

Extraordinary accumulations of antioxidants in *Ammopiptanthus mongolicus* (Leguminosae) and *Tetraena mongolica* (Zygophyllaceae) distributed in extremely stressful environments

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ABSTRACT. *Ammopiptanthus mongolicus* (Leguminosae) and *Tetraena mongolica* (Zygophyllaceae) are distinctively distributed in the northwestern Gobi and desert areas of China. Their environments involve seasonally extreme drought and temperatures, extraordinarily high UV radiation and poor soil qualities with high salinity. *Ammopiptanthus mongolicus* remains evergreen for all four seasons and has been traditionally used as anti-inflammatory, anti-infectious and pain-killing medicines. All the extracts prepared from the two species exhibited significantly higher scavenging activities against the $\cdot\text{O}_2^-$ than a control. Five selected extracts also showed wider spectra of antioxidative capacities. An activity-guided fractionation led to identification of four major compounds. Resveratrol, a super strong antioxidant, accounted for as high as 0.05% of the dried weight of *A. mongolicus*. Two isoflavones isolated are also reported to be antioxidative and anti-inflammatory. Our results imply that plant species living in the extremely stressful environments may become an abundant natural resource of strong antioxidants. Considering the fact that oxidation is involved in the processes of infections, inflammation and other disorders, these results collectively suggest that efficacies of *A. mongolicus* in treating infections, inflammatory disorders and in killing pains may be attributed, at least partly, to its significantly larger non-enzymatic antioxidative capacities.

Keywords: 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical; Active-oxygen scavenging activity; *Ammopiptanthus mongolicus*; Electron paramagnetic resonance (EPR); Lipid peroxidation; Resveratrol; Superoxide anion radical ($\cdot\text{O}_2^-$); *Tetraena mongolica*.

Abbreviations: DMSO, Dimethyl Sulfoxide; DPPH, 2,2-Diphenyl-2-picrylhydrazyl hydrate; EPR, Electron paramagnetic resonance; MDA, Malondialdehyde; $\cdot\text{O}_2^-$, Superoxide anion radical; ROS, Reactive oxygen species.

INTRODUCTION

Ammopiptanthus mongolicus (Maxim.) S. H. Cheng [Leguminosae] is the relics of the Tertiary Period, distinctively distributed in the northwestern desert area of China, where is marked by seasonally extreme drought and temperatures (over 40°C in the summer and under -30°C in the winter), poor soil quality with high salinity, and extraordinarily high ultraviolet-irradiation. The unique for *A. mongolicus* is that it remains evergreen for all four seasons in the desert area. It is catalogued in Chinese Tra-

ditional Medicine as an anti-inflammatory, anti-infectious and pain-killing medicinal plant since it has long been used to treat respiratory disorders (lung diseases, cough, and infected throat), to kill stomachache, and to cure cold-caused wounds and chronic rheumatism (Li et al., 2004). We initially assumed that its efficacy in treating the diseases could be somehow related to its harsh environment, possibly through the accumulation of some kinds of secondary compounds in its aerial parts.

It has been understood that, sessile plants, particularly those living in the extremely adverse environment, are under constant attack of excess reactive oxygen species (ROS), and have evolved efficient anti-oxidation defense systems, including antioxidative enzymes or non-enzymatic antioxidants. The level of the antioxidants, which

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may account for the efficacies of medicinal plants, varies widely among species (Reinert et al., 1982; Bennett et al., 1984). On the other hand, more and more studies demonstrate that ROS are involved in the pathogenesis during infections and various degenerative disorders, such as cardiovascular disease and neuro-degenerative diseases (Harman, 1994; Cox and Cohen, 1996; Ames, 1998; Finkel and Holbrook, 2000). Oxidation is also involved in the early stages of carcinogenesis (Vuillaume, 1987). Based on these understandings and findings, a working hypothesis was further postulated that the efficacy of *A. mongolicus* in the traditional treatments of the various diseases might be attributed to its extraordinary antioxidative capacity derived from extraordinarily accumulated antioxidants under the extremely stressful environment. To justify this hypothesis, we set out to analyze the antioxidative capacities of various extracts from its aerial parts, with a hope to identify distinctive antioxidants.

To further verify the assumption that an extraordinary non-enzymatic antioxidative capacity might be a characteristics of plants living in extremely stressfully environments, extracts from a super xerophyte, *Tetraena mongolica* Maxim. (Zygophyllaceae) were also analyzed in parallel. *Tetraena mongolica*, predominantly distributed in Gobi-like dry area, is an otherwise similar bushy plant species. Due to its limited distribution, it has been listed as an endangered species by the State. To make the analyses experimentally justifiable, *Daphniphyllum paxianum* Rosenth., a species naturally distributed in the milder environment of southern part of China, was analyzed as a negative reference.

In this study, all the extracts from *A. mongolicus* as well as from *T. mongolica* exhibited significantly higher antioxidative capacities against superoxide anion radical than those from *D. paxianum*. Five selected extracts also showed different magnitude of counteracting capacities against DPPH and lipid peroxyl radicals. Three major antioxidants were identified, and resveratrol, in particular, accounted for as high as 0.05% of the dried weight of *A. mongolicus*.

MATERIALS AND METHODS

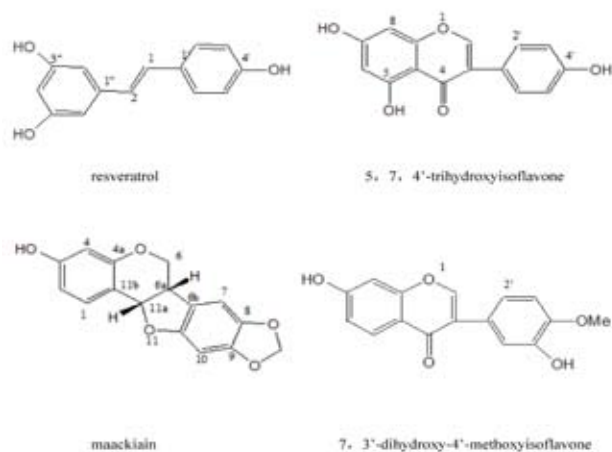
Plant materials

Ammopiptanthus mongolicus and *T. mongolica*, were collected from the northern Gobi and desert areas of the Ningxia Hui and Inner Mongolia Autonomous Regions, and *D. Rosanth* from the milder environment of northern Guangdong province. They were identified by Dr. Yulong Ding (Department of Botany, Nanjing Forestry University).

Preparation of extracts and isolation of pure compounds

Powdered aerial parts of *A. mongolicus*, *T. mongolica* and *D. paxianum* were exhaustively extracted four times with ethanol, and the resultant extracts (A₀, T₀,

and D₀) were then partitioned within ethyl acetate and H₂O. The ethyl acetate-soluble portions were repeatedly chromatographed over silica gel and Sephadex LH-20 column, and samples A₁, A₃, A₄, A₅, A₆, T₁, T₃, T₄ and D₁ were subsequently obtained. The H₂O-soluble portion was further partitioned within *n*-Butanol and H₂O. The *n*-Butanol-soluble portion was repeatedly chromatographed over silica gel and Sephadex LH-20 column, and samples A₂, A₇, T₂, T₅ and D₂ were then obtained. Finally, 5, 7, 4'-trihydroxyisoflavone, maackiain and 7, 3'-dihydroxy-4'-methoxyisoflavone were isolated from A₄, and resveratrol from A₅ (with structure formula). The whole procedure was outlined in Figure 1.



Supplements. 5, 7, 4'-trihydroxyisoflavone: C₁₅H₁₀O₅, ¹H NMR (CD₃OD, 400 MHz) δ: 12.0 (1H, s, 5-OH) 8.13 (1H, s, H-2), 7.72 (2H, d, J= 8.5 Hz, H-2', 6'), 7.29 (2H, d, J= 8.5 Hz, H-3', 5'), 6.76 (1H, d, J= 2.1 Hz, H-8), 6.68 (1H, d, J= 2.1 Hz, H-6); ¹³C NMR (CD₃OD, 100 MHz) δ: 153.5 (d, C-2), 122.5 (s, C-3), 181.4 (s, C-4), 158.8 (s, C-5), 94.7 (d, C-6), 166.1 (s, C-7) 100.3 (d, C-8), 159.4 (s, C-9), 106.0 (s, C-10), 124.1 (s, C-1'), 131.2 (d, C-2', 6'), 116.4 (d, C-3', 5'), 163.8 (s, C-4'); **maackiain:** C₁₆H₁₂O₅, ¹H NMR (CD₃OD, 400 MHz) δ: 7.24 (1H, d, J= 8.4 Hz, H-1), 6.47 (1H, dd, J= 8.4, 2.3 Hz, H-2), 6.29 (1H, d, J= 2.3 Hz, H-4), 4.18 (1H, d, J= 11 Hz, H-6α), 3.53 (1H, t, J= 11, 4.8 Hz, H-6β), 3.43 (1H, m, H-6a), 6.77 (1H, s, H-7), 6.35 (1H, s, H-10), 5.85 (1H, d, J= 11 Hz, H-OCH₂O-), 5.84 (1H, d, J= 11 Hz, H-OCH₂O-), 5.42 (1H, d, J= 6.9 Hz, H-11a); ¹³C NMR (CD₃OD, 100 MHz): 133.6 (d, C-1), 111.2 (d, C-2), 160.6 (s, C-3), 104.6 (d, C-4), 158.5 (s, C-4a), 67.9 (t, C-6), 42.1 (d, C-6a), 120.3 (s, C-6b), 106.4 (d, C-7), 143.6 (s, C-8), 149.9 (s, C-9), 94.7 (d, C-10), 156.1 (s, C-10a), 80.5 (d, C-11a), 113.4 (s, C-11b), 103.0 (t, C-OCH₂O-); EIMS m/z 284 (100) 162 (20), 267 (15), 175 (13); **7, 3'-dihydroxy-4'-methoxyisoflavone:** C₁₆H₁₂O₅, ¹H NMR (C₅D₅N, 400 MHz) δ: 8.52 (1H, d, J= 8.5 Hz, H-5), 8.26 (1H, s, H-2), 7.88 (1H, d, J= 2.3 Hz, H-2'), 7.41 (1H, dd, J= 8.5, 2.3 Hz, H-6'), 7.30 (1H, dd, J= 8.5 Hz, H-6), 7.18 (1H, d, J= 2.3 Hz, H-8), 7.13 (1H, d, J= 8.5 Hz, H-5'), 3.85 (3H, s, H-OCH₃); ¹³C NMR (C₅D₅N, 100 MHz) δ: 175.8 (s, C-4), 164.2 (s, C-7), 159.6 (s, C-9), 152.9 (d, C-2), 148.8 (s, C-3'), 148.2 (s, C-4'), 128.4 (d, C-6'), 126.5 (s, C-3), 125.1 (s, C-1'), 120.6 (d, C-5), 118.2 (s, C-10), 118.0 (d, C-6), 116.0 (d, C-5'), 112.5 (d, C-2'), 103.2 (d, C-8), 56.1 (q, C-OCH₃).

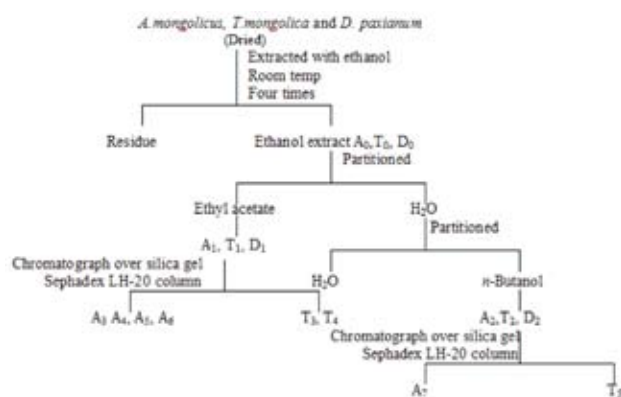


Figure 1. Preparations of extracts from *A. mongolicus*, *T. mongolica* and *D. paxianum*. Powdered aerial parts were exhaustively extracted four times with ethanol, and the resultant extracts (A_0 , T_0 , and D_0) were then partitioned within ethyl acetate and H_2O . The ethyl acetate-soluble portions were repeatedly chromatographed over silica gel and Sephadex LH-20 column, and samples A_1 , A_3 , A_4 , A_5 , A_6 , T_1 , T_3 , T_4 and D_1 were subsequently obtained. The H_2O -soluble portion was further partitioned within *n*-Butanol and H_2O . The *n*-Butanol-soluble portion was repeatedly chromatographed over silica gel and Sephadex LH-20 column, and samples A_2 , A_7 , T_2 , T_5 and D_2 were then obtained. A, T and D stand for *A. mongolicus*, *T. mongolica* and *D. paxianum* respectively.

Determination of scavenging activities against superoxide anion radical with EPR

EPR assay was carried out as described by Arudi with some modifications (1981). One hundred μ l sample dissolved in DMSO at different concentrations was added into a test tube containing 890 μ l of DMSO. Ten μ l of 50 mmol/L NaOH were then added. Forty μ l of the mixture were added into a long aqueous quartz tube, which was allowed to incubate at room temperature for 30 minutes before it was transferred into liquid nitrogen immediately. Inhibition ratio was determined by comparing with a control group. EPR spectra were obtained with a Bruker ER 200D SRC spectrometers and ER 4111 VT temperature controller using micro-sampling pipettes under the following conditions: SF, 9.59 GHz; SP, 20 mW; MA, 5 G; MF, 100 KHz; GN, 2.5×10^4 ; CF, 3360 G; SW, 300 G; TC, 200 mS; TI, 100 S; TE, 130 K. The inhibition percentage was calculated as: inhibition % = $100 \times (\text{ref-extract})/\text{ref}$, where ref is the reference signal (DMSO-NaOH), extract is the test signal. Each value was the mean of five measurements.

DPPH assay

A method similar to Cotelle's was employed except that methanol was used instead of ethanol (Cotelle et al., 1996). Two ml of 0.2 mmol/L DPPH in methanol were gently mixed with 2 ml solutions of the extracts at different concentrations (diluted to final concentrations of 500, 100, 50 and 10 μ g/ml). L-Ascorbic acid was used as positive control. The inhibition percentage was

calculated as: inhibition % = $100 \times [1 - (A_i - A_j)/A_c]$, where A_c is the absorbance of DPPH without extracts, A_j is the absorbance of extracts, and A_i is the absorbance of DPPH with extracts.

Lipid peroxidation assay

Lipid peroxidation assay was carried out basically as described by Buege and Aust with some modifications (Buege and Aust, 1978). Sprague-Dawley mice (σ or f , 220 ± 20 g) were purchased from the Centre of Experimental Animals, The Second Military Medical University. The liver tissue of SD mice was prepared as 10% tissue homogenate. One volume of homogenate was mixed thoroughly with two volumes of the stock solution of 10% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 mol/L hydrochloric acid. The absorbance of the clear supernatant was determined at 535 nm. The amount of MDA produced by hydrolyzing TEP was used as standard (Csallany et al., 1984). L-Ascorbic acid was used as positive control. The inhibition percentage was calculated as: inhibition % = $100 \times (\text{Abs}_{\text{standard}} - \text{Abs}_{\text{extract}})/\text{Abs}_{\text{standard}}$.

Statistical analysis

All values were expressed as mean \pm SD. Student's t-test was used to determine the significance of differences in all analyses. p values of less than 0.05 were considered to be statistically significant.

RESULTS

Preparations of extracts from *A. mongolicus*, *T. mongolica* and *D. paxianum*

Three hundred and fifty g A_1 , 190 g T_1 and 200 g D_1 were obtained from 6400 g A_0 , 5600 g T_0 , and 5000 g D_0 respectively. Four extracts were further eluted from A_1 fraction by chromatography, A_3 (petroleum ether 100%, 30 g), A_4 (petroleum ether : ethyl acetate 6:4, v/v, 35 g), A_5 (petroleum ether : ethyl acetate 1:1, v/v, 45 g) and A_6 (petroleum ether : ethyl acetate 2:8, v/v, 20 g) respectively; and two extracts from T_1 , T_3 (petroleum ether : ethyl acetate 8:2, v/v, 37 g) and T_4 (petroleum ether : ethyl acetate 4:6, v/v, 40 g) respectively. From the H_2O soluble fractions, three *n*-BuOH soluble fractions were derived, A_2 (190 g), T_2 (120 g) and D_2 (150 g) respectively. A_7 (chloroform : methanol 1:1, v/v, 35 g) and T_5 (chloroform : methanol 1:1, v/v, 28 g) were finally eluted from A_2 and T_2 respectively by chromatography (Figure 1).

Scavenging activities of the extracts against superoxide anion radical

Upon the addition of a scavenger, a decrease in $\cdot O_2^-$ signal intensity is a reflection of its scavenging activity. In this study, it was expressed as the IC_{50} value (w/v) of the scavenger, at which 50% $\cdot O_2^-$ generated was counteracted. The scavenging activities of eight extracts from *A. mongolicus* and six extracts from *T. mongolica*, as well as three from *D. paxianum*, were determined accordingly,

and their IC_{50} values were summarized in Table 1. All of the extracts exhibited significantly scavenging activities against the $\cdot O_2^-$ by EPR. Generally, the extracts from *A. mongolicus* showed higher scavenging activities than the extracts from *T. mongolica*, and, as expected, the extracts from *D. paxianum* the lowest.

Interestingly, upon further partitioning and/or eluting of the initial ethanol extract, a general tendency of enhanced scavenging activity was observed (Table 1), particularly as the ratio of ethyl acetate to petroleum ether in eluting fluids was raised (Figure 3). A_5 showed the lowest IC_{50} value among the all, implicating that more and/or stronger $\cdot O_2^-$ scavengers were concentrated in the extract. With A_5 , a scavenging activity against $\cdot O_2^-$ could still be detected at a concentration as low as 0.006 g/ml. An EPR spectrum of A_5 scavenging activities against $\cdot O_2^-$ was shown in Figure 2.

Scavenging activities of selected extracts against DPPH radical

The inhibition percentages of five selected extracts against stable DPPH radical were determined accordingly and their inhibition percentages listed in Table 2. Different

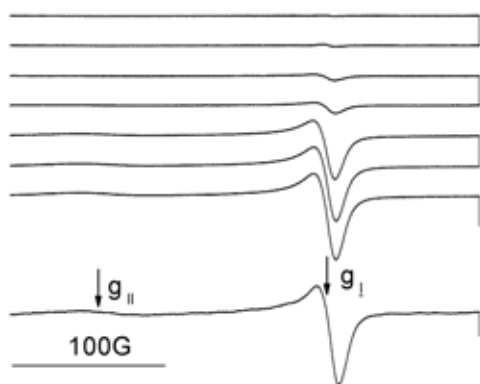


Figure 2. Scavenging activities of diluted A_5 against $\cdot O_2^-$ detected by EPR. A series of dilutions (50%, 40%, 30%, 20%, 15% and 10%) was made from its initial concentration of 0.06 g/ml, and their respective scavenging capacities (100%, 96.38%, 92.57%, 88.22%, 46.38%, 42.75% and 22.46%) against superoxide anion radical ($\cdot O_2^-$) were determined accordingly, with 100% counteracted by the undiluted initial A_5 (the top), and 0% by blank (the bottom).

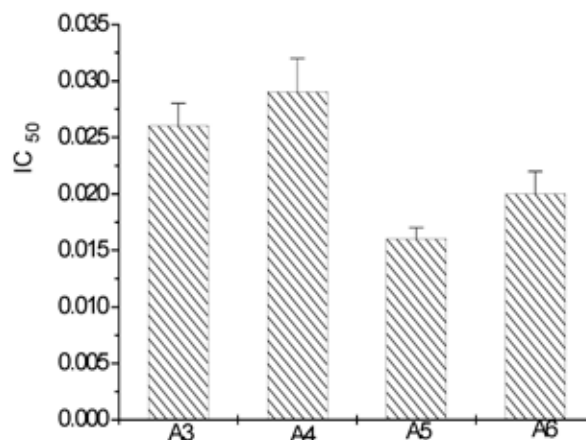


Figure 3. Comparison of IC_{50} values of scavenging $\cdot O_2^-$ activities of four extracts from *A. mongolicus* by EPR. The IC_{50} values of A_3 , A_4 , A_5 and A_6 were 0.026, 0.029, 0.016 and 0.020 respectively. A stands for *A. mongolicus*.

Table 1. The IC_{50} values of all the extracts against superoxide anion radical with EPR.

| | Concentrations (g/ml) |
|---|-----------------------|
| A_0 ethanol extract | 0.088±0.015 |
| A_1 ethyl acetate extracts | 0.075±0.010 |
| A_2 <i>n</i> -BuOH -eluted extracts | 0.151±0.008 |
| A_3 (petroleum ether 100%) | 0.026±0.005 |
| A_4 (petroleum ether : ethyl acetate 6:4) | 0.029±0.005 |
| A_5 (petroleum ether : ethyl acetate 1:1) | 0.016± 0.009 |
| A_6 (petroleum ether : ethyl acetate 2:8) | 0.020±0.005 |
| A_7 (chloroform : methanol 1:1) | 0.024± 0.010 |
| T_0 ethanol extract | 0.110± 0.010 |
| T_1 ethyl acetate extracts | 0.093±0.009 |
| T_2 <i>n</i> -BuOH-eluted extracts | 0.085± 0.013 |
| T_3 (petroleum ether : ethyl acetate 8:2) | 0.035±0.011 |
| T_4 (petroleum ether : ethyl acetate 4:6) | 0.070±0.008 |
| T_5 (chloroform : methanol (1:1) | 0.020±0.005 |
| D_0 ethanol extract | 0.182±0.020 |
| D_1 ethyl acetate extracts | 0.210±0.015 |
| D_2 <i>n</i> -BuOH-eluted extracts | 0.156±0.011 |

Table 2. Inhibition percentages of selected extracts to DPPH radical.

| Concentrations (μ g/ml) | Extracts | | | | | |
|---------------------------------|----------|----------|----------|----------|----------|-----------------|
| | A_1 | A_2 | T_1 | T_2 | A_5 | L-Ascorbic acid |
| 10 | 11.7±2.0 | 5.42±0.3 | 0 | 0 | 15.8±1.3 | 74.03±2.3 |
| 50 | 29.0±3.6 | 11.7±2.0 | 24.5±1.1 | 12.3±3.6 | 41.4±4.2 | 80.25±3.0 |
| 100 | 45.7±2.5 | 16.2±2.3 | 60.8±3.5 | 33.8±2.8 | 72.2±2.5 | 96.30±2.1 |
| 500 | 92.2±1.2 | 47.2±3.4 | 95.8±2.9 | 92.2±3.5 | 96.0±3.1 | 99.64±0.9 |

The scavenging effects were expressed as the percentage inhibition (means±S.D., n=4) compared to the blank (buffer instead of extract). L-Ascorbic acid was used as a positive control.

extracts showed varied inhibition percentages to DPPH radical, with the A₁ and A₅ being relatively stronger. A₅ showed stronger scavenging capacities particularly at concentrations higher than 10 µg/ml. However, its scavenging capacities were smaller than those of L-ascorbic acid at comparable concentrations.

Lipid peroxidation assay on selected extracts

Inhibitory effects of five selected extracts to the production of MDA were determined using the mouse liver tissue, with L-ascorbic acid as a positive control, and their inhibition percentages at different concentrations were shown in Figure 4. Although all the extracts examined exhibited significantly antioxidative capacities at most concentrations, their capacities were generally slightly or significantly smaller than that of L-Ascorbic acid at comparable concentrations. Only at concentrations lower than 1 mg/ml, did A₅ and T₂ show significantly or slightly higher anti-oxidative capacities than those of L-Ascorbic acid, being 6.57% and 1.75% higher respectively at 0.1 mg/ml, and 11.53% and 9.84% at 0.01 mg/ml (Figure 4).

Identification of major pure compounds and scavenging activity of resveratrol against ·O₂⁻

Since A₅ consistently exhibited the highest scavenging activities or antioxidative capacities in the previous assays, an attempt was made to obtain pure scavengers or antioxidants from the extract. We isolated a major compound from A₅, which was identified as resveratrol according to its IR absorption, ¹H NMR (Nuclear Magnetic Resonance) spectrum and ¹³C NMR signal information. Purified resveratrol from A₅ accounted for 0.05% of the dried weight of *A. mongolicus*. Other three pure compounds (5, 7, 4'-trihydroxyisoflavone, maackiain and 7, 3'-dihydroxy-4'-methoxyisoflavone) were isolated and identified from A₄. In the scavenging activity assay against ·O₂⁻, resveratrol exhibited a large magnitude of antioxidative potency. At concentrations from 0.0375 to

Table 3. Scavenging activities of resveratrol against ·O₂⁻.

| | Concentration (g/mL) | Inhibition (%) |
|-------------|----------------------|----------------|
| Resveratrol | 0.0375 | 100 |
| | 0.0300 | 97.86 |
| | 0.0225 | 95.72 |
| | 0.0113 | 93.58 |
| | 0.0038 | 90.37 |
| Vitamin E | 0.0521 | 99.93 |
| | 0.0208 | 97.74 |
| | 0.0156 | 93.26 |
| | 0.0104 | 90.26 |

0.0038 g/ml, over 90% of ·O₂⁻ generated was counteracted, compared to Vitamin E with which a two to three times higher concentration (0.0521 to 0.0104 g/ml) was required to achieve a similar inhibition ratio (Table 3). However, when we tried to dilute them further, both of them exhibited non-characteristic spectra of ·O₂⁻, which might be due to the presence of excess hydroxyl groups in both of the compounds. Further analysis is under way.

DISCUSSION

Over-generation of ROS is observed under diversified stress conditions (Elstner et al., 1988; Foyer and Harbinson, 1994). ROS can break and destruct bio-molecules and membranes. Some small molecules, such as phenolic and flavonoid compounds, play a big part in scavenging ROS (Foyer et al., 1994), and it has been observed that UV-B radiation causes an increase in the synthesis of UV-absorbing flavonoids (Gusman et al., 2001). However, to our best knowledge, the correlation between the severity of environmental conditions, with which plants grow naturally, and their non-enzymatic scavenging activity against ROS has not been meaningfully explored. In this study, we found that various extracts from two distinctive species, *A. mongolicus* and *T. mongolica*, stressed under seasonally extreme environmental conditions and constant poor soil qualities with high salinity in their lives, exhibited significantly higher scavenging activities against the ·O₂⁻, than those of the extracts from *D. paxianum*, a species growing in the much milder environment with relatively fertile soil in the northern mountain area of Guangdong province, the southeast part of China. The results indicated a preliminary positive correlation between the severity of plant environmental conditions and their non-enzymatic scavenging activities against the ·O₂⁻.

The concentration-dependent scavenging activities of A₅ dilutes implied the existence of strong active oxygen scavengers in the extract, which lead to the isolation of a strong antioxidant, resveratrol. It accounted for as high as

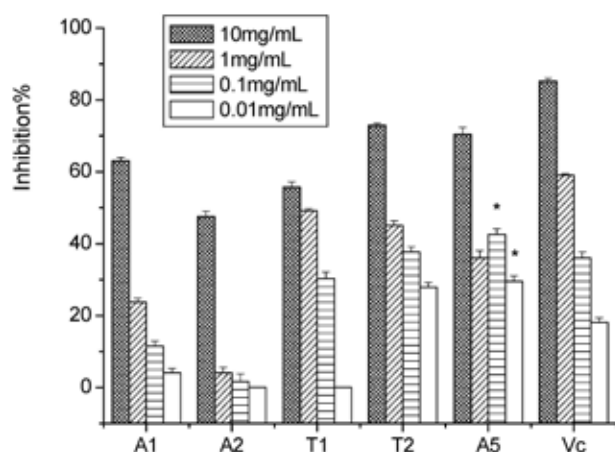


Figure 4. Inhibition percentages of five selected extracts to the production of MDA at different concentrations. Each bar represents mean ± S.D. (n=3). (*p<0.05, vs. Vc).

0.05% of the dried weight of *A. mongolicus*. The extraordinarily high content is in a sharp comparison with that (0.001-0.0005%) in the *Vitis vinifera*, which was reported to be the most abundant natural source of resveratrol (Langcake and Pryce, 1976). Resveratrol is a kind of non-flavonoid polyphenol, and has been identified as a natural antioxidant in red and white wine (Soleas et al., 1997; German and Walzem, 2000). Plants synthesize resveratrol in response to fungal infections and UV irradiation stress (Langcake and Pryce, 1976; Jang et al., 1997), as well as to nutrient limitation (Soleas et al., 1997). Taken together, these results help to explain the toughness of *A. mongolicu*.

Resveratrol has been found to suppress NMBA-induced rat esophageal tumorigenesis by targeting COXs and PGE₂ (Li et al., 2002). The two major isoflavone compounds isolated have been reported to have strong antioxidative and anti-inflammatory activities (Cao, 1997). Another major compound, maackiain, has been known to be anti-bacterial/fungal (Cachinero et al., 2002). In one of our separate experiments, significant effects of these extracts on the extensions of life span of *D. melanogaster* were observed, with adequate concentrations varying from 1 mg to 50 mg/100 g (data to be published elsewhere). All these data support our working hypothesis about the efficacy of *A. mongolicus* in the traditional treatment of respiratory disorders, stomachache, cold-caused wounds and chronic rheumatism.

DPPH and lipid peroxidation have been used as convenient tools for radical scavenging assays. Five selected extracts with the lowest IC₅₀ against the ·O₂⁻ also showed antioxidative capacities against DPPH radical and lipid peroxidation radical at different magnitudes of potency, with the A₅ still being the strongest antioxidant. The consistency of antioxidative capacities of these extracts against different radicals indicates that these extracts have wider spectra of scavenging activities.

In conclusion, the accumulation of antioxidants could be stimulated to an extraordinarily high level in plant species distributed in extremely stressful environments. And the medicinal efficacy of *A. mongolicus* in the treatments of respiratory disorders, stomachache, cold-caused wounds and chronic rheumatism may be fundamentally related to the antioxidative process. One implication of our results is that some plant species living in the harsh environment could become an abundant natural resource for the development of medicines for anti-inflammatory and anti-infectious, as well as for anti-degenerative disorders and even anti-tumorigenesis. It is also advisable to develop new types of healthy food and / or food additives from the edible parts of plants living in stressful environments or to make food crops more nutritional and/or healthy by stressing them before harvesting.

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分佈於沙漠和戈壁極端環境下的沙冬青和四合木體內 抗氧化劑的超量積聚

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沙冬青和四合木分佈於我國西北和蒙古的沙漠和戈壁地區，是耐極度乾旱、極端溫度、貧瘠和鹽鹼的子遺植物。沙冬青在夏季攝氏四十度高溫和冬季零下攝氏三十度低溫的沙漠上能維持常綠不落葉，被當地居民製成傳統藥物治療呼吸道疾病、凍瘡、類風濕和止痛。本研究的主要目的是分析極端逆境下植物體內抗氧化劑的積累，並據此推測沙冬青用於多種傳統疾病治療的可能機理。利用化學分離的手段，借助於電子順磁共振波譜法、DPPH 染色法和脂質過氧化的方法，通過對各組份浸膏抗氧化活性的分析測定，進而對主要抗氧化成分進行追蹤分離。利用不同有機溶劑及其不同配比組合分離獲得的浸膏與對照相比都顯示出了較高的清除自由基活性，其中以 1 份石油醚與 1 份乙酸乙酯的配比從沙冬青中提取出的浸膏清除自由基活性最高。從高活性組份中繼續分離獲得了大量的白藜蘆醇（占總幹重植株的 0.05%）以及兩種異黃酮。這一結果表明，極端惡劣的生態環境有利於植物中強抗氧化劑的累積；同時也表明沙冬青對多種傳統疾病的治療功效可能與其強抗氧化作用有關。

關鍵詞：活性氧；沙冬青；四合木；白藜蘆醇；超氧陰離子；DPPH 自由基；脂質過氧化；電子順磁共振波譜法。