NIAGARA BASED BY'S APITHERAPY BROWN PROPOLIS HAS STRONGER ANTIOXIDANT QUALITIES THAN BRAZILIAN GREEN PROPOLIS

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ABSTRACT

Propolis is a complex mixture of natural sticky and resinous components produced by honeybees from living plant exudates. Globally, research has been dedicated to studying the biological properties and chemical composition of propolis from various geographical and climatic regions. However, the chemical data and biological properties of Ontario, Canada based brown propolis is minimal at best and the industry has had to rely on international studies used to support their regions propolis production.

INTRODUCTION

Propolis also known as "***bee glue***" is a nontoxic hive product accumulated by bees from diverse plants containing compounds such as flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols, ketones, sesquiterpenes, coumarins, steroids, amino acids, and inorganic compounds. It functions in sealing holes, cracks, reconstruction, and smothering the inner surfaces of the beehive. Propolis and its extracts have application in treating diseases due its anti-inflammatory, antioxidant, antibacterial, antimycotic, antifungal, antiulcer, anticancer, and immunomodulatory properties. With progress being made in analytical methods, more than 300 compounds have been identified in propolis to date, including flavonoids, terpenoids, phenolic acids and phenolic esters and sugars.

A large body of evidence highlights that propolis exerts many biological functions that can be ascribed to its antioxidant and anti-inflammatory components, including different polyphenol classes.

The aim of this study is to investigate the mechanisms at the basis of propolis anti-inflammatory and antioxidant activities in relation to Canadian based BY's Apitherapy Brown Propolis sourced from Niagara on the Lake, Brazilian Green Propolis and lastly generic Ontario based raw propolis.

The results showed that BY's Niagara based brown Propolis, whose major polyphenolic components are flavonoids, induced changes in the expression levels of all miRNAs, and was more active than green propolis (whose main polyphenolic components are hydroxycinnamic acid derivatives) which caused changes only in the expression levels of miR-19a-3p and miR-27a-3p.

In addition, only brown propolis was able to modify the expression levels of mRNAs, the target of the reported miRNAs, which code for Tumor Necrosis Factor-α (TNF-α), Nuclear Factor, Erythroid 2 Like 2 (NFE2L2) and Glutathione Peroxidase 2 (GPX2), and (2) the protein levels of TNF-α and NFE2L2.

ANTIOXIDANT, ANTI-INFLAMATORY & ANTIMICROBIAL PROPERTIES OF PROPOLIS

In regard to biological activities, there are hundreds of studies present in the scientific literature supporting the healthy properties of propolis, such as gastroprotective, hepatoprotective, immunomodulatory, wound healing, antidiabetic, and antineoplastic properties. **These are ascribed to the three main activities of propolis, namely antioxidant, anti-inflammatory, and antimicrobial activities.**

One of the most studied properties of propolis is its antioxidant capacity. The main compounds responsible for this activity are caffeoylquinic acid derivates, which show higher radical scavenging activity than most common antioxidants, such as vitamin C and vitamin E. In addition, caffeic acid phenethyl ester (CAPE) exerts protective effects on the lipid peroxidation of erythrocyte membranes. The strong antioxidant activity of propolis suggests that it could be used as an ingredient in the preparation of functional foods and food supplements and may be useful in the prevention and dietary management of patients with chronic diseases caused by oxidative stress. For instance, in 2004, Lahouel et al. found that propolis can also have protective effects against drug side effects and cancer chemotherapeutic agent toxicity.

As an anti-inflammatory agent, propolis has been shown to inhibit the synthesis of prostaglandins, activate the thymus, help the immune system by promoting phagocytic activity, stimulate cellular immunity and improve curative effects in epithelial tissues. Based on literature data, CAPE blocks the release of interleukin 1β (IL-1β) through the inhibition of Nuclear Factor kB (NF-kB) activity. Propolis flavonoids and CAPE have been compared to the cyclooxygenase (COX) inhibitor, indomethacin (IM), and the lipoxygenase (LOX) inhibitor, nordihydroguaiaretic acid (NDGA), and were found to have the same effects as IM and NDGA. In addition, a study showed that CAPE inhibits the release of inflammatory cytokines and simultaneously increases the production of anti-inflammatory cytokines, such as IL-10 and IL-4. The same research showed that CAPE decreases the infiltration of inflammatory cells, such as neutrophils and monocytes.

Regarding epigenetic mechanisms, microRNAs (miRNAs) play a very important role in the regulation of gene expression. They are a class of endogenous non-coding RNA, consisting of about 22 nucleotides, which can regulate gene expression at the post-transcriptional level. They exert their functions by binding complementary sequences on messenger RNA (mRNA) targets, interfering with the translation process and preventing or altering gene expression. There are some studies on the epigenetic effects of propolis in the current scientific literature. In a 2014 research article, Kumazaki et al. showed that two propolis cinnamic acid derivatives, baccharin and drupanin, induce apoptosis in human drug-resistant colon cancer cells by increasing the expression level of anti-oncogenic miR-143, which leads to down-regulation of its target gene, Erk5, and consequently contributes to cell cycle arrest.

CLINICAL STUDY CRITERIA

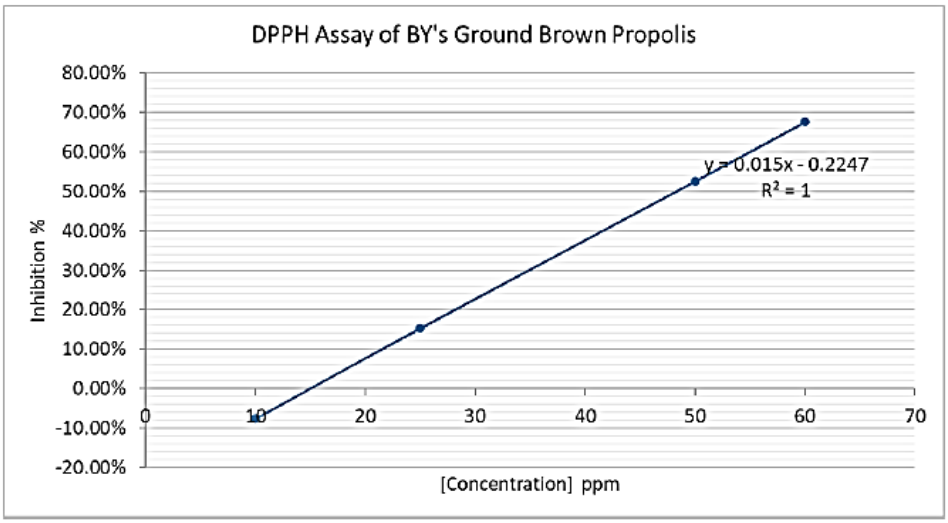
Testing Apparatus and Process:

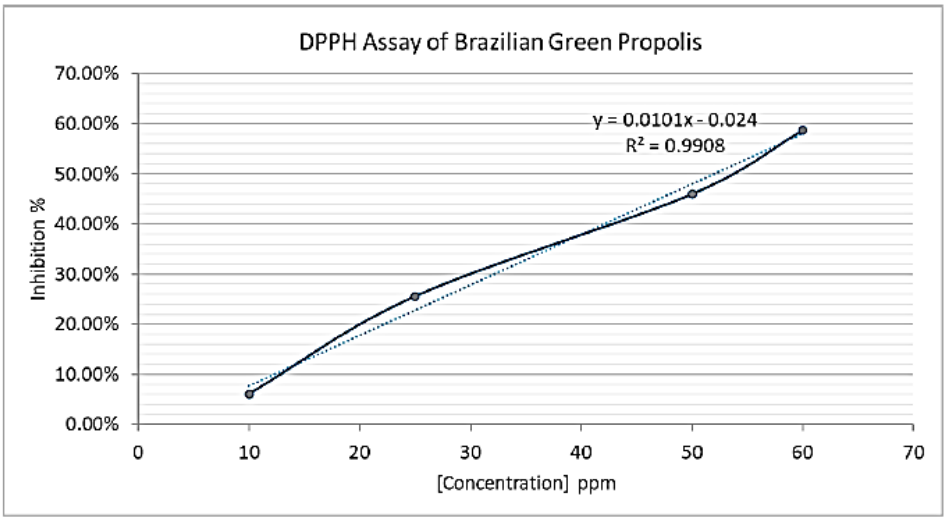
DPPH (Antioxidant) Assay

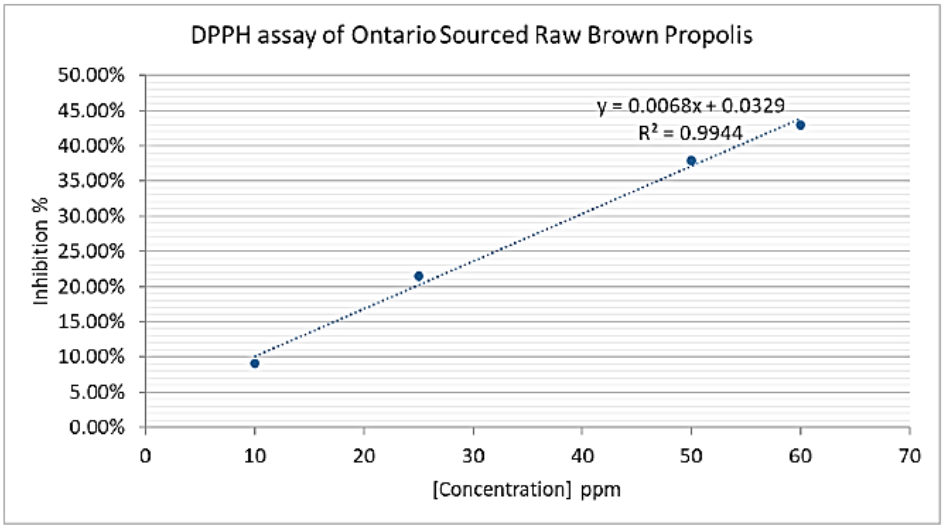
The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is a popular approach for the measurement of antioxidant properties that assesses the potential molecules in a natural product that may serve as free-radical scavengers. The technique is associated with the elimination of DPPH, a stabilized free radical. The free-radical DPPH strongly absorbs 517 nm, and therefore the reduction of the absorbance at 517 is associated with an antioxidant reacting with DPPH, and subsequent antioxidant capacity. In other words, the stronger the antioxidant capacity of a given sample, the stronger the inhibition of DPPH. Ascorbic acid (vitamin C) is typically used as a comparison, given its strong antioxidant effects. The IC50 of a sample, is the concentration at which 50% of has been reacted with. Therefore, the lower the IC50, the stronger the antioxidant.

This work showed that when compared, BY's Ground Brown Propolis had the highest antioxidant capacity, followed by Brazilian Green Propolis, then Ontario Raw Brown Propolis as is noted in the outcome chart analysis that follows.

Chemical Composition and Results:







Chemical Profiling

Propolis samples were prepared by dissolving approximately 10 mg of propolis into 10 mL of LC/MS grade acetonitrile. Samples were acquired in MS2 mode, collecting tandem mass spectra for any compounds which reached an abundance threshold of 20,000 counts. Data is first collected on the instrument, followed by a compound discovery algorithm that pulls out a set of suspected compounds from the data. A molecular feature extraction score is generated, demonstrating the likelihood that the feature extracted is a real compound. The compound list is then matched against library entries and assigned a score for any 'hits'. If no hits are registered on the library, the compound is matched against the database by accurate mass. If the compound is not matched with any database entries, the software uses user input into the possible elements present to generate a chemical formula based on the mass of the compound. The level of confidence proceeds highest to lowest from library hit, to database hit, to molecular formula generation.

SUMMARY OF FINDINGS

The IC50 value is a parameter widely used to measure the antioxidant activity of test samples. It is calculated as the concentration of antioxidants needed to decrease the initial DPPH concentration by 50% [23]. Thus, the lower IC50 value the higher antioxidant activity. IC50 is the amount of propolis required to "quench" a free radical. The lower the IC50, the stronger the antioxidant. **The highest rating and strength of antioxidant was the BY's Apitherapy Brown Ground Propolis followed by the Brazilian Green Propolis and then the raw propolis sourced in Ontario.**

The most notable difference in the chemical compositions of brown and green propolis is the higher content of flavonoids found in BYs Niagara Based brown propolis, relative to hydroxycinnamic acid derivatives. In particular, brown propolis showed higher levels of chrysin and apingenin. A large body of evidence suggests that flavones exert anti-inflammatory and antioxidant activities.

Conclusions as based on the final report summary and findings dated Oct 2nd/2023 included the following:

1. BY's Apitherapy and Wellness Center of Niagara Ground Brown Propolis had the highest antioxidant capacity of the three propolis samples tested.
2. Hundreds of bioactive compounds were identified in the BYs Niagara Based brown propolis sample, including flavonoids, terpenoids, phenolic acids, and catechins.
3. Several unique flavonoids were characterized in the BY's Ground Brown Propolis. Flavonoids are well known for their antioxidant and anti-inflammatory properties.
4. Many unique bioactive compounds known as terpenoids (secondary plant metabolites), which are typically affiliated with antioxidant activity, were found in BY's Ground Brown Propolis.
5. Another group of bioactive compounds, catechins, were uncovered in the BY's Ground Brown Propolis sample. Catechins are natural polyphenolic compounds which serve as antioxidants in food and medicinal plants.
6. All samples were quite chemically distinct from one another, reinforcing the fact that regionality (BYs Farm Niagara on The Lake location and onsite lack of chemicals and pesticides) has a significant affect on the chemical nature of propolis.

These results suggest that brown propolis has greater epigenetic activity, probably due to the higher contents of flavanone and flavone. The same considerations can be made with regards to their ability to induce changes in the expression levels of mRNAs. In this case, BY's Apitherapy Brown Propolis has also been shown to possess a superior modulatory capacity; it is able to modify the expression levels of mRNAs coding for TNF-α, NFE2L2, GPX2 and TNF-α and NFE2L2 protein levels. In conclusion, brown and green propolis, which showed different metabolite profiles, exert their biological functions through different mechanisms of action. The BY's Apitherapy Brown Propolis displayed higher levels of all active ingredients for inducing a positive reaction to antioxidant, anti-inflammatory, and antimicrobial activities over the Brazilian Green Propolis and the generic Ontario based raw propolis. This study displayed the unique importance of the sourcing of the Brown Propolis and it's resulting therapeutic effectiveness.

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