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Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae)

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Abstract

This study was designed to examine the in vitro antimicrobial and antioxidant activities of the essential oil and various extracts (prepared by using solvents of varying polarity) of *Salvia tomentosa* (Miller). The essential oil was particularly found to possess strong antimicrobial activity while other non-polar extracts and subfractions showed moderate activities while polar extracts remained almost inactive. GC and GC/MS analyses of the oil resulted in the identification of 44 compounds, representing 97.7% of the oil; β-pinene (39.7%), α-pinene (10.9%) and camphor (9.7%) were the main components. The samples were also subjected to screening for their possible antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene-linoleic acid assays. In the first case, the free radical scavenging activity of aqueous methanol extract (MW) was superior to all other extracts ($IC_{50} = 18.7$ μg/ml). Polar extracts exhibited stronger activities than non-polar extracts. In the case of the linoleic acid system, oxidation of the linoleic acid was effectively inhibited by the polar subfraction of the MW extract, while the oil was less effective. The MW extract showed 90.6% inhibition, that is close to the synthetic antioxidant BHT.

Keywords: *Salvia tomentosa*; Essential oil; Antimicrobial activity; Antioxidant activity; GC-MS

1. Introduction

Essential oils and extracts obtained from many plants have recently gained popularity and scientific interest. Many plants have been used for different purposes, such as food, drugs and perfumery (Heath, 1981). Researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics (Essawi & Srour, 2000).

Plant products are also known to possess potential for food preservation (Baratta et al., 1998a; Baratta, Dorman, Deans, Biondi, & Ruberto, 1998b; Deans, 1991; Deans & Ritchie, 1987; Halendar et al., 1998). Oxidation of lipids, which occurs during raw material storage, processing, heat treatment and further storage of final products, is one of the basic processes causing rancidity of food products, leading to their deterioration. Due to undesirable influences of oxidized lipids on the human organism, it seems to be essential to decrease contact with products of lipid oxidation in food (Karpinska, Borowski, & Danowska-Oziewicz, 2001). In order to prolong the storage stability of foods, synthetic antioxidants are used for industrial processing. But, according to toxicologists and nutritionists, the side effects of some synthetic antioxidants used in food processing such as, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have already been documented. For example, these substances can show carcinogenic effects in living organisms (Ames, 1983; Baardseth, 1989). From this point of view, governmental authorities and consumers are concerned about the

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safety of their food and about the potential effects of synthetic additives on health (Reische, Lillard, & Eintenmiller, 1998).

Salvia, the largest genus of Lamiaceae, includes about 900 species, widespread throughout the world. This genus is represented, in Turkish flora, by 88 species and 93 taxa, 45 of which are endemic (Guner, Ozhatay, Ekim, & Baser, 2000). Some members of this genus are of economic importance since they have been used as flavouring agents in perfumery and cosmetics. Sage (*S. officinalis*) has been credited with a long list of medicinal uses: e.g. spasmolytic, antiseptic, astringent (Newall, Anderson, & Philipson, 1996). Some of the phenolic compounds of plants belonging to this genus have also shown excellent antimicrobial activity, as well as scavenging activity of active oxygen, as in superoxide anion radicals, hydroxyl radicals and singlet oxygen (Masaki, Sakaki, Atsumi, & Sakurai, 1995), inhibiting lipid peroxidation (Hohmann et al., 1999), consequently, the corresponding extracts have been widely used to stabilize fat and fat-containing foods (Ternes & Schwarz, 1995).

Despite the medicinal potential of plants in Turkey being considerable, knowledge of this area and studies on these plants are scarce (Digrak, Alma, & Ilcim, 2001). As far as our literature survey could ascertain, antimicrobial activities of *S. tomentosa* have been previously published (Haznedaroglu, Karabay, & Zeybek, 2001) on limited number of microorganisms, but no information is available on the antioxidative nature of this plant. The aim of the present study was to investigate the antimicrobial and antioxidant potential of the essential oil and various extracts of *S. tomentosa*, as well as to establish the best procedure to obtain extracts containing active principles.

2. Materials and methods

2.1. Collection of plant material

S. tomentosa was collected from Söğütlügöl plateau (1000 m), Düziçi-Osmaniye, Turkey, when flowering (late July, 2001). The voucher specimen was identified by Dr. Erol Donmez at the Department of Biology, Cumhuriyet University, Sivas-Turkey and deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas-Turkey (CUFH-Voucher no: ED 6363).

2.2. Isolation of the essential oil

A portion (100 g) of the aerial parts of *S. tomentosa* was submitted for 3 h to water-distillation, using a Clevenger-type apparatus (British type) (yield 0.51% v/w). The obtained essential oil (EO) was dried over anhydrous sodium sulphate and, after filtration, stored at +4 °C until tested and analysed.

2.3. Preparation of the extracts

2.3.1. General

Extracts of air-dried and ground plant materials were prepared by using solvents of varying polarity and the extraction protocol of each is given below:

2.3.2. Preparation of the deodorized hot water extract

After completion of hydro-distillation, the liquid retentate was collected and lyophilized. This procedure gave deodorized hot water extract (DeHW) in a yield of 21.09% (w/w) (Dapkevicius, Venskutonis, Van Beek, & Linssen, 1998).

2.3.3. Preparation of the deodorized methanol extract

The solid retentate of the hydrodistillation was dried and re-extracted with methanol. The resulting extract (DeMeOH) (11.57%, w/w) was fractionated with water and chloroform ($CHCl_3$) to give deodorized water-soluble (DeMW) (2.62%, w/w) and water-insoluble (deodorized chloroformic) (DeMC) (7.47%, w/w) subfractions (Dapkevicius et al., 1998).

2.3.4. Preparation of the hexane, dichloromethane and methanol extracts

A portion (100 g) of dried plant material was extracted with hexane (HE) (5.36%, w/w), followed by dichloromethane (DCM) (1.67%, w/w) and methanol (MeOH) (7.83%, w/w) in a Soxhlet apparatus (6 h for each solvent). The latter extract was suspended in water and partitioned with chloroform ($CHCl_3$) to obtain water soluble (MW) (6.01%, w/w) and water insoluble (MC) (1.42%, w/w) subtractions. (Sokmen, Jones, & Erturk, 1999).

All extracts obtained were lyophilized and kept in the dark at +4 °C until used.

2.4. Gas chromatography/mass spectrometry analysis conditions

2.4.1. Gas chromatography analysis

The essential oil was analysed using a Hewlett Packard 5890 II GC equipped with a FID detector and HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Injector and detector temperatures were set at 220 and 290 °C, respectively. Oven temperature was kept at 50 °C for 3 min, then gradually raised to 160 °C at 3 °C/min, held for 10 min and finally raised to 240 °C at 3 °C/min. Helium was the carrier gas, at a flow rate of 1 ml/min. Diluted samples (1/100 in acetone, v/v) of 1.0 μl were injected manually and in the splitless mode. Quantitative data were obtained electronically from FID area percent data without the use of correction factors.

2.4.2. Gas chromatography/mass spectrometry analysis

Analysis of the oils was performed using a Hewlett Packard 5890 II GC, equipped with a HP 5972 mass selective detector and a HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). For GC/MS detection, an electron ionization system, with ionization energy of 70 eV, was used. Helium was the carrier gas, at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. Oven programme temperature was the same with GC analysis. Diluted samples (1/100 in acetone, v/v) of 1.0 μl were injected manually and in the splitless mode. The components were identified by comparison of their relative retention times and mass spectra with those of standards (for the main components), NBS75K library data of the GC/MS system and literature data, as described by Adams (2001). The results were also confirmed by comparison of the compounds elution order with their relative retention indices on non-polar phases as reported by Adams (2001).

2.5. Antimicrobial activity

2.5.1. Microbial strains

The essential oil and extracts were individually tested against a panel of microorganisms, including *Staphylococcus aureus* ATCC 25923 and ATCC 29213 (for minimum inhibitory concentration (MIC) test), S*treptococcus pneumoniae* ATCC 49619, *Moraxella catarrhalis* ATCC 49143, *Bacillus cereus* ATCC 11778, *Acinetobacter lwoffii* ATCC 19002, *Enterobacter aerogenes* ATCC 13043, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 7002, *Pseudomonas aeruginosa* ATCC 27853, *Clostridium perfringens* KÜKENS-Turkey, mycobacter, *Mycobacterium smegmatis* CMM 2067, *Candida albicans* ATCC 10239 and *C. krusei* ATCC 6258. Bacterial strains were cultured overnight at 37 °C in Mueller Hinton agar (MHA), with the exception of *S. pneumoniae* (MHA containing 50 ml citrate blood/l) and *C. perfringens* (in anaerobic conditions). Yeasts were cultured overnight at 30 °C in Sabouraud dextrose agar.

2.5.2. Antimicrobial screening

Two different methods were employed for the determination of antimicrobial activities: agar well-diffusion method for the extracts and disc diffusion method for the essential oil (NCCLS, 1999). The MICs of the essential oil against the test microorganisms were determined by the broth microdilution method (NCCLS, 1997). The MICs of netilmicin and amphotericin B were also determined in parallel experiments in order to control the sensitivity of the test microorganisms. All tests were performed in duplicate.

2.5.3. Agar-well diffusion method

In the agar-well diffusion method, all extracts were weighed and dissolved in phosphate buffer saline (PBS; pH 7.0–7.2) and dimethylsulphoxide (DMSO), respectively, 10 mg/ml, followed by sterilization using a 0.45 μm membrane filter. Each microorganism was suspended in sterile saline and diluted at $\approx 10^6$ colony forming unit (cfu) per ml. They were "flood-inoculated" onto the surface of MHA. The wells (8 mm in diameter) were cut from the agar and 0.06 ml of extract solution was delivered into them. After incubation for 24 h at 37 °C, all plates were examined for any zones of growth inhibition, and the diameters of these zones were measured in millimetres. All tests were performed in duplicate.

2.5.4. Disc diffusion method

The agar disc diffusion method was employed for the determination of antimicrobial activities of the essential oil in question (NCCLS, 1997). Briefly, a suspension of the tested microorganism (0.1 ml of 10^8 cells per ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 15 μl of the oil and placed on the inoculated plates. These plates, after remaining at 4 °C for 2 h, were incubated at 37 °C for 24 h for bacteria and, at 30 °C for 48 h, for yeasts. The diameters of the inhibition zones were measured in millimetres. All tests were performed in duplicate.

2.5.5. Determination of minimum inhibitory concentration

A broth microdilution broth susceptibility assay was used, as recommended by NCCLS, for the determination of the MIC (NCCLS, 1999). All tests were performed in Mueller Hinton Broth (MHB; BBL) supplemented with Tween 80 detergent (final concentration of 0.5% (v/v), with the exception of the yeasts (Sabouraud dextrose broth-SDB + Tween 80). Bacterial strains were cultured overnight at 37 °C in MHA and the yeasts were cultured overnight at 30 °C in SDB. Test strains were suspended in MHB to give a final density of 5×10^5 cfu/ml and these were confirmed by viable counts. Geometric dilutions, ranging from 0.036 to 72.0 mg/ml of the essential oil, were prepared in a 96-well microtitre plate, including one growth control (MHB + Tween 80) and one sterility control (MHB + Tween 80 + test oil). Plates were incubated under normal atmospheric conditions, at 37 °C for 24 h for bacteria, and at 30 °C for 48 h for yeasts. The bacterial growth was indicated by the presence of a white "pellet" on the well bottom.

2.6. Antioxidant activity

2.6.1. DPPH assay

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds

were measured from the bleaching of the purple-coloured methanol solution of 2,2′-diphenylpicrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent (Burits & Bucar, 2000; Cuendet, Hostettmann, & Potterat, 1997). Fifty microlitre of various concentrations of the extracts in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent ($I\%$) was calculated in following way:

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

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated form the plot of inhibition percentage against extract concentration. Tests were carried out in triplicate.

2.6.2. DPPH assay on TLC

This procedure was applied for all extracts and the essential oil. Five microlitre of a 1:10 dilution of the extracts in methanol were applied to the TLC plate and methanol–ethyl acetate (1:1) mixture was used as developer. The plate was sprayed with a 0.2% DPPH reagent in methanol and left at room temperature for 30 min. As explained above, yellow spots formed from bleaching of the purple colour of DPPH reagent, were evaluated as positive antioxidant activity.

2.6.3. β-Carotene-linoleic acid assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius et al., 1998). A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade) and 25 μl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml distilled water saturated with oxygen (30 min 100 ml/min) were added with vigorous shaking. Two thousand five hundred microlitres of this reaction mixture were dispensed into test tubes and 350 μl portions of the extracts, prepared at 2 g/l concentrations, were added and the emulsion system was incubated for 48 h at room temperature. The same procedure was repeated with synthetic antioxidant, BHT, as positive control, and a blank. After this incubation period, absorbances of the mixtures were measured at 490 nm. Antioxidative capacities of the extracts were compared with those of BHT and blank.

2.7. Assay for total phenolics

Total phenolic constituents of the aforesaid extracts of *S. tomentosa* were determined by the literature methods involving the Folin–Ciocalteu reagent and gallic acid as standard (Chandler & Dodds, 1983; Slinkard & Singleton, 1977). 0.1 ml of extract solution, containing 1000 μg extract, was taken in a volumetric flask, 46 ml distilled water and 1 ml Folin-Ciocalteu reagent were added, and flask was shaken thoroughly. After 3 min, 3 ml of a solution of 2% Na_2CO_3 were added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated for all standard gallic acid solutions and a standard curve was obtained by the equation given below:

$$\text{Absorbance} : 0.0012 \times \text{Gallic acid } (\mu g) + 0.0033.$$

3. Results and discussion

3.1. Chemical composition of the essential oils

About 44 compounds, representing 97.7% of the oil, were identified. GC and GC/MS analyses revealed that the major constituents of the oil were β-pinene (39.7%), α-pinene (10.9%) and camphor (9.7%) as listed in Table 1.

To the best of our knowledge, there are many reports on the chemical composition of the oils isolated from the plants belonging to the genus *Salvia* (Ahmadi & Mirza, 1999; Baser, Ozek, Kirimer, & Tumen, 1993; Baser, Beis, & Ozek, 1995a; Baser, Kurkcuoglu, Ozek, & Sarikardasoglu, 1995b; Baser, Demircakmak, & Ermin, 1996; Baser, Duman, Vural, Adiguzel, & Aytac, 1997; Baser, Kurkcuoglu, & Aytac, 1998; Couladis, Tzakou, Stojanovic, Mimica-Dukic, & Jancic, 2001; Perry et al., 1999; Rustaiyan, Masoudi, Monfared, & Komeilizadeh, 1999; Sefidkon & Khajavi, 1999; Torres, Velasco-Negueruela, Perez-Alonso, & Pinilla, 1997; Tumen, Baser, Kurkcuoglu, & Duman, 1998). Most of these reports indicate that 1,8-cineole (eucalyptol) and borneol are the main and/or characteristic constituents of *Salvia* oils.

According to a study carried out by Haznedaroglu et al. (2001), cyclofenchene, 1,8-cineole, borneol and β-caryophyllene were the major constituents of *S. tomentosa* oil. These findings are not in agreement with the results presented here, except for borneol, which was found to be 4.3% in our study. The changes in the essential oil compositions might have arisen from several differences (climatical, seasonal, geographical, geological), as mentioned by Perry et al. (1999).

Table 1
Chemical composition of *S. tomentosa* essential oil (%)

	Compounds[a]	Rt.[b] (min)	KI[c]	%
1	α-Thujene	9.491	930	0.7
2	α-Pinene	9.781	939	10.9
3	Camphene	10.407	954	2.4
4	β-Pinene	11.931	979	39.7
5	β-Myrcene	12.475	991	0.7
6	α-Terpinene	13.645	1017	0.4
7	p-Cymene	14.071	1025	1.1
8	Limonene	14.262	1029	2.2
9	Eucalyptol	14.344	1031	1.1
10	(Z)-β-Ocimene	14.761	1037	0.7
11	(E)-β-Ocimene	15.269	1050	0.2
12	γ-Terpinene	15.758	1060	1.4
13	*cis*-Sabinene hydrate	16.221	1070	0.4
14	Terpinolene	17.228	1089	0.5
15	Linalool	17.917	1097	0.9
16	*trans*-Sabinene hydrate	18.960	1098	0.2
17	Camphor	20.185	1146	9.7
18	Borneol	21.291	1169	4.3
19	Terpinen-4-ol	21.763	1177	0.7
20	α-Terpineol	22.434	1189	0.3
21	Linalyl acetate	25.518	1257	0.3
22	Bornyl acetate	26.951	1289	0.5
23	Thymol	27.486	1290	0.7
24	Carvacrol	27.930	1299	0.9
25	α-Cubebene	29.935	1351	0.5
26	α-Ylangene	30.942	1375	0.3
27	α-Copaene	31.159	1377	0.4
28	β-Bourbonene	31.577	1388	0.8
29	β-Cubebene	31.794	1388	0.2
30	α-Gurjunene	32.674	1410	0.3
31	Caryophyllene	33.155	1419	2.3
32	β-Copaene	33.545	1432	0.6
33	Aromadendrene	33.980	1441	0.3
34	α-Caryophyllene	34.660	1455	1.9
35	Allo-Aromadendrene	34.933	1460	0.2
36	γ-Muurolene	35.649	1480	1.5
37	Germacrene D	35.821	1485	0.6
38	β-Selinene	36.066	1490	0.4
39	γ-Amorphene	36.420	1496	1.2
40	γ-Cadinene	37.245	1514	0.5
41	δ-Cadinene	37.626	1523	1.4
42	Ledol	39.495	1569	0.7
43	(-)-Spathulenol	39.912	1578	0.4
44	Viridiflorol	40.583	1593	2.3
	Total			97.7

[a] Compounds listed in order of elution from a HP-5 MS column.
[b] Retention time (as minutes).
[c] Kovats Index on DB-5 column in reference to *n*-alkanes (Adams, 2001).

3.2. Antimicrobial activity

As can be seen in Table 2, water-soluble extracts (DeHW and DeMW) did not exhibit antimicrobial activity, but water-insoluble extracts were found to have moderate activity against *S. aureus*, *S. pneumoniae*, *M. catarrhalis*, *B. cereus*, *A. lwoffii*, *C. perfringens*, *M. smegmatis* and *C. albicans*. Except for four test microorganisms (*M. catarrhalis*, *B. cereus*, *A. lwoffii* and C. albicans), the essential oil of *S. tomentosa* exhibited stronger antimicrobial activity against the microorganisms than those of extracts. In general, weaker activity was observed against gram-negative ones.

Results obtained from disc diffusion method, followed by measurements of MIC, indicate that *C. perfringens*, an anaerobic microorganism, is the most sensitive microorganism tested, with the lowest MIC values (0.54 mg/ml) in the presence of the oil isolated from *S. tomentosa* (Table 3). *S. pneumoniae* and *M. smegmatis* were other sensitive ones against the oil with an MIC value at 2.25 mg/ml. No activity was observed against three gram-negative microorganisms (*E. coli*, *P. mirabilis* and *P. aeruginosa*).

As far as our literature survey could ascertain, there was only one report on the antibacterial activity of the essential oil of *S. tomentosa*. As far as this report is concerned, weak antibacterial activity was observed against microorganisms including *E. coli*, *S. aureus* and *P. aeruginosa* (Haznedaroglu et al., 2001). The antimicrobial activity of the major compounds of the oil studied here was previously well defined by several researchers (Dorman & Deans, 2000; Knobloch, Pauli, Iberi, Wegand, & Weis, 1989; Tabanca, Kirimer, Demirci, Demirci, & Baser, 2001; Vardar-Unlu et al., 2003). Based on one report, pinene-type monoterpene hydrocarbons (α-pinene and β-pinene) and borneol (oxygenated monoterpene) had slight activity against a panel of microorganisms (Dorman & Deans, 2000). Antimicrobial activity of borneol was also reported by other investigators (Knobloch et al., 1989; Tabanca et al., 2001; Vardar-Unlu et al., 2003). On the other hand, camphor is also known to possess slight antifungal (Alvarez-Castellanos, Bishop, & Pascual-Villalobos, 2001) and antibacterial activity (Demetzos, Angelopoulou, & Perdetzoglou, 2002). Despite slight activity capacities, pinene-type monoterpenes, camphor and borneol, could be responsible for the total activity spectrum. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds (Cowan, 1999). Moreover, moderate activities of water-insoluble extracts could also be attributed to the presence of several types of compounds belonging to different classes, such as oleoresins in hexane extract (HE) (Dapkevicius et al., 1998), sterols and their derivatives, flavones and flavonoids in dichloromethane extract (Guillen & Manzanos, 1998), and more polar thermolabile and/or thermo-stable phenolics in the hydrophobic subfractions of methanol extract (Sokmen et al., 1999).

3.3. Antioxidant activity

The essential oil and various extracts were subjected to screening for their possible antioxidant activity. Two

Table 2
Antimicrobial activity of the various extracts of *S. tomentosa* using agar well diffusion method[a]

Microorganisms	Extracts						
			MeOH			DeMeOH	
	HE	DCM	MC	MW	DeHW	DeMC	DeMW
Staphylococcus aureus	12.50	NA	NA	NA	NA	11.50	NA
Streptococcus pneumoniae	17.00	13.00	12.00	NA	NA	19.50	NA
Moraxella catarrhalis	11.00	NA	NA	NA	NA	NA	NA
Bacillus cereus	14.50	10.50	NA	NA	NA	13.00	NA
Acinetobacter lwoffii	9.50	15.00	10.50	NA	NA	14.00	NA
Enterobacter aerogenes	NA	NA	NA	NA	NA	NA	NA
Escherichia coli	NA	NA	NA	NA	NA	NA	NA
Klebsiella pneumoniae	NA	NA	NA	NA	NA	NA	NA
Proteus mirabilis	NA	NA	NA	NA	NA	NA	NA
Pseudomonas aeruginosa	NA	NA	NA	NA	NA	NA	NA
Clostridium perfringens	13.00	12.50	9.00	NA	NA	14.50	NA
Mycobacterium smegmatis	14.00	NA	NA	NA	NA	14.50	NA
Candida albicans	14.50	13.50	11.50	NA	NA	12.00	NA
Candida krusei	NA	NA	NA	NA	NA	NA	NA

NA, not active.
[a] Diameter of inhibition zone (mm) including well diameter of 8 mm.

Table 3
Antimicrobial activity of the essential oil of *S. tomentosa* using agar disc diffusion and MIC methods

Microorganisms	Essential oil		The MIC's of the antibiotics[c]	
	DD[a]	MIC[b]	NET	AMP B
Staphylococcus aureus	14.75	18.00	8×10^{-3}	NT
Streptococcus pneumoniae	18.00	2.25	NT[d]	NT
Moraxella catarrhalis	7.25	72.00	NT	NT
Bacillus cereus	11.00	9.00	NT	NT
Acinetobacter lwoffii	10.25	18.00	NT	NT
Enterobacter aerogenes	7.00	72.00	NT	NT
Escherichia coli	NA[e]	NA	1×10^{-2}	NT
Klebsiella pneumoniae	6.50	72.00	1×10^{-2}	NT
Proteus mirabilis	NA	NA	NT	NT
Pseudomonas aeruginosa	NA	NA	1×10^{-2}	NT
Clostridium perfringens	15.50	0.54	NT	NT
Mycobacterium smegmatis	18.75	2.25	NT	NT
Candida albicans	12.50	18.00	NT	1×10^{-3}
Candida krusei	12.50	36.00	NT	1×10^{-3}

[a] DD, agar disc diffusion method. Diameter of inhibition zone (mm) including disk diameter of 6 mm.
[b] MIC, minimum inhibitory concentration; values given as mg/ml for the essential oils and as μg/ml for antibiotics.
[c] NET, netilmycine; AMP B, amphotericin B.
[d] NT, not tested.
[e] NA, not active.

complementary test systems, namely DPPH free radical scavenging and beta carotene/linoleic acid systems. Free radical scavenging capacities of the extracts, measured by DPPH assay, are shown in Fig. 1. Since the reaction followed a concentration-dependent pattern, only concentrations of active extracts providing 50% inhibition were included in the table. The free radical scavenging activity of aqueous methanol extract (MW) was superior to all other extracts ($IC_{50} = 18.7$ μg/ml). Polar extracts exhibited stronger activity than non-polar extracts. When compared to BHT, the MW is the most effective radical scavenger. Activity should be related to its phenolic content since gallic acid equivalent of total phenolics was estimated as 200 ± 4.00 μg/mg dry weight extract (20%, w/w, see Table 4).

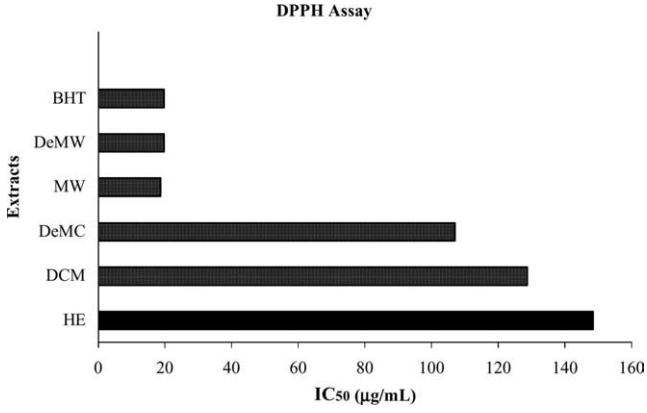

Fig. 1. Free radical scavenging capacities of the extracts measured in DPPH assay.

Table 4
Amounts of total phenolic compounds in *S. tomentosa* extracts

Extracts	Gallic acid equivalents (μg mg^{-1})
Control	–
The oil (EO)	–
HE	275[a] ± 3.06[b]
Dichloromethane extract (DCM)	51 ± 2.08
Methanol/H_2O extract (MW)	200 ± 4.00
Methanol/$CHCl_3$ extract (MC)	72 ± 2.05
Deodorized methanol/H_2O extract (DeMW)	150 ± 3.50
Deodorized methanol/$CHCl_3$ extract (DeMC)	10 ± 0.98
Deodorized hot water extract (DeHW)	149 ± 2.70

Results are given as mean ± standard deviation of three different experiments.
[a] Values are the means of three replicates.
[b] Standard deviation.

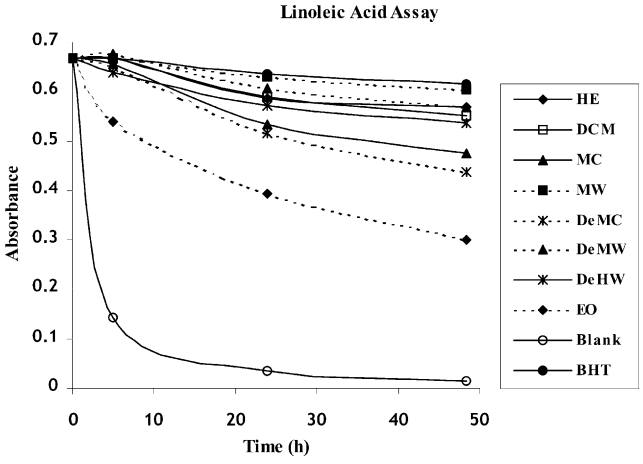

Fig. 2. Absorbance change of β-carotene at 490 nm in the presence of *S. tomentosa* extracts.

The extracts applied on silica gel TLC plates and main zones were determined for each extract. With DPPH reagent, at least three spots appeared immediately after spraying the TLC trace of MW extract. Although isolation and the structural determination have not yet been completed, preliminary results show that these components are polar phenolic acids or polyphenols.

In the linoleic acid system, oxidation of linoleic acid was effectively inhibited by the polar subfraction of MW extract (Fig. 2) while the oil was less effective. MW extract shows 90.6% inhibition, that is close to the synthetic antioxidant reagent BHT. Antioxidants minimize the oxidation of lipid components in cell membranes or inhibit the volatile organic compounds and the conjugated diene hydroperoxides, arising from linoleic acid oxidation, that are known to be carcinogenic. Polar extracts exhibited stronger activity than non-polar extracts, indicating that polyphenols or flavanones and flavonoids may also play important roles in the activity. Therefore, any extraction procedure is suitable for obtaining *S. tomentosa* active components inhibiting lipid oxidation.

3.4. Amount of total phenolics

Based on the absorbance values of the various extract solutions, reacting with Folin–Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents, as described above, results of the colorimetric analysis of total phenolics are given in Table 4. Total phenolics was highest in the HE (27.5%), followed by polar subfraction of methanol extract (20.0 %), polar subfraction of deodorized methanol extract (DeMW) and deodorized hot water extract (15.0% and 14.9%, respectively). The lowest amount of total phenolics was recorded in the non-polar subfraction of the deodorized methanol extract (1.0%).

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