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Original article:

CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OIL FROM WEDELIA PROSTRATA

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ABSTRACT

The following study deals with the chemical composition, antioxidant and antimicrobial activity of essential oils of *Wedelia prostrata* and their main constituents in vitro. A total of 70 components representing 99.26 % of the total oil were identified. The main compounds in the oil were limonene (11.38 %) and α-pinene (10.74 %). Antioxidant assays (1,1-diphenyl-2-picrylhydrazyl, superoxide anion radical, and reducing power test) demonstrate moderate activities for the essential oil and its main components (limonene and α-pinene). The essential oil (1000 µg/disc) exhibited promising antimicrobial activity against 10 strains of test microorganisms as a diameter of zones of inhibition (20.8 to 22.2 mm) and MIC values (125 to 250 µg/ml). The activities of limonene and α-pinene were also determined as main components of the oil. α-Pinene showed higher antimicrobial activity than the essential oil with a diameter of zones of inhibition (20.7 to 22.3 mm) and MIC values (62.5 to 125 µg/ml). The antioxidant and antimicrobial properties of the essential oil may be attributed to the synergistic effects of its diverse major and minor components.

Keywords: *Wedelia prostrata,* chemical composition, essential oil, antioxidant activity, antimicrobial activity

INTRODUCTION

Microorganisms and oxidation are the major causes of food deterioration. In particular, lipid peroxidation of food lipid components produced during the manufacturing process and food storage is the main cause food quality deterioration, leading to rancidity and changes in the taste, smell, and colour, and eventually the loss of food quality (Mau et al., 2004). The subsistence and growth of microorganisms in food may also lead to spoilage, toxin formation, and quality deterioration of food products (Celiktas et al., 2007). Moreover, the consumption of spoiled food encompass a wide spectrum of illnesses and is a growing public health problem worldwide.

For many years, different synthetic preservatives have been widely used as antioxidants and antimicrobial agents in the food industry to increase the storage and marketing shelf life of food. However, although synthetic preservatives have been proven highly effective and less expensive than natural substances, these compounds exhibit mutagenic activity against non-target organisms (Tripathi et al., 2007) and cause

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environmental pollution (Misra and Pavlostathis, 1997). The growing interest in the substitution of synthetic food preservatives has fostered research on the screening of new antioxidants and antimicrobial preservatives from natural sources (Bajpai et al., 2008). At present, interest in the effective use of essential oils from plants in food preservation has been increasing.

The genus *Wedelia* comprises approximately 60 species that are distributed in tropical and warm temperate regions, including India, Burma, Ceylon, China, and Japan (Li et al., 2007). Several species are used as folk medicine in many countries to treat a variety of diseases, such as headaches, fevers, infections, and pathologies of the respiratory tract (Li et al., 2007; Miles et al., 1990). Five of these species, namely, *Wedelia biflora*, *W. urticifolia*, *W. wallichii*, *W. prostrata*, and *W. chinensis*, are found and used as folk medicines in the southern provinces of China. *W. prostrata* is mainly distributed in tropical and subtropical areas in Asia. In traditional Chinese medicine, *W. prostrata* is used for the treatment of inflammation and ulcer.

A series of studies has demonstrated the potential medicinal effect of essential oils from various *Wedelia* species. For example, essential oil from *W. trilobata* leaves exhibited antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* (Nirmal et al., 2005); essential oil from *W. chinensis* was found effective against gram positive bacteria and fungi; and essential oils from *W. chinensis* and *W. biflora* exhibited significant antibacterial and antifungal activities when compared to that of standard ciproflacin (Sureshkumar et al., 2007).

To the best of our knowledge, no report on the phytochemical and biological studies of *W. prostrata* has been published so far. The aim of the current study is to determine the chemical composition of the essential oil of *W. prostrata* via gas chromatography-flame ionisation detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS), evaluate its antimicrobial activity against pathogens and the antioxidant activity.

MATERIALS AND METHODS

Plant material

W. prostrata plants were collected in the Danxia Mountains, Guangdong Province, China on June 2009, and identified by Dr. Xun Gong. The plants were dried in the shade (at room temperature). The voucher specimen (No. 499993) was deposited in the Kuming Institute of Botany, Chinese Academy of Sciences.

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH, 95 %), limonene, α-pinene, α-tocopherol, streptomycin, tetracycline, riboflavin, methionine, and nitro blue tetrazolium were purchased from Sigma-Aldrich (St. Louis, MI, USA). Potassium ferricyanide, trichloroacetic acid, methanol, and all other reagents were of analytical grade and were obtained from Jinhuada Chemical Reagent Co. (Guangzhou, China).

Isolation of the essential oil

The air-dried plant materials (500 g) of *W. prostrata* was chopped and subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The obtained oils were dried over sodium sulphate for 24 h, filtered, and then stored at 4° C in sealed brown glass vials until tested.

GC–FID analysis

An Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5 5 % phenylmethylsiloxane capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and equipped with an FID detector was used for the GC-FID analysis. Helium gas at a constant flow rate of 1 ml/min was used as the carrier gas. The injector and mass transfer line temperatures were set at 250 and 280 °C, respectively. The essential oil solution (1 μL) in hexane was injected and analysed under the following column conditions: initial column tem-

perature at 40 °C for 1 min, which was then increased to 250 °C at a 3 °C/min heating ramp, and then subsequently kept at 250 °C for 20 min.

GC–MS analysis

Quantitative and qualitative analysis of the essential oil was performed using a GC-MS 6890-5975 system (Agilent Technologies, Palo Alto, CA, USA) equipped with a HP-5 MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). For GC-MS detection, an electron ionisation system with a 70 eV ionisation energy was used. Helium gas was used as the carrier gas at a constant flow rate of 1 ml/min. The injector and mass transfer line temperatures were set at 250 and 280 °C, respectively. Essential oil solution (1 μL) in hexane was injected and then analysed under the following column conditions: initial column temperature at 40 °C for 1 min, which was then increased to 250 °C at a 3 °C/min heating ramp, and then subsequently kept at 250 °C for 20 min. The Kovats indices were calculated for all volatile components using a homologous series of n-alkanes (C_8–C_{25}) on the HP-5 MS column. The major oil components were identified via coinjection with standards (whenever possible) and confirmed through the Kovats indices using the Wiley (V.7.0) and National Institute of Standards and Technology (NIST) V.2.0 GC–MS library. The relative concentration of each compound in the essential oil was quantified based on the peak area integrated in the analysis program.

Antioxidant activity determination

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

0.1 ml of 25, 50, 75 and 100 μg/ml essential oil and its main components (limonene and α-pinene) were each mixed with 1 ml of 0.2 mM DPPH (dissolved in methanol). The reaction mixture was incubated for 20 min at 28 °C in a dark environment. The control solution, which contained all the reagents except the sample, was used as a blank. The DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm using a spectrophotometer and calculating using the following equation:

$$\text{DPPH scavenging effect \%} = (A_{control} - A_{sample}/A_{control}) \times 100$$

where $A_{control}$ and A_{sample} are the absorbance of the control sample and the test compound, respectively. The DPPH radical scavenging activity of α-tocopherol was also assayed for comparison.

Superoxide anion radical scavenging activity

All solutions were prepared in a 0.2 M phosphate buffer (pH 7.4). 0.1 ml of 25, 50, 75 and 100 μg/ml samples were each mixed with 3 ml of the reaction buffer solution (pH 7.4) containing 1.3 μM riboflavin, 0.02 M methionine, and 5.1 μM nitro blue tetrazolium. The reaction solution was illuminated by exposing them to two 30 W fluorescent lamps for 20 min, and the absorbance was measured at 560 nm. The reaction mixture without any test sample was used as the control. The superoxide anion radical scavenging activity (%) was calculated using the equation:

$$\text{Superoxide anion radical scavenging activity \%} = (A_{control} - A_{sample}/A_{control}) \times 100$$

The superoxide anion radical scavenging activity of α-tocopherol was also assayed for comparison.

Determination of the reducing power

0.1 ml of 25, 50, 75 and 100 μg/ml samples were each mixed with a phosphate buffer (2.5 ml, 0.2 M, pH 6.6), and potassium ferricyanide (2.5 ml, 1 %), and the resulting mixtures were incubated at 50 °C for 20 min. Trichloroacetic acid (2.5 ml, 10 %) was added to each sample, and the mixtures were centrifuged at 3000 r/min for 10 min. A 5 ml aliquot of the upper layer was mixed with distilled water (5 ml), followed by the addition of ferric chloride (1 ml, 0.1 %). The absorbance was then measured at 700 nm against a control that

consisted of all the reagents without the test sample. A higher absorbance would indicate higher reducing power. The reducing power of α-tocopherol was also determined for comparison.

Antimicrobial activity

Test microorganisms

The in vitro antimicrobial activities of the essential oil and its main components were evaluated against a panel that included laboratory control strains obtained from the China Centre for Type Culture Collection (CCTCC). These strains are

- two Gram-negative bacteria (*Pseudomonas aeruginosa* CCTCC AB93066 and *Escherichia coli* CCTCC AB91112),
- two Gram-positive bacteria (*Bacillus subtilis* CCTCC AB92068 and *Staphylococcus aureus* CCTCC AB91053),
- two yeast strains (*Hansenula anomala* CCTCC AY92046 and *Saccharomy cescerevisiae* CCTCC AY92042), and
- four moulds (*Aspergillus niger* CCTCC AF91004, *Chaetomium globosum* CCTCC AF 200039, *Mucor racemosus* CCTCC AF 93209, and *Monascus anka* CCTCC AF93208).

All strains were maintained on an agar slant at 4 °C. The bacterial strains were cultured in a Muller-Hinton broth (MHB) at 37 °C for 24 h, whereas the yeast strains were cultured on a Sabouraud dextrose agar (SDA) at 28 °C for 48 h. The fungal strains were cultured on SDA at 28 °C for 120 h prior to testing.

Inhibitory effect via the disc diffusion method

The disc diffusion method was used to determine the antimicrobial activities of the essential oils. Petri plates were prepared by pouring 20 ml MBH or SDA and allowing the solution to solidify. The plates were then dried, and 0.1 ml of the standardised inoculum containing 10^6 to 10^7 CFU/ml of the bacterial suspension was poured, uniformly spread, and allowed to dry for 5 min. A Whatman No. 1 sterile filter paper disc (6 mm diameter) was impregnated with 1000 µg/disc of the essential oils. Negative controls were prepared using the same solvent employed to dissolve the samples. The standard reference antibiotics, namely, streptomycin and tetracycline (10 µg/disc) were used as the positive controls for the test bacteria. The plates were incubated for bacteria at 37 °C for 24 h, for yeasts at 28 °C for 48 h, and for fungi at 28 °C for 120 h. The antimicrobial activity was evaluated by measuring the diameter of the zones of inhibition against the test organisms. The experiments were repeated in triplicate and the results are expressed as average values.

Determination of the minimum inhibitory concentration (MIC)

The MICs of the essential oils against the test microorganisms were determined using the broth microdilution method. Dilutions of the essential oils, ranging from 0.25 to 1000 µg/ml, were prepared in MHB or SDA. Exactly 0.5 MacFarland standard suspensions of the test microorganisms were inoculated in the tubes. A control test was also performed using inoculated broth or agar supplemented only with dimethyl sulphoxide under identical conditions, with streptomycin as the reference. The bacteria were incubated at 37 °C for 24 h, the yeast strains at 28 °C for 48 h, and fungi at 28 °C for 120 h.

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Statistical analysis

All tests were performed in triplicate, and the results were calculated as the mean ± SD.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The steam distillation of 500 g dried plant material yielded 2.8 ml (0.56 % v/w) greenish oil with a distinct smell. The oil sample was analysed via GC-FID and GC-MS, and the components were identified on the basis of their RI values as well as by comparison of their mass spectra with those reported in literature. The GC-MS analysis of the essential oil of *W. prostrata* indicated 70 components representing 99.26 % of the oil (Table 1). The composition of the essential oil was as follows: 49.03 % monoterpene hydrocarbon fraction, 26.52 % sesquiterpene hydrocarbon fraction, 4.77 % oxygenated monoterpene fraction, 6.15 % oxygenated sesquiterpenoid fraction, 7.20 % phenylpropanoids fraction and 5.59 % others. The main components in the oil were d-limonene (11.38 %) and α-pinene (10.74 %).

Previous reports (Craveiro et al., 1993; Koheil, 2000) on the *Wedelia* species showed that monoterpene hydrocarbons are the major compounds in their essential oils. The *W. paludosa* oils contain β-pinene (10.3 %), limonene (21.3 %), and γ-murolene (11.8 %), whereas the major components of the *W. trilobata* oils are α-phellandrene (17.4 %) and limonene (16.3 %) (Craveiro et al., 1993). The major components of the essential oils obtained from the flowerheads of *W. trilobata* are β-phellandrene (25.65 %), limonene (8.93 %), γ-terpinene (5.90 %), trans-β-caryophyllene (4.83 %) and α-pinene (4.72 %) (Koheil, 2000). The current results indicate that the essential oil of *W. prostrata* contains components relatively similar to those of other *Wedelia* species.

Antioxidant activity

Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation (Hatano et al., 1989). In addition, scavenging activity on DPPH radicals has been widely used to determine the free radical-scavenging activity. The DPPH radical scavenging activity can be reduced by the hydrogen donating ability (Prasad et al., 2005). Under oxidative stress, the concentration of superoxide radical can dramatically increase in all cells, thereby inducing several pathophysiological processes, because of its transformation into a more reactive species (Wickens, 2001). Therefore, the measurement of the comparative interceptive ability of antioxidant extracts by determining their ability to scavenge the superoxide radical has been proposed (Vani et al., 1997). The superoxide anion scavenging activity may be due to the action of a free hydroxyl group (Siddhuraju et al., 2002). The reducing power, which is associated with and may be a major indicator of antioxidant activity, is widely used to evaluate the antioxidant activity of polyphenols (Hsu et al., 2006). Most nonenzymatic antioxidant activity, such as the scavenging of free radicals and the inhibition of peroxidation, is mediated by redox reactions (Zhu et al., 2002).

The essential oil of *W. prostrata* and its main components (limonene and α-pinene) were screened for possible antioxidant activities using three different test systems, namely, the DPPH, superoxide anion, and reducing power assays. The essential oil and its main components exhibited moderate antioxidant activity at all the concentrations tested (Figures 1–3). The highest percentage of DPPH radical scavenging activity (88.1 %) and superoxide anion scavenging activity (86.2 %) and the highest absorbance of reducing power (0.92) were exhibited by the 100 µg/ml essential oil. The order of antioxidant activity was determined as α-tocopherol > limonene > essential oil > α-pinene.

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Table 1: Chemical composition of the essential oil of *Wedelia prostrata*

Peak no.	RI[a]	Components	% RA[b]	Identification Methods[c]
		Monoterpene hydrocarbons	49.03	
1	928	α-Thujene	0.52	MS, RI
2	936	α-Pinene	10.74	MS, RI, Co
3	949	Camphene	2.10	MS, RI
4	973	Sabinene	1.14	MS, RI
5	978	β-Pinene	3.23	MS, RI
6	986	β-Myrcene	1.43	MS, RI
7	998	α-Phellandrene	7.65	MS, RI, Co
8	1012	3-Carene	1.08	MS, RI
9	1030	Limonene	11.38	MS, RI, Co
10	1032	β-Phellandrene	4.22	MS, RI, Co
11	1040	(Z)-β-Ocimene	0.85	MS, RI
12	1051	(E)-β-Ocimene	0.34	MS, RI
13	1059	γ-Terpinene	4.35	MS, RI
		Oxygenated monoterpenes	4.77	
14	1078	(Z)-Linalool oxide	0.65	MS, RI
15	1099	Linalool	0.56	MS, RI
16	1137	trans-Pinocarveol	0.29	MS, RI
17	1168	Borneol	0.43	MS, RI
18	1178	Terpinene-4-ol	0.43	MS, RI
19	1191	α-Terpineol	1.26	MS, RI
20	1229	Nerol	0.34	MS, RI
21	1255	Geraniol	0.46	MS, RI
22	1270	Geranial	0.35	MS, RI
		Sesquiterpene hydrocarbons	26.52	
23	1351	α-Cubebene	1.28	MS, RI
24	1375	α-Copaene	0.24	MS, RI
26	1384	β-Bourbonene	0.68	MS, RI
27	1390	β-Elemene	1.25	MS, RI
28	1405	α-Cedrene	1.04	MS, RI
29	1409	α-Gurjunene	0.63	MS, RI
30	1413	β-Panasinsene	0.47	MS, RI
31	1414	Longifolene	2.18	MS, RI
32	1418	β-Caryophyllene	6.08	MS, RI, Co
33	1419	β-Cedrene	0.57	MS, RI
34	1428	β-Copaene	0.34	MS, RI
35	1436	β-Gurjunene	0.43	MS, RI
36	1437	γ-Elemene	2.04	MS, RI
37	1454	α-Humulene	1.07	MS, RI
38	1458	(E)-β-Farnesene	0.75	MS, RI
39	1475	γ-Muurolene	1.81	MS, RI

Table 1 (cont.): Chemical composition of the essential oil of *Wedelia prostrata*

Peak no.	RI[a]	Components	% RA[b]	Identification Methods[c]
40	1486	Germacrene D	1.66	MS, RI
41	1502	α-Muurolene	1.35	MS, RI
42	1506	(E)-α-Farnesene	1.25	MS, RI
43	1524	δ-Cadinene	0.34	MS, RI
44	1543	Selina-3,7(11)-diene	0.58	MS, RI
45	1556	Germacrene B	0.48	MS, RI
		Oxygenated sesquiterpenes	6.15	
46	1562	(E)-Nerolidol	1.28	MS, RI
47	1578	Spathulenol	1.49	MS, RI
48	1578	Caryophyllene oxide	0.56	MS, RI
49	1598	Guaiol	0.58	MS, RI
50	1621	Fonenol	0.33	MS, RI
51	1639	Isospathulenol	0.16	MS, RI
52	1646	α-Muurolol	0.67	MS, RI
53	1652	α-Cadinol	0.37	MS, RI
54	1653	Pogostol	0.21	MS, RI
55	1653	β-Eudesmol	0.50	MS, RI
		Phenylpropanoids	7.20	
56	1017	p-Cymene	2.12	MS, RI
57	1020	o-Cymene	2.20	MS, RI
58	1235	Methyl thymyl ether	0.63	MS, RI
59	1297	Carvacrol	0.15	MS, RI
60	1357	Eugenol	0.69	MS, RI
61	1402	Methyl eugenol	1.41	MS, RI
		Others	5.59	
62	854	3-Hexen-1-ol	1.22	MS, RI
63	862	(E)-2-Hexen-1-ol	1.51	MS, RI
64	902	Heptanal	0.59	MS, RI
65	982	1-Octen-3-ol	1.14	MS, RI, Co
66	1286	Bornyl acetate	0.23	MS, RI
67	1905	(Z)-7-Hexadecenoic acid, methyl ester	0.24	MS, RI
68	1928	Methyl palmitate	0.18	MS, RI
69	1948	Isophytol	0.12	MS, RI
70	1971	n-Hexadecanoic acid	0.36	MS, RI
		Total identified (%)	99.26	

[a] Retention index (RI) relative to n-alkanes on the HP-5 MS capillary column
[b] Relative area (peak area relative to the total peak area)
[c] MS = mass spectrum, Co = co-injection with standard compound

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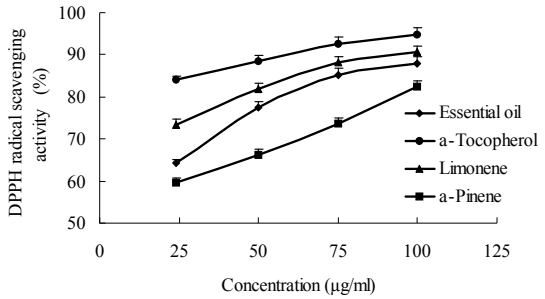

Figure 1: DPPH radical scavenging activity of *W. prostrata* essential oil and its main components

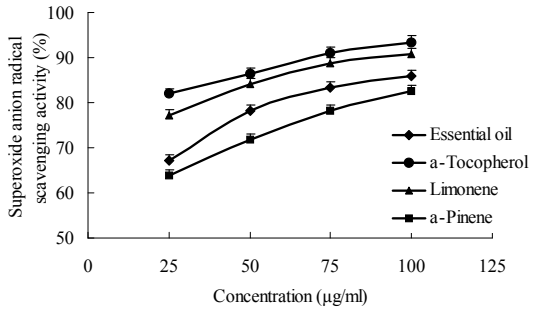

Figure 2: Superoxide radical scavenging activity of *W. prostrata* essential oil and its main components

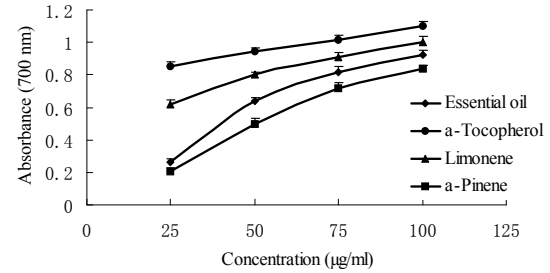

Figure 3: Reducing power of *W. prostrata* essential oil and its main components

Previous studies also revealed that α-pinene (Wang et al., 2008) and limonene (Maróstica et al., 2009) possess antioxidant activities, which were confirmed by our results. The current results further show that the antioxidant activity of essential oil can be attributed to the synergistic activities of multiform unsaturated compounds such as limonene and α-pinene.

Antimicrobial activity

The antimicrobial activity of *W. prostrata* essential oil and its main components were evaluated against a set of 10 microorganisms, and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zones, zone diameters (Table 2), and MIC values (Table 3).

Table 2 shows that the oil has a definite antimicrobial activity against all the test organisms. Limonene and α-pinene also showed considerable antimicrobial activities. In each case, tetracycline showed the highest antimicrobial effect, whereas the essential oil, limonene, and α-pinene were more effective compared to streptomycin. As for the negative control, the concentration of the solvent used in the current study did not affect the growth of the sample strains.

Table 2: Zones of growth inhibition (mm) showing antimicrobial activity for *W. prostrata* essential oil and its main components

Microorganism	Diameter of the inhibition zones				
	[a]Essential oil and its main components			[b]Standard	
	Essential oil	Limonene	α-Pinene	SM	TC
Pseudomonas aeruginosa	20.9 ± 0.9	21.8 ± 0.8	21.4 ± 0.5	20.7 ± 0.6	21.7 ± 0.6
Escherichia coli	21.8 ± 1.1	22.2 ± 0.8	22.3 ± 0.5	21.2 ± 0.7	22.6 ± 0.7
Bacillus subtilis	22.2 ± 0.5	22.8 ± 0.6	21.7 ± 1.0	20.6 ± 0.6	23.6 ± 0.7
Staphylococcus aureus	22.0 ± 0.6	22.5 ± 0.7	21.5 ± 0.7	20.7 ± 0.5	23.5 ± 0.6
Hansenula anomala	21.3 ± 0.7	22.8 ±0.8	20.9 ± 0.6	20.8± 0.6	23.2 ± 0.4
Saccharomy cescerevisiae	20.8 ± 0.7	22.5 ± 0.7	21.5 ± 0.8	20.3 ± 0.5	23.1 ± 0.5
Aspergillus niger	21.5 ± 0.6	23.1 ± 0.7	21.4 ± 0.7	21.0 ± 0.6	23.2 ± 0.6
Chaetomium globosum	20.8 ± 0.5	21.8 ± 0.5	20.7 ± 0.6	20.4 ± 0.4	22.8 ± 0.4
Mucor racemosus	20.8 ± 0.7	21.9 ± 0.7	20.8 ± 0.5	20.5 ± 0.5	22.3 ± 0.7
Monascus anka	21.2 ± 0.6	22.5 ± 0.6	20.9 ± 0.6	20.6 ± 0.5	22.6 ± 0.5

The diameter of the inhibition zones (mm), including the disc diameter (6 mm), are given as mean ± SD of triplicate experiments. [a]Diameter of the inhibition zones of the essential oil (tested volume, 1000 µg/disc); [b]Standard antibiotics: SM, streptomycin; TC, tetracycline (tested volume, 10 µg/disc)

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As shown in Table 3, the essential oil exhibited moderate to high antimicrobial effect against all test microorganisms, with MIC values ranging from 125 to 250 µg/ml. Limonene and α-pinene exhibited high antimicrobial effect, with MIC values ranging from 62.5 to 125 µg/ml.

Earlier papers on the analysis and antibacterial properties of the essential oils of *W. trilobata* have shown that they have varying degrees of growth inhibitory effects against some bacteria because of their chemical constituents, including limonene, β-phellandrene, α-phellandrene, γ-terpinene, β-caryophyllene, and α-pinene (Nirmal et al., 2005; Craveiro et al., 1993; Koheil, 2000). The current study shows that the antimicrobial activity of the oils from *W. prostrata* could, in part, be associated with its major components (limonene, α-pinene, α-phellandrene, and β-caryophyllene). Limonene has been demonstrated to have bacteriostatic activity against several microorganisms (Bakkali et al., 2008; Sokovic and van Griensven, 2006; Donsì et al., 2011; Singh et al., 2010). Pinene has been previously shown active against many organisms (Bakkali et al., 2008; Sokovic and van Griensven, 2006; Jiang et al., 2011). Pinene can destroy the cellular integrity, thereby inhibit the respiration and ion transport processes. Moreover, pinene can also increase the membrane permeability in yeast cells and isolated mitochondria (Uribe et al., 1985). The antimicrobial activities of α-phellandrene and β-caryophyllene have also been reported (Simic et al., 2002). Our results on the antimicrobial activity of limonene and α-pinene are similar to these reports.

In addition, the components with lower concentrations, such as γ-terpinene, β-phellandrene, β-pinene, camphene, *p*-cymene, o-cymene, longifolene, and γ-elemene, may also be contributing to the antimicrobial activity of the oil. Therefore, the synergistic effects of the diverse major and minor components of the essential oils should be taken into consideration to account for the oil biological activity (Burt, 2004).

The mechanism of action of this class of compounds has not been completely elucidated to date; however, these chemical components may be exerting their toxic effects against these microorganisms through the disruption of bacterial or fungal membrane integrity (Filipowicz et al., 2003). Conner and Beuchat (1984) suggested that the antimicrobial activity of the essential oils of herbs and spices or their components could be the result of damage to or disturbance of several enzymatic cell systems, including energy production and synthesis of structural components.

Table 3: Minimum inhibitory concentrations (MIC) of the *W. prostrata* essential oil and its main components against the growth of microorganisms

Microorganism	[a]MICs			
	Essential oil	Limonene	α-Pinene	Streptomycin
Pseudomonas aeruginosa	250	125	125	-
Escherichia coli	250	125	62.5	-
Bacillus subtilis	125	62.5	62.5	-
Staphylococcus aureus	125	62.5	62.5	-
Hansenula anomala	250	125	125	-
Saccharomy cescerevisiae	250	62.5	125	-
Aspergillus niger	125	62.5	125	-
Chaetomium globosum	125	125	62.5	-
Mucor racemosus	125	62.5	62.5	-
Monascus anka	250	62.5	125	-

[a]MIC, minimum inhibitory concentration (values in µg/ml)

CONCLUSION

The antioxidative and antimicrobial properties of the essential oils from many plants are of great interest to both the academe and the food, cosmetic, and pharmaceutical industries because of their possible use as natural additives to replace synthetic antimicrobial agents. For the first time, we demonstrate that the essential oil of *W. prostrata* exhibits antioxidant activity and successfully inhibits the growth of different pathogens that can cause food spoiling as well as health problems. The results obtained in this study show that the essential oil of *W. prostrata* may be a new potential source of natural antioxidants and antimicrobial agents for the food industry. However, further studies need to be conducted to understand the mechanism of the activity and obtain more information on the safety and toxicity of the oil.

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