Antioxidant Evaluation of Three Adaptogen Extracts

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Abstract: Adaptogens are harmless herbs which have pharmaceutical benefits due to their balancing, regulative and tonic functions. However, despite these medicinal effects, the antioxidant potential of adaptogens is rarely mentioned. This study investigated the antioxidant potential of 3 adaptogen extracts, Rhodiola rosea (golden root), Eleutherococcus senticosis (Siberian ginseng) and Emblica officinalis (Indian gooseberry, Amla). The results of this study showed that R. rosea had the highest potential for singlet oxygen scavenging, hydrogen peroxide scavenging, ferric reducing, ferrous chelating and protein thiol protection than either of the other 2 extracts. E. senticosis, on the other hand, showed the best potential for hypochlorite scavenging. In addition, the polyphenol content in the 3 adaptogen extracts followed the order: R. rosea, E. officinalis and E. senticosis. Our data suggest that the antioxidant potential of the 3 adaptogen extracts was proportional to their respective polyphenol content. The supplementation of adaptogen extracts containing high levels of polyphenols may not only have adaptogen properties, but may decrease the risk of complications induced by oxidative stress.

Keywords: Adaptogen; Antioxidant; Chemiluminescence; FRAP; Oxidative Stress.

Introduction

Many herbs with physiological and pharmaceutical effects have been studied both in vitro and in vivo. Due to their medicinal properties, these herbs are usually manufactured as functional foods or supplements in order to improve quality of life. Adaptogen herbs, also called adaptogens, are well accepted as functional foods.

The word “adaptogen” is derived from the Greek word “adapto,” which means “to adjust.” Herbs described as adaptogens are harmless and usually have balancing, regulative and tonic functions. However, despite these medicinal effects, the antioxidant potential of adaptogens is rarely mentioned. This study investigated the antioxidant potential of 3 adaptogen extracts, Rhodiola rosea (golden root), Eleutherococcus senticosis (Siberian ginseng) and Emblica officinalis (Indian gooseberry, Amla). The results of this study showed that R. rosea had the highest potential for singlet oxygen scavenging, hydrogen peroxide scavenging, ferric reducing, ferrous chelating and protein thiol protection than either of the other 2 extracts. E. senticosis, on the other hand, showed the best potential for hypochlorite scavenging. In addition, the polyphenol content in the 3 adaptogen extracts followed the order: R. rosea, E. officinalis and E. senticosis. Our data suggest that the antioxidant potential of the 3 adaptogen extracts was proportional to their respective polyphenol content. The supplementation of adaptogen extracts containing high levels of polyphenols may not only have adaptogen properties, but may decrease the risk of complications induced by oxidative stress.

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functions in those who take them (Wiston, 2004; Brown et al., 2002). A number of herbs are classified as adaptogens; the most common are ginsengs, *Rhodiola rosea* (golden root), *Eleutherococcus senticosis* (Siberian ginseng) and *Emblica officinalis* (Indian gooseberry, Amla). In previous studies of *R. rosea*, *E. senticosis* and *E. officinalis* were shown to exhibit not only adaptogen properties, but also anti-inflammatory activity, cardioprotection, hepatoprotection as well as immune enhancing effects (Chen and Chen, 2004; Rege et al., 1993; Dorbinyan and Kteyan, 2000). In addition, these adaptogen herbs also have antioxidant potential which is rarely mentioned.

This study aimed to investigate the antioxidant properties of *R. rosea*, *E. senticosis* and *E. officinalis* by screening their ability to scavenge singlet oxygen, hypochlorite and hydrogen peroxide by using chemiluminescent analysis. Furthermore, their ferric reducing/antioxidant power (FRAP), iron chelating potential and protein thiol protective effect were also examined to determine whether these adaptogen herbs were also capable of preventing oxidative stress-induced complications (Babior, 2000; Droge, 2002; Chapple, 1997).

**Materials and Methods**

**Adaptogen Extracts**

The 3 adaptogen extracts, *R. rosea*, *E. senticosis* and *E. officinalis*, were obtained from Numen Biotech Co., Ltd. (Taipei, Taiwan) with batch numbers of 20040407-12, 040419 and 608291, respectively.

**Chemicals and Reagents**

Acetonitrile, methanol and ethanol were purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA 08865), and phosphate buffered saline (PBS) was purchased from the Invitrogen Corporation (Grand Island, NY, USA 14072). Other chemicals and reagents were from Sigma-Aldrich Inc. (St. Louis, MO, USA).

**Singlet Oxygen Scavenging Potential**

The first step in this experiment was to produce singlet oxygen in a chemiluminescent analyzer which was achieved by mixing Na2CO3 buffer, H2O2 and acetonitrile in basic conditions (Lu et al., 2002). Then 0.5 ml of Na2CO3 buffer (100 mM), 0.05 ml of H2O2 (1.7%) and 0.05 ml of adaptogen extract were added to the chemiluminescent analyzer (CLA-FS1, Tohoku Electronic Industrial Co. Ltd., Miyagi, Japan). After mixing for 20 sec, 0.1 ml of luminol solution (20 mg of luminol in 20 ml of 30% acetonitrile) was added by injection. Intensive chemiluminescence was detected during this step due to singlet oxygen production. Measurement of chemiluminescence was terminated after 60 sec. During this experiment, distilled water, trolox (6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water-soluble vitamin E-like antioxidant) and ascorbic acid were used as the blank, standard and control, respectively. The scavenging potential of the 3 adaptogen extracts for singlet oxygen were expressed as trolox equivalents.
Hypochlorite Scavenging Potential

The experimental protocol was modified as described by (Li et al., 2002). In the experiment, 1 ml of distilled water, 0.02 ml of NaOCl (0.037%) and 0.1 ml of adaptogen extract were added to the chemiluminescent analyzer. After mixing for 20 sec, 0.1 ml of luminol solution (44 mg of luminol in 10 ml of 0.01 N sodium hydroxide) was added by injection. Intensive chemiluminescence was observed during this step. Measurement of chemiluminescence was terminated after 90 sec. During this experiment, distilled water, trolox and ascorbic acid were used as the blank, standard and control, respectively. The scavenging potential of the 3 adaptogen extracts for hypochlorite were expressed as trolox equivalents.

Hydrogen Peroxide Scavenging Potential

The chemiluminescence induced by H2O2 was measured by mixing H2O2 and lucigenin in Tris buffer (Yeung et al., 2002). In this study, 0.1 ml of H2O2 (1.7%), 1 ml of Tris buffer (50 mM, pH 7.2) and 0.1 ml of adaptogen extract were added to the chemiluminescent analyzer. After mixing for 20 sec, 0.1 ml of lucigenin solution (1.96 mM) was added by injection. Intensive chemiluminescence was measured during this step. Measurement of chemiluminescence was terminated after 90 sec. During this experiment, distilled water, trolox and ascorbic acid were used as the blank, standard and control, respectively. The scavenging potential of the 3 adaptogen extracts for hydrogen peroxide were expressed as trolox equivalents.

Ferric Reducing/Antioxidant Power (FRAP) Assay

The FRAP assay was modified from that described by Othman et al. (2007). The FRAP reagent was prepared by mixing acetate buffer (246.1 mg of sodium acetate in 10 ml of 10% acetic acid), TPTZ solution (31.23 mg of TPTZ and 0.044 ml of 37% HCl in 10 ml of distilled water) and FeCl3 solution (54.06 mg of FeCl3·6H2O in 10 ml of distilled water) in a ratio of 10:1:1. The FRAP reagent (0.5 ml), distilled water (0.05 ml) and 0.016 ml of adaptogen extract were transferred into vials and incubated at room temperature. After 4 min incubation, the absorbance (λ = 593 nm) in all vials was measured. During this experiment, distilled water, FeSO4 and ascorbic acid were used as the blank, standard and control, respectively. The FRAP data for the 3 adaptogen extracts were expressed as FeSO4 equivalents.

Iron Chelating Analysis

The method was modified from that described by Dziezak et al. (1986). Briefly, 0.925 ml of PBS, 0.025 ml of FeCl2 (2.5 mg of FeCl2 and 1.05 ml of 12 N HCl in 8.95 ml of distilled water) and 0.2 ml of adaptogen extract were pipetted into vials. After mixing for 30 sec, 0.05 ml of ferrozine solution (25 mg of ferrozine in 10 ml of distilled water) was added. All vials were incubated for 10 min at room temperature and absorbance (λ = 562 nm) was then measured. PBS, EDTA and ascorbic acid were used as the blank, standard and control, respectively. The iron chelating potential of the 3 adaptogens was expressed as EDTA equivalents.
HSA Thiol Group Determination

The presence of the protein (human serum albumin, HSA) thiol group was determined by the method described by Hu (1994). The HSA stock solution (20 mg/ml) was divided into 5 and included a control (C), C with glutaraldehyde (C + G), C + G with R. rosea (C + G + R), C + G with E. senticosis (C + G + E), and C + G with E. officinalis (C + G + A). For the analysis, 100 µl of C, 2 µl of G (2.5%) and 5 µl of each adaptogen extract were transferred into appropriate vials. Then 900 µl of Tris-EDTA buffer (6.06 mg of Tris base and 1488 mg of EDTA•2Na in 200 ml of distilled water, pH 8.2) was added to the vials and the absorbance was read at 412 nm (A1). A 25 µl aliquot of DTNB reagent (39.63 mg of DTNB in 1 ml of methanol) was added to all the vials and incubated at room temperature. After 15 min incubation, the absorbance at 412 nm for all the vials (A2) and the blank (B, DTNB reagent) was measured. The protein thiol concentration was calculated according to (A2 - A1 - B) and the data were expressed as cysteine equivalents.

Polyphenol Analysis

Analysis of the polyphenol content of the 3 adaptogen extracts was carried out by using the Folin-Ciocalteu reagent (Marinova et al., 2005). In this analysis, 15 ml of the adaptogen extract was diluted with 80% ethanol to give a final concentration of 0.094 mg/ml, 0.188 mg/ml and 0.375 mg/ml, respectively. Then, 0.5 ml of the diluted extract solution, 6.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu reagent (2 N) and 5 ml of Na2CO3 buffer (7% w/v) were mixed in test tubes. After 90 min incubation at room temperature, the absorbance (λ = 620 nm) in all samples was measured.

Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD). Data analysis was carried out using ANOVA. Values of p < 0.05 were considered statistically significant.

Results

Chemiluminescent analysis detects ultra-weak luminescence when an oxidant oxidizes light-emitting materials, such as luminol or lucigenin, and the luminescence intensity is proportional to the concentration of the oxidants. Antioxidants scavenge oxidants which lead to a decrease in luminescence intensity. Table 1 shows the results of chemiluminescent analysis in determining the antioxidant potential of the 3 adaptogen extracts for scavenging singlet oxygen, hydrogen peroxide and hypochlorite. The order of antioxidant potential for scavenging singlet oxygen was R. rosea (R, 39.25 ± 1.43) > E. officinalis (A, 6.03 ± 0.14) > E. senticosis (E, 5.45 ± 0.24). Furthermore, the order of antioxidant potential for scavenging hypochlorite was E (5.45 ± 0.24) > R (3.33 ± 0.22) > A (3.13 ± 0.19), and the order of the antioxidant potential for scavenging hydrogen peroxide was R (0.69 ± 0.03) > A (0.092 ± 0.01) > E (0.051 ± 0.02). The data showed that R. rosea exhibited excellent
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Table 1. Chemiluminescent Analysis of Reactive Oxygen Species (ROS) Scavenging Power of Three Adaptogen Extracts

<table>
<thead>
<tr>
<th></th>
<th>Singlet Oxygen</th>
<th>Hypochlorite</th>
<th>Hydrogen Peroxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>39.25 ± 1.43</td>
<td>33.3 ± 0.22</td>
<td>0.69 ± 0.03</td>
</tr>
<tr>
<td>E</td>
<td>4.68 ± 0.17</td>
<td>5.45 ± 0.24</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>A</td>
<td>6.03 ± 0.34</td>
<td>3.13 ± 0.19</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Vit. C</td>
<td>1.82 ± 0.03</td>
<td>16.16 ± 0.30</td>
<td>1.02 ± 0.01</td>
</tr>
</tbody>
</table>

*Data were expressed as mean (n = 6) ± SD and trolox equivalents (mg trolox/mg extract).

Antioxidant potential for scavenging singlet oxygen and hydrogen peroxide compared to the other 2 adaptogen extracts. *E. senticosis*, on the other hand, showed the highest antioxidant potential for scavenging hypochlorite.

An antioxidant can donate electrons to terminate the chain reaction induced by free radicals. This is one reason why antioxidants are capable of scavenging free radicals. The FRAP assay is a method used to investigate the electron-donating ability of an antioxidant. The principle of the FRAP assay is that electrons offered by an antioxidant will convert the Ferric-TPTZ complex into a blue colored Ferrous-TPTZ complex (Othman *et al.*, 2007), making the antioxidant potential proportional to the FRAP value. Figure 1 shows the FRAP data for the 3 adaptogen extracts. At a high concentration (0.1 mg/ml), the order of FRAP data was R (368 ± 12) > A (241 ± 48) > E (148 ± 25). At a low concentration (0.01 mg/ml), the order was R (133 ± 12) > A (29 ± 2) > E (undetectable). We observed that *R. rosea* showed the highest FRAP data in both low and high concentrations.

Under normal physiological conditions, the ferrous ion is capable of initiating free radical production via the Fenton reaction (Dziezak, 1986). Therefore, the iron chelating potential of antioxidants is important in order to block the production of free radicals. Figure 2 shows the iron chelating potential of the 3 adaptogen extracts. At a high concentration (1 mg/ml), the

![Figure 1. FRAP analysis of 3 adaptogen extracts. *Data were expressed as mean (n = 3) ± SD and FeSO₄ equivalents (µM).](image-url)
Figure 2. Iron chelating analysis of three adaptogen extracts. *Data were expressed as mean (n = 3) ± SD and EDTA equivalents (mg).

Iron chelating potential of the 3 adaptogen extracts followed the order: R (0.03 ± 0.002) > A (0.027 ± 0.005) > E (0.024 ± 0.004). At a low concentration (0.1 mg/ml), the iron chelating potential of the 3 adaptogen extracts followed the order: R (0.022 ± 0.001) > E (0.02 ± 0.002) > A (0.013 ± 0.001). *R. rosea exhibited excellent potential in chelating the ferrous ion in both low and high concentrations.

In the presence of oxidants, position 34 (Cys-34) of HSA is easily oxidized, thereby reducing the thiol (SH group) content of HSA (Mera et al., 2005). On the other hand, antioxidants can scavenge oxidants and reduce the depletion of the thiol content of HSA. Figure 3 shows that the thiol content of HSA was depleted in the presence of glutaraldehyde (C vs. C + G = 18.9 ± 0.3 vs. 10.8 ± 1.0). In the presence of the adaptogen extracts, the thiol content of HSA was elevated due to the termination of HSA oxidation by glutaraldehyde. These results were statistically significant: (C + G + R > C + G = 16.7 ± 0.5 > 10.8 ± 1.0, p < 0.05; C + G + E > C + G = 15.4 ± 1.5 > 10.8 ± 1.0, p < 0.05; C + G + A > C + G = 14.8 ± 1.6 > 10.8 ± 1.0, p < 0.05).

Figure 3. Human serum albumin (HSA) thiol analysis. *Compared to C + G, p < 0.05. **Data were expressed as mean (n = 3) ± SD and Cysteine equivalents (µg/ml). ***AS represented the ascorbic acid and functioned as control.
Polyphenols contribute to the antioxidant defense properties of herbs. Therefore the level of polyphenols is proportional to the antioxidant potential of herbs. Figure 4 shows the polyphenol content of the 3 adaptogen herbs. *R. rosea* (R) exhibited the highest polyphenol content (41.4 ± 3.41), followed by *E. officinalis* (A, 9.2 ± 0.79) and *E. senticosis* (E, 4.4 ± 0.79).

**Discussion**

*R. rosea* exhibited excellent potential in singlet oxygen and hypochlorite scavenging as well as FRAP potential, iron chelating ability and protection of protein thiol groups than the other 2 adaptogen extracts (*E. senticosis* and *E. officinalis*). This was due to the high percentage of polyphenols in *R. rosea*. Polyphenols are capable of neutralizing oxidative and chain reactions induced by free radicals because they are excellent donors of protons (Frei et al., 2003) and electrons (Othman et al., 2007). Furthermore, polyphenols decrease oxidative...

Figure 5. Diagrammatic representation of antioxidant properties of polyphenols. I. Polyphenols function as electron and hydrogen donors (modified from Bors et al., 2000). II. Polyphenols function as metal chelators (modified from Le Nest et al., 2004).
stress induced by transition metals (such as iron and copper) due to their metal chelating effects (Brown et al., 1996). The results of this study illustrated that the level of polyphenols was proportional to the antioxidant potential of the 3 adaptogen herbs. These findings are consistent with a previous report (Othman et al., 2007). Figure 5 outlines the mechanism of reactive oxygen species (ROS) scavenging and metal chelation by polyphenols.

In general, adaptogen supplements containing high levels of polyphenols offer not only physiological and pharmaceutical benefits but also elevate antioxidant defense. This could decrease the risk of complications associated with oxidative stress and improve the quality of life in those who take them.

References


