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Antioxidant activity of polysaccharides from fermented *Meconopsis* Vig. endophytic fungi

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Abstract: 24 endophytic fungi were isolated from four kinds of plants of the *Meconopsis* genus: *M. chelidonifolia, M. punicea, M. henrici and M. integrifolia*. Intracellular and extracellular polysaccharides were extracted from the 24 fungi after fermentation. Hydroxyl radical scavenging, DPPH test and reducing power assays were used to screen the antioxidant activity of the polysaccharide samples. The results showed that 8 strains of extracellular polysaccharides produced by fermentation were in amounts higher than 100 mg per 500 ml fermented liquid; the 48 kinds of polysaccharide samples possess low antioxidant activity.

Key words: *Meconopsis*, Endophytic fungi, Polysaccharides, Antioxidant.

INTRODUCTION

Meconopsis is a genus of flowering plants of the family *Papaveraceae*. There are about 40 species grown in China. *Meconopsis integrifolia* and *Meconopsis nepaulensis* are important herbal plants, which have been used in traditional folk medicine for the treatment of cough, detoxication, etc. So far, its endophytic fungi antioxidant activity has not been investigated [1-3]. We isolated 24 fungus strains from four kinds of the *Meconopsis* plant. In order to obtain strains of strong antioxidant activity, we subjected the fungi to liquid fermentation, extracted the polysaccharides and compared their antioxidant activity**.**

MATERIALS AND METHODS

24 strains of fungi were isolated from *Meconopsis chelidonifolia*, *Meconopsis punicea*, *Meconopsis henrici* Bur. et Franch and *Meconopsis integrifolia* (Maxim.) Franch.

Extraction of polysaccharides

The fungi liquid and thallus was separated and was fermented in 500 ml PD medium, vacuum spin steaming the liquid from 500 to 60 ml. Three volumes of 95% ethanol were added. After the keeping the mixture in a refrigerator at 5-10^{o}C for 12 h, the active polysaccharide fully precipitated. The polysaccharide was washed 2 times with anhydrous ethanol, acetone and ether, respectively and was dried at 60^{o}C for 2 h. The dry thallus was grinded and passed through a 60 mesh sieve. A 20 fold volume of water was added to extract intracellular polysaccharide at 98^{o}C for 2 h. The procedure was repeated twice and the extracts were merged. Impurities in the protein were removed by the Sevag method.

Analysis of the content of polysaccharides

Add 0.1 mL, 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL and 1. mL, respectively, of a glucose solution (0.1mg/mL) into tubes, and fill with water until 1 mL. Use 1 mL water as a blank. Add 1 mL phenol solution (5%) and 5 mL concentrated sulfuric acid to each tube. Heat in a boiling water bath for 15 min, then cool to room temperature. Measure the OD value of each tube at 490 nm. In this way the standard curve was prepared (Figure 1). Each sample was analyzed in triple. Polysaccharide content (PD)=x50mL/5mg×100%

y=8.538x-0.027

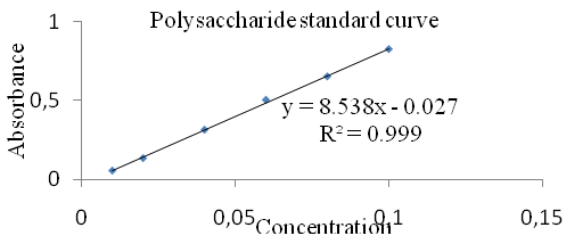

Fig. 1. Polysaccharide standard curve.

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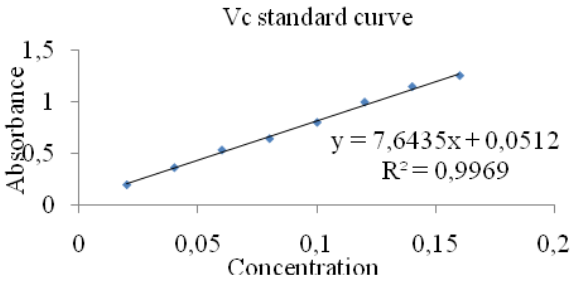

Fig. 2. Vc standard curve.

Screening for antioxidant activity. Determination of hydroxyl radical scavenging activity

To a polysaccharide sample (1 mg/L) in a test tube add 1 mL $FeSO_4$ solution (6mmol/L), 1 mL salicylic acid-ethanol solution (6mmol/L), 1 mL sample solution, 1mL 0.1% H_2O_2 and 4 mL distilled water, shake well in a 37°C water bath for 30 min. Cool to room temperature and measure the absorbance value of each tube at 51 nm.

Clearance (%)=[1-(A_{sample}- $A_{contrast}$)/ A_{blank}]×100%.

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

To a polysaccharide sample (1mg/L), in a test tube add 2 ml of 0.025 mg/L ethanol solution of DPPH, shake well at room temperature for 30 min. Measure the absorbance value of each tube at 517 nm [4].

Inhibition of DPPH radical (%)=[1-(A_{sample}- $A_{contrast}$)/ A_{blank}]×100%.

Table 1. Results of endophytic fungi polysaccharide content determination

Sample	QMP(mg)	Ā	PC(%)	QP(mg)	Sample	QMP(mg)	Ā	PC(%)	QP(mg)
YL101	96.6	0.82	99.99	96.6	QL204	121.3	0.38	47.80	58.0
YL101 I	12.0	0.82	99.56	12.0	QL204	4.2	0.57	70.10	2.9
YL201	347.2	0.78	95.34	331.0	QL205	136.2	0.81	98.28	133.9
YL201 I	10.7	0.78	95.35	10.2	QL205	0.010.6	0.08	12.90	0.0014
YL202	390.5	0.13	18.75	73.2	QS102	0.296.4	0.70	86.21	0.26
YL202 I	12.3	0.12	18.28	2.2	QS102 I	0.103.6	0.68	83.05	0.086
YL203	436.5	0.19	25.66	112.0	BS101	423.2	0.25	33.39	141.3
YL203 I	13.7	0.78	95.35	13.1	BS101 I	15.9	0.11	16.88	2.7
YL204	16.5	0.19	25.78	4.3	BS102	54.5	0.41	52.13	28.4
YL204 I	25.4	0.61	75.67	19.2	BS102 I	18.0	0.22	29.88	5.4
YL205	35.1	0.03	7.74	2.7	RL201	196.7	0.53	65.60	129.0
YL205 I	27.1	0.11	16.88	4.6	RL201 I	16.7	0.43	54.06	9.0
YL1 E	36.1	0.25	33.04	11.9	RL202	29.4	0.45	56.58	16.6
YL1 I	7.0	0.14	19.81	1.4	RL202 I	16.6	0.42	52.72	8.8
YL2 E	34.7	0.42	53.07	18.4	RL203	30.8	0.82	99.99	30.8
YL2 I	26.0	0.30	39.36	10.2	RL203 I	28.4	0.82	99.80	28.4
YS101 E	887.4	0.82	99.80	885.6	RL204	574.6	0.12	17.81	102.3
YS101 I	12.2	0.79	96.40	11.8	RL204 I	15.7	0.03	7.51	1.2
YR202	44.5	0.13	18.40	8.2	RS202	55.4	0.62	76.14	42.3
YR202I	31.1	0.09	14.41	4.5	RS202 I	13.4	0.41	51.83	6.9
QL201	212.9	0.16	22.38	47.6	RS203	14.7	0.69	84.22	12.4
QL201 I	26.0	0.05	9.85	2.6	RS203 I	16.5	0.72	87.85	14.5
QL202	68.4	0.33	41.94	28.7	RS302	2302	0.40	50.37	116.0
QL202 I	17.6	0.19	26.25	4.6	RS302 I	34.3	0.68	83.29	28.6

Notes: I intracellular polysaccharide, E extracellular polysaccharide; MP quantity of mixture polysaccharide; PC polysaccharide concentration; QP quantity of polysaccharide; Ā average absorbance of three parallel sets.

Table 2. Results of the determination of hydroxyl radical scavenging activity.

Sample	A_{sample}	$A_{contrast}$	A_{blank}	Clearance(%)	Sample	A_{sample}	$A_{contrast}$	A_{blank}	Clearance(%)
YL101 E	0.431	0.001		30.86	QL204 E	0.1213	0.381		20.58
YL101 I	0.564	0.023		13.02	QL204 I	0.0042	0.571		19.29
YL201 E	0.578	0.000		7.07	QL205 E	0.1362	0.812		19.29
YL201 I	0.570	0.012		10.13	QL205 I	0.0106	0.083		7.56
YL202 E	0.538	0.001		13.67	QS102 E	0.2964	0.709		19.13
YL202 I	0.600	0.009		4.98	QS102 I	0.1036	0.682		6.59
YL203 E	0.597	0.004		4.66	BS101 E	0.4232	0.258		11.25
YL203 I	0.534	0.023		17.85	BS101 I	0.0159	0.117		12.22
YL204 E	0.307	0.013		52.73	BS102 E	0.0545	0.418		36.82
YL204 I	0.555	0.016		13.34	BS102 I	0.0180	0.228		14.31
YL205 E	0.433	0.017		33.12	RL201 E	0.1967	0.533		11.58
YL205 I	0.512	0.004	0.622	18.33	RL201 I	0.0167	0.434	0.622	3.54
YL1 E	0.543	0.006		13.17	RL202 E	0.0294	0.456		30.55
YL1 I	0.527	0.016		17.88	RL202 I	0.0166	0.423		8.68
YL2 E	0.547	0.007		22.19	RL203 E	0.0308	0.826		46.30
YL2 I	0.534	0.022		8.20	RL203 I	0.0284	0.825		9.81
YS101 E	0.455	0.007		27.97	RL204 E	0.5746	0.125		7.88
YS101 I	0.575	0.021		10.93	RL204 I	0.0157	0.037		-
YR202 E	0.506	0.012		20.58	RS202 E	0.0554	0.623		-
YR202I	0.520	0.000		16.40	RS202 I	0.0134	0.415		-
QL201 E	0.447	0.013		30.23	RS203 E	0.0147	0.692		7.23
QL201 I	0.530	0.019		17.85	RS203 I	0.0165	0.723		-
QL202 E	0.354	0.004		43.73	RS302 E	0.2302	0.403		26.37
QL202 I	0.568	0.013		10.77	RS302 I	0.0343	0.684		10.61

Note:- no hydroxyl radical scavenging activity

Reducing power assay

Different extracts and a standard (1 mg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 mol/L, pH 6.6) and potassium ferricyanide (2.5 mL, 1% w/v) was added to the mixture, which was then centrifuged for 10 min at 3000 r/min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1% w/v) and the absorbance was measured at 700 nm on a spectrophotometer [5]. High absorbance value of the reaction mixture indicates a high reductive potential. Draw the standards of different concentration of vitamin C curve. (Figure 2.)

RESULTS AND DISCUSSION

10 kinds of polysaccharide contents are below 20% of the samples. The polysaccharide samples still contain a lot of impurities which should be removed before application. Polysaccharide contents are shown in Table1.

The experimental results showed that the polysaccharide from the fermented liquid of YL204 has the highest activity of hydroxyl radical scavenging (52.73%). Thallus polysaccharide in RL204,RS202 and RS203 has no activity of hydroxyl radical scavenging. Results are shown in Table 2.

The results showed that thallus polysaccharide and polysaccharide from fermented liquid in YL202 have higher DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity (45.50% and 42.78%, respectively). 12 samples displayed no activity (Table 3).

The results showed that all 48 samples have ability of reducing power. YL202 and YL2 have stronger ability equivalent V_c (0.0210 mg/ml and 0.0216 mg/ml, respectively). Results are shown in Table 4.

Table 3. Results of DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity

Sample	A_{sample}	$A_{contrast}$	A_{blank}	Clearance(%)	Sample	A_{sample}	$A_{contrast}$	A_{blank}	Clearance(%)
YL101 E	0.315	0.017		18.80	QL204 E	0.308	0.062		32.97
YL101 I	0.473	0.084		-	QL204 I	0.370	0.019		4.36
YL201 E	0.356	0.037		13.08	QL205 E	0.304	0.016		21.53
YL201 I	0.288	0.018		26.43	QL205 I	0.701	0.285		-
YL202 E	0.208	0.008		45.50	QS102 E	0.397	0.103		19.89
YL202 I	0.306	0.096		42.78	QS102 I	0.489	0.113		-
YL203 E	0.292	0.005		21.80	BS101 E	0.302	0.002		18.26
YL203 I	0.287	0.049		35.15	BS101 I	0.449	0.160		21.25
YL204 E	0.458	0.025		-	BS102 E	0.284	0.017		27.25
YL204 I	0.381	0.031		4.63	BS102 I	0.257	0.025		36.78
YL205 E	0.480	0.052		-	RL201 E	0.280	0.010		26.43
YL205 I	0.350	0.017	0.367	9.26	RL201 I	0.380	0.015	0.367	0.54
YL1 E	0.299	0,029		26.43	RL202 E	0.433	0.160		25.61
YL1 I	0.352	0.026		11.17	RL202 I	0.367	0.017		4.63
YL2 E	0.417	0.067		4.63	RL203 E	0.286	0.006		23.71
YL2 I	0.496	0.103		-	RL203 I	0.346	0.022		11.72
YS101 E	0.631	0.152		-	RL204 E	0.303	0.006		19.07
YS101 I	0.366	0.042		11.71	RL204 I	0.401	0.027		-
YR202 E	0.531	0.051		-	RS202 E	0.554	0.177		-
YR202I	0.431	0.038		-	RS202 I	0.664	0.241		-
QL201 E	0.336	0.019		13.62	RS203 E	0.342	0.010		9.54
QL201 I	0.331	0.037		19.89	RS203 I	0.330	0.011		13.08
QL202 E	0.346	0.049		19.07	RS302 E	0.483	0.197		22.71
QL202 I	0.278	0.014		28.07	RS302 I	0.296	0.040		30.25

Notes: - no DPPH free radical scavenging activity

Table 4. Results of reducing power assay.

Sample	A_{sample}	Equivalent V_c(mg/ml)	Samples	A_{sample}	Equivalent V_c(mg/ml)	Sample	A_{sample}	Equivalent V_c(mg/ml)
YL101 E	0.158	0.0140	YS101 E	0.139	0.0115	BS102 E	0.150	0.0129
YL101 I	0.147	0.0125	YS101 I	0.130	0.0103	BS102 I	0.175	0.0162
YL201 E	0.114	0.0082	YR202E	0.142	0.0119	RL201 E	0.191	0.0183
YL201 I	0.129	0.0102	YR202I	0.117	0.0086	RL201 I	0.158	0.0139
YL202 E	0.125	0.0097	QL201 E	0.180	0.0169	RL202 E	0.184	0.0174
YL202 I	0.212	0.0210	QL201 I	0.149	0.0128	RL202 I	0.151	0.0131
YL203 E	0.172	0.0158	QL202 E	0.177	0.0165	RL203 E	0.153	0.0133
YL203 I	0.148	0.0126	QL202 I	0.133	0.0107	RL203 I	0.149	0.0128
YL204 E	0.165	0.0149	QL204 E	0.157	0.0138	RL204 E	0.156	0.0137
YL204 I	0.140	0.0116	QL204 I	0.133	0.0107	RL204 I	0.140	0.0116
YL205 E	0.173	0.0159	QL205 E	0.149	0.0128	RS202 E	0.125	0.0096
YL205 I	0.172	0.0158	QL205 I	0.141	0.0117	RS202 I	0.129	0.0102
YL1 E	0.144	0.0121	QS102 E	0.165	0.0148	RS203 E	0.174	0.0161
YL1 I	0.132	0.0106	QS102 I	0.158	0.0139	RS203 I	0.162	0.0145
YL2 E	0.216	0.0216	BS101 E	0.137	0.0112	RS302 E	0.185	0.0175
YL2 I	0.135	0.0110	BS101 I	0.131	0.0104	RS302 I	0.143	0.0120

From the three different assays used, we got some previous information of endophytic fungi in the *Meconopsis* plant. Especially the endophytic fungi in *Meconopsis chelidonifolia* displayed high quality of polysaccharides and excellent antioxidant potential. Intracellular and extracellular polysaccharides in YL202 fungus possess the highest antioxidant capacity in DPPH free radical scavenging activity. This method is considered as the most accurate method used to assess the antioxidant activity of samples and the widely used assay. Different samples showed inconformable antioxidant capacity in the different assays. Besides, we found that samples' hydroxyl radical scavenging activity is due to more than 30% to extracellular polysaccharide. The results of this study showed that *Meconopsis* endophytic fungi may become a potential source of natural antioxidants. This first report study provides information and basic work on the bioactivity and compounds of *Meconopsis* endophytes.

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АНТИОКСИДАНТНА АКТИВНОСТ НА ПОЛИЗАХАРИДИ ОТ ФЕРМЕНТИРАНИ *Meconopsis* Vig. ЕНДОФИТНИ ФУНГИ

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(Резюме)

Двадесет и четири ендофитни гъбички (фунги) бяха изолирани от 4 вида растения от рода *Meconopsis*: *M. chelidonifolia, M. punicea, M. henrici* и *M. integrifolia*. От гъбичките са извлечени вътре-клетъчни и извънклетъчни полизахариди след 24-часова ферментация. Анализи по отстраняване на хидроксилните радикали, DPPH-тест и намалена мощност са използвани за скрийнинг на антиоксидантната активност на образците от полизахаридите. Резултатите показват, че при 8 щама екстрацелуларните полизахариди, получени при ферментация са повече от 100 mg за 500 ml ферментационна среда; 48 вида полизахариди притежават ниска антиоксидантна активност.