Lichen as a Source of Vitamin D$_2$

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**Introduction**

- Vitamin D is essential for maintaining overall health, and a deficiency of Vitamin D has been associated with various chronic illnesses such as cancer and cardiovascular disease.
- Vitamin D can be acquired through sun exposure, diet, or supplementation; supplementation is often necessary as adequate levels of vitamin D are typically not attained otherwise.
- There are two forms of vitamin D: vitamin D$_{2}$ which originates from provitamin D$_{2}$ (ergosterol), and vitamin D$_{3}$ which originates from provitamin D$_{3}$ (7-dehydrocholesterol).
- Unlike vitamin D$_{3}$, vitamin D$_{2}$ is derived from animals and so is not desirable to certain groups such as vegans.
- Lichen, a symbiotic organism of fungi and algae, has previously been marketed on the internet as a non-animal source of both vitamin D$_{2}$ and vitamin D$_{3}$.
- Lichen is also a major food source for vertebrates such as caribou in Northern Canada and other areas of the world; as these vertebrates also require vitamin D to maintain bone health and are unlikely to get it from sun exposure due to their fur preventing the formation of vitamin D in the skin, lichen could prove to be their source of vitamin D.

**Objectives**

- To determine if vitamin D and its precursor, provitamin D$_{2}$, are present in lichen and sphagnum moss collected in the wild
- To determine if lichen exposed to artificial ultraviolet radiation can produce vitamin D
- To determine what forms of vitamin D are present in lichen, i.e. vitamin D$_{2}$, vitamin D$_{3}$, or both

**Methods**

**Collection**
- Two types of lichen (Stereocaulon & Cladonia) and a sphagnum moss were collected June 2019 in Saskatchewan - 53°N

**Saponification** – extracts vitamin D related compounds
- 1g samples were dissolved in ethanol and potassium hydroxide (KOH) and shaken overnight at room temperature
- After being extracted in hexane, samples were dried under nitrogen and re-dissolved in the High-Performance Liquid Chromatography (HPLC) solvent

**Irradiation**
- Samples of lichen irradiated with solar simulated ultraviolet radiation for 4.5 min from a distance of 12 cm under Speriti UV lamp
- Saponified

**Straight Phase HPLC** – separates pro-D from vitamin D
- Saponified samples were re-dissolved in 0.8% isopropl alcohol in hexane and chromatographed on an HPLC at 1.5 mL/min in column 4.6x250 mm, 5 μm Zorbax RX-SIL

**Reverse Phase HPLC** – separates vitamin D$_{2}$ & D$_{3}$
- Vitamin D peaks were collected from straight phase HPLC, dried under nitrogen, and re-dissolved in 25% methanol in acetonitrile; samples were applied to a reverse phase column (Vydac Reverse C18) and run at 1.5 mL/min

**Results**

Figure 2. HPLC Chromatographs and UV Absorption Spectra. Lichen samples (bottom) were compared against a standard (top) to identify vitamin D and provitamin D. The chromatograms were evaluated for peaks based on their elution times and the UV absorption spectra. Vitamin D was found in both species of lichen, but not the moss. Provitamin D was found in all three species.

Figure 3. Vitamin D Content in Non-Irradiated Lichen Compared to Irradiated Lichen. For each species, there was an increase in vitamin D after irradiation. For both Cladonia and Sphagnum moss, this increase was statistically significant (P<0.05).

Figure 4. HPLC Reverse Phase Chromatographs. The vitamin D peak regions from the straight phase HPLC were analyzed on reverse phase. The three species were compared against a standard, and no vitamin D$_{3}$ was found in any.

**Conclusions**

- Vitamin D was found in lichen collected from the wild demonstrating that lichen do produce vitamin D and are likely a major source for some vertebrates such as caribou and reindeer in the areas of Northern Canada and other northern regions.
- The amount of vitamin D found in one gram of the non-irradiated lichen was an average of 92.2 ± 55.4 ng (25 ng=1 IU of vitamin D$_{3}$) and since a caribou typically eats 3 kg of lichen a day, they would get an average of about 11,064 IU of vitamin D a day.
- The solar UV irradiated moss also produced vitamin D, and therefore could potentially serve as an additional source of vitamin D.
- Supplements derived from lichen have been largely marketed as a vegan source of vitamin D$_{3}$; however, our results showed only vitamin D$_{2}$ present.
- Therefore, lichen can be utilized as an alternative natural source of vitamin D$_{2}$, but not vitamin D$_{3}$.

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**References**