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Research Article

Comparison of Xanthine Oxidase-Inhibiting and Free Radical-Scavenging Activities Between Plant Adaptogens of *Eleutherococcus senticosus* and *Rhodiola rosea*

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ABSTRACT The present study employed 2,2-diphenyl-l-picrylhydrazyl (DPPH) radical-scavenging and xanthine–xanthine oxidase (XO) assays to compare the antioxidant capacity between two plant adaptogens, *Eleutherococcus senticosus* (Araliaceae) and *Rhodiola rosea* (Crassulaceae). The IC_{50} value for XO activity for *Rhodiola* was 355.4 μg/ml, while that for *Eleutherococcus* was >1,000 μg/ml. *Eleutherococcus* inhibited DPPH generation by 58.3 ± 2.8% at 1,000 μg/ml, whereas *Rhodiola* inhibited DPPH radical by 91.1 ± 2.6% at the same concentration. The results suggested that *Rhodiola* inhibited not only XO but also served as a potent radical scavenger. *Rhodiola* has potential as a natural source of antioxidants. Drug Dev Res 71:249–252, 2010.

Key words: adaptogens; *Eleutherococcus senticosus*; *Rhodiola rosea*

INTRODUCTION

The importance of reactive oxygen species (ROS) and free radicals in human disease states has attracted increasing attention over the past decade. ROS can initiate a wide range of toxic oxidative reactions [Mircescu, 2006]. ROS released by phagocytic cells are involved in the link between inflammation and cancer. Excessive and persistent formation of ROS by inflammatory cells is a key factor in their genotoxic effects. Additionally, ROS have been implicated in many degenerative diseases, including aging, arthritis, and Parkinson's disease [Carreras et al., 2004; Droge, 2002; Hogg, 1998; Inoue et al., 2003]. Therapeutic strategies thus should aim at reducing free-radical formation and scavenging free radicals [Berg et al., 2004]. Natural compounds with antioxidant actions such as vitamins and minerals, polyphenols, and other non-nutrient compounds of plants, which inhibit generation of ROS, or which scavenge free radicals, are may be beneficial for human health [Badami et al., 2003; Braca et al., 2002].

Adaptogens are harmless herbs that have pharmaceutical benefits due to their balancing, regulative and tonic functions [Boon-Niermeijer et al., 2000; Panossian and Wagner, 2005]. Special attention was

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paid to the reported pharmacological effects of the adaptogen-containing plant, *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. (Araliaceae), referred to by some as "Siberian ginseng," and to its secondary chemical composition [Davydov and Krikorian, 2000]. *Rhodiola rosea* (Crassulaceae), also known as golden root, can increase the resistance of a variety of organisms against the damaging effect of different stress conditions [Brown et al., 2002; Khanum et al., 2005]. Based on these observations, it is suggested that adaptogens are experienced as mild stressors at the lifespan enhancing concentrations and thereby induce increased stress resistance and a longer lifespan [Boon-Niermeijer et al., 2000; Panossian and Wagner, 2005]. Despite these effects, the antioxidant potential of adaptogens is rarely mentioned.

Intracellular ROS production is associated with a number of cellular events including activation of NAD(P)H oxidase, xanthine oxidase (XO, EC 1.2.3.2), and the cellular mitochondrial respiratory chain. Novel inhibitors of XO may be beneficial in various diseases [Perez-Ortiz et al., 2007]. The XO [Nguyen et al., 2005] and 2,2-diphenyl-l-picrylhydrazyl radical (DPPH) assays [Acuna et al., 2002; Braca et al., 2002] have been routinely used to evaluate antioxidant activity due to free radical generation and scavenging and were employed to compare the antioxidant activity *E. senticosus* and *R. rosea*

MATERIALS AND METHODS

Reagents

2,2-Diphenyl-picrylhydrazyl radical (DPPH), xanthine oxidase (XO), xanthine, and allopurinol were obtained from the Sigma-Aldrich (St. Louis, MO). All other reagents were of first grade.

Plant Adaptogens

The concentrated powders from *E. senticosus* and *R. rosea* were supplied by Sheng Chang Pharmaceutical Co., Ltd. (Taipei, Taiwan) under internationally certified Good Manufacturing Practices guidelines. The experienced botanists and chemists at the supplier used macroscopic and microscopic examinations as well as thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) identification to authenticate the plants, plant parts used, and processed raw herbs. The reference specimens were deposited at the herbarium of the supplier. The powder was dissolved in sterile water and stored as a stock concentration of 100 mg/ml at 4°C until use.

Assay for Inhibition of Xanthine Oxidase Activity

The activity of XO with xanthine as a substrate, was measured spectrophotometrically [Nguyen et al., 2005]. The final concentration of XO was 250 μU/ml in a 0.1 mmol/L phosphate buffer (pH 7.4). Xanthine and XO were mixed in a cuvette with either the compounds tested or vehicle. The difference of absorbance was measured at 295 nm for 3 min, and the enzyme activity was calculated with references:

$$(\text{activity of control} - \text{activity of the mixture})/(\text{activity of control}) \times 100.$$

The extent of inhibition was expressed as the concentration of the chemical required to inhibit 50% of enzyme activity (IC_{50}).

DPPH Free Radical Scavenging Activity

The free radical scavenging activity of *E. senticosus* or *R. rosea* was measured using DPPH. This activity was measured by the method of Blois [1958] wherein the bleaching rate of a stable free radical, DPPH was monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH absorbed at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreased. Briefly, 0.1 mmol/L solution of DPPH in ethanol was prepared, and 1 ml of this solution was added to 3 ml of *E. senticosus* or *R. rosea* solution in ethanol at different concentrations. After 30 min, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_{\text{Control}} - A_{\text{Sample}}/A_{\text{Control}}) \times 100],$$

where A_{Control} is the absorbance of the control reaction and A_{Sample} is the absorbance in the presence of *E. senticosus* or *R. rosea*.

Statistics

Data were expressed as the mean ± standard deviation (SD) of the number (n) of animals in the group, as indicated in the Tables and Figures. Statistical differences among groups were determined by using two-way repeated-measures analysis of variance (ANOVA). The Dunnett range post hoc comparisons were used to determine the source of significant differences where appropriate. A P-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

XO Inhibition

XO is the enzyme responsible for the formation of uric acid from hypoxanthine and xanthine. It serves as an important biological source of oxygen-derived free

radicals that contribute to oxidative damage to tissues involved in many pathological processes of ROS-induced diseases, e.g., inflammation, atherosclerosis, cancer, and aging [Perez-Ortiz et al., 2007]. Additionally, an inhibitor of XO has the potential to be a therapeutic agent for hyperuricemia [Pacher et al., 2006]. We further measured the antioxidant activity of two plant adaptogens using XO assay.

Allopurinol, a potent inhibitor of XO, is clinically used for gout treatment to prevent urate from accumulating in joints [Connor, 2009]. Figure 1 shows that allopurinol as well as *R. rosea* and *E. senticosus* inhibited the XO activity in a dose-dependent manner. Allopurinol inhibited XO with an IC_{50} value of 51 μg/ml; *R. rosea* was also an inhibitor against XO with an IC_{50} value of 355 μg/ml (Table 1). In contrast, *E. senticosus* was less potent with an IC_{50} value of $>1{,}000$ μg/ml (Table 1). Although *R. rosea* was not as effective in inhibiting XO as allopurinol, it was more potent than that of *E. senticosus*. Since there are numerous ROS sources in addition to XO additional experiments were performed.

DPPH Free Radical Scavenging Activity

The role of antioxidants lies in their interaction with oxidative free radicals [Berg et al., 2004]. The imbalance between ROS and antioxidant defense mechanisms leads to oxidative modification in cellular membrane or intracellular molecules. The DPPH assay measured hydrogen atom (or one electron) donating activity and hence provided an evaluation of antioxidant activity due to free radical scavenging. DPPH, a purple-colored stable free radical, was reduced into the yellow-colored diphenylpicryl hydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant sample/conserves [Özcelik et al., 2003].

In this study, the free radical scavenging activities of *E. senticosus* and *R. rosea* were determined using DPPH. Figure 2 illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of *E. senticosus* and *R. rosea*. Moreover, the free radical scavenging activity of *E. senticosus* and *R. rosea* were concentration dependent. At 1000 μg/ml, *E. senticosus* inhibited DPPH generation by $58.3 \pm 2.8\%$. *R. rosea* at 1000 μg/ml inhibited DPPH by $91.1 \pm 2.6\%$, showing a strong scavenging activity towards DPPH indicating that *R. rosea* inhibited not only XO but also served as a potent radical scavenger, whereas *E. senticosus* had only a single role of radical suppression. *R. rosea* exhibited greater antioxidant activity, which may explain why it has been used in folk medicine as novel, natural and economic antioxidant were it may be helpful in preventing or slowing the progress of various oxidative stress-related diseases. From these results, it can be concluded that *R. rosea* might be better than *E. senticosus* in clinical applications to detoxify inflammatory oxidants and to prevent oxidative damage to cells and tissues.

TABLE 1. Concentration of Allopurinol, *Eleutherococcus senticosus* and *Rhodiola rosea* Required to Inhibit 50% of XO Enzyme Activity (IC_{50})

	IC_{50} (μg/ml)
Allopurinol	51.3 ± 8.6
Eleutherococcus senticosus	2847.6 ± 35.4
Rhodiola rosea	355.4 ± 20.4

Values (mean ± SD) were obtained for each group of five experiments.

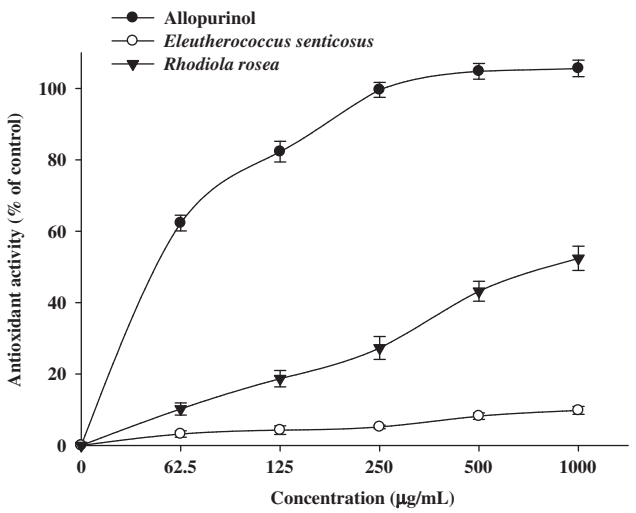

Fig. 1. Antioxidant activities of allopurinol (●), *Eleutherococcus senticosus* (○), and *Rhodiola rosea* (▼) in XO assay. Values (mean ± SD) were obtained for each group of five experiments.

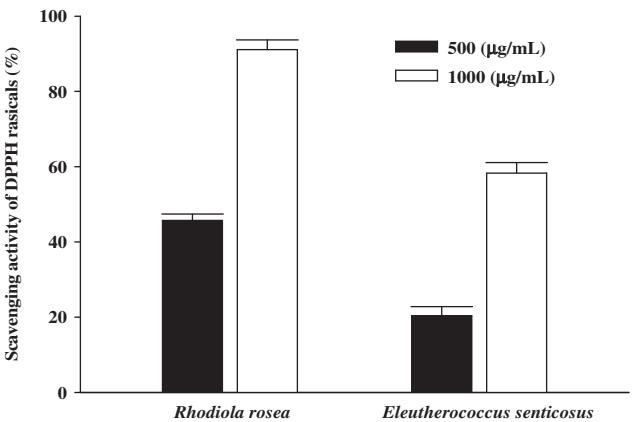

Fig. 2. Radical scavenging activity of *Eleutherococcus senticosus* and *Rhodiola rosea* using DPPH system. Values (mean ± SD) were obtained for each group of five experiments.

Currently, the various compounds in the extracts are being studied to identify those responsible for the activity reported. A number of major compounds in extracts from plant adaptogens have been identified, isolated and characterized. The major active constituents in *Acanthopanax senticosus* include acanthoside, eleutheroside, daucosterine, β-sitosterol, sesamine, and savinine, etc. [Park et al., 2000], while *R. rosea* contains phenylpropanoids, proanthocyanidins, and flavonoids. The most unique active chemical constituents are the phenylpropanoids, rosavin (the most active), rosin, rosarin, rhodiolin, salidroside, and its aglycon, *p*-tyrosol [Linh et al., 2000]. Apart from a search for responsible single components, a parallel approach aimed at identifying synergy between single compounds is of interest and may lead to additional benefit.

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