Evidence of Possible Evolutionary Divergence in Plant Genera Based on Antioxidant Properties

Student paper by Elizabeth Asai and Sharon Cao

Abstract

The purpose of this investigation was to determine if three Western species of the Panax, Lycium, and Astragalus genera had antibacterial and/or antioxidant properties, and how their properties compared to Eastern herbs in the same genera. The group hypothesized that when compared, the corresponding herbs would have identical antibacterial and antioxidant properties. An antibacterial disc test was used to determine each herb's ability to inhibit the growth of *Escherichia coli and Micrococcus luteus*. Herb and bacteria pairs which were not antibacterial were tested in a UV exposure test, a method used to qualify antioxidant properties. Mixtures of bacterial broth and herbal teas were exposed to UV light, and plated onto agar. Survival of the bacteria was observed. In addition, to determine if color of the herb impacted the protection of the bacteria, Nigrosin, a black stain, was tested under the same UV exposure procedure. In a DPPH test, the color change of the DPPH was used to calculate radical scavenging activity as a measure of antioxidant strength. The corresponding herbs' data were then compared. For the antibacterial test, only the known antibiotic control, Streptomycin, yielded a zone of inhibition. All of the herb and bacteria pairs showed antioxidant properties after UV exposure. The DPPH test found that the herb from the *Lycium* genus had the most radical scavenging activity, followed by the *Astragalus* and *Panax* genera. When compared to the data from Eastern species of these herbs, all three of the Western herbs were found to have greater antioxidant properties, especially in the Lycium genus. In the wolfberry trials, the Eastern wolfberry had a radical scavenging activity of 65.0% and the Western herb had 93.1% scavenging activity (Har & Seetoh, 2008).

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Introduction

Since the first drug was isolated from a plant in 1803, science has progressed to the point that a majority of our most effective drugs have herbal active ingredients. In an experiment done at the University of Exeter, Martin and Ernst's showed that tea products had matching or better bacterial treatment results when compared to those of generic antibiotics (2003). Another research investigation that used essential oils was done at the University of Utrecht. In this experiment, discs were coated in the aqueous mixtures of thyme oil, oregano oil, bay, and clove and were placed in a zone of bacteria (Burt & Reinders, 2003). The antibacterial progress was measured by taking the diameter of the zone of inhibition. An experiment done at the Jadavpur University in Calcutta, India also analyzed the diameter of the zone of inhibition (Mandal et al., 2000). In this study, Asparagus roots were finely chopped and then made into an aqueous mixture. The zone of inhibition was measured after different concentrations of asparagus were placed on a range of different bacteria cultures. The asparagus was found to have greater antibacterial properties as concentrations increased. One common trait of these investigations is that the majority tested the herbal properties on a range of different bacteria.

While extensive research has been done on the antibacterial properties of very common herbs, little has been done to compare antibacterial properties of herbs native to different parts of the world. This investigation aims to directly compare both the antibacterial and antioxidant properties of Eastern and Western Hemisphere herbs. This comparison can be made possible by putting together data collected by students in Virginia, USA, for the Western herbs, and students from Singapore for the Eastern herbs. Both groups had similar experiments so that there was little variation in the experiment method. It was hypothesized that if Western and Eastern herbs were

compared, then herbs with corresponding genera would have similar antibacterial and antioxidant properties. This was thought because it did not seem reasonable that two isolated yet highly related herbs would evolve different antibacterial or antioxidant properties unless there was more stress due to bacteria or oxidative stress on one, but not the other.

Materials and Method

Phase I – Antibacterial Test: Antibiotic Discs
Mueller Hinton agar plates were poured. Two 10 μL loops of *E. coli* (Sargent Welch WL23601) and two 10 μL loops of *M. luteus* (Microbiologics 0242P) were plated on agar. The bacteria were incubated for 48 hours at 37 degrees Celsius. Herbal extracts were made with 1g crushed herb and 10 ml 100 degrees Celsius sterile water using each of the three herbs, *Panax quinquefolius, Lycium berlandieri*, and *Astragalus newberryi*. Five filter discs per herb received 40 Ìl of herbal extract. For the control, Streptomycin discs were used. Blank discs with 40 Ìl of sterile water served as the negative control. One colony of bacteria was plated onto each Mueller Hinton agar plate. At the same time, prepared filter discs were placed on each plate. After 48 hours, the zone of inhibition's diameter was measured. If no zone of inhibition was present in all trials of a particular herb and bacteria pair, that combination was used in the next phase, the UV exposure test.

Phase II – Antioxidant Test: UV Exposure
Bacterial broths of *Escherichia coli* and *Micrococcus luteus* were made with 30 ml nutrient broth and two 10 μL transferred loops of bacteria. Broths were incubated at 30 degrees Celsius for 48 hours. Herbal extracts were prepared with 1g crushed herb and 10 ml 100 degrees Celsius sterile water. Mixtures of 1 ml herbal extract and 3 ml bacterial broth were made in separate Petri dishes. For the controls, 1 ml sterile water or Nigrosin was used instead of the herbal teas. All of the plates were exposed to a UV transilluminator for 3 minutes at 300l. Mueller-Hinton agar plates were poured. One inoculating loop of each post-exposure mixture was plated onto five plates. Observance of survival was recorded after 48 hours of incubation at 30 degrees Celsius.

Phase III – Antioxidant Test: DPPH Test
Herbal extracts were prepared using 1g crushed herb and 10 ml 100 degrees Celsius sterile water. Mixtures of 2.9 ml methanol and 0.1 ml of each herbal extract served as blanks. A mixture of 1 ml DPPH, 1.9 ml methanol, and 0.1 ml 50 % ethanol was made in a cuvette to serve as the control without extract. The color change that occurred in this sample was measured by the spectrophotometer at 517 nm. The radical scavenging activity, a direct measure of antioxidant strength, was calculated using the following formula taken from Cheng Wai Goh-Yip (oral communication, November 2, 2007).

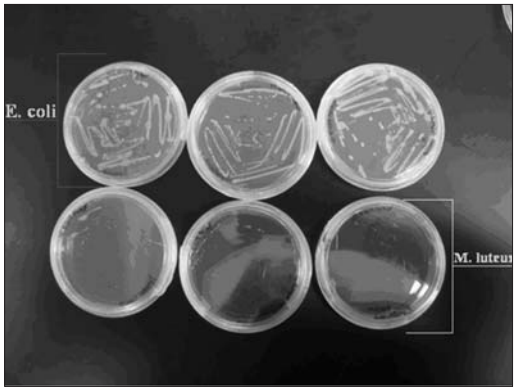
$((A_{517}(\text{control without extract}) - A_{517}(\text{sample with extract})) / A_{517}(\text{control without extract})) \times 100\ \%$
(where A_{517} indicates the absorbance measured at 517 nm)

At a wavelength of 517 nm, a color change of the DPPH is detectable in relation to the radical scavenging activity of the herb. This formula compares the color with herbal antioxidant to the original color without antioxidants of any kind in a percent difference calculation.

Results

Phase I – Antibacterial Test: Antibiotic Discs
No zones of inhibition from the experimental trials formed around the antibiotic discs when placed in contact with either gram negative or gram positive bacteria, indicating that none of the herbs tested had antibacterial properties.

Phase II – Antioxidant Test: UV Exposure
All herbs tested showed post exposure growth for both gram negative and positive bacteria. As expected, the sterile water control showed no post exposure growth.



Post Exposure Growth Dish of E. coli and M. luteus with Western Lycium torreyi

Phase II Continued – Antioxidant Test: Color Control
The Nigrosin and sterile water trials showed no post exposure growth, indicating that neither gave the bacteria an advantage against ultraviolet damage.

Phase III – Antioxidant Test: DPPH Test

	Western Results			Eastern Results		
Genus Tested	**DPPH Blank**	**Average Absorbance**	**Radical Scavenging Activity**	**DPPH Blank**	**Average Absorbance**	**Radical Scavenging Activity**
Panax	0.678	0.354	47.79%	0.868	0.675	18.50%
Lycium	0.657	0.047	92.89%	0.873	0.283	65.00%
Astragalus	0.717	0.303	57.74%	0.858	0.730	11.20%

Table A: Radical Scavenging Activity Computed for Each Herb

All of the western herbs tested showed considerable radical scavenging activity, especially when compared to those of the eastern species. The *Lycium* genus, or wolfberry, was found to have the greatest levels of antioxidant activity in both species.

Discussion and Conclusion

Our results show that none of the herbs tested were antibacterial due to the lack of the formation of a zone of inhibition. The known antibiotic, streptomycin had a greater effect on the gram-positive bacteria *M. luteus*, rather than the gram-negative bacteria, *E. coli*, which could suggest that the *E. coli* was more resistant to the antibiotic. However, the lack of antibacterial properties allowed the group to use the herbs in phase II since it was known that the herbs didn't inhibit the growth of the bacteria. It was indicated that all the herbs tested had some antioxidant properties due to the fact that the bacteria survived when mixed with the three separate herbal teas, while the control trials all perished with only sterile water. The wolfberry trials showed especially vigorous growth as the colonies were too numerous to count. The results from the Nigrosin experiment showed that color did not provide protection for the bacteria as no bacteria survived in the trial. By using this DPPH test, we were able to find more quantitative results by calculating the radical scavenging activity. After calculating the radical scavenging activity, we were able to observe that the Lycium genus showed the strongest antioxidant properties, followed by the *Astragalus* and *Panax genera*. In comparing to a study done by Singaporean students that occurred simultaneously to our experimentation, radical scavenging activity for same genus herbs were found to be greater in all three Western herbs. The radical scavenging activities of ginseng, astragalus and wolfberry, as quantified using the chemical DPPH were 18.5%, 11.2%, and 65.0% for the Asian species, and 47.8%, 55.3%, and 93.1% for the Western species respectively (Har & Seetoh, 2008). Our group is hypothesizing that different environmental conditions such as a greater risk of oxidation damage posed by sunlight exposure caused the Western species to evolve greater antioxidant properties. This is supported by the fact that the Western species used were native to desert climates, such as in New Mexico and Arizona, while the Eastern species were commonly found in forested area such as Tibet.

The *Lycium genera* had the highest amount of antioxidant properties. All three Western herbs had higher antioxidant properties than the Eastern herbs. Work is currently underway to characterize the proteins, flavonoids, etc. that might be involved in the antioxidant properties leading to evolutionary divergence in these genera.

References

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