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RESEARCH ARTICLE

Antimicrobial activity of essential oil and aqueous and ethanol extracts of *Teucrium polium* L. subsp. *gabesianum* (L.H.) from Tunisia

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Abstract The antimicrobial effects of essential oil, ethanol and aqueous extracts of *Teucrium polium* L. were investigated against 13 microorganisms. Extracts and essential oil were obtained from maceration, decoction and hydrodistillation respectively. Samples were tested for their antimicrobial activity using the disk diffusion, the agar dilution and the agar incorporation method. Essential oil was analysed using GC/MS, results showed that β-pinene (35.97%) and α-pinene (13.32%) were the main components. Furthermore, essential oil exhibited the highest antimicrobial activity, it was most effective against *Proteus mirabilis, Staphylococcus aureus* and *Citrobacter freundei* where inhibition zone ranged between 15 and 25 mm, and with the microbial inhibitory concentration (MIC) values of 0.078–0.156 mg/ml. The oil and ethanol extract showed the best antifungal activity against *Microsporum canis*, *Scopulariopsis brevicaulis*, and *Trichophyton rubrum* with the inhibition percentage (I%) ranging from 18.94 to 100%. However, none of the samples exhibited antifungal activity against *Aspergillus fumigatus*. In this study, the obtained results showed significant effects of essential oils and ethanol extracts of *T. polium* which may used as a substitute to the synthetic drugs against certain microbial diseases.

Keywords *Teucrium polium* L. subsp. *gabesianum* · Essential oil · Antibacterial activity · Antifungal activity

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Introduction

Aromatic and medicinal plants were used to prevent or cure infectious diseases. Previous studies demonstrated their richness in polyphenols such as tannins, alkaloids and flavonoids which shown antimicrobial activities (González-Lamothe et al. 2009; Habbal et al. 2011).

Teucrium polium L. is called in Tunisia "Al-Ja'adeh", "Khayata" and "Gattaba" which means "cicatrisant". The genus *Teucrium* belongs to the *Lamiaceae* family and is represented by more than 340 species, from these, only 19 species are found in Tunisia. *T. polium* is mainly found in the Mediterranean and Western Irano-Turanian regions (Tepe et al. 2011). It is widely distributed in Jordan and Palestine (Afifi et al. 2005) and well known in Arab traditional medicine for its anti-diabetic, anti-inflammatory, hypotensive, anorexic, antispasmodic, antiulcer, antipyretic and antinociceptive properties (Darabpour et al. 2010; Tourandokht et al. 2005; Nosrati et al. 2010). According to Le Floc'h (1983), *T. polium* is regarded as an excellent depurative and febrifuge, it is also employed like tonic and digestive in the treatments of gastralgies and enteritis. De Marino et al. (2012) announce that this species is used as stimulants, tonics, diaphoretics, diuretics and in the treatments of stomach pain, asthma, amenorrhea, leucorrhoea, chronic bronchitis and gout by local nomadic populations. On the other

hand, it was reported that essential oils and alcoholic extracts of *T. polium* had an antibacterial activities against both Gram negative and Gram positive bacteria. In this context, Djabou et al. (2013) demonstrated that essential oils of *T. polium* inhibit the growth of toxi-infectious and foodborne bacteria. Moreover, ethanolic and methanolic extracts of *T. polium* showed antibacterial properties against some bacterial strains (Darabpour et al. 2010; Khaled-Khodja et al. 2014). This work aims to study the antibacterial and antifungal activities of ethanol and aqueous extracts and essential oils of *T. polium*.

Materials and methods

Plant material

Aerial parts of *T. polium* were collected from Zarroug Mountain, Gafsa (South-west of Tunisia) in May 2010. After collection, the plant material was dried for 7–10 days in the shade at room temperature. The dried matter was then ground and stored in cloth bags at 5 °C before being used in experiments to prepare the plant extracts.

Extracts preparation

Aqueous extracts

Dried matter (100 g) was stirred in 1 l distilled water for 15 min at 95 °C, followed by a rapid filtration through four layers of gauze and a second filtration through filter paper (Whatman No. 1). The percent (w/w) extraction yield was 9%. The resulting solution was freeze-dried and the powder was stored at −20 °C in a desiccant until required.

Ethanol extracts

Dried powder matter (100 g) was extracted by continuous mixing in 1 l ethanol (95%) during 24 h at room temperature. After filtration, ethanol was evaporated using a rotary vacuum evaporator (K IKA-WERKE). The extract was stored at −20 °C. The percent (w/w) extraction yield was 7% on a dry matter basis.

Extraction and chemical analyses of essential oils

Essential oils were extracted, from powdered plant, by hydrodistillation method using the apparatus described in the IXth edition of French Pharmacopoeia according to Okoh et al. (2010) (similar to a Clevenger-type apparatus). The percentage yield based on the dried plant was 0.29% (v/w). The essential oil was stored at +4 °C until tested and analysed.

Gas chromatography analysis The oil was analysed on Hewlett Packard (HP) 6890 using the following experimental conditions: flame ionization detector (FID), HP-5 fused silica capillary column temperature 50°–250 °C at 5 °C/min; injector and detector temperature 250 and 280 °C, respectively; carrier gas: nitrogen 1.2 ml/min; volume injected: 1 µl of the oil diluted in hexane (10%).

Gas chromatography–mass spectrometry analysis The samples were assayed by GC/MS using a HP 5972 gas chromatograph equipped with a fused silica capillary column (HP-5, 25 m × 0.2 mm, film thickness 0.25 mm). The GC/MS was operated under the following operating conditions: injector temperature: 250 °C; oven temperature: programmed from 50 to 280 °C at 5 °C/min; carrier gas: He at 20 psi: 1 µl of 1/dilution solution in 100 hexane. The oil components were identified by comparison of their retention indices [relatives to C_{26}–C_{28} alkanes on the BP-5 column] and mass spectra with those of authentic standards of library searches of the oil library LIBR Tped using the Finnegan library search routine based on fit and purity of mass spectra (Hossain et al. 2012).

Antimicrobial activities

Antibacterial activity

Bacterial strains Both extracts (aqueous and ethanol) and essential oil were used against a range of bacteria, namely *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Citrobacter freundei* and *Proteus mirabilis*. Microorganisms were obtained from the culture collection of the Laboratory of transmissible diseases and biologically active substances, Tunisia.

Susceptibility tests Susceptibility of the bacterial strains to the extracts and essential oils was investigated using the disk diffusion method (Teixeira et al. 2012) and by comparing their antibiogram inhibition zones to those reported by the National Committee for Clinical Laboratory Standards. Organisms were maintained on Muller-Hinton agar (MH). Inoculate were prepared by diluting overnight (24 h at 37 °C) cultures in Muller-Hinton Broth medium to approximately 10^6 CFU/ml. Whatman disks No. 3 of 6 mm diameter were impregnated with 10 µl of oil or aqueous and ethanol extracts at 20 mg/ml and then placed on to the surface of inoculated plates (90 mm). The diameters of inhibition zones were measured after incubation at 37 °C for 24 h and compared with positive control disk of Gentamycin 10 µg/ml. All experiments were performed in triplicate. The susceptibility of the strains to the extracts

and essential oils was further evaluated by a microdilution method (Zander et al. 2010). This method aims to determine the minimum inhibitory concentration (MIC), which was applied only to the extracts that have shown inhibition zones ≥8 mm. The initial concentration was 10 mg/ml. Essential oils and ethanol extract were dissolved in 10% of ethanol 99% (v/v). This method was applied using 96-well plate with round bottom according to Marzouk et al. (2010). Column 2–11, wells were filled with 100 µl of Muller Hinton Browth (MHB). The wells of column 12, containing only solvent were taken as control for sterility. The first column contains only extracts used as sterility control. The wells in column 10 were taken as control bacterial and column eleven's wells containing only MHB. The final volume in each well was 200 µl. The dilutions were made in 1/2. Gentamycin at the concentration range of 1 mg was prepared in MHB and used as standard drug for positive control. The agar plates were prepared and incubated at 37 °C for 24 h. MIC was defined as the concentration at which no colony was observed after incubation. The assays were performed in triplicate.

Antifungal activity

Microbial strains The extracts and essential oils were tested against seven strains of fungi which can be classified into three groups; two opportunist pathogenic yeast (*Candida albicans* and *Cryptococcus neoformans*), three dermatophytes (*Trichophyton rubrum*, *T. soudanense* and *Microsporum canis*) and two hyphamycets (*Aspergillus fumigatus* and *Scopulariopsis brevicaulis*). The microorganisms were preserved and maintained in the Laboratory of transmissible diseases and biologically active substances, Tunisia.

Antifungal testing Susceptibility of the fungi strains to the extracts and essential oils was investigated using the agar incorporation method (dilution in a solid medium). These phytopathogens were cultivated on sabouraud-chloramphenicol agar (SCA) in 6 cm Petri dishes. The essential oils, the ethanol extract or the aqueous extract was mixed with 100 ml of SCA to give final concentrations of 0.5–1 mg/ml. The ethanol extract and essential oils were dissolved in 10% of ethanol 99%, and this solvent was used as a negative control. After cooling and solidification, the medium was inoculated with 5 mm in diameter of a 7-day old mycelium culture of the tested dermatophytes, a 3-day culture suspension adjusted to 10^5 conidies/ml for *A. fumigatus* and a 3-day yeast culture suspended in sterile distilled water and adjusted to 10^5 spores/ml. The Petri dishes were then incubated at 24 °C for 7 days for dermatophytes, 24 h at 37 °C for *C. albicans* and *A. fumigatus, and* 48 h at 37 °C for *C. neoformans*. Three replicates for each concentration and microorganisms were carried out.

The antifungal activity was evaluated using two methods:

1. By calculating the percentage inhibition (I %) from the diameter values of the colonies in the control plate (dC) and the colonies in the plate added with the assayed extracts (dE), I% = (dC − dE)/dC, according to the method of Singh et al. (1993).
2. By determining the minimal inhibitory concentration (MIC): the lowest concentration which inhibits the visible growth of fungi during the incubation period adapted to each species (Mahlo et al. 2010).

Results

Chemical composition of essential oils

Pale yellow essential oils were extracted with yield of 0.29% for aerial parts of *T. polium* (v/w) on a dry matter basis. The oils were analyzed by GC and GC/MS and the qualitative and quantitative composition are presented in Table 1. The GC/MS analyses showed that the essential oils of *T. polium* were mainly represented by β-pinene (35.97%), α-pinene (13.32%), α-thujene (8.46%), p-cymene (5.25%) and Verbenone (5.03%).

Antibacterial activity

As shown in Table 2, all extracts exhibited an antibacterial activity against both Gram-negative and Gram-positive bacteria at different levels. *P. aeruginosa* was the most resistant (9–13 mm), whereas *C. freundei* (13–25 mm) and *S. aureus* (10–17 mm) were the most sensitive. These results were confirmed by the agar dilution method (Table 2), (0.156–0.625, 0.078–0.156 and 0.078–0.625 mg/ml, respectively, against *P. aeruginosa, C. freundei* and *S. aureus*).

Antifungal activity

The essential oils and both extracts (aqueous and ethanol) of *T. polium* had interesting activities against fungi with a percentage of inhibition varying from 0 to 100% (Table 3). All extracts had no adverse effects on *A. fumigatus* growth. For the three tested dermatophytes, *M. canis* was markedly inhibited (48.88–100%), whereas *S. brevicaulis* was slightly inhibited (8.6–58%). For the two opportunist pathogenic yeasts, *C. albicans* was weakly inhibited (2.03–9.63%), in contrast *C. neoformans* was only inhibited by the essential oils.

Table 1 Components of the essential oil of *Teucrium polium* L

Components	RI	Percentage
α-Thujene	1019	8.46
α-Pinene	1019	13.32
Camphene	1060	1.03
6-Methylhept-5-en-2-one	1343	NI
β-Pinene	1105	35.97
1.8-Dehydro-cineole	1197	NI
Myrcene	1150	4.67
p-Cymene	1257	5.25
Limonene	1190	0.49
1.8-Cineole	1203	T
Terpinolene	1269	1.64
Linalool	1529	0.49
α-Thujone	1412	0.52
Isophorone	1544	0.19
Fenchol	1570	0.67
β–Thujone	1426	0.25
Chrysanthenone	1489	0.39
α-Campholenal	1472	0.53
Nopinone	1562	0.21
Camphor	1501	0.3
Trans-Pinocarveol	1629	0.31
Cis-Verbenol	1629	0.32
Trans-Verbenol	1654	0.48
Myrtenal	1602	1.37
α-Terpinéol	1672	0.41
Myrtenol	1764	0
Verbenone	1679	5.03
Trans-Carveol	1805	0.38
Fenchyl-acétate	1459	0.36
Nerol	1782	0.21
Spathulenol	1571	0.17
α-cubenol	1600	2.59
T-cadinol	1608	0.78
α-cadinol	1626	0.2
Terpinen-4-ol	1581	1.2
p-Cymen-8-ol	1817	2.31
4-Methylacetophenone	1752	0.6
Cis-Pinocamphone	1507	T
Pinocarvone	1546	1.09
p-Mentha-1,5-dien-8-ol	1698	0.32
Umbellulone	1616	0.23
Borneol	1672	1.24
Trans-Pinocampone	1627	T
Sabina ketone	1606	T

T trace less than 0.1%; *NI* not identified; *RI* retention indices

Discussion

The unavoidable appearance every now and then of new antibiotic-resistant strains explains the perpetual demand on more efficient medicines. Under this conjuncture, medicinal plants represent a major part in the drug research programs. They can be found either as a natural drug component like in many developing countries, or as a raw material for pure chemical derivatives processing like in industrialized countries.

Environmental factors such as sunshine duration, temperature, soil, ground water, availability of nutrients are known to affect the presence and/or the concentrations of the active principles, including the volatile oils of various medicinal herbs. Sampling site and the time of collection of plant material are additional factors that may influence the phytochemical composition (Robbers et al. 1996). In Jordan, Aburjai et al. (2006), working on *T. polium* showed that the major components of its aerial part essential oils are 8-cedren-13-ol, β-caryophyllene, germacrene D and sabinene with a percentage of 24.8, 8.7, 6.8 and 5.2% respectively. For the same species, it was shown (Afifi et al. 2009), that there are differences in the chemical composition and concentration of volatile constituents between collected from different regions in Jordan. Our data are different from those found by Boulila et al. (2008) on Tunisian *T. polium*. These authors showed that main volatile components are myrcene (15.3%), germacrene D (9.0%), α-pinene (6.6%), β-pinene (5.8%), and α-cadinol (5.1%). In addition, Turkish *T. polium* essential oils obtained from the aerial parts have shown 37 components with β-pinene (18.0%), β-caryophyllene (17.8%), α-pinene (12.0%), caryophyllene oxide (10.0%), myrcene (6.8%), germacrene D (5.3%), limonene (3.5%) and spathulenol (3.3%) were the major identified components (Çakir et al. 1998). Working on the same species originated from Saudi Arabia, Hassan et al. (1979) affirmed that *T. polium* essential oil rich in terpenoid compounds including β-pinene, limonene, α-cadinene, α-phellandrene and the alcohols linalool, terpinen-4-ol, guaiol, cedrenol and cedrol. In addition, it was free from esters. Egyptian *T. polium* mainly contains Myrcene, OC-pinene, menthofuran, ocimene, pulegone and menthone (Wassel and Ahmed 1974). In Spain, Velasco-Negueruela and Perez-Alonso (1990) reported that the major compounds of *T. polium* were α-pinene (15.8%), β-pinene (11.7%), sabinene (7.2%), trans-pinocarveol (4.3%), terpinen-4-ol (4.5%) and β-bisabolene (2.5%).

Table 2 Antibacterial activity of the essential oil, ethanol and aqueous extracts of *Teucrium polium* L. using percentage inhibition (I%) of bacterial growth and minimal inhibitory concentration (MIC, mg/ml)

Microorganisms	Standard		Essential oil		Ethanol extract		Aqueous extract	
	I%	MIC	I%	MIC	I%	MIC	I%	MIC
Escherichia coli	30	0.031	14	0.156	10	0.312	–	NT
Enterococcus faecalis	21	0.125	13	0.156	10	0.312	7	NT
Staphylococcus aureus	30	0.015	17	0.078	11	0.312	10	0.625
Pseudomonas aeruginosa	27	0.062	13	0.156	9	0.625	–	NT
Citrobacter freundei	31	0.015	25	0.078	16	0.156	13	0.156
Proteus mirabilis	29	0.31	15	0.156	12	0.156	10	0.312

Standard: gentamycin, 10 μg/ml, (–): no inhibition, *NT* not tested

Table 3 Antifungal activity of the ethanol and aqueous extracts, and essential oil of *Teucrium polium* L. using percentage inhibition (I%) of microorganisms and minimal inhibitory concentration (MIC, mg/ml)

Microorganisms	Essential oil		Ethanol extract		Aqueous extract	
	I%	MIC	I%	MIC	I%	MIC
Trichophyton rubrum	42.1	>1	55.78	>1	0	NT
Microsporum canis	100	0.062	100	0625	48.88	>1
Trichophyton soudanense	18.94	>1	49	>1	0	NT
Scopilariopsis brevicaulis	27.95	>1	58	>1	8.6	>1
Cryptococcus neoformans	5	>1	0	NT	0	NT
Candida albicans	9.63	>1	3.94	>1	2.03	>1
Aspergillus fumigatus	0	NT	0	NT	0	NT

NT not tested

In the present study, the essential oils of *T. polium* were rich in α-pinene (13.32%), β-pinene (35.97%), α-thujene (8.46%), p-cymene (5.25%) and Verbenone (5.03%). This difference in essential oil compositions of *T. polium*, may be explained by the difference of the origin of plants (Bourgou et al. 2010). α-pinene has been previously known for its antibacterial activity (Kabouche et al. 2005). Moreover, Ultee et al. (2002) demonstrated that p-cymene had a very low antibacterial activity, however could induce a bacterial membrane swelling. By this mechanism p-cymene probably facilitates the transport of α-pinene and β-pinene into the cell. The mechanism of action of terpenes which were active against bacteria is still unknown, but probably some lipophilic compounds caused a membrane interruption (Cowan 1999). The oil antimicrobial activity can be explained by the presence of borneol, linalool and terpinen-4-ol, which were proven to have bacteriostatic activity against many microorganisms (Barel et al. 1991, Cosentino et al. 1999). At lower concentrations, these components can also have synergic interactions with other active compounds (Marino et al. 2001).

Previous studies on the composition of *T. polium* showed the presence of phenolic compounds (Djabou et al. 2012). Our data show interesting antibacterial and antifungal activities of the ethanol extract from *T. polium,* this is probably due to polyphenol substances present in the extract. Furthermore, many authors have focused in their studies on the effect of phenolic compounds on cellular membranes. In this context, Tsuchiya et al. (1996) demonstrated the interaction of phenolic compounds and extracellular proteins with the cell membrane. Indeed, phenolics leak out intracellular constituents by altering cells walls and membranes, which increase cells permeability. Critical membrane functions regulating cells exchange and metabolite synthesis are also affected by these alterations. Consequently, the multilevel cellular effect of the phenolic compounds may block the bacterial activity. Tassou et al. (2000) noticed a loss of intracellular material from *E. coli* and *S. aureus* which was related to phenol concentration. Ethanol extract of *T. polium* may be considered a well inhibitory of *Trichophyton rubrum* which is the main cause of athlete's foot and onichomycoses in human beings.

As a result of the variability in chemical composition of *T. polium*, there was variability in antimicrobial activity. According to Darabpour et al. (2010), *Bacillus anthracis* was the most sensitive species when treated by the ethanol extract (50–400 mg/ml), while *Escherichia coli* and *Proteus mirabilis* were more resistant. These authors reported that the MIC of *Staphylococcus aureus* and *Salmonella typhi* was 40 mg/ml; while in *Bordetella bronchiseptica* and *Bacillus anthracis* was 10 mg/ml.

The effect of ethanol extract against *S. aureus* in this work (11 mm inhibition zone) was similar to that found by Sarac and Ugur (2007) (9 mm).

Overall, the contemporary presence of antibacterial and antifungal activities in the essential oil and ethanol extract from *T. polium* proves that this plant contains bioactive compounds and may be utilized for several activities.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Aburjai T, Hudaib M, Cavrini V (2006) Composition of the essential oil from Jordanian germander (*Teucrium polium* L.). J Essent Oil Res 18:97–99

Afifi FU, Al-Khalidi B, Khalil E (2005) Studies on the in vivo hypoglycemic activities of two medicinal plants used in the treatment of diabetes in Jordanian traditional medicine following intranasal administration. J Ethnopharmacol 100:314–318

Afifi FU, Abu–Irmaileh BE, Al-Noubani RA (2009) Comparative analysis of the essential oils of *Teucrium polium* L. grown in different arid & semi arid habitats in Jordan. Jordan J Pharm Sci 2:42–52

Barel S, Segal R, Yashphe J (1991) The antimicrobial activity of the essential oil from *Achillea fragrantissima*. J Ethnopharmacol 33:187–191

Boulila A, Béjaoui A, Messaoud C, Boussaid M (2008) Variation of volatiles in Tunisian populations of *Teucrium polium* L. (*Lamiaceae*). Chem Biodivers 5:1389–1400

Bourgou S, Pichette A, Marzouk B, Legault J (2010) Bioactivities of black cumin essential oil and its main terpenes from Tunisia. S Afr J Bot 76:210–216

Çakir A, Emin Duru M, Harmandar M, Cirimlnna R, Passannanti S (1998) Volatile constituents of *Teucrium polium* L. from Turkey. J Essent Oil Res 10:113–115

Cosentino S, Tuberoso CIG, Pisano B, Satta M, Mascia V, Arzedi E (1999) In vitro antimicrobial activity and chemical composition of *Sardinian thymus* essential oils. Lett Appl Microbiol 29:130–135

Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12:564–582

Darabpour E, Motamedi H, Seyyed Nejad SM (2010) Antimicrobial properties of *Teucrium polium* against some clinical pathogens. Asian Pac J Trop Med 3:124–127

De Marino S, Festa C, Zollo F, Incollingo F, Raimo G, Evangelista G, Iorizzi M (2012) Antioxidant activity of phenolic and phenylethanoid glycosides from *Teucrium polium* L. Food Chem 133:21–28

Djabou N, Muselli A, Allali H, Dib MEA, Tabti B, Varesi L, Costa J (2012) Chemical and genetic diversity of two Mediterranean subspecies of *teucrium polium* L. Phytochemistry 83:51–62

Djabou N, Lorenzi V, Guinoiseau E et al (2013) Phytochemical composition of Corsican *teucrium* essential oils and antibacterial activity against foodborne or toxi-infectious pathogens. Food Control 30:354–363

González-Lamothe R, Mitchell G, Gattuso M, Moussa SD, Malouin F, Bouarab K (2009) Plant antimicrobial agents and their effects on plant and human pathogens. Int J Mol Sci 10:3400–3419

Habbal O, Hasson SS, El-Hag AH, Al-Mahrooqi Z, Al-Hashmi N, Al-Bimani Z, Al-Balushi MS, Al-jabri AA (2011) Antibacterial activity of *Lawsonia inermis* Linn (henna) against *Pseudomonas aeruginosa*. Asian Pac J Trop Biomed 1:173–176

Hassan MMA, Muhtadi FJ, Al-Badr AA (1979) GLC-mass spectrometry of *Teucrium polium* oil. J Pharm Sci 68:800–801

Hossain MA, Shah MD, Sang SV, Sakari M (2012) Chemical composition and antibacterial properties of the essential oils and crude extracts of Merremia borneensis. J King Saud Univ Sci 24:243–249

Kabouche Z, Boutaghane N, Laggoune S, Kabouche A, Ait-Kaki Z, Benlabed K (2005) Comparative antibacterial activity of five *Lamiaceae* essential oils from Algeria. Int J Aromather 15:129–133

Khaled-Khodja N, Boulekbache-Makhlouf L, Madani K (2014) Phytochemical screening of antioxidant and antibacterial activities of methanolic extracts of some Lamiaceae. Ind Crops Prod 61:41–48

Le Floc'h E (1983) Contribution à une étude ethnobotanique de la flore tunisienne, vol 2. p 205

Mahlo SM, McGaw LJ, Eloff JN (2010) Antifungal activity of leaf extracts from South African trees against plant pathogens. Crop Prot 29:1529–1533

Marino M, Bersani C, Comi G (2001) Impedance measurements to study the antimicrobial activity of essential oils from *lamiaceace* and *compositae*. Int J Food Microbiol 67:187–195

Marzouk B, Marzouk Z, Décor R, Mhadhebi L, Fenina N, Aouni M (2010) Antibacterial and antifungal activities of several populations of Tunisian *Citrullus colocynthis* Schrad. immature fruits and seeds. J Mycol Med 20:179–184

Nosrati N, Aghazadeh S, Yazdanparast R (2010) Effects of *Teucrium polium* on insulin resistance in nonalcoholic steatohepatitis. J Acupunct Meridian Stud 3:104–110

Okoh OO, Sadimenko AP, Afolayan AJ (2010) Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis* L. obtained by hydrodistillation and solvent free microwave extraction methods. Food Chem 120:308–312

Robbers JE, Speedie MK, Tyler V (1996) Pharmacognosy and pharmacobiotechnology. Williams and Wilkins, Baltimore, pp 80–104

Sarac N, Ugur A (2007) Antimicrobial activities and usage in folkloric medicine of some *Lamiaceae* species growing in Mugla, Turkey. Eur J Biosci 4:28–37

Singh G, Upadhyay RK, Narawaynan CS, Padmkumari KP, Rao GP (1993) Chemical and fungitoxic investigation on the essential oil of *Citrus cinensis* (L) Per. Z. deutsche Zeitschrift fur Planzenfrankeiten und Pflanzenschutz 100:69–74

Tassou C, Koutsoumanis K, Nychas GJE (2000) Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. Food Res Int 33:273–280

Teixeira B, Marques A, Ramos C et al (2012) European pennyroyal (*Mentha pulegium*) from Portugal: chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. Ind Crops Prod 36:81–87

Tepe B, Degerli S, Arslan S, Malatyali E, Sarikurkcu C (2011) Determination of chemical profile, antioxidant, DNA damage protection and antiamoebic activities of *Teucrium polium* and *Stachys iberica*. Fitoterapia 82:237–246

Tourandokht B, Mehrdad R, Farshad RD (2005) Antinociceptive effect of *Teucrium polium* leaf extract in the diabetic rat formalin test. J Ethnopharmacol 97:207–210

Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Linuma M (1996) Comparative study on the antimicrobial activity of phytochemical flavones against Methicillin-resistant *Staphylococcus aureus*. J Ethnopharmacol 50:27–34

Ultee A, Bennink MH, Moezelaar R (2002) The phenolic hydroxyl group of carvacrol is essential for action against the foodborne pathogens *Bacillus cereus*. Appl Environ Microbiol 68:1561–1568

Velasco-Negueruela A, Perez-Alonso MJ (1990) The volatile of six *Teucrium* species from the Iberianpeninsula and the—Balearic island. Phytochemistry 29:1165–1169

Wassel GM, Ahmed SS (1974) On the essential oil of *Teucrium polium*. Pharmazie 29:351–352

Zander J, Besier S, Faetke S, Saum SH, Müller V, Wichelhaus TA (2010) Antimicrobial activities of trimethoprim/sulfamethoxazole, 5-iodo-2′-deoxyuridine and rifampicin against *Staphylococcus aureus*. Int J Antimicrob Agents 36:562–565