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3 **Chemistry of California *Lycium cooperi* and**
4 ***Lycium andersonii***
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10 **ABSTRACT**
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Aims: To examine the chemistry of two California *Lycium* species and evaluate the possible use of California *Lycium* species as dietary supplements.

Study design: Samples of *Lycium andersonii* and *Lycium cooperi* were 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.

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13 *Keywords: Lycium, Lycium cooperi, Lycium andersonii, zeaxanthin, age related macular*
14 *degeneration*

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

29 Chinese plants in the genus *Lycium* have been used for thousands of years to treat age
30 related diseases [5]. The berries of these plants, called wolfberries or goji berries, contain
31 zeaxanthins and other antioxidants [5]. The major source of goji berries from *Lycium*
32 *barbarum* and *Lycium chinense* is China, which exports more than 95,000 tons every year
33 [6].

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

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

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44 2. METHODOLOGY

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46 Leaf and berry samples from *L. andersonii* and *L. cooperi* are prepared by the following
47 procedures. A few grams of fresh fruit were crushed in 10 mL of ethanol. Hexane-ethanol-
48 acetone-toluene (10:6:7:7, v/v/v/v), 40 mL, was added with stirring for 1 h in a light protected
49 beaker. Hexane, 30 mL, was added with stirring. The top layer was collected. This hexane
50 extraction was repeated 5 times. The combined hexane extracts were reduced to dryness.
51 The residue was dissolved in 5 mL of acetonitrile and analyzed by HPLC/MS. Leaves, 50 g,
52 were extracted into 300 mL of 80% ethanol with 20 sec of microwave heating. The solvent
53 was evaporated. The residue was dissolved in 5 mL of acetonitrile and subjected to
54 HPLC/MS.

55 HPLC/MS depended on a C₃₀ column eluted with methanol-acetonitrile-water (84:14:5, v/v/v)
56 at 1 mL/min. The capillary voltage was 2000 volts. The corona current was 4 uA. The
57 vaporizer temperature was 330 degrees.

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59 3. RESULTS AND DISCUSSION

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61 Plant extracts were screened for the molecular weights and UV max values of various
62 compounds known to be present in other species of *Lycium* (Table 1). Table 2 shows the
63 compounds found in plant extracts. A number of compounds were found based on
64 molecular weight, but could not be confirmed based on retention times or UV spectral data,
65 due to limitations of the equipment. These compounds are: campesterol,
66 lycibarbarspermidine H and lycibarbarspermidine N. Other lycibarbarspermidines were
67 found in the extracts (Table 2). Since these compounds have 4 similar isomers A, B, C, and
68 D, with identical molecular ions and UV spectra (Table 1), it is not possible to tell which
69 isomer is present in our extracts.

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73 **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, 267, 442	250, 290, [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]

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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

78 The zeaxanthin found was all-trans-zeaxanthin. The cryptoxanthin found was all-trans- β -
79 cryptoxanthin. Zeaxanthin monopalmitate was identified based on its molecular ion and its

80 most abundant fragment at m/z 551, which formed by loss of palmitic acid. Lyciumoside 1
81 was identified in negative ion mode as (M-H)⁻ and in positive ion mode as (M+Na)⁺ and
82 (M+K)⁺.

83 The amount of zeaxanthin in plant material was calculated based on the extinction
84 coefficient, 2340 100 mL/g/cm [11]. The amount found in *L. andersonii* berries is about 2.74
85 mg/g. A slightly lower value is found for *L. cooperi* berries (1.53 mg/g). Leaves of both
86 species contain a bit less zeaxanthin (1.46 mg/g for *L. andersonii* leaves and 1.35 mg/g for
87 *L. cooperi* leaves). These data are similar to the amount of zeaxanthin found in *L. barbarum*
88 berries [12].

89 In addition, the molar extinction coefficient of kaempferol is 15,849 L/mol/cm in 96% ethanol
90 [13]. Hence, the amount of kaempferol is about 10.84 and 4.62 mg/g for *L. andersonii* berries
91 and leaves, respectively. In *L. cooperi* leaves the amount is about 4.41 mg/g.

92 The molar extinction coefficient of quercetin is 20,892 L/mol/cm or 691.2
93 100mL/g/cm [13]. Quercetin is present in *L. andersonii* leaves (3.30 mg/g), *L. cooperi* leaves
94 (3.07 mg/g), *L. andersonii* berries (1.45 mg/g) and *L. cooperi* berries (0.87 mg/g).

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

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

Species	GPS location	Habitat
<i>L. andersonii</i>	Latitude: 34.27670 Longitude: -116.45834	Desert
<i>L. brevipes</i>	Latitude: 33.74412 Longitude: -118.41055	Coast
<i>L. californicum</i>	Latitude: 33.46107 Longitude: -117.70801	Coast
<i>L. cooperi</i>	Latitude: 34.27670 Longitude: -116.45834	Desert
<i>L. fremontii</i>	Latitude: 32.96931 Longitude: -116.26030	Desert

<i>L. pallidum</i>	Latitude: 34.98494 Longitude: -117.18605	Desert
<i>L. parishii</i>	Latitude: 32.87254 Longitude: -116.22209	Desert
<i>L. torreyi</i>	Latitude: 34.12025 Longitude: -114.51474	Desert
<i>L. verrucosum</i>	extinct	

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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. andersonii*, produce zeaxanthin and other antioxidant compounds that are apparently beneficial in the prevention and treatment of macular degeneration. 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.

135 **COMPETING INTERESTS**

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137 Authors have declared that no competing interests exist.

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140 **CONSENT**

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

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145 **ETHICAL APPROVAL (WHERE EVER APPLICABLE)**

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147 Animals were not used in this study.

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