Chemistry of California *Lycium cooperi* and *Lycium andersonii*

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ABSTRACT

Aims: To examine the chemistry of two California *Lycium* species and evaluate the possible use of California *Lycium* species as dietary supplements especially for age related macular degeneration.
Study design: This exploratory analytical research used samples of *Lycium andersonii* and *Lycium cooperi* collected in the field and analyzed in the lab.
Place and Duration of Study: University of Southern California School of Pharmacy, 1985 Zonal Avenue, Los Angeles, CA USA 90089.
Methodology: Plant extracts were analyzed by high pressure liquid chromatography mass spectrometry with ultraviolet photodiode array detection in order to identify the chemical characteristics of compounds found in the plants.
Results: Several known compounds were found in extracts of *Lycium cooperi* and *Lycium andersonii* foliage and fruit including: zeaxanthin, zeaxanthin monopalmitate and β-cryptoxanthin. The various California species of *Lycium* are discussed as possible alternatives to Chinese *Lycium barbarum*.
Conclusion: California *Lycium* berries may be suitable substitutes for Chinese *Lycium* berries.

Keywords: age related macular degeneration, Lycium, Lycium andersonii, Lycium cooperi, zeaxanthin

1. INTRODUCTION

Damage to the macula of the retina can increase with aging and results in macular degeneration in one or both eyes. Patients with diabetic retinopathy are at increased risk of developing macular degeneration [1]. The incidence of type 2 diabetes increases yearly due to obesity [2]. As diabetic retinopathy increases so will macular degeneration. Loss of central vision is the hallmark of the disease [3, 4]. Aging and smoking increase the progression of the disease. Treatment includes anti-vascular endothelial growth factor antibodies, such as ranibizumab, aflibercept and bevacizumab that can save the eye sight of some people and slow down the progression of the disease [3, 4]. These are very expensive medicines that are not used in the general aging population. Instead, antioxidant vitamin supplements including supplements containing zeaxanthin have been shown to slow down the progression of macular degeneration [3, 4].

Chinese plants in the genus *Lycium* have been used for thousands of years to treat age related diseases [5]. The berries of these plants, called wolfberries or goji berries, contain zeaxanthins and other antioxidants [5]. The major source of goji berries from *Lycium barbarum* and *Lycium chinense* is from China, which exports more than 95,000 tons every year [6].

California has 9 species of *Lycium* [7]. These plants have been used by California Indians for thousands of years as food and as medicine [8, 9, 10]. They are currently being used as food and medicine by only a small number of people, in part due to lack of knowledge about Californica *Lycium* plants. There have been no investigations of the chemistry of these plants. The current report is the first investigation of the chemistry of *L. cooperi* and *L. andersonii*.

The authors traveled extensively in California to find every species of *Lycium* and studied the Botany and palatability of these plants. Distinguishing characteristics of *L. cooperi* and *L. andersonii* were found to separate these species from other species in the field.

2. METHODOLOGY

Leaves and berries of *L. andersonii* and *L. cooperi* were collected in the field and stored on ice for transport back to the laboratory. Frozen leaf and berry extracts from *L. andersonii* and *L. cooperi* are prepared by the following procedures. Two grams of fresh fruit were crushed with a mortar and pestle in 10 mL of ethanol. Hexane-ethanol-acetone-toluene (10:6:7:7, v/v/v/v), 40 mL, was added with stirring for 1 h in a light protected beaker. Hexane, 30 mL, was added with stirring to form two layers. The top layer was collected. This hexane extraction was repeated 5 times. The combined hexane extracts were reduced to dryness. The residue was dissolved in 5 mL of acetonitrile and analyzed by HPLC/MS. Leaves, 50 g, were extracted into 300 mL of 80% ethanol with 20 sec of microwave heating. The solvent was evaporated at reduced pressure. The residue was dissolved in 5 mL of acetonitrile and subjected to HPLC/MS.

HPLC/MS depended on a C_{30} reverse phase column (2.1 by 250 mm) eluted with methanol-acetonitrile-water (84:14:5, v/v/v) at 1 mL/min. The capillary voltage was 2000 volts. The corona current was 4 uA. The vaporizer temperature was 330 degrees.

3. RESULTS AND DISCUSSION

Plant extracts were screened for the molecular weights and UV max values of various compounds known to be present in other species of *Lycium* (Table 1). Compound identities were confirmed when the UV spectrum and characteristic MS ions matched published results. For some compounds, published HPLC retention times were used to confirm or eliminate possible identities. Table 2 shows the compounds found in plant extracts. A number of compounds were found based on molecular weight, but could not be confirmed based on retention times or UV spectral data, due to limitations of the equipment. These compounds are: campesterol, lycibarbarspermidine H and lycibarbarspermidine N. Other lycibarbarspermidines were found in the extracts (Table 2). Since these compounds have 4 similar isomers A, B, C, and D, with identical molecular ions and UV spectra (Table 1), it is not possible to tell which isomer is present in our extracts.

Table 1: Molar weights and UV maximum absorption values for selected molecules

Compound	$(M+H)^+$ $(g.mol^{-1})$	$(M+K)^+$ $(g.mol^{-1})$	$(M+Na)^+$ $(g.mol^{-1})$	UV_{max}(nm)	References
Kaempferol	287	325	309	265, 365	[16]
Quercetin	303	341	325	258, 269, 375	[16]
Alkaloid I	192	230	214	271, 321	[17]
Lycibarbar spermidine A-D	634	672	656	290, 325	[18]
Emodin	271	309	293	223, 250, 267, 290, 442	[19]
Lyciumoside I (M-H)-	629	669	653	-	[20]
Zeaxanthin	570	608	592	450	[21]
Zeaxanthin monopalmitate	807	845	829	450	[21]
B-Cryptoxanthin	554	592	576	454	[11]
Sitosterol	416	454	438	206	[22]
α-tocopherol	432	470	454	280	[23]

Table 2: Molecules found in plant extracts

Compound	Andersonii berries	Andersonii leaves	Cooperi berries	Cooperi leaves
Kaempferol	X	X		X
Quercetin	X	X	X	X
Alkaloid I	X		X	

Lycibarbar spermidine	X	X	X	X
Emodin	X	X	X	X
Lyciumoside I	X		X	X
Zeaxanthin	X	X	X	X
Zeaxanthin monopalmitate	X	X	X	X
β-Cryptoxanthin	X	X	X	X
B-Sitosterol	X	X	X	X
α-Tocopherol			X	X

The zeaxanthin found was all-trans-zeaxanthin. The cryptoxanthin found was all-trans-β-cryptoxanthin. Zeaxanthin monopalmitate was identified based on its molecular ion and its most abundant fragment at m/z 551, which formed by loss of palmitic acid. Lyciumoside 1 was identified in negative ion mode as $(M-H)^-$ and in positive ion mode as $(M+Na)^+$ and $(M+K)^+$.

The amount of zeaxanthin in plant material was calculated based on the extinction coefficient at 452 nm, 23,400 L/g/cm [11]. The amount found in *L. andersonii* berries is about 2.74 mg/g. A slightly lower value is found for *L. cooperi* berries (1.53 mg/g). Leaves of both species contain a bit less zeaxanthin (1.46 mg/g for *L. andersonii* leaves and 1.35 mg/g for *L. cooperi* leaves). These data are similar to the amount of zeaxanthin found in L. barbarum berries [12].

In addition, the molar extinction coefficient of kaempferol is 15,849 L/mol/cm in 96% ethanol [13]. Hence, the amount of kaempferol is about 10.84 and 4.62 mg/g for *L. andersonii* berries and leaves, respectively. In *L. cooperi* leaves the amount is about 4.41 mg/g.

The molar extinction coefficient of quercetin is 20,892 L/mol/cm [13]. Quercetin is present in *L. andersonii* leaves (3.30 mg/g), *L. cooperi* leaves (3.07 mg/g), *L. andersonii* berries (1.45 mg/g) and *L. cooperi* berries (0.87 mg/g).

The locations of the various species of *Lycium* found in this study are shown in Table 3. The *L. cooperi* and *L. andersonii* used in this study were found in the same location. Some of the California *Lycium* species can be difficult to distinguish in the field. The characteristics and locations indicated in the Jepson Manual are usually useful [7]. The most troubling identification is *Lycium brevipes* and *Lycium parishii*. The two plants are very similar in appearance and have been reported to grow in the same locations in western desert regions near San Diego. They can be usually distinguished by the number of lobes on the calices. *L. brevipes* has 4 calyx lobes. *L. parishii* has 5 calyx lobes. William Hoyer, Botanist on San Nicolas Island for the US Navy informed the authors that *L. verrucosum* is extinct. *L. verrucosum* has only been reported on San Nicolas Island and nowhere else. On San Nicolas Island he and other botanists have found *L. brevipes* and *L. californicum*.

Table 3. Locations of the species of *Lycium* found in this study.

Species	GPS location	Habitat

L. *andersonii*	Latitude: 34.27670 Longitude: -116.45834	Desert
L. *brevipes*	Latitude: 33.74412 Longitude: -118.41055	Coast
L. *californicum*	Latitude: 33.46107 Longitude: -117.70801	Coast
L. *cooperi*	Latitude: 34.27670 Longitude: -116.45834	Desert
L. *fremontii*	Latitude: 32.96931 Longitude: -116.26030	Desert
L. *pallidum*	Latitude: 34.98494 Longitude: -117.18605	Desert
L. *parishii*	Latitude: 32.87254 Longitude: -116.22209	Desert
L. *torreyi*	Latitude: 34.12025 Longitude: -114.51474	Desert
L. *verrucosum*	extinct	

L. andersonii, *L. cooperi*, *L. fremontii* and probably other desert species are summer dormant, according to the author's observations. The coastal species are perennial and only lose their leaves when there is not enough water. *L. andersonii* was found to produce the most fruit compared to other species. The berries of *L. andersonii*, *L. brevipes*, *L. parishii* and *L. fremontii* produce soft berries that have a mildly bitter, peach taste very similar to *L. barbarum*. *L. cooperi* berries are firm and have a mild peach flavor. *L. pallidum* berries are firm and sour. Berries from other species were not eaten. The dried berries of *L. barbarum* are larger than the dried berries of any California *Lycium*.

4. CONCLUSION

California *Lycium* plants, *L. cooperi* and *L. andersonnii*, produce zeaxanthin and other antioxidant compounds that are reported to be beneficial in the prevention and treatment of macular degeneration (24). These plants should be further investigated for use in the treatment of macular degeneration.

Lycium barbarum is difficult to grow commercially in California due to the high summer heat [14]. Temperatures above 27 degrees may decrease fruiting. *Lycium barbarum* can be grown in protected gardens in the Los Angeles area and does bear fruit [15]. It is not clear if these plants can be grown commercially in the Los Angeles area. Commercial goji berry cultivation in California may have to depend on native California species.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Author Wong was responsible for growing and tending *L. barbarum* over the last several years. All other authors were involved in traveling in California, hiking to find plants of interest, making and analyzing extracts. Authors Navarrete, Bracquemond and Adams wrote the manuscript. All authors read and approved the final manuscript.

CONSENT

Human subjects were not used in this study. Consent was not necessary.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Animals were not used in this study.

REFERENCES

1. He M, Chang F, Lin H, Wu J, Hsieh T, Lee Y. The association between diabetes and age-related macular degeneration among the elderly in Taiwan. Diabetes Care. 2018; 41: 2202-2211.
2. Adams J, Parker K. Extracellular and intracellular signaling. Royal Society of Chemistry, London. 2011.
3. Lim L, Mitchell P, Seddon J, Holz F, Wong T. Age-related macular degeneration. Lancet. 2012; 379: 1728-38.
4. Ong B, Ah-Fat F. Age-related macular degeneration. British Journal of Hospital Medicine. 2016; 77: C18-C21.
5. Gao Y, Wei Y, Wang Y, Gao F, Chen Z. Lycium barbarum: a traditional chinese herb and a promising anti-aging agent. Aging and Disease. 2017; 8: 778-91.
6. Wolfberry festival to be held in Ningxia. China Daily. 2004, July 19.
7. Baldwin B, Goldman D, Keil D, Patterson R, Rosatti T, Wilkin D. The Jepson Manual Vascular Plants of California, *second edition*. University of California Press, Berkeley. 2012, 1252-3.
8. Strike S. Ethnobotany of the California Indians. volume 2. Aboriginal uses of California's indigenous plants. Koeltz Scientific Books, Champaign. 1994, 87.

9. Moerman D. Native american ethnobotany. Timber Press, Portland. 1998.
10. Bean L, Saubel K. Temalpakh. Cahuilla indian knowledge and usage of plants. Malki Museum Press, Morongo Indian Reservation. 1972.
11. Sarungallo Z, Harivadi P, Andarwulan N, Purnomo E, Wada M. Analysis of α-cryptoxanthin, β-cryptoxanthin, α-carotene, and β-carotene of Pandanus conoideus oil by high-performance liquid chromatography (HPLC). Procedia Food Science. 2015; 3: 231-243.
12. Niro S, Fratianni A, Panfili G, Falasca L, Cinquanta L, Rizvi Alam M. Nutritional evaluation of fresh and dried goji berries cultivated in Italy. Italian Journal of Food Science. 2017; 29: 398-408.
13. Franke D, Lai J, Halm B. Absorption, distribution, metabolism, and excretion of isoflavonoids after soy intake. Archives of Biochemestry and Biophysics. 2014; 559: 24-28.
14. Karp D. Goji taunts North American farmers. Los Angeles Times. 2009, August 5.
15. Spurrier J. Super goji berry takes care to grow. Los Angeles Times. 2013, May 4.
16. O'Neil M. The merck index - an encyclopedia of chemicals, drugs, and biologicals. Merck and Co, Whitehouse Station. 2006, 913
17. Qian D, Zhao Y, Yang G, Huang L. Systematic review of chemical constituents in the genus Lycium (Solanaceae). Molecules. 2017; 22: 911-34.
18. Zhou Z, Fan H, He R, Xiao J, Tsoi B, Lan K, Kurihara H, So K, Yao X, Gao H. Lycibarbarspermidines A-O, new dicaffeoylspermidine derivatives from wolfberry, with activities against Alzheimer's disease and oxidation. Journal of Agricultural and Food Chemistry. 2016; 64: 2223-37.
19. Wells J, Cole R, Kirksey J. Emodin, a toxic metabolite of Aspergillus wentii isolated from weevil-damaged chestnuts. Applied Microbiology. 1975; 30: 26-28.
20. Yahara S, Shigeyama C, Ura T, Wakamatsu K, Yasuhara T, Nohara T. Cyclic peptides, acyclic diterpene glycosides and other compounds from Lycium chinense Mill. Chemical and Pharmaceutical Bulletin (Tokyo). 1993; 41: 703-9.
21. Sajilata M, Singhal R, Kamat M. The carotenoid pigment zeaxanthin - a review. Comprehensive Reviews in Food Science and Food Safety. 2008; 7: 29-49.
22. Saeidnia S, Manayi A, Gohari A, Abdollahi M. The story of beta-sitosterol-a review. European Journal of Medicinal Plants. 2014; 4: 590-609.
23. NIST chemistry webBook, SRD 69, 2019. Accessed September 2019.
24. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss. Archives of Ophthalmology. 2001; 119: 1417–1436.