

CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITIES OF EXTRACTS FROM *Pteris multifida*

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The aerial parts of *Pteris multifida* were collected at the flowering stage, in July to August 2002, from Pingjiang district of Hunan Province (China). The air-dried and finely ground samples were extracted successively with 1 L of methanol using a Soxhlet apparatus for about 72 h at a temperature not exceeding the boiling point of the solvent [1]. The resulting extracts were suspended in water and partitioned with chloroform to obtain water-soluble (polar) (7.69%, w/w) and water-insoluble (nonpolar) subfractions (4.08%, w/w), which were then lyophilized and kept in the dark at -20°C until further tested. The same samples (250 g) were placed in a Clevenger-type apparatus with 2 L double distilled water (previously saturated with NaCl) and hydrodistilled for 4.5 h; after that, the yellowish oils were extracted from the condensate (saturated with NaCl) with diethyl ether. The obtained essential oils (EOs) were dried over anhydrous sodium sulfate after filtration and stored in sealed glass tubes at -20°C until further tested and analyzed (Table 1 and 2).

The microorganisms listed in Table 3 were provided by the Biology Department of Longdong University, Qingyang (China). Two different methods were employed for the determination of antimicrobial activities: agar-well diffusion method for the methanol extracts (MEs) and agar-disc diffusion method for the EOs [2, 3]. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the EOs against the test microorganisms were determined by the broth microdilution method [4, 5]. The MICs and MBCs of levofloxacin were also determined in parallel experiments in order to control the sensitivity of the test microorganisms. All tests were performed in duplicate.

TABLE 1. Organoleptic and Physico-chemical Properties of the EOs from *P. multifida*

Properties	Leaf oil	Stem oil	Properties	Leaf oil	Stem oil
Color	Pale yellow	Light yellow	Optical rotation		
Aspect	Liquid, movable	Liquid, movable	(20° , pentane, c 0.51)	+2.10°	+1.98°
Odor	Perfume	Perfume	Solubility in ethanol		
Refractive index (n_{D}^{20})	1.4586	1.4595	60% (v/v)	1:30	1:30
Specific gravity (d_{D}^{20})	0.934 g/cm ³	0.956 g/cm ³	70% (v/v)	1:20	1:15
Acid number	0.87	0.92	80% (v/v)	1:10	1:8
Ester number	12.18	19.35	90% (v/v)	1:3	1:2
Iodine number	152	138	Absolute	1:1	1:1

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TABLE 2. Chemical Composition of the Hydrodistilled EOs of *P. multifida*

Compounds	RI ^{calc.a} /RI ^{lit.b}	Percentage, %		Compounds	RI ^{calc.a} /RI ^{lit.b}	Percentage, %	
		leaf	stem			leaf	stem
1-Hexanal	806/800	1.29	1.34	(<i>epi</i>)- α -Cedrane	1438/1441	0.15	0.81
<i>n</i> -Hexanol	868/867	0.19	-	α -Humulene	1451/1454	1.21	2.14
Camphene	951/953	6.03	4.21	Alloaromadendrene	1458/1461	1.32	-
<i>p</i> -Cymene	1025/1026	5.98	6.10	n.i.	1462/N.d.	-	<0.10
Limonene	1028/1031	1.82	1.03	Germacrene D	1484/1480	<0.10	-
1,8-Cineole	1029/1033	1.96	2.64	β -Selinene	1488/1485	1.08	0.91
γ -Terpinene	1054/1062	3.08	3.62	α -Muurolene	1493/1499	2.23	2.17
<i>cis</i> -Linalool oxide	1069/1074	0.77	0.56	γ -Cadinene	1508/1513	3.18	2.85
Linalool	1101/1098	4.14	2.45	α -Agarofuran	1540/1545	1.57	2.24
α -Thujone	1100/1102	0.92	1.77	Spathulenol	1572/1576	0.26	1.47
β -Thujone	1116/1114	2.47	1.97	Caryophyllene oxide	1575/1581	1.83	1.85
Camphor	1141/1143	1.84	-	β -Copaen-4-ol	1580/1585	0.89	2.34
<i>endo</i> -Borneol	1160/1165	2.34	3.14	n.i.	1590/N.d.	<0.10	0.36
Terpinen-4-ol	1174/1176	3.46	2.87	n.i.	1595/N.d.	0.24	-
<i>p</i> -Cymen-8-ol	1186/1183	2.06	1.34	Humulene epoxide	1600/1606	2.02	1.98
α -Terpineol	1188/1189	3.48	3.74	τ -Cadinol	1642/1640	2.16	1.85
Myrtenal	1191/1193	0.96	1.24	α -Muurolol	1642/1645	1.07	2.37
<i>trans</i> -Piperitol	1202/1205	2.37	1.98	6-Hydroxycaryophyllene	1648/1646	-	1.74
Geraniol	1254/1255	3.17	2.95	Tetracosane	2398/2400	0.82	2.94
Bornyl acetate	1281/1285	2.60	2.74	Pentacosane	2494/2500	2.31	2.24
δ -Elemene	1336/1339	-	0.45	Total of grouped components			
α -Cubebene	1355/1351	0.96	0.47	Monoterpene hydrocarbons		16.91	14.96
Eugenol	1359/1356	10.72	8.64	Oxygenated monoterpenes		33.59	31.34
α -Ylangene	1368/1372	-	1.99	Sesquiterpene hydrocarbons		14.57	15.72
α -Copaene	1374/1376	1.17	-	Oxygenated sesquiterpenes		9.80	15.84
Geranyl acetate	1386/1383	1.35	1.95	Other compounds		15.33	15.16
β -Bourbonene	1382/1384	2.05	-	Total identified		90.50	93.02
β -Cubebene	1393/1390	-	1.28	Unidentified		0.24	0.36
β -Caryophyllene	1415/1418	1.22	2.65	Yield (mL/100 g dried material)		0.94	1.03

^{a,b}Retention index (%): Kovats Index on HP-5MS column in reference to C₈-C₂₂ *n*-alkanes; N.d.: not determined; n.i.: not identified; -: not detected.

TABLE 3. Antimicrobial Activity of the EOs and the MEs of *P. multifida*

Test microorganisms	Essential oils						MeOH extracts	Levofloxacin		
	leaf			stem			CHCl ₃			
	IZD	MIC	MBC	IZD	MIC	MBC	IZD	IZD	MIC	MBC
<i>Staphylococcus aureus</i> ATCC 25923	18.00	4.50	9.00	18.75	4.50	9.00	12.25	32.00	0.30	0.30
<i>Streptococcus pneumoniae</i> ATCC 49619	20.50	4.50	4.50	20.25	4.50	4.50	8.50	30.00	0.61	0.61
<i>Enterococcus faecalis</i> ATCC 29212	18.70	9.00	18.00	20.00	9.00	9.00	N.a.	26.50	1.22	1.22
<i>Bacillus cereus</i> ATCC 11778	19.50	9.00	9.00	18.25	18.00	18.00	9.00	12.50	4.87	9.75
<i>Bacillus subtilis</i> ATCC 10907	24.00	0.56	0.56	23.25	0.56	0.56	10.50	10.75	9.75	9.75
<i>Mycobacterium smegmatis</i> CMM 2067	21.00	2.25	2.25	22.75	1.13	1.13	10.00	8.75	19.50	19.50
<i>Acinetobacter lwoffii</i> ATCC 19002	11.50	72.00	72.00	16.50	36.00	36.00	N.a.	8.00	>19.50	>19.50
<i>Escherichia coli</i> ATCC 25922	N.a.	>72.00	>72.00	N.a.	>72.00	>72.00	N.a.	32.00	0.30	0.61

TABLE 3. (continued)

Test microorganisms	Essential oils						MeOH extracts	Levofloxacin		
	leaf			stem			CHCl ₃	IZD	MIC	MBC
	IZD	MIC	MBC	IZD	MIC	MBC	IZD			
<i>Enterobacter aerogenes</i> ATCC 13043	15.50	36.00	36.00	17.25	36.00	36.00	N.a.	28.50	0.61	1.22
<i>Klebsiella pneumoniae</i> ATCC 13883	18.25	18.00	18.00	20.25	9.00	9.00	N.a.	15.75	4.87	4.87
<i>Proteus mirabilis</i> ATCC 7002	N.a.	>72.00	>72.00	N.a.	>72.00	>72.00	N.a.	24.00	2.44	4.87
<i>Pseudomonas aeruginosa</i> ATCC 27853	N.a.	>72.00	>72.00	N.a.	>72.00	>72.00	N.a.	32.25	0.15	0.30
<i>Candida albicans</i> ATCC 10239	20.00	4.50	9.00	18.50	9.00	9.00	N.a.	N.t.	N.t.	N.t.
<i>Candida krusei</i> ATCC 6258	22.50	2.25	2.25	21.50	4.50	4.50	14.75	N.t.	N.t.	N.t.
<i>Saccharomyces cerevisiae</i> ATCC 2365	10.50	36.00	36.00	8.75	72.00	72.00	N.a.	N.t.	N.t.	N.t.
<i>Aspergillus niger</i> ATCC 16401	8.50	72.00	72.00	9.50	72.00	72.00	N.a.	N.t.	N.t.	N.t.

IZD: agar disc diffusion method, diameter of inhibition zone (mm) including disk diameter of 6 mm. MIC, MBC: values given as mg/mL (for the essential oils) and mg/mL (for levofloxacin). N.a.: not active; N.t.: not tested.

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