Chemical Characterization of Honeybee Propolis: Antioxidant Analysis and Component Identification

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Introduction

What is propolis?

Propolis is a sticky, resinous substance produced by honeybees from various plant resins and other botanic sources. Known as "bee glue," it is a structural component of beehives that also acts as a disinfectant. In previous work, propolis has been found to have antioxidant, antiseptic, antimicrobial, antifungal, antiviral, immunomodulatory, and anti-inflammatory properties, making it a subject of interest for its potential applications in human medicine and health.[1]

Figure 1: Propolis trap. Bees fill the gaps of the trap with reddish-orange propolis, which hardens when cold and can be collected by picking it out with a small tool.

In this research

The goals of this project were to assess the antioxidant potential of local honeybee propolis and to identify the compounds present in the mixture. Because propolis is made from plant products, its chemical components, and therefore its efficacy as an antioxidant, vary with season and geographical location.[2] We performed a DPPH radical scavenging assay to assess antioxidant potential, and we identified components in the propolis by several analytical techniques, including HPLC and GC-MS.[3]

Experimental Methods

Preparation of Propolis Extracts

Collect crude propolis from propolis trap (Figure 1). → Dissolve in 95% ethanol for one hour at 40°C. → Filter solution and calculate the concentration in micrograms per milliliter.

All propolis samples were collected from Professor Chris Smart' s hive in Wappingers Falls, NY. Ethanol extracted propolis (EEP) samples were prepared from propolis collected in 2019 (EEP1) as well as from a fresh sample harvested on 8 July 2020 (EEP2 and EEP3). We developed a new method for propolis extraction by adjusting previous methods.[3,4]

HPLC and GC-MS analysis

Components of the propolis extracts were separated by high performance liquid chromatography (HPLC). Fractions containing individual compounds were collected and freeze-dried. These fractions were dissolved in methanol and injected into a gas chromatography-mass spectrophotometer (GC-MS) for identification.

Antioxidant Potential

One measure of antioxidant potential is radical scavenging activity (RSA), which is how effectively a compound donates an electron to reduce free radicals to stable, unreactive products (Eq. 1).

$$RSA\% = \frac{A_o - (As - Ab)}{A_o} * 100 \qquad \text{Eq. 1}$$

Where RSA% is the percent radical scavenging activity, A_o is the original absorbance of the DPPH solution, A_s is the absorbance of the sample, and A_b is the absorbance of the blank.

An ethanolic solution of a violet powder called 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to determine the radical scavenging activity of the EEP3 sample in comparison to a known antioxidant and preservative, butylated hydroxytoluene (BHT). We monitored the reaction with a UV-VIS spectrophotometer which detects the wavelength of the solution.

Figure 2: Test tubes for DPPH radical scavenging assay. From left to right: increasing concentrations of EEP, with constant concentration of DPPH. When the DPPH free radical is scavenged, or trapped, by a single electron donor, the solution turns yellow, losing its purple color. The more purple the solution, the less effective the antioxidant.

Results and Discussion

Component Identification

Retention times were consistent between samples, as seen in Figure 3, with the exceptions of two regions during which there are peaks in the new propolis but not in the older propolis. These compounds are likely either volatile components, which would evaporate or decrease over time, or they were sourced from different plants. Some of the compounds identified in the new propolis are listed in Table 1.

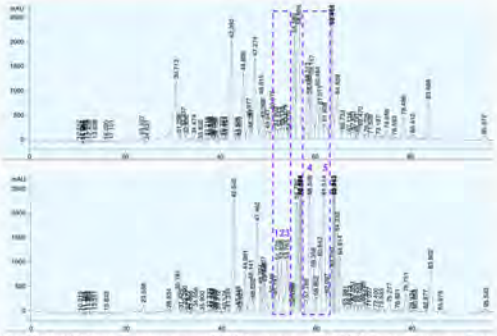

Figure 3: HPLC chromatograms of EEP1, above, and EEP2, below. The regions indicated by rectangles show the five distinct peaks which do not appear in the older sample (EEP1) but do appear in the newer sample (EEP2).

Table 1: Components in 8 July (EEP2) propolis sample

Antioxidants	Sesquiterpenes	Monoterpenes and other compounds
trans-cinnamic acid	γ-eudesmol	α-pinene
cis-cinnamic acid	α-bisabolol	β-myrcene
p-coumaric acid	β-cadinene	camphene
pinocembrin	β-caryophyllene	*l*-limonene
cinnamyl caffeate	α-curcumin	vanillin
	α-copaene	

DPPH Radical Scavenging Assay

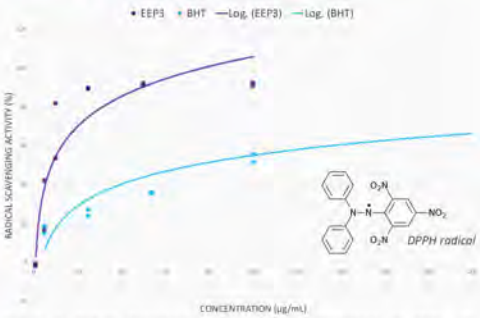

Figure 4: Radical Scavenging Activity vs. Concentration of EEP3. All measurements were performed in duplicate. The equation of the EEP3 radical scavenging effect (purple), is y = 22.281ln(x) + 3.469. The BHT (green) scavenged the DPPH radical according to the equation y = 16.159ln(x) - 19.089.

The half maximal inhibitory concentration (IC50) of the reference antioxidant, BHT, was 71.9 µg/mL. The propolis had an IC50 of 8.1 µg/mL, making it over 3 times more effective as a radical scavenger than BHT.

Conclusion

We isolated and identified numerous compounds within the propolis samples, including several known antioxidants and many compounds that are also present in local flora. The radical scavenging activity of the new propolis suggests that locally-sourced propolis acts as a strong antioxidant.

More work is required to identify each of the HPLC peaks shown in Figure 3. These compounds can also be analyzed in comparison to local vegetation to find the sources used by the bees.

Additional antioxidant assays can be carried out to determine how season and time elapsed affect the antioxidant potential of propolis, as well as to compare propolis samples to other known antioxidants.

Acknowledgements + References

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