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Antioxidant, Anti-5-lipoxygenase and Antiacetylcholinesterase Activities of Essential Oils and Decoction Waters of Some Aromatic Plants

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Abstract: The scavenging of free radicals and superoxide anion, the inhibition of 5-lipoxygenase and the antiacetylcholinesterase activities of essential oils and decoction waters of eight aromatic plants (*Dittrichia viscosa*, *Foeniculum vulgare*, *Origanum vulgare*, *Salvia officinalis*, *Thymbra capitata*, *Thymus camphoratus*, *Thymus carnosus* and *Thymus mastichina*) were studied. The essential oils were dominated by 1,8-cineole in *S. officinalis* (59%), *T. mastichina* (49%) and *T. camphoratus* (21%); borneol (20%) in *T. carnosus*; carvacrol in *Thymbra capitata* (68%); γ-terpinene (49%) in *O. vulgare*; α-pinene (26%) in *F. vulgare*; and *trans*-nerolidol (8%) + β-oplopenone (7%) in *D. viscosa*. *O. vulgare* decoction waters had the highest amount of phenols (45±3 mg GAE/mL) while *F. vulgare* only had 5±0 mg GAE/mL. The decoction waters showed higher radical scavenging activity than the essential oils. *O. vulgare* decoction water showed the best antioxidant activity (IC_{50}=3±0 μg/mL), while the most effective essential oils were those of *Thymbra capitata* (IC_{50}=61±2 μg/mL) and *O. vulgare* (IC_{50}=156±5 μg/mL). *Thymbra capitata* (IC_{50}=6±0 μg/mL) decoction water showed the best superoxide anion scavenging activity. *F. vulgare* decoction water and essential oil revealed the best 5-lipoxygenase inhibition capacity (IC_{50}=27±1 μg/mL and IC_{50}=68±2 μg/mL, respectively). *T. mastichina* (IC_{50}=46±4 μg/mL), *S. officinalis* (IC_{50}=51±4 μg/mL), *Thymbra capitata* (IC_{50}=52±1 μg/mL) and *T. camphoratus* (IC_{50}=137±2 μg/mL) essential oils showed the best antiacetylcholinesterase activity.

Keywords: Essential oils; decoction water; biological activities; Apiaceae; Asteraceae; Lamiaceae.

1. Introduction

Oxidation induced by reactive oxygen species (ROS) can damage membranes, lipids, lipoproteins, and induce DNA mutation. This type of cell or tissue injuries has been associated with

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aging, atherosclerosis, carcinogenesis, cardiovascular- and Alzheimer's diseases. Preventing or minimizing these oxidation-related diseases may involve the use antioxidant substances that scavenge and eradicate ROS, namely the superoxide- $(O_2^{\bullet-})$, hydroxyl- $(HO^{\bullet})$, peroxyl- $(ROO^{\bullet})$, and nitric oxide radicals $(NO^{\bullet})$ [1,2].

In addition to the beneficial effects of these antioxidants in human health, some of them are also used in food industry as preservatives for preventing or delaying the oxidation process. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are synthetic antioxidants commonly used for this purpose. Nevertheless, given the fact that they may be dangerous for animal health, there is an increasing interest in natural food additives, such as spices or spice extracts, which can have the dual role of natural antioxidants and seasoning. In addition, studies have demonstrated that antioxidant rich plant preparations can prevent cancer, as well as cardiovascular-, neurodegenerative-, inflammation- and other aging-related diseases [2 and references therein].

Although the antioxidant activity of several herbs and spices essential oils has been extensively reported [3-8], much less is known on the antioxidant capacity of their decoction waters, which are usually discarded.

In the present work, the essential oils and decoction waters of eight aromatic plants from three different families [*Foeniculum vulgare* (Apiaceae), *Dittrichia viscosa* (Asteraceae), *Thymus camphoratus*, *T. carnosus*, *T. mastichina*, *Thymbra capitata*, *Origanum vulgare* and *Salvia officinalis* (Lamiaceae)] were evaluated for antioxidant (scavenging of free radicals and superoxide anion), anti-inflammatory (inhibition of 5-lipoxygenase) and antiacetylcholinesterase (acetylcholinesterase inhibitor) activities.

2. Materials and Methods

2.1. Plant Material

The flowering aerial parts of *Thymus mastichina*, *T. carnosus*, *T. camphoratus*, *Thymbra capitata* and *Origanum vulgare* were provided by Direcção Regional de Agricultura e Pescas do Algarve (DRAPALG, Portugal). *Dittrichia viscosa* aerial parts were collected, in the flowering phase, in the Campus de Gambelas (Universidade do Algarve). All species were dried in a dark ventilated place, at room temperature, until weight stabilization. Voucher specimens of all these species have been deposited in the Herbarium of the Museu, Laboratório e Jardim Botânico de Lisboa (LISU) and at the Herbarium of the Faculdade de Ciências e Tecnologia (Universidade do Algarve). *Salvia officinalis* and *Foeniculum vulgare* dried aerial parts phtytoceuticals were purchased in local herbal shops (brand name "Segredo da Planta").

2.2. Isolation procedure

2.2.1. Isolation of essential oils

The essential oils were isolated from the dried plant material (50 g) by hydrodistillation for 3 h using a Clevenger-type apparatus according to the European Pharmacopoeia method [9]. The isolation procedure was run at a distillation rate of 3mL/min. The essential oils were stored at -20 ºC in the dark until analysis. A minimum essential oil volume was diluted in distilled *n*-pentane prior to GC analysis.

2.2.2. Preparation of the extracts

After hydrodistillation, each decoction water (the remaining hydrodistillation aqueous phase) was collected and concentrated under vacuum at 70 ºC. This extract was re-dissolved in Dimethyl Sulfoxide (DMSO) 99.8% : water (3:1). This fraction was stored at -20ºC in the dark prior to analysis.

2.3. Chemical analysis of the essential oils

2.3.1. Gas chromatography (GC)

Gas chromatographic analyses were performed using a Perkin Elmer Autosystem XL (Perkin

Elmer, Shelton, Connecticut, USA) gas chromatograph equipped with two flame ionization detectors (FIDs), a data handling system and a vaporizing injector port into which two columns of different polarities were installed: a DB-1 fused-silica column (30 mx0.25 mm i. d., film thickness 0.25 µm) (J & W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column (30mx0.25mm i. d., film thickness 0.15µm) (J & W Scientific Inc.). Oven temperature was programmed, 45-175 °C, at 3 °C/min, subsequently at 15 °C/min up to 300 °C, and then held isothermal for 10 min; injector and detector temperatures, 280 °C and 300 °C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30 cm/s. The samples were injected using split sampling technique, ratio 1:50. The volume of injection was 0.1 µL of a pentane-oil solution. The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as mean values of two injections from each oil, without using response factors.

2.3.2. Gas chromatography-mass spectrometry (GC-MS)

The GC-MS unit consisted of a Perkin Elmer Autosystem XL (Perkin Elmer, Shelton, Connecticut, USA) gas chromatograph, equipped with DB-1 fused-silica column (30 mx0.25 mm i.d., film thickness 0.25 µm) (J & W Scientific, Inc.), and interfaced with a Perkin-Elmer Turbomass mass spectrometer (software version 4.1, Perkin Elmer, Shelton, Connecticut, USA). Injector and oven temperatures were as above; transfer line temperature, 280 °C; ion source temperature, 220 °C; carrier gas, helium, adjusted to a linear velocity of 30 cm/s; split ratio, 1:40; ionization energy, 70 eV; scan range, 40-300 u; scan time, 1 s. The identity of the components was assigned by comparison of their retention indices, relative to C_9-C_{21} *n*-alkane indices and GC-MS spectra from a home-made library, constructed based on the analyses of reference oils, laboratory-synthesised components and commercial available standards. Whenever needed mass spectra were compared with available literature [10].

2.4. Antioxidant activity evaluation

2.4.1. Free radical scavenging activity (DPPH)

A methanolic stock solution (50 µL) of each sample (essential oils and extracts) at different concentrations was placed in a cuvette, and 2 mL of 60 µM methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich, Steinheim, Germany) was added [11]. Absorbance measurements were made at 517 nm using a Shimadzu 160-UV spectrophotometer (Tokyo, Japan) after 60 min of reaction at room temperature. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as negative control. Butylated hydroxytoluene (BHT) Sigma-Aldrich, Steinheim, Germany) was used as positive control. The percentage inhibition of the DPPH radical by the samples was calculated according to the following formula: *Scavenging effect % = [(A_0 – A_1) / A_0] * 100* where A_0 was the absorbance of the control without extract or essential oil and A_1 was the absorbance of the sample. Tests were carried out in triplicate. Sample concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against sample (essential oil or extract solution) concentrations.

2.4.2. Superoxide anion scavenging activity

Measurements of superoxide anion scavenging activity of samples were based on the method described by Payá *et al.* [12]. Superoxide anions were generated in an enzymatic hypoxanthine/xanthine oxidase system assayed by reduction of nitroblue tetrazolium (NBT). The superoxide anion was generated in 666 µL of phosphate buffer (KH_2PO_4) (50 mM, pH 7.4), containing 100 µL hypoxanthine 1 mM, 100 µL EDTA 1 mM, 100 µL NBT (1 mM), and different concentrations of samples. The reaction was started with the addition of 31.5 µL of xanthine oxidase from bovine milk (EC 232-657-6, Sigma-Aldrich, Steinheim, Germany) (0,73 U/mL) (to the mixture. The absorbance was recorded at 560 nm against blank samples in a Shimadzu 160-UV spectrophotometer. The percentage of inhibition was calculated using the following equation: *Inhibition % = [(A_0 – A_1) / A_0] * 100*, where A_0 was the absorbance of the control (without extract) and A_1 was the

absorbance of the sample. Tests were carried out in triplicate. Sample concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against sample (essential oil or extract solution) concentrations.

2.5. 5-Lipoxygenase assay

The 5-lipoxygenase assay followed the procedure described by Frum and Viljoen [13]. The standard assay mixture contained 12.5 μL of each essential oil or extract dissolved in DMSO dimethylsulfoxide), 50 μL of linoleic acid (0.003 g/10mL) (Sigma-Aldrich, Steinheim, Germany) and made up to 1 mL with 0.1 M phosphate buffer with Tween 0.005%. The reaction was initiated with the addition of 1.5 μL 5-lipoxygenase from soybean (EC 1.13.11.12, Sigma-Aldrich, Steinheim, Germany) (0.054 g/mL). The increase in absorbance at 234 nm was recorded for 5 min in a Shimadzu 160-UV spectrophotometer. Nordihydroguaiaretic acid (NDGA) (Sigma-Aldrich, Steinheim, Germany) was used as positive control. The percentage inhibition of enzyme activity was calculated by comparison with the negative control: $\% = [(A_0 - A_1) / A_0] * 100$, where A_0 was the absorbance of the control without extract or essential oil and A_1 was the absorbance of the sample. Tests were carried out in triplicate. Sample concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against sample (essential oil or extract solution) concentrations.

2.6. Acetylcholinesterase inhibition

The acetylcholinesterase inhibition assay was adapted from that described by Mata *et al*. [18]. Briefly, in a total volume of 1 mL, 415 μL of Tris-HCl buffer 0.1 M (pH 8), 10 μL of a buffer solution of sample (in methanol for essential oil or water for aqueous extracts) with different concentrations and 25 μL of enzyme (electric eel acetylcholinesterase, type-VI-S, EC 3.1.1.7, Sigma-Aldrich, Steinheim, Germany) solution containing 0.28 U/mL were incubated for 15 min at room temperature. 75 μL of a solution of AChI (acetylthiocholine) (Sigma-Aldrich, Steinheim, Germany) 1.83 mM and 475 μL of DTNB (5,5'-dithiobis[2-nitrobenzoic acid]) 3 mM (Sigma-Aldrich, Steinheim, Germany) were added and the final mixture incubated, for 30 min, at room temperature. Absorbance of the mixture was measured at 405 nm in a Shimadzu 160-UV spectrophotometer. Galanthamine (Sigma-Aldrich, Steinheim, Germany) was used as positive control. The percentage inhibition of enzyme activity was calculated by comparison with the negative control*: $\% = [(A_0 - A_1) / A_0] * 100$* where A_0 was the absorbance of the control without extract or essential oil and A_1 was the absorbance of the sample. Tests were carried out in triplicate. Sample concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against sample (essential oil or extract solution) concentrations.

2.7. Determination of total phenols

The total phenol contents in the extracts were determined using the Folin-Ciocalteu reagent and gallic acid as standard as described by Slinkard *et al*. [14]. The sample (0.5 mL) and 2 mL of sodium carbonate (75 g/L) were added to 2.5 mL of 10 % (v/v) Folin-Ciocalteu reagent (Sigma-Aldrich, Steinheim, Germany). After 30 min of reaction at room temperature, the absorbance was measured at 765 nm in a Shimadzu 160-UV spectrophotometer. Tests were carried out in triplicate.

2.8. Statistical analysis

Statistical comparisons were made with one-way ANOVA followed by Tukey multiple comparison test. The level of significance was set at $P < 0.05$. Statistical calculation was performed using SPSS 15.0 software.

3. Results and Discussion

3.1. Essential oils

3.1.1. Chemical composition

The oil yields were ranked into three main groups: <1% (*Dittrichia viscosa* and *Foeniculum vulgare*), >1% and <3% (*Origanum vulgare*, *Salvia officinalis* and *Thymus camphoratus*) and >3% (*T. carnosus*, *T. mastichina* and *Thymbra capitata*). These yields are within the values usually reported for these species, in spite of some variations depending on several factors, namely part of plant used, collection site, harvesting period, time of extraction, fertilization type, among other factors [15-20].

The chemical composition of the essential oils isolated from the eight species, as well as their yields, is reported in Table 1, in order of their elution on DB-1 column. Considering the grouped components, the essential oils could be sorted in: a) monoterpene hydrocarbons-rich (*F. vulgare* and *O. vulgare*); b) oxygen-containing monoterpenes-rich (*S. officinalis*, *Thymbra capitata*, *T. camphoratus* and *T. mastichina*); c) monoterpene hydrocarbons/oxygen-containing monoterpenes-rich (*T. carnosus*); and d) oxygen-containing sesquiterpenes-rich (*D. viscosa*). *F. vulgare* oil showed also a relative high percentage of phenylpropanoids that were not detected in the remaining oils, Table 1.

Despite sharing similar main grouped components, the dominant oil components differed. In *F. vulgare*, α-pinene (26%), *p*-cymene (12%) and limonene (17%) contributed to >50% of the total of the essential oil; whereas in *O. vulgare*, γ-terpinene (49%), *p*-cymene (14%) and thymol (15%) together exceeded 70% of the oil. In *S. officinalis* 1,8-cineole dominated (59%), whereas in *Thymbra capitata* oil, carvacrol (68%) was the main component. 1,8-Cineole (49%) and camphor (6%) attained >50% of *T. mastichina* oil. *T. camphoratus* oil was dominated by 1,8-cineole (21%) and borneol (13%). Borneol (20%) and terpinen-4-ol (13%) were the main components in *T. carnosus* oil. *trans*-Nerolidol (8%), β-oplopenone (7%), T-cadinol (6%) were among the main *D. viscosa* oil components.

The results herewith reported for *Thymbra capitata*, *T. camphoratus*, *T. carnosus* and *T. mastichina* essential oils fit within the previously observed chemotypes for these species [21]. High relative amounts of 1,8-cineole were also detected in one Italian and three Greek *S. officinalis* samples [6,19,22,23]. Piccaglia and Marotti [24] considered five chemical groups in the oils isolated from fresh aerial parts of wild fennel collected in thirteen Italian localities, one which was characterized by relative high amounts of *trans*-anethole, α-pinene and limonene such as the presently studied oils. In contrast to previously *O. vulgare* thymol / γ-terpinene rich oils from plants collected in Portugal [17,25], in the present study γ-terpinene (49%) and similar amounts of thymol (15%) and *p*-cymene (14%) were dominant. Although fokienol constituted the main *Dittrichia viscosa* oil component reported by Blanc *et al.* [26], oxygen-containing sesquiterpenes were also reported to be present in high relative amounts.

3.1.2. Antioxidant activity

3.1.2.1. Free radical scavenging activity (DPPH assay)

Thymbra capitata (IC$_{50}$=61.1±1.9 µg/mL) and *O. vulgare* (IC$_{50}$=156.3±5.1 µg/mL) oils showed an antioxidant activity significantly higher than the remaining essential oils (Table 2), although lower than that found for BHT (IC$_{50}$=13±1.0 µg/mL). Carvacrol, present in relative high amounts in *Thymbra capitata* oil can be partly responsible for such activity; whereas in *O. vulgare* oil the activity may be attributed to two components, γ-terpinene and thymol. The relative good capacity of the carvacrol- or thymol and γ-terpinene-rich oils for scavenging free DPPH radicals was also reported in essential oils of the same species but from different origins [4,27,28]. DPPH radical scavenging activities of these essential oils support the view that not only phenol compounds are good antioxidants. According to Ruberto and Baratta [29] some structural features, such as the presence of strongly activated methylene group in the molecule, are probably the reason for antioxidant activity of monoterpene hydrocarbons.

3.1.2.2. Superoxide anion scavenging activity

Under the experimental conditions, the essential oils superoxide anion scavenging activity determination was impaired, because of the development of a purple colour before the addition of the enzyme, due to interference between the essential oils and hypoxanthine.

3.1.3. 5-Lipoxygenase inhibition

All essential oils were able to inhibit 5-lipoxygenase, particularly *F. vulgare* oil (IC_{50}=67.7±2.3 µg/mL), in contrast to *T. mastichina* oil (IC_{50}=1084± 146.1 µg/mL), which showed the poorest activity (Table 2). *F. vulgare* oil IC_{50} was close to that found for the positive control [Nordihydroguaiaretic acid (NDGA) IC_{50}=63.7±2.3 µg/mL]. Several essential oils and their components have shown to be effective as 5-lipoxygenase inhibitors [30-32], namely limonene, 1,8-cineole, γ-terpinene and α-pinene [12,33,34]. The IC_{50} value found in the present work for *F. vulgare* oil fits within of those limonene- and α-pinene rich oils, reported by Viljoen *et al*. [34].

3.1.4. Acetylcholinesterase inhibition

The essential oils of *T. mastichina* (45.8±4.1 µg/mL), *S. officinalis* (50.8±3.8 µg/mL), and *Thymbra capitata* (51.9±0.9 µg/mL) possessed the highest acetylcholinesterase inhibiting capacity (Table 2). Several essential oils, as well as some of their components, have been reported as having acetylcholinesterase inhibitor ability, including those isolated from some species of *Salvia*, *Thymus* and *Origanum* [35-40]. However, it is the first time that this property is reported for *T. mastichina* oil. In spite of these IC_{50} values, they were far from that found for galanthamine positive control (IC_{50}=8.6±0.2 µg/mL). 1,8-Cineole, the dominant constituent of the essential oil of *S. officinalis* and *T. mastichina* and carvacrol in *Thymbra capitata* oil can partly explain the best activity of these oils, since some studies have revealed that these monoterpenes are potent acetylcholinesterase inhibitors, with a IC_{50}=49 µg/mL for 1,8-cineole [41] and IC_{50}=69 µg/mL for carvacrol [42].

3.2. Decoction water extracts

3.2.1. Extraction yields and total phenols

The best decoction water extract yield was obtained from *T. mastichina* and *Thymbra capitata* (41% both). The lowest percentages were found in *T. camphoratus* (16%) and *F. vulgare* (17%) (Table 3). In spite of *Thymbra capitata* high extract yield, the total phenol content was low (11 mg/mL) when compared to that of the remaining extracts. These results agree with those found by Hinneburg *et al*. [43] in which no significant association was detected between the extraction yields and total phenols from selected culinary herbs and spices. The results obtained in the present work suggest that those plants having good yield extracts but low total phenols may contain water–soluble nonphenolic compounds in relative high amounts. *O. vulgare* and *T. camphoratus* had the best ratio of total phenol to extraction yield (17 and 13, respectively) in contrast to *Thymbra capitata* and *F. vulgare* (3 in both cases). Hinneburg *et al*. [43] also reported large differences in those ratios with other species.

3.2.2. Antioxidant activity

3.2.2.1. Free radical scavenging activity (DPPH assay)

The best scavenging free radical effect was recorded with *O. vulgare* (IC_{50}=2.8±0.0 µg/mL) and *T. carnosus* (IC_{50}=2.9 ±0.0 µg/mL) decoction waters, in opposite to those of *Thymbra capitata* (IC_{50}=4.4±0.0 µg/mL) and *T. mastichina* (IC_{50}=4.2 ±0.0 µg/mL) (Table 3). However the poorest IC_{50} values found for these samples were higher than those of the positive control (BHT IC_{50}=13±1.0 µg/mL).

Typical phenol compounds presenting antioxidant activity include phenolic acids and flavonoids and within each group, the diversity of chemical structures is huge with the consequent diversity of activities. In spite of the diversity and complexity of the natural mixtures of phenolic compounds in the extracts, there was a small association between total phenols and free radical

scavenging (r=0.456; p<0.05). Similar, but higher, relationships were reported by Dorman *et al*. [44,45] for aqueous extracts of Lamiaceae plants. The absence of any correlation between total phenols and DPPH scavenging reported by Hinneburg *et al*. [43] was explained based on the variety of the material used that belonged to six diverse plant families.

3.2.2.2. Superoxide anion scavenging activity

All tested plant decoction waters showed superoxide anion radicals scavenging capacity, *Thymbra capitata* (IC$_{50}$=6.4±0.2 µg/mL) significantly exceeding such ability in comparison to the other samples (Table 3). *O. vulgare* (IC$_{50}$=20.4±1.0 µg/mL) and *F. vulgare* (IC$_{50}$=18.8±1.0 µg/mL), on the contrary, showed the lowest scavenging ability. Gallic acid, used as positive control, showed an IC$_{50}$=35 µg/mL lower than that of the decoction waters.

Interestingly, *Thymbra capitata* extract showed low efficacy as DPPH free radical scavenging, but high superoxide anion scavenging activity. This may be due to the different mechanisms of scavenging of DPPH and superoxide. Kabouche *et al*. [46] studying the antioxidant activity of abietane diterpenes reported that those with good DPPH scavenging ability were not necessarily the same for scavenging superoxide anion radicals. Those results were dependent on the mechanisms involved in two methods: a H-transfer method for DPPH assay and electron-transfer method for superoxide assay.

No correlation between total phenols and superoxide anion radical scavenging was detected. The present results support the view that scavenging activity of an extract cannot be predicted on the basis of its total phenolic content [47].

3.2.3. 5-Lipoxygenase inhibition

F. vulgare decoction water was the most effective as 5-lipoxygenase inhibitor (IC$_{50}$=27.4±0.6 µg/mL) (Table 3). Methanolic fruit extract of *F. vulgare* had been already reported as possessing anti-inflammatory activity [47]. In many plant species, this activity has been attributed to flavonoids, among other groups of compounds [48].

3.2.4. Acetylcholinesterase inhibition

Decoction water extracts could not be assessed for acetylcholinesterase inhibition capacity due to negative interferences between DTNB and the extracts.

In conclusion, the decoction water extracts were significantly more effective than the essential oils, suggesting that the compounds present in extracts are more active than essential oils. Further studies are needed to determine the detailed chemical composition of these extracts.

Table 1. Chemical composition of the essential oils isolated from the species under study. *Dittrichia viscosa* (*Dv*) *Foeniculum vulgare* (*Fv*) *Origanum vulgare* (*Ov*) *Salvia officinalis* (*So*) *Thymbra capitata* (*Tc*) *Thymus camphoratus* (*Thcamp*) *Thymus carnosus* (*Thc*) *Thymus mastichina* (*Thm*).

Components	RI[a]	Dv	Fv	Ov	So	Tc	Thcamp	Thc	Thm
Tricyclene	921				0.1		0.5	0.4	0.3
α-Thujene	924	0.1	0.2	2.4	t	0.7	0.2	5.1	0.5
α-Pinene	930	0.2	25.8	1.1	8.4	1.6	11.9	4.9	7.0
Camphene	938	t	1.3	0.1	2.8	0.2	10.6	11.4	6.9
Thuja-2,4(10)-diene*	940	0.1	0.1				1.2	0.3	
Sabinene	958	t	0.2	0.1			0.2	2.0	2.0
1-Octen-3-ol	961		t	0.1	t	0.1			t
β-Pinene	963	0.3	6.8	t	3.2	0.1	0.9	2.8	5.3
Caproic acid (hexanoic acid)	968	t							
Dehydro-1,8-cineole	973	1.2					0.2		t
2-Pentyl furan	973	t							

Components	RI[a]	Dv	Fv	Ov	So	Tc	Thcamp	Thc	Thm
2,4-Heptadienal	973	t							
3-Octanol	974			0.9		t			
β-Myrcene	975	t	5.4	1.7	2.2	3.0		0.7	1.2
cis-Dehydroxy linalool oxide	995	t							
α-Phellandrene	995	t	6.9	0.4	0.1	0.5	0.1	0.2	0.1
δ-3-Carene	1000			0.1		0.1			
Benzene acetaldehyde	1002		t						
α-Terpinene	1002	0.2	t	4.7	t	2.2	1.0	3.8	0.6
p-Cymene	1003	0.3	11.5	14.1	1.9	12.7	1.5	0.9	0.4
1,8-Cineole	1005	5.6			59.1		21.3		49.4
β-Phellandrene	1005		1.9	0.2		0.4		0.4	
Limonene	1009	0.1	16.6	0.5	1.1	0.5	0.9	1.5	1.6
cis-β-Ocimene	1017		1.0	1.6				t	t
trans-β-Ocimene	1027			0.4		t		0.9	0.6
γ-Terpinene	1035	0.4	0.1	49.1	0.1	6.1	1.6	6.3	1.1
Camphenilone	1036						0.2		
trans-Sabinene hydrate	1037			t		t		4.2	0.4
cis-Linalool oxide	1045	t					1.2		
Fenchone	1050		6.3						
trans-Linalool oxide	1059						0.4		
p-Cymenene	1059	t					0.4		
2,5-Dimethyl styrene	1059					t			
6-Methyl-3,5-heptadien-2-one	1064	t							
p-Mentha-2,4(8)-diene	1064	t							
Terpinolene	1064			t		0.2	0.3	1.3	0.2
cis-Sabinene hydrate	1066			t		t		7.3	0.1
n-Nonanal	1073	0.1					0.1		
α-Thujone	1074				1.1				
Linalool	1074	0.5		t	0.2	0.8	3.5	0.1	2.2
1,3,8-p-Menthatriene	1074	t							
β-Thujone	1081				2.1				
trans-p-2-Menthen-1-ol	1095	t		t		t		0.7	
α-Campholenal	1098				t		0.8		0.1
trans-Sabinol	1101	t							
Camphor	1102		0.1		5.7		8.1	0.3	5.8
cis-Sabinol	1102				t				
trans-Pinocarveol	1106						0.8	0.2	0.1
cis-p-2-Menthen-1-ol	1110					t			
allo-Ocimene	1110		0.1	t					
cis-Verbenol	1110	t					2.3	0.6	
Sabina ketone*	1114								t
trans-Verbenol	1114		0.1					1.7	t
trans-Pinocamphone	1116				0.2		0.1		
Menthone	1120		t						
Pinocarvone	1121						0.3		t
Isomenthone	1126		t						
Nerol oxide	1127	t							
Mentha-1,5-dien-8-ol	1134	0.4							
δ-Terpineol	1134				0.9				3.1
Borneol	1134			0.1	0.9	0.2	13.3	20.2	3.1
Thuj-3-en-10-al	1144	t							
Terpinen-4-ol	1148	1.1	0.1	0.4	0.5	0.8	3.4	13.1	2.4
Myrtenal	1153	t					0.6	t	t
cis-Dihydrocarvone	1159							0.2	

Components	RI[a]	*Dv*	*Fv*	*Ov*	*So*	*Tc*	*Thcamp*	*Thc*	*Thm*
α-Terpineol	1159	0.4	1.3	t	1.5	0.1	0.5	0.8	3.4
Methyl chavicol	1163		0.5						
trans-Dihydrocarvone	1164							0.1	
Verbenone	1164						1.0	0.1	
Myrtenol	1168	t			0.1		0.4	0.1	t
trans-Carveol	1189						0.6	0.1	
Borneol formate	1199						0.1	0.1	t
Cuminaldehyde	1200				t				
Thymol methyl ether	1208	t							
Carvone	1210			0.9		t	0.2		
Pulegone	1210		t						
cis-Anethole	1220		t						
Carvacrol methyl ether	1224			0.9					
Geraniol	1236	0.1				t			
Geranial	1240					t			
Linalyl acetate	1245				0.1				
trans-Anethole	1254		11.8		t				
Thymol formate	1262					t			
p-Cymen-7-ol	1265	0.1							
Bornyl acetate	1265	0.1			0.4		0.4	4.1	0.1
Thymol	1275	t		14.7	0.1	0.1	0.2	0.1	
Carvacrol	1286	t	1.6	0.1	1.0	68.1		t	
trans-Sabinene hydrate acetate	1286							t	
cis-Theaspirane	1286	0.2							
trans-Theaspirane	1300	0.1							
Hexyl tiglate	1316	t							
Eugenol	1327	t							
α-Terpenyl acetate	1334				0.5			0.4	
α-Cubebene	1345	0.1		t					
Carvacrol acetate	1348					t			
cis-β-Damascenone	1356	0.1							
α-Ylangene	1371	0.5							
α-Copaene	1375	0.7		t	0.1				
β-Bourbonene	1379			0.1				t	
Isogermacrene D	1385	t							
β-Elemene	1388			t					
7-*epi*-Sesquithujene	1406	t							
trans-β-Caryophyllene	1414			1.7	0.9	1.1		0.4	0.5
β-Copaene	1426			t					
allo-Aromadendrene	1428	0.6			0.1				
trans-α-Bergamotene	1434			0.1		t			
Borneol butyrate	1451						0.1		
Thymol isobutyric ester	1465	0.1							
γ-Muurolene	1469	1.2		t					
α-Amorphene	1469	0.6							
Germacrene-D	1474			0.7				0.1	t
cis-β-Guaiene	1478	0.9							
trans-Muurola-4(14),5-diene*	1479	0.3		0.5					
Bicyclogermacrene	1487			t					
Viridiflorene	1487			0.1					
α-Muurolene	1494	1.3		0.1	0.3	t			t
Borneol 2-methyl butyrate	1495						0.2		

Components	RI[a]	Dv	Fv	Ov	So	Tc	Thcamp	Thc	Thm
β-Bisabolene	1500			1.3		0.1			
γ-Cadinene	1500	3.7					0.7		
trans-Calamenene	1505	0.3			t				
δ-Cadinene	1505	5.7		0.2	0.1				
α-Calacorene	1525	0.4							
Elemol	1530							1.1	0.5
trans-α-Bisabolene	1536					0.1			
Geranyl butyrate	1544								0.2
trans-Nerolidol	1549	8.4							
Spathulenol	1551								0.1
β-Caryophyllene oxide	1561			0.2	0.6	0.1			
Globulol	1566				0.7		1.0		
Viridiflorol	1569						0.5	0.2	
β-Oplopenone*	1576	7.2							
10-epi-γ-Eudesmol	1593	4.2							
γ-Eudesmol	1609							0.1	0.1
τ-Cadinol	1616	5.5					1.2		0.1
α-Muurolol	1618	1.1							
β-Eudesmol	1620							0.1	0.2
Intermedeol	1626	1.0		t					
α-Cadinol	1626	5.3							
α-Eudesmol	1634							0.1	t
3-Methoxy cummin alcohol isobutyric ester	1678	1.6							
3-Methoxy cummin alcohol isovaleric ester	1759	0.1							
Rosadiene*	1993					t			
Abietatriene	2027					t		0.1	
% Identification		62.5	99.7	99.6	97.1	99.9	95.0	99.6	99.7
Grouped Components									
Monoterpene hydrocarbons		1.7	77.9	76.5	19.9	28.3	31.3	42.9	27.8
Oxygen-containing monoterpenes		11.3	9.5	17.1	74.4	70.1	60.2	54.5	70.4
Sesquiterpene hydrocarbons		16.3		4.8	1.5	1.3	0.7	0.5	0.5
Oxygen-containing sesquiterpenes		32.7		0.2	1.3	0.1	2.7	1.6	1.0
Diterpenes						t		0.1	
Phenylpropanoids		t	12.3		t				
Fatty acids		t							
C13 Compounds		0.4							
Others		0.1	t	1.0	t	0.1	0.1		t
Yield (% w/w, dw)		0.4	0.2	1.8	1.3	3.9	1.3	3.1	6.3

RI[a], Calculated retention index relative to C_9-C_{21} n-alkanes on the DB1 column; t, trace (<0.05%); UI = unidentified compounds.

*identification based on mass spectra only.

Table 2. IC$_{50}$ values of the essential oils assessed for free radical scavenging, anti-inflammatory and antiacetylcholinesterase activities.

Plant species	IC$_{50}$ (µg/mL)[*]		
	DPPH	5-Lipoxygenase inhibition	Acetylcholinesterase inhibition
Dittrichia viscosa	1011.2±12.2[e]	291.2±22.0[de]	916.9±40.9[b]
Foeniculum vulgare	2342.0±101.6[c]	67.7±2.3[f]	215.0±50.4[a]
Origanum vulgare	156.3±5.1[f]	264.2±20.7[def]	699.3±14.7[c]
Salvia officinalis	2020.9±49.8[d]	827.9±60.6[b]	50.8±3.8[d]
Thymbra capitata	61.1±1.9[f]	93.3±10.5[ef]	51.9±0.9[d]
Thymus camphoratus	1794.0±53.8[d]	334.3±43.6[cd]	137.1±1.6[d]
Thymus carnosus	3904.2±84.1[b]	544.3±64.5[c]	721.7±33.9[c]
Thymus mastichina	6706.8±173.7[a]	1084.5±146.1[a]	45.8±4.1[d]
BHT	13.0±1.0[g]	nd	nd
NDGA	nd	63.7±2.3[f]	nd
Galanthamine	nd	nd	8.6[e]

[*]Results are given as mean ± standard deviation of three different experiments. Values with different letters in the same experiment and same column are significantly different. p≤0.05.
nd: not determined

Table 3. Decoction waters extracts yield (%), phenol content (mg/mL) and free radical scavenging, anti-inflammatory and superoxide anion scavenging activities given as IC$_{50}$ (µg/mL).

Plant species	Extraction yield	Phenols content	DPPH	5-Lipoxygenase inhibition	Superoxide anion
	(%)	(mg/mL)[a]	IC$_{50}$ (µg/mL)[*]		
Dittrichia viscosa	29.4	14.3±0.6[de]	4.0±0.0[d]	41.9±0.4[c]	7.9±0.4[ef]
Foeniculum vulgare	17.1	5.3±0.0[f]	3.7±0.0[f]	27.4±0.6[g]	18.8±1.0[b]
Origanum vulgare	26.9	45.2±3.2[a]	2.8±0.0[i]	37.6±0.3[e]	20.4±1.0[b]
Salvia officinalis	35.6	34.9±0.3[b]	3.8±0.0[e]	45.2±0.5[b]	9.7±2.0[ef]
Thymbra capitata	40.8	11.0±0.9[e]	4.4±0.0[c]	40.0±0.2[d]	6.4±0.2[f]
Thymus camphoratus	15.6	19.6±1.6[cd]	3.5±0.0[g]	33.1±0.3[f]	13.5±1.0[cd]
Thymus carnosus	24.3	16.6±0.9[d]	2.9±0.0[h]	41.7±0.2[c]	10.1±0.3[de]
Thymus mastichina	41.1	22.9±2.1[e]	4.2±0.0[b]	66.7±0.6[a]	14.8±1.0[c]
BHT			13.0±1.0[a]	nd	nd
NDGA			nd	63.7±2.3[a]	nd
Gallic acid			nd	nd	35.0±1.1[a]

[*]Results are given as mean±standard deviation of three different experiments. Values with different letters in the same experiment and same column are significantly different, *p*≤0.05.
nd: not determined

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