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Antimicrobial properties of selected propolis samples

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Abstract

Propolis, a natural resinous substance collected by honeybees from buds and exudates of plants, is known for its use in the beehive as a protective barrier against intruders. It is widely used as a popular remedy in folk medicine and as a constituent of bio-cosmetics. Recently, it is extensively used in food and beverages to improve health and prevent diseases. Depending on the season, bee species, vegetation and the area of collection, the chemical composition of propolis are qualitatively and quantitatively variable, resulting in diverse biological properties. In this project, the antimicrobial activity of several propolis samples obtained from various parts of the world (Europe, Australia, USA and the Philippines) was determined. The propolis samples were extracted with ethanol and the extracts were assayed with Gram-positive and Gram-negative microorganisms. Results demonstrate that propolis is effective against a range of opportunistic pathogens, with the samples from Russia and Washington showing the most potent antibacterial activity, inhibiting seven microorganisms each. The propolis from the Philippines (Bicol and Laguna) also exhibited notable antimicrobial effects, although to a slightly lesser extent.

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Introduction

Propolis is a natural resinous substance collected by honeybees from the buds and exudates of plants. In the hive, propolis serves a crucial role as a defensive barrier, repairing combs by sealing holes and cracks, and reinforcing the comb borders to prevent pathogenic invasions.

Additionally, bees use propolis to trap intruders that breach the hive, further safeguarding the colony (Sawaya *et al.*, 2011).

Propolis is renowned for its wide array of bioactive properties, including anti-inflammatory, anti-tumor, cytotoxic, hepatoprotective, and immunomodulatory effects. However, its most vital property is its ability to inhibit pathogenic microbial invasions (Liberio *et al.*, 2011). The chemical composition of propolis is highly variable, influenced by factors such as the season, type of bee, local vegetation, and the geographical area of collection (Lotti *et al.*, 2011). This diversity in composition results in varying degrees of bioactivity among different propolis samples. The synergistic effects of these diverse compounds make propolis particularly effective against microorganisms, as the variability in its composition makes it difficult for pathogens to develop resistance. Each propolis sample presents a unique combination of bioactive compounds, reducing the likelihood that microorganisms can adapt to and recognize the antimicrobial agents within it.

Given the substantial evidence of propolis's antimicrobial properties and the diversity of its chemical makeup, a key question arises: which propolis samples are the most potent in inhibiting microbial growth, and what specific properties contribute to their enhanced efficacy against pathogens? By analyzing propolis samples from various regions around the world, we can identify which areas produce the most antimicrobial-rich propolis. This information could pave the way for a more targeted search for antimicrobial agents that not only have potent effects but also pose a lower risk of fostering pathogen resistance.

In this study, we surveyed bee propolis samples from different global regions to evaluate their antimicrobial activity against both Gram-positive and Gram-negative bacteria. Through this comparative analysis, we aim to determine which propolis samples exhibit the strongest antimicrobial properties and to explore the underlying factors that contribute to their effectiveness. This research could lead to the discovery of new, natural antimicrobial agents with the potential to combat infections while mitigating the growing problem of antibiotic resistance.

Materials and methods

Bacterial cultures

There were fourteen microorganisms (seven Gram positive and seven Gram-negative) that were used in the experiment. The gram positive bacteria used were *Bacillus megaterium, Micrococcus luteus, Rhodococcus rhodochrous, Saphyloccus epidermidis, Sporosarcina urease, Staphylococcus aureus* and *Streptomycs griseus,* while the Gram-negative bacteria used were *Branhamella catarrhalis, Escherichia coli, Pseudomonas aeroginosa*, *Pseudomonas putida*, *Rhodospirillum rubrum*, *Sarcina aurantiaca* and *Serratia marcesens*. These bacterial cultures were all purchased from Carolina. These cultures were inoculated into tryptic soy broth (TSB) using sterile technique and incubated for 48 hours at 37°C.

Propolis extracts

Propolis samples were obtained from eight different places around world. These places are Washington and California in the US, Australia, Latvia, Russia, and the Philippines (Quezon, Bicol and Laguna). The collected propolis sample was air-dried, weighed and macerated with absolute ethanol (10 mL for 1 g sample) a week. The macerated extract was filtered through Whatman

#41 filter paper to obtain the liquid extract to be used in the assay.

Microbial assays

Autoclaved disks comprised of filter paper soaked into each individual propolis sample extract and then left to air dry to remove the ethanol. Each microorganism was inoculated into a Petri-plate containing tryptic soy agar (TSA) using a lazy L spreader for even bacterial distribution. Each autoclave disk soak with propolis extract was placed in its appropriately labeled section on the plate that were then incubated at 37°C for 48 hours. The microbial zone of inhibition around the soaked disks were analyzed to determine the efficacy of the propolis extract in hindering bacterial growth. The extract was not effective if any growth appeared around the disk. If the zone of inhibition was less than a ½ cm, it indicated slight bacterial inhibition by the extract. If the zone of inhibition was greater than ½ cm, it denoted great bacterial inhibition. Ampicillin solution was used as the positive control while autoclaved disk soaked in ethanol was used as negative control.

Results and discussion

In the present study, the antimicrobial properties of propolis samples from different geographical regions, including Russia, Washington, Bicol, and Laguna, were assessed against both Gram-negative and Gram-positive bacteria. The results, presented in Table 1 and illustrated in Fig. 1, show that propolis is effective against a wide range of opportunistic pathogens. The data indicate that the most potent antimicrobial effects were observed with the propolis samples from Russia and Washington, which produced the largest zones of inhibition on the agar plates. These samples demonstrated strong activity against both Gram-positive and Gram-negative bacteria without showing a preference for either type, confirming the broad-spectrum nature of their antimicrobial properties.

The antimicrobial activity of propolis has been extensively documented in the literature, with numerous studies highlighting its effectiveness against a variety of microorganisms. Previous research has demonstrated that propolis exhibits significant antibacterial activity against *Staphylococcus aureus* (Krol *et al.*, 1993), *Streptococcus mutans* (Koo *et al.*, 2002), *Streptococcus pyogenes* (Bosio *et al.*, 2000), *Salmonella* spp. (Orsi *et al.*, 2005), anaerobic bacteria in the human oral cavity (Santos *et al.*, 2002), and other pathogens, including *Mycobacterium* species (Bankova *et al.*, 2000). The underlying mechanism of propolis's antimicrobial activity is complex, involving at least 200 different compounds identified in various propolis samples. These compounds include fatty acids, phenolic acids and esters, flavonoids, terpenes, β-steroids, aromatic aldehydes and alcohols, sesquiterpenes, naphthalene, and stilbene derivatives (Walker and Crane, 1987; Greenway *et al.*, 1991; Bankova *et al.*, 2000). Among these, phenolic compounds, particularly flavonoids such as pinocembrin, galangin, and pinobanksin, have been frequently cited as key contributors to the antimicrobial activity of propolis (Krol *et al.*, 1993; Burdock, 1998; Castaldo and Capasso, 2002).

Specifically, the propolis from Russia and Washington exhibited significant inhibition against seven microorganisms, surpassing the performance of other samples. This aligns with previous studies that have highlighted the powerful antimicrobial effects of flavonoid-rich propolis, as these compounds are known to disrupt microbial cell membranes, inhibit enzyme activity, and interfere with bacterial metabolism (Wang, 2000). The strong inhibition observed with these samples suggests that their flavonoid content, particularly pinocembrin and galangin, may be responsible for their heightened antimicrobial activity.

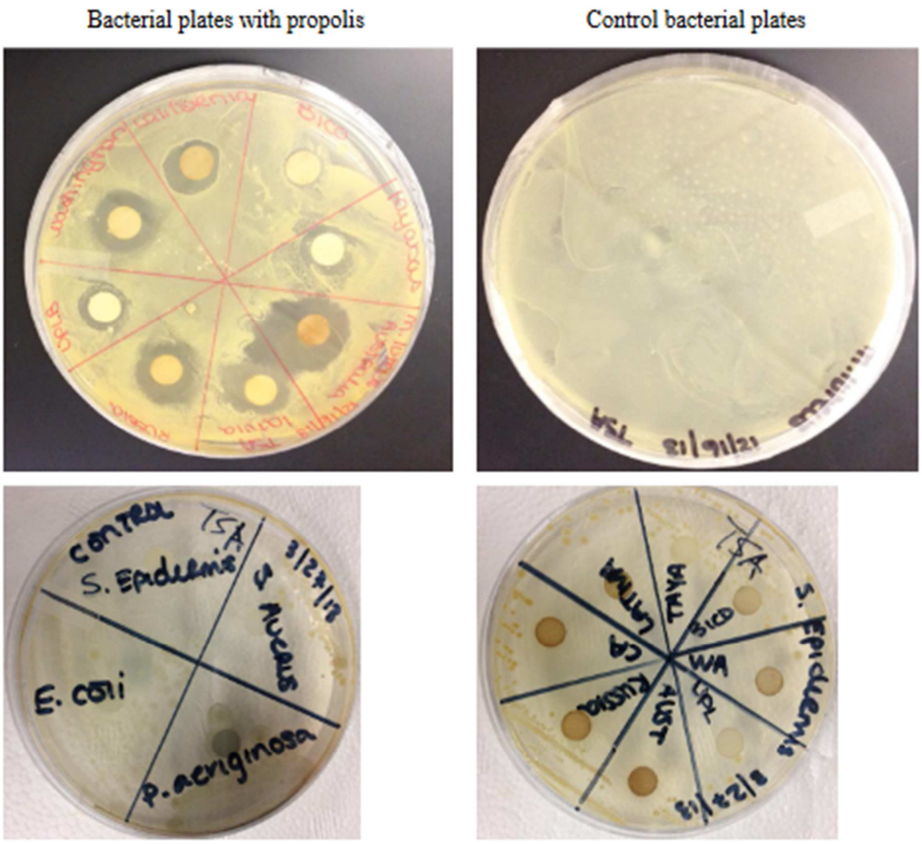

Fig. 1. Antimicrobial activity of propolis samples on microorganisms used in this study

The propolis sample from Bicol also showed substantial antimicrobial activity, inhibiting six different microorganisms (Table 1). However, it exhibited only slight inhibition against four additional microorganisms, suggesting a slightly narrower spectrum of activity compared to the Russian and Washington samples. This could be attributed to variations in the chemical composition of the Bicol propolis, potentially reflecting differences in the local flora and environmental conditions that influence the types and concentrations of bioactive compounds present.

The propolis from Laguna demonstrated significant inhibition against five microorganisms, with slight inhibition observed in four others. While this sample was not as potent as those from Russia and Washington, it still exhibited a notable level of antimicrobial activity, reinforcing the idea that propolis from different regions possesses unique antimicrobial properties due to its diverse chemical composition. This diversity in activity underscores the importance of regional factors in shaping the biological properties of propolis, as the variation in local plant sources directly impacts the types and concentrations of bioactive compounds available to the bees.

In terms of microbial resistance, certain bacteria, such as *Serratia marcescens*, *Rhodococcus rhodochrous*, and *Staphylococcus aurantiaca*, were notably resistant to the propolis samples, showing minimal to no inhibition. This resistance could be due to the inherent biological characteristics of these microorganisms, such as robust cell walls or efflux mechanisms that reduce the efficacy of antimicrobial agents. The resistance observed in these strains highlights the need for further research to understand the specific mechanisms by which certain bacteria resist propolis, and whether higher concentrations or alternative formulations of propolis could overcome this resistance.

Table 1. Antimicrobial activity of different propolis samples against Gram-positive and Gram-negative bacteria. (Propolis sample used: 1- California; 2- Washington; 3- Australia; 4- Latvia; 5-Russia; 6-Bicol; 7-Laguna; 8- Quezon)

Microorganisms	Propolis samples							
	1	2	3	4	5	6	7	8
Gram-positive								
B. megaterium	+	+	+	+	+	–	+	–
M. luteus	++	++	++	++	++	–	+	+
R. rhodochrous	–	–	–	–	–	–	–	–
S. epidermidis	–	++	+	++	–	–	–	–
S. urease	+	++	++	+	++	+	++	–
S. aureus	–	–	+	–	–	+	–	+
S. griseus	–	++	++	–	++	++	++	++
Gram-negative								
B. catarrhalis	++	++	++	++	++	+	++	–
E. coli	–	++	–	–	++	–	++	++
P. aeruginosa	++	+	++	+	++	+	+	-
P. putida	+	-	+	-	+	-	+	-
R. rubrum	++	++	++	++	++	++	++	-
S. aurantiaca	-	-	-	-	-	-	-	-
S. marcesens	-	-	-	-	-	-	-	-

++ shows significant microbial inhibition

\+ shows slight microbial inhibition

– shows no microbial inhibition

Interestingly, the study found that Gram-positive bacteria were generally more susceptible to inhibition by propolis compared to Gram-negative bacteria. This is consistent with existing literature, which often reports greater sensitivity of Gram-positive bacteria to phenolic compounds due to their simpler cell wall structure, which lacks the outer membrane present in Gram-negative bacteria (Harborn and Williams, 2000). Specifically, four Gram-positive bacteria - *Streptomyces griseus*, *Micrococcus luteus*, *Sporosarcina ureae*, and *Staphylococcus epidermidis* - were effectively inhibited by various propolis samples. Conversely, four Gram-negative bacteria - *Rhodospirillum rubrum*, *Branhamella catarrhalis, Escherichia coli*, and *Pseudomonas aeruginosa* -were also affected by the propolis extracts, albeit to varying degrees. This differential susceptibility suggests that while propolis is broadly effective, certain structural features of bacteria, such as the presence of an outer membrane in Gram-negative bacteria, may influence the degree of inhibition.

A crucial observation from this study is that no single propolis sample demonstrated a clear preference for either Gram-negative or Gram-positive organisms. Despite variations in the level of inhibition, all samples were capable of inhibiting both types of bacteria to some extent. This finding supports the potential of propolis as a versatile antimicrobial agent, capable of addressing a wide range of bacterial infections. The broad-spectrum activity of propolis, coupled with its ability to inhibit both Gram-positive and Gram-negative bacteria, highlights its potential application in developing new antimicrobial therapies.

These results contribute to the growing body of evidence supporting the use of propolis as an effective antimicrobial agent. The study also emphasizes the importance of continuing research into the diverse biological activities of propolis from different regions. By exploring the full spectrum of microbial species affected by propolis, and by analyzing the specific chemical components responsible for its antimicrobial effects, researchers can better understand how to harness the potential of propolis in medical and environmental applications.

Moreover, the development of environmentally friendly antibacterial agents derived from propolis presents a significant opportunity to address the challenges posed by chemically synthesized antibiotics, which contribute to environmental degradation and the rise of antibiotic-resistant bacteria. The diverse chemical composition of propolis, which includes compounds that act synergistically, reduces the likelihood of microbial resistance developing. This is particularly important in the current global context, where the overuse and misuse of antibiotics have led to an alarming increase in resistant strains. Propolis, with its natural origin and multifaceted antimicrobial properties, represents a promising alternative to traditional antibiotics.

Conclusion

Results of this study underscore the significant potential of propolis as a natural antimicrobial agent. The propolis samples from Russia, Washington, Bicol, and Laguna have all demonstrated varying degrees of effectiveness against a range of microorganisms, with the Russian and Washington samples showing particularly strong activity. These findings suggest that propolis could be developed into a viable alternative to synthetic antimicrobial agents, offering a sustainable and effective solution to combating bacterial infections while mitigating the environmental impact and reducing the risk of antibiotic resistance. Further research into the chemical composition and biological activities of propolis will be essential in unlocking its full potential for medical and environmental applications.

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