INTRODUCTION

**What is propolis?**

Propolis is a sticky, resinous substance produced by honeybees from various plant resins and other botanical 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, anti-inflammatory, immunomodulatory, and anti-inflammatory properties, making it a subject of interest for its potential applications in human medicine and health.¹

![Image of propolis](image)

**IN THIS RESEARCH**

The goals of this project were to assess the antioxidant potential of local honeypropolis 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 PROPSI EXTRACTS**

Collect crushed propolis from propolis traps (Figure 1).

1. Combine propolis and 0.5 ml of acetone in a screw-capped test tube, and incubate for one hour at 40°C.
2. Collect the filtered solution and add 4 ml of anhydrous pyridine to the precipitate.
3. Dissolve in 65% ethanol for one hour at 40°C.
4. Filter solution and calculate the concentration in micromoles 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 1999 (EEP1) as well as from a fresh sample harvested on 8 July 2000 (EEP2 and EEP3). We developed a new method for propolis extraction by adjusting previous methods.¹³

**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 spectrometry (GC-MS) for identification.

![HPLC chromatograms](image)

**ANTIOXIDANT POTENTIAL**

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

\[
RSA\% = \frac{A_0 - (As - Ab)}{A_0} \times 100 \tag{Eq. 1}
\]

Where RSA is the percent radical scavenging activity, \(A_0\) is the absorbance of the DPPH solution, \(As\) is the absorbance of the sample, and \(Ab\) 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.

**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.

![HPLC chromatograms](image)

**TABLE 1: COMPONENTS IN 8 JULY (EEP2) PROPSI SAMPLE**

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Sesquiterpenes</th>
<th>Monoterpenes and other compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-geraniol</td>
<td>α-pinene</td>
<td></td>
</tr>
<tr>
<td>cis-geraniol</td>
<td>β-myrcene</td>
<td></td>
</tr>
<tr>
<td>β-caryophyllene</td>
<td>limonene</td>
<td></td>
</tr>
<tr>
<td>chrysanthemol</td>
<td>camphor</td>
<td></td>
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</tbody>
</table>

**DPPH RADICAL SCAVENGING ASSAY**

![Graph of DPPH assay](image)

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.

**ACKNOWLEDGMENTS + REFERENCES**

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4. Silva M. et al., "Chemical Composition and Antioxidant Activity of Bia Hopkinsia, a New Type of Brazilian Propolis," Evidence-based Complementary and Alternative Medicine, 2008