19.3±0.5	210.3±9.4	26.3±1.0	15.6±0.2
Bacillus cereus (NCTC 7464)	24.3±1.2	130.0±5.0	24.5±0.9	260.4±6.3	34.0±1.6	8.0±0.3
Bacillus subtilis (NCTC 10400)	27.0±1.3	40.9±1.4	25.4±1.1	70.0±2.1	32.1±1.0	4.5±0.1
Bacillus pumilis (wild type)	16.5±0.5	511.3±16.3	19.6±0.9	620.3±19.0	22.6±1.0	62.2±1.1
Pseudomonas aeruginosa (NCTC 1662)	19.5±0.6	1250.3±36.0	15.3±0.5	2081.0±55.2	30.1±1.8	30.2±1.7
Salmonella Poona (NCTC 4840)	18.0±0.5	1040.3±21.2	20.5±0.7	620.2±19.0	24.7±1.1	2.5±0.1
Escherichia coli (ATCC 8739)	19.5±0.9	1250.0±33.0	17.5±0.4	1040.0±36.0	31.0±1.7	2.2±0.2
Ampicillin resistant Escherichia coli (NCTC 10418)	22.0±1.2	830.9±26.6	19.5±0.6	1250.2±39.0	33.0±1.5	5.5±0.2

[a]Values are mean±standard deviation of three samples of each *Origanum* species, analyzed individually in triplicate; [b]Zone of inhibition in mm including the disc diameter (6 mm); [c]MIC, minimal inhibitory concentration (µg mL^{-1}).

antimalarial activity of the *Origanum* essential oils. Of the two *Origanum* essential oils tested, the *O. vulgare* essential oils showed anti-heme biocrystallization activity (Table 3). The *O. vulgare* essential oils at a concentration of 10 mg/mL exhibited 91.7% inhibition (IC50=0.04 mg/mL). However, *O. majorana* showed only 49.2% inhibition at the same concentration. The positive control chloroquine showed 90% inhibition at 0.005 mg/mL. The ability of any compound to inhibit the biocrystallization of ferriprotoporphyrin IX (FPIX) is believed to be directly connected to their antimalarial activity (Raynes et al., 1996).

The antioxidant activity of *Origanum* essential oils was assessed by different *in-vitro* tests. In the DPPH assay, the radical scavenging capacity of the tested essential oils increased in a concentration dependent manner. The values for 50% scavenging (IC50) are given in Table 3. The *O. vulgare* essential oils showed better radical scavenging activity (IC50: 65.5 µg/mL) than that of *O. majorana* essential oil (IC50: 89.2 µg/mL). However, the activity of both oils was less than that of the positive control, BHT (IC50: 9.9 µg/mL), a well known free radical scavenger. Table 3 also shows the level of percent inhibition of linoleic acid oxidation as exhibited by the tested essential oils. Both *O. vulgare* and *O. majorana* essential oils depicted statistically similar inhibition, *i.e*., 77.3 and 72.8%, respectively. The activity was slightly less than that of the positive control BHT (90.9%). The antioxidant activity of the essential oils of *O. vulgare* and *O. majorana* was also assessed qualitatively by bleaching β-carotene in linoleic acid system assay. In this assay, the greater the effectiveness of an antioxidant, the slower will be the color depletion, and less decrease in absorbance indicates a lower rate of oxidation of linoleic acid reflecting higher antioxidant activity. The *O. vulgare* essential oil exhibited better antioxidant activity than *O. majorana* oil. The control showed the highest rate of color depletion and thus least antioxidant activity.

Lipid peroxidation is an important issue in food industries and while comparing the present findings with the literature, not even a single report showing the percent inhibition of peroxidation by *Origanum* essential oils could be found. However, there are some reports available in the recent literature on the radical scavenging activity of other Lamiaceae essential oils (Puertas-Mejiia et al., 2002; Ruberto et al., 2002; Hussain et al., 2008; Kelen & Tepe, 2008; Hussain et al., 2010). The antioxidant activity of essential oils may be attributed to the presence of phenolic compounds as was observed in *Salvia officinalis* (Lu & Foo, 2000). Singh & Marimuthu (2006) found that essential oils effectively suppress the peroxide formation in linoleic acid system during incubation.

The MTT assay is a sensitive, simple and reliable method for evaluating cytotoxicity of plant-based products. The effect of increasing amounts of the tested *Origanum* essential oils on the cell proliferation of two human cancer (MCF-7 and LNCaP) and one fibroblast (NIH-3T3) cell lines was appraised. The inhibitory effect of *Origanum* essential oils on cancer cell viability ranged from 79-88% at 0.5 mg/mL (data not shown). The IC50 values, calculated from the graphs, are presented in Table 3, indicating that the tested

Table 3. Antimalarial, antioxidant and cytotoxic activities of *O. majorana* and *O. vulgare* essential oils[a]

Materials	Heme biocyrstallization assay		Antioxidant activity		Cytotoxicity assay (IC50, µg/mL)		
	Inhibition (%) at 10 mg/mL	(IC50, mg/mL)	DPPH, IC50, (µg mL-1)	Inhibition of linoleic acid peroxidation (%)	Breast cancer (MCF-7)	Prostate cancer (LNCaP)	Fibroblast (NIH-3T3)
Origanum majorana	49.2±1.8	> 10.0	89.2±2.4	72.8±4.9	70.0±2.5	85.3±2.8	300.5±12.0
Origanum vulgare	91.7±5.3	0.04±0.01	65.5±2.0	77.3±3.5	100.0±4.5	90.1±3.2	320.3±9.7
Chloroquine diphosphate	90% with 0.005 mg/mL	<0.005	NT	NT	NT	NT	NT
BHT	NT	NT	9.9±0.2	90.9±2.7	NT	NT	NT
Doxorubicin	NT	NT	NT	NT	28.8±1.2	33.3±1.1	NT

[1]Values are mean±standard deviation of three samples of each *Origanum* species, analyzed individually in triplicate. NT=not tested.

Origanum essential oils showed prominent cytotoxicity against both the cancer cell lines. The cytotoxicity of *O. majorana* essential oil against MCF-7, LNCaP and NIH-3T3 cell lines in terms of IC50 values were 70.0, 85.3 and 300.5 µg/mL, respectively, and were notably stronger than those of *O. vulgare* essential oil (Table 3). Doxorubicin was used as the positive control in the case of cytotoxicity test against MCF-7 and LNCaP cell lines, and the IC50 values were 28.8 and 33.3 µg/mL, respectively. These essential oils were comparatively less toxic against fibroblast (NIH-3T3) cell line. The analysis of variance showed significant (p<0.05) variation in the toxicity of both essential oils tested. Generally, it is believed that the major bioactive components of essential oils determine their biological properties. According to published guidelines, the IC50<10 µg/mL represents potentially very toxic; IC50 10-100 µg/mL represents potentially toxic; IC50 100-1000 µg/mL represents potentially harmful and IC50>1000 µg/mL represents potentially non toxic (Gad-Shayne, 1999). There are only a few reports in literature on the cytotoxicity of some Lamiaceae essential oils (Sivropoulou et al., 1996; Sivropoulou et al., 1997; Legault et al., 2003; DeSousa et al., 2004).

Finally, the composition and profile of the essential oils of *O. majorana* and *O. vulgare* as observed in the present study were comparable but not exactly the same with other published data. The bioassay results demonstrated that the essential oils of both *O. majorana* and *O. vulgare* had antioxidant, antibacterial and cytotoxic potentials. However, only *O. vulgare* essential oils exhibited potential antimalarial activity.

Acknowledgements

Grants supports by the Higher Education Commission, Islamabad, Pakistan under the International Research Initiative Support Program and the Indigenous PhD Fellowship Program schemes are gratefully acknowledged.

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